Medical Genetics Summaries

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Medical Genetics Summaries is a growing collection of summaries which describe the impact that specific sequence variations have on health. The summaries review genetic variants that underlie inherited conditions, affect the risk of developing a disease in the future, or influence how an individual may respond to a specific drug.
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Introduction

Laura Dean, MD

Medical Genetics Summaries (MGS) is a collection of articles that feature conditions with a genetic component, for which information useful at the point of care is limited. Topics fall into two broad categories: diseases and drug responses.

The intended audience of MGS is clinicians who seek practical, evidence-based information to use in clinical care settings. The summaries are guideline-driven, drawn from authoritative sources, undergo a formal review process, and are regularly updated.

Genetic variants and disease

Pitt-Hopkins syndrome has a clear genetic component. A variant in the TCF4 gene results in the syndrome, and genetic testing of the TCF4 gene confirms the diagnosis. However, for many other diseases, the underlying genetics is complex. For example, although schizophrenia is highly heritable, many genes have been implicated as contributing to the disease, and genetic testing is not currently available.

A person’s blood group is determined by genetics—the four common blood groups (A, B, AB, and O) are encoded by ABO alleles. Serological testing is commonly used to determine an individual’s blood type, e.g., before receiving a blood transfusion. However, in other settings, genetic testing may be used to determine an individual’s ABO genotype, such as in the research setting, e.g., investigating the associations between ABO blood groups and the risk of diseases such as pancreatic cancer and thromboembolic disease.

Genetic variants and drug responses

There is often a wide variability in how different individuals respond to standard doses of the same drug. This is because a drug response can be influenced by age, gender, drug-drug interactions, drug-food interactions, comorbidity, liver and renal function, pregnancy, and genetic factors. For an increasing number of drugs, genetic testing (also known as pharmacogenetic testing) can be used to optimize drug therapy.

Currently, about 10% of drug labels approved by the U.S. Food and Drug Administration (FDA) contain pharmacogenetic information. However, actionable information on genetic variants can be hard to find, and sources often differ in their recommendations. MGS draws together information from different authoritative sources to one place, and includes a summary—thus providing accessible information at the point of care.

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Corresponding author.
To avoid confusion, only generic drug names are used. Nomenclature tables include both the official and commonly used terms for alleles, and phenotypes are termed “drug responses”, e.g., omeprazole drug response. Finally, each summary links to the NIH’s Genetic Testing Registry, which provides information about laboratories that offer genetic tests and details about the tests, including ordering information.

**Genetic testing to ensure the drug has a therapeutic target**

A small number of drugs are prescribed after genetic testing has been performed. One reason for this is that the drug is effective for specific genotypes. These drugs include trastuzumab—a chemotherapy agent only indicated for specific tumors that overexpress HER2, and maraviroc—an antiviral agent that is only indicated for a specific strain of the HIV virus (CCR-5 trophic HIV-1).

**Genetic testing can help avoid idiosyncratic drug reactions**

Another reason for genetic testing is to avoid severe, and potentially fatal, drug reactions. A category of drug reactions are idiosyncratic—they are unpredictable, severe, and not related to the dose and duration of the drug therapy.

The FDA recommends that all individuals be screened for the HLA-B*57:01 allele before starting treatment with abacavir, a drug used in the treatment of HIV. This is because around 6% of Caucasians of European origin carry this variant allele, placing them at high risk of abacavir-induced hypersensitivity reaction. Symptoms include fever, rash, and acute respiratory symptoms.

**An individual’s ancestry may be important**

For the epilepsy drug carbamazepine, the FDA states that patients with ancestry in “genetically at-risk populations” should be screened for the presence of HLA-B*15:02 prior to initiating treatment. Carriers of this variant, which is most commonly found in individuals of Han Chinese descent, are at a high risk of developing Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)—both potentially fatal conditions—during carbamazepine therapy.

Also common in individuals with Han Chinese ancestry is the HLA-B*58:01 allele, which is strongly associated with severe cutaneous adverse reactions (SCAR) triggered by allopurinol therapy, which is used to treat gout.

**A wide range of gene variants are associated with idiosyncratic drug reactions**

Idiosyncratic drug reactions are not just limited to variant HLA-B alleles. For the antibiotic gentamicin, genetically predisposed individuals who carry a variant in a mitochondrial gene (MT-RNR1) may suffer from irreversible hearing loss after just a single dose of gentamicin. And for individuals who require treatment with thiopurines (e.g., azathioprine), the FDA recommends TPMT genotyping or phenotyping prior to treatment. This is because patients who carry two non-functional TPMT alleles universally experience life-threatening myelosuppression when treated with thiopurines.
**Genetic testing can help optimize the drug dose**

Drug labels always provide standard dosing information. But a growing number of labels also include recommendations for adjusting the dose, or selecting an alternative drug, based on a patient’s genotype (if known). Generally, dose adjustment is recommended for variants in genes that are known to influence drug metabolism, leading to altered plasma levels of active drugs and metabolites.

**Cytochrome P450 (CYP) genes influence drug levels**

The “CYP” gene family encodes enzymes that metabolize over a quarter of commonly prescribed drugs. One of these genes, CYP2D6, is particularly complex. Over 100 variants are known, many of which encode enzymes with different levels of activity. Depending on the level of CYP2D6 activity, individuals may respond poorly to the analgesics codeine and tramadol. A standard dose of codeine may provide inadequate pain relief in some, and severe toxicity, such as respiratory depression, in others.

In addition, standard doses of a wide range of drugs (e.g., atomoxetine—used in ADHD, venlafaxine—an antidepressant, clozapine—an antipsychotic, and tamoxifen—used to treat breast cancer) will lead to higher than expected active drug plasma levels in individuals who have low or absent CYP2D6 activity. This can increase the risk of side effects, and may contribute to non-compliance and treatment failure.

**Barriers to genetic testing**

Ordering a genetic test to help determine whether a particular drug will be effective or safe is a relatively new area for doctors and genetic counselors. The field is rapidly evolving, evidenced by an increasing panorama of genetic tests becoming available. And there are potential legal concerns, such as a cause for liability in cases where the optimal dose of a drug was not given. Education and training are needed.

More prospective randomized trials are needed to investigate the clinical outcomes when drug therapy or a specific dose is selected on the basis of genotype. The effectiveness data can be used for cost-effectiveness analysis, and be summarized into actionable clinical guidelines with prescribing recommendations.

Sometimes, genetic testing has not been possible because of the acute nature of the clinical scenario (e.g., gentamicin and neonatal sepsis). However, as technology improves and turn-around time is reduced, the use of genetic testing can be expected to increase.

For example, clopidogrel is an antiplatelet agent that is used in patients presenting with acute coronary syndrome, and patients who may need to undergo percutaneous intervention. Because clopidogrel is a pro-drug, it must first be metabolized by CYP2C19 before it becomes effective. However, in the 3% of Caucasians and 15 to 20% of Asians who have low or absent CYP2C19 activity, clopidogrel will have a smaller or no effect on platelet function. Fortunately, the advent of “bedside testing” and a faster turn-around of results means that more of these patients can be identified and offered alternative antiplatelet agents.
The use of genetic testing is often not clear-cut

In the case of warfarin, the FDA-approved drug label provides a dosing table, allowing for the adjustment of initial doses of warfarin based on \textit{CYP2C9} and \textit{VKORC1} genotypes. Warfarin is an anticoagulant, given to prevent the formation of blood clots. If the dose of warfarin is too low, the risk of thrombosis remains, but if the dose is too high, there is an increased risk of bleeding. And both outcomes can be a cause of a stroke.

Despite the drug label's dosing table, it is thought that less than 1% of patients commence warfarin therapy with their \textit{CYP2C9} and \textit{VKORC1} genotypes known. Interestingly, however, the most recent evidence suggests that \textit{CYP2C9} and \textit{VKORC1} variants may have less of an effect on warfarin levels than previously thought, with many other clinical factors having more of an impact.

The future

Genetic testing is important—it can help avoid drug toxicity and help optimize drug efficacy. As the number of genetic tests grows, \textit{Medical Genetics Summaries} will expand to help ensure that healthcare providers have the information they need to provide evidence-based care.

Helpful Links

- NIH Genetic Testing Registry
- FDA Table of Pharmacogenomic Biomarkers in Drug Labeling
- CPIC Dosing Guidelines
Genetic variants and drug responses
Abacavir Therapy and *HLA-B*57:01 Genotype

Laura Dean, MD

Created: September 1, 2015.

Introduction

Abacavir is a nucleoside reverse transcriptase inhibitor used in the treatment of human immunodeficiency virus (HIV) infection. It is used in combination with other medications as part of highly active antiretroviral therapy (HAART).

The human leukocyte antigen B (HLA-B) plays an important role in how the immune system recognizes and responds to pathogens. HLA-B belongs to a class of molecules that are found on the surface of most cells. These molecules are responsible for presenting peptides to immune cells. Peptides derived from normal human proteins are recognized as such, whereas foreign peptides derived from pathogens trigger an immune response.

Abacavir specifically interacts with HLA-B*57:01 and alters the repertoire of self peptides that are presented to T lymphocytes, which activates an immune reaction known as a hypersensitivity reaction. Around 6% of Caucasians of European origin have the variant allele, *HLA-B*57:01, and this places them at high risk of having a hypersensitivity reaction to abacavir (1-5). The association between *HLA-B*57:01 and abacavir hypersensitivity has also been found in Hispanics and individuals with African origins (6).

The FDA recommends screening for the *HLA-B*57:01 allele in all patients before starting abacavir therapy, and before restarting abacavir therapy in a patient who has previously tolerated the drug if their *HLA-B*57:01 status is unknown (7). The Clinical Pharmacogenetics Implementation Consortium (CPIC) also recommends that *HLA-B*57:01 screening should be performed (see Table 1) (8, 9).

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### Table 1. HLA-B phenotypes and the therapeutic recommendations for abacavir therapy

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Examples of diplotypes</th>
<th>Phenotype</th>
<th>Therapeutic recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncarrier of HLA-B*57:01</td>
<td>*X/*Xb</td>
<td>Low or reduced risk of abacavir hypersensitivity. Found in ~94% of patients.</td>
<td>Use abacavir per standard dosing guidelines</td>
</tr>
<tr>
<td>Carrier of HLA-B*57:01</td>
<td>*57:01/*Xb *57:01/</td>
<td>Significantly increased risk of abacavir hypersensitivity. Found in ~6% of patients.</td>
<td>Abacavir is not recommended</td>
</tr>
<tr>
<td></td>
<td>*57:01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The strength of therapeutic recommendations is "strong".

*HLA-B*, human leukocyte antigen B

* X, any HLA-B genotype other than HLA-B*57:01

* Xb, any HLA-B genotype other than HLA-B*57:01

Table is adapted from Martin M.A. et al. *Clinical pharmacogenetics implementation consortium guidelines for HLA-B genotype and abacavir dosing*. Clinical pharmacology and therapeutics. 2012;91(4):734–8 (8).

### Drug: Abacavir

Abacavir is a nucleoside reverse transcriptase inhibitor (NRTI) that targets HIV. It inhibits the conversion of the viral genome from RNA to DNA, thus suppressing the ability of the virus to insert its viral DNA into the host cell's genome.

Abacavir is a nucleoside analog. It is phosphorylated by intracellular enzymes to form the active metabolite carbovir triphosphate, which is an analog of deoxyguanosine-5’-triphosphate (dGTP). Carbovir triphosphate competitively inhibits HIV reverse transcriptase by competing with its natural substrate (dGTP) to be incorporated into viral DNA. Once incorporated, the nucleoside analog terminates DNA chain elongation, preventing further synthesis of viral DNA (10).

During the first 6 weeks of treatment with abacavir, around 5-8% of patients develop a hypersensitivity reaction. Symptoms include fever, rash, fatigue, gastrointestinal symptoms (e.g., nausea, vomiting, abdominal pain) and acute respiratory symptoms (e.g., cough and dyspnea). If a hypersensitivity reaction is suspected, treatment must be stopped immediately. Usually symptoms worsen if treatment is not stopped, and improve within 24 hours after treatment is stopped. Re-introduction of abacavir in a patient who had a prior hypersensitivity reaction is contraindicated due to the risk of severe symptoms, life-threatening hypotension, and death (11). Data from the PREDICT-1 study suggest that 100% of individuals with immunologically confirmed (abacavir patch test positive) abacavir hypersensitivity present within 3 weeks of initial dosing (12).

Cytotoxic (CD8+) T cells mediate the hypersensitivity reaction to abacavir. It is thought that short peptide fragments, derived from either the drug or its metabolites, form a peptide-HLA complex, specifically with HLA-type HLA-B*57:01. This complex activates the T cell to release inflammatory cytokines, signaling the start of the hypersensitivity response. More recently, it has been shown that abacavir might occupy a space below the region of HLA that presents peptides—this may lead to altered peptide presentation and
trigger an autoimmune reaction. Whatever immune mechanism(s) are involved, the hypersensitivity reaction to abacavir is thought to be maintained over the lifetime of an individual (13).

A significant body of work has now accumulated to shed light on the specificity of HLA-B*57:01 in the immunopathogenesis of abacavir hypersensitivity (14-17). The crystal structure of abacavir bound to peptide and HLA-B*57:01 has recently been solved by independent investigators (1, 3). Approximately 45% of those carrying HLA-B*57:01 are tolerant of abacavir and ongoing research is studying the immunopathogenesis of HLA-B*57:01 positive abacavir tolerance (12).

**Gene: HLA-B*57:01**

The HLA-B gene is a member of the major histocompatibility complex (MHC) gene family, which includes more than 200 genes. The HLA region has been subdivided into 3 subgroups: Class I, Class II, and Class III.

The class I region contains the “classical” HLA molecules, HLA-A, HLA-B, and HLA-C. These molecules are expressed on the surfaces of almost all cells and play an important role in processing and presenting of antigens. The Class I region also contains a variety of other genes, many of which are not known to be involved in immune function.

An important role of HLA-B is to present peptide fragments to immune cells (CD8+ T cells). Most of these peptides originate from the normal breakdown of normal cell proteins (“self”). However, if foreign peptide fragments are presented, e.g., from a pathogen, the CD8+ T cells will recognize the peptides as “non-self” and be activated to release inflammatory cytokines and launch an immune response.

Because the HLA genes need to present such a wide variety of “self” and “non-self” peptides, the HLA genes are both numerous and highly polymorphic—more than 1,500 HLA-B alleles have been identified (8). Variations in the HLA genes play an important role in determining susceptibility to autoimmune disease and infections; they are also critical in the field of transplant surgery where the donor and recipient must be HLA-compatible.

The HLA-B*57:01 allele is associated with an increased risk of hypersensitivity reaction to abacavir. The allele is co-dominant, so an individual needs to carry only one copy of the HLA-B*57:01 allele to be at risk. The US FDA recommendations for screening for the presence of HLA-B*57:01 before initiating abacavir therapy and several studies support that this has significantly reduced the incidence of abacavir-induced hypersensitivity (18). To-date, HLA-B*57:01 screening has had a 100% negative predictive value for abacavir hypersensitivity (confirmed by a positive skin patch test), since hypersensitivity has only been found in individuals carrying HLA-B*57:01 (12, 19).

HLA-B*57:01 has also been linked to an increased risk of liver damage in individuals taking flucloxacillin, an antibiotic that is no longer available in the US. Genotype screening is not routinely done because this adverse reaction is rare (<1 in 5,000) and the
positive predictive value of HLA-B*57:01 carriage for development of flucloxacillin associated hepatitis is significantly less than 1% (20).

In addition to its role in hypersensitivity reactions, HLA-B*57:01 has an important role in HIV infection. In Caucasians with HIV, HLA-B*57:01 has been linked with a lower viral load set point (the amount of viral RNA detected in blood during the asymptomatic phase of HIV infection) (21). In addition, HLA-B*57:01 has been overrepresented in a small group of individuals who have HIV which has not progressed to AIDS, despite lack of treatment with antiretroviral therapy (22).

The frequency of the HLA-B*57:01 allele varies significantly by population (6). It is relatively common in European populations (6–7%). Approximately 2-3% of African American and admixed American populations carry HLA-B*57:01; however, it is uncommon in homogenous South-Asian and African populations, being absent in some African populations, and in the Japanese. The allele is most common in Northern Thai and Indian populations (up to 20%) (8).

**Genetic Testing**

Genetic testing is available for HLA-B*57:01 through commercial laboratories in the US that typically offer single allele testing with a short turnaround time. The genotype results are either “positive” (HLA-B*57:01 being present in one or both copies of the HLA-B gene) or “negative” (no copies of HLA-B*57:01 are present). There are no intermediate phenotypes because HLA-B is expressed in a codominant manner (8).

**Therapeutic Recommendations based on Genotype**

This section contains excerpted¹ information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**Statement from the US Food and Drug Administration (FDA):**

Serious and sometimes fatal hypersensitivity reactions have been associated with abacavir. Hypersensitivity to abacavir is a multi-organ clinical syndrome usually characterized by a sign or symptom in 2 or more of the following groups: (1) fever, (2) rash, (3) gastrointestinal (including nausea, vomiting, diarrhea, or abdominal pain), (4) constitutional (including generalized malaise, fatigue, or achiness), and (5) respiratory (including dyspnea, cough, or pharyngitis). Discontinue abacavir as soon as a hypersensitivity reaction is suspected.

¹ The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
Patients who carry the *HLA-B*5701 allele are at high risk for experiencing a hypersensitivity reaction to abacavir. Prior to initiating therapy with abacavir, screening for the *HLA-B*5701 allele is recommended; this approach has been found to decrease the risk of hypersensitivity reaction. Screening is also recommended prior to reinitiation of abacavir in patients of unknown *HLA-B*5701 status who have previously tolerated abacavir. *HLA- B*5701-negative patients may develop a suspected hypersensitivity reaction to abacavir; however, this occurs significantly less frequently than in *HLA-B*5701-positive patients.

Regardless of *HLA-B*5701 status, permanently discontinue abacavir if hypersensitivity cannot be ruled out, even when other diagnoses are possible.

Following a hypersensitivity reaction to abacavir, NEVER restart abacavir or any other abacavir-containing product because more severe symptoms can occur within hours and may include life-threatening hypotension and death.

Reintroduction of abacavir or any other abacavir-containing product, even in patients who have no identified history or unrecognized symptoms of hypersensitivity to abacavir therapy, can result in serious or fatal hypersensitivity reactions. Such reactions can occur within hours.

Please review the complete therapeutic recommendations that are located here (7).

**Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC):**

*HLA-B*57:01 screening should be performed in all abacavir-naive individuals before initiation of abacavir-containing therapy. In abacavir-naive individuals who are *HLA-B*57:01- positive, abacavir is not recommended (see Table 1) and should be considered only under exceptional circumstances when the potential benefit, based on resistance patterns and treatment history, outweighs the risk. *HLA-B*57:01 genotyping is widely available in the developed world and is considered the standard of care prior to initiating abacavir.

Patients testing negative for *HLA-B*57:01 also have a 3% risk of developing a clinically diagnosed hypersensitivity reaction, and standard practice includes patient counseling and careful monitoring for signs and symptoms of a hypersensitivity reaction. The development of signs and symptoms of a hypersensitivity reaction warrants that serious consideration be given to discontinuing abacavir, regardless of the HLA-B genotyping results.

Please review the complete therapeutic recommendations that are located here (8).

**Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):** To date, the association between *HLA-B*5701 genotype and the hypersensitivity reaction to abacavir remains the only example of a randomized clinical trial of pharmacogenetics. The advice regarding selection of an alternative drug for treating *HLA- B*5701-positive patients is in
agreement with the recommendations of the Food and Drug Administration and the European Medicines Agency.

Please review the complete therapeutic recommendations that are located here (23).

**Nomenclature**

<table>
<thead>
<tr>
<th>Allele name</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-B*57:01</td>
<td>rs2395029 is a tag SNP for HLA-B*57:01</td>
</tr>
</tbody>
</table>

* For the MHC region, variations in genes such as HLA-B occur across the whole sequence of the gene, not a single locus. Therefore, the HLA-B*57:01 allele is defined by its sequence (GenBank: AF196183.1) rather than single coding or protein variants. If there is strong linkage disequilibrium between one or more SNPs, the presence of these SNPs (tag SNPs) may be used for HLA typing (24). In the case of HLA-B, the presence of the rs2395029 allele (a SNP in the HLA complex P5 gene) is 99.9% predictive of the presence of an HLA-B*57:01 allele (25).

Guidelines on nomenclature of the HLA system are available from HLA Nomenclature: [http://hla.alleles.org/](http://hla.alleles.org/)

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**References**


Related Summaries by Gene

Allopurinol Therapy and HLA-B*58:01 Genotype
Carbamazepine Therapy and HLA Genotypes
Phenytoin Therapy and HLA-B*15:02 and CYP2C9 Genotypes

Related Summaries by Drug Class

Maraviroc Therapy and CCR5 Genotype

Tests in GTR by Condition

Abacavir hypersensitivity

Tests in GTR by Gene

HLA-B gene
Allopurinol Therapy and HLA-B*58:01 Genotype

Laura Dean, MD

Created: March 26, 2013; Updated: March 16, 2016.

Introduction

Allopurinol is a xanthine oxidase inhibitor that decreases the production of uric acid. It is most commonly used in the management of gout and hyperuricemia (high levels of uric acid).

The human leukocyte antigen B (HLA-B) plays an important role in how the immune system recognizes and responds to pathogens. The variant HLA-B*58:01 allele is strongly associated with severe cutaneous adverse reactions (SCAR) during treatment with allopurinol. This allele is most commonly found in Asian subpopulations, notably in individuals of Korean, Han Chinese, or Thai descent (1-3).

At this time, the FDA-approved drug label does not discuss HLA-B genotype (4). However, the Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends that allopurinol should not be prescribed to patients who have tested positive for HLA-B*58:01, and that an alternative medication should be considered to avoid the risk of developing SCAR (see Table 1) (1, 2).

Table 1. HLA-B phenotypes and the therapeutic recommendations for allopurinol therapy, adapted from CPIC

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Examples of diplotypes</th>
<th>Phenotype</th>
<th>Therapeutic recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncarrier of HLA-B*58:01</td>
<td>*X/*Xb</td>
<td>Low or reduced risk of allopurinol-induced SCAR</td>
<td>Use allopurinol per standard dosing guidelines</td>
</tr>
</tbody>
</table>

The strength of therapeutic recommendations is “strong” (1).

HLA-B, human leukocyte antigen B
SCAR, severe cutaneous adverse reaction
*X, any HLA-B genotype other than HLA-B*58:01
*Xb, any HLA-B genotype other than HLA-B*58:01


Table 1. continues on next page...
Table 1. continued from previous page.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Examples of diplotypes</th>
<th>Phenotype</th>
<th>Therapeutic recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier of HLA-B*58:01</td>
<td>*5801/*Xb, *5801/5801</td>
<td>Significantly increased risk of allopurinol-induced SCAR</td>
<td>Allopurinol is contraindicated</td>
</tr>
</tbody>
</table>

The strength of therapeutic recommendations is “strong” (1).

*HLA-B*, human leukocyte antigen B

SCAR, severe cutaneous adverse reaction

*X*, any HLA-B genotype other than HLA-B*58:01

*Xb*, any HLA-B genotype other than HLA-B*58:01


**Drug: Allopurinol**

Allopurinol is a commonly prescribed drug for the management of gout and hyperuricemia. Uric acid is produced by the breakdown of purine nucleotides, and high concentrations of uric acid can lead to gout and uric acid kidney stones.

Allopurinol is an analogue of the purine hypoxanthine. Allopurinol decreases the production of uric acid by inhibiting xanthine oxidase, which catalyzes the conversion of hypoxanthine and xanthine to uric acid. In addition, allopurinol facilitates the incorporation of hypoxanthine and xanthine into DNA and RNA, and the resulting increase in nucleotide concentration leads to a feedback inhibition of de novo purine synthesis, which in turn leads to a decrease in uric acid levels (5).

Allopurinol is rapidly oxidized in the liver to the active metabolite oxypurinol, which also inhibits xanthine oxidase. Allopurinol has a short plasma half-life of ~1-2 hours, whereas oxypurinol has a half-life of ~15 hours. After the rapid oxidation of allopurinol, any remaining drug is promptly filtered and excreted by the kidneys. However, after oxypurinol is filtered by the kidneys, it is reabsorbed in a manner similar to how uric acid is reabsorbed. Therefore, it is thought that the effective inhibition of xanthine oxidase over a 24-hour period after a single dose of allopurinol is largely brought about by the effects of oxypurinol (4).

In general, allopurinol is well tolerated; however, allopurinol is one of the most common causes of severe cutaneous adverse reactions (SCAR), and the HLA-B*58:01 allele is strongly associated with allopurinol-induced SCAR.

**Allopurinol-induced Adverse Drug Reactions**

In general, there are two categories of adverse drug reactions. Type A reactions account for up to 85-90% of all adverse drug reactions. They are predictable based on the known properties of the drug, and they can affect any individual, if their exposure to the drug is
high enough. For allopurinol, one of the most common type A adverse effects is an acute attack of gout after starting allopurinol therapy (4).

Type B reactions account for the remaining 10-15% of adverse drug reactions. These include hypersensitivity reactions that occur in susceptible individuals. Such idiosyncratic hypersensitivity reactions can occur at any dose and develop through a mechanism that is unrelated to the mechanism of action of the drug. For this reason, it is difficult to predict in whom a drug-induced hypersensitivity reaction is likely to occur.

Severe cutaneous adverse reactions are type B reactions, which include Stevens-Johnson syndrome (SJS), or the more severe toxic epidermal necrolysis (TEN); as well as drug reaction with eosinophilia and systemic symptoms (DRESS), and allopurinol hypersensitivity syndrome (AHS).

Allopurinol is the most common cause of SJS/TEN in Europe (6). SJS/TEN are life-threatening conditions that are primarily characterized by lesions of the skin (detachment of the epidermis) and mucus membranes (severe erosions). SJS/TEN is also associated with fever, raised white cell count, hepatitis, and acute renal failure.

The underlying mechanisms for allopurinol-induced SCARs remain unclear, but cytotoxic T cells (CD8+ T cells) are involved. In the case of allopurinol, although the presence of HLA-B*58:01 substantially increases the risk of SCAR, it is not an absolute requirement, indicating that other variables also contribute to its etiology (1, 7).

One theory, known as the p-I concept, is that there is a direct pharmacological reaction of the drug (e.g., allopurinol) with the immune receptors (activated drug-specific T cells) and this provides an initial signal to induce T-cell activation and trigger a T cell–mediated hypersensitivity reaction. The signal may be strengthened by the additional interaction with HLA molecules (e.g., HLA-B*58:01) (7-11).

Although allopurinol induced-SCAR is rare (the risk is estimated to be 0.1–0.4%), allopurinol is one of the most serious causes of SCAR, which carries a mortality rate of up to 25% (1, 2).

The FDA-approved dose of allopurinol for the management of gout or hyperuricemia is to start with a daily dose of 100 mg, and titrate the dose upwards to a maximum daily dose of 800 mg, until the uric acid concentrations are less than 6.0 mg/dl. Allopurinol is often prescribed in doses that may be too low to achieve a therapeutic goal, an approach taken in part to reduce the risk of drug hypersensitivity (12). One study has found that a lower starting dose of allopurinol may reduce the risk of allopurinol hypersensitivity syndrome (13).

**HLA Gene Family**

The human leukocyte antigen (HLA) genes are members of the MHC gene family, which includes more than 200 genes. The MHC family has been subdivided into 3 subgroups
based on the structure and function of the encoded proteins: Class I, Class II, and Class III.

The class I region contains the genes encoding the HLA molecules HLA-A, HLA-B, and HLA-C. These molecules are expressed on the surfaces of almost all immune cells and play an important role in processing and presenting antigens. The class I gene region also contains a variety of other genes, many of which are not known to be involved in immune function.

An important role of HLA class I molecules is to present peptide fragments to immune cells (CD8+ T cells). Most of these peptides originate from the breakdown of normal cellular proteins (“self”). However, if foreign peptide fragments are presented (e.g., from a pathogen), CD8+T cells will recognize the peptides as “non-self” and will be activated to release inflammatory cytokines and launch an immune response to dispose of the pathogen or foreign body (14).

Because HLA molecules need to present such a wide variety of “self” and “non-self” peptides, the HLA genes are both numerous and highly polymorphic. More than 1,500 HLA-B alleles have been identified. Each HLA allele has a name that is prefixed by HLA, followed by the gene name, an asterisk and a two digit number that corresponds to antigen specificity, and the assigned allele number (15). For example, the HLA-DRB1*13:01 allele is composed of:

- HLA: the HLA prefix (the HLA region on chromosome 6)
- DRB1: the DRB1 gene (a particular HLA gene in this region)
- 13: the allele group (historically determined by serotyping, i.e., a group of alleles that share the same serotype)
- 01: the specific HLA allele (a specific protein sequence; determined by genetic analysis).

Additional digits have recently been added to the nomenclature to discriminate alleles that do not differ in the protein amino acid sequence, but differ in their genetic sequence (i.e., due to synonymous and noncoding genetic variants).

Variation in the HLA genes plays an important role in the susceptibility to autoimmune disease and infections and they are also critical in the context of transplant surgery where better outcomes are observed if the donor and recipient are HLA-compatible (1, 2). More recently, specific HLA variants have been associated with susceptibility to adverse drug reactions, including allopurinol-induced hypersensitivity reactions.

**Gene: HLA-B**

The HLA-B*58:01 allele is associated with an increased risk of severe hypersensitivity reactions to allopurinol, such as SJS/TEN. The allele is codominant, so an individual needs to carry only one copy of the HLA-B*58:01 allele to be at increased risk.
The association between *HLA-B*58:01 and allopurinol-induced adverse effects was first discovered in the Han Chinese population, where a study found that all patients who had allopurinol-induced SJS/TEN (51/51, 100%) carried *HLA-B*58:01, compared with only 15% of the allopurinol-tolerant patients (20/135, 15%) (16).

Further studies also found an association with *HLA-B*58:01 and severe allopurinol-induced adverse effects in other populations, including Thai, Korean, European, and Japanese populations (17-19). The association is stronger in the Han Chinese than in European and Japanese populations, which is most likely due to differences in *HLA-B*58:01 allele frequencies between racial and ethnic populations (20).

The *HLA-B*58:01 allele is most common in individuals of Asian descent, with a frequency of ~10-15% in the Han Chinese, ~12% in Koreans, and ~6-8% in individuals of Thai descent (3, 21-25). The risk allele is less common among Europeans and Japanese with a frequency of only ~1-2% (26, 27).

Although the risk of SCAR due to allopurinol is generally low (0.1–0.4%) and certain populations have a low frequency of the *HLA-B*58:01 risk allele (e.g., Europeans), the risk of allopurinol-induced SCAR is substantially elevated in *HLA-B*58:01 carriers. The odds ratio for allopurinol-induced SCAR among *HLA-B*58:01 carriers in a meta-analysis was 73 using healthy controls and 165 using allopurinol-tolerant controls (5).

**Genetic Testing**

Genetic testing is available for several *HLA-B* alleles, including *HLA-B*58:01. The genotype results are either “positive” (*HLA-B*58:01 being present in one or both copies of the *HLA-B* gene) or “negative” (no copies of *HLA-B*58:01 are present). There are no intermediate phenotypes because *HLA-B* is expressed in a codominant manner (1, 2).

Several studies have looked into the cost-effectiveness of *HLA-B*58:01 testing to guide urate-lowering therapy (ULT). A 2012 American College of Rheumatology guideline recommended that prior to treatment with allopurinol, the *HLA-B*58:01 genotype of gout patients at high risk for SCARs, including Korean patients with chronic renal insufficiency, should be determined (3). One study reported that in Korean patients with kidney disease, ULT guided by *HLA-B*58:01 genotyping was less costly and more effective than treatment without genotyping, and that *HLA-B*58:01 genotyping could considerably reduce the occurrence of allopurinol-induced SCARs and related deaths (28). Cost-effectiveness analysis of treating patients with chronic gout (without additional risk factors) in Singapore and in Portugal found that *HLA-B*58:01-guided ULT was not cost-effective at this time.

A potential alternative to costly HLA genotyping, may be to test for single nucleotide variants that are tightly associated with *HLA-B*58:01. A number of variants have been found to be in linkage disequilibrium (LD) with *HLA-B*58:01, for example, the rs9263726 variant in the *PSORS1C1* gene is strongly associated with *HLA-B*58:01 in the Japanese population (20).
Therapeutic Recommendations based on Genotype

This section contains excerpted¹ information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Given the high specificity for allopurinol-induced SCAR, allopurinol should not be prescribed to patients who have tested positive for HLA-B*58:01. Alternative medication should be considered for these patients to avoid the risk of developing SCAR. For patients who have tested negative, allopurinol may be prescribed as usual (see Table 1). However, testing negative for HLA-B*58:01 does not totally eliminate the possibility of developing SCAR, especially in the European population.

Please review the complete therapeutic recommendations that are located here (1, 2).

2012 Statement from the American College of Rheumatology (ACR): Prior to initiation of allopurinol, rapid polymerase chain reaction-based HLA-B*5801 screening should be considered as a risk management component in subpopulations where both the HLA-B*5801 allele frequency is elevated and the HLA-B*5801-positive subjects have a very high hazard ratio ("high risk") for severe allopurinol hypersensitivity reaction (e.g., Koreans with stage 3 or worse chronic kidney disease and all those of Han Chinese and Thai descent).

Please review the complete therapeutic recommendations that are located here (3).

Nomenclature

<table>
<thead>
<tr>
<th>Allele name</th>
<th>Other name(s)</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-B*58:01</td>
<td>Not applicable*</td>
<td>Not applicable*</td>
<td>Not applicable*</td>
</tr>
</tbody>
</table>

* For the MHC region, variations in genes such as HLA-B occur across the whole sequence of the gene, not a single locus. Therefore, the HLA-B*58:01 allele is defined by its sequence (GenBank: EU499350.1) rather than single coding or protein variants.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Guidelines on nomenclature of the HLA system are available from HLA Nomenclature: [http://hla.alleles.org/](http://hla.alleles.org/)

¹ The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
Acknowledgments

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Version history

To view an earlier version (26 March 2013) of this summary, please click here.

References


Related Summaries by Gene
Abacavir Therapy and HLA-B*57:01 Genotype
Carbamazepine Therapy and HLA Genotypes
Phenytoin Therapy and HLA-B*15:02 and CYP2C9 Genotypes

Tests in GTR by Gene
HLA-B gene
Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype

Laura Dean, MD

Introduction

Amitriptyline is a tricyclic antidepressant used in the treatment of several psychiatric disorders, including major depression, obsessive-compulsive disorder, panic attacks, generalized anxiety disorder, post-traumatic stress disorder, and bulimia. Amitriptyline also has different off-label uses, including migraine prevention, neuropathic pain management, fibromyalgia, and enuresis (bedwetting) (1).

Tricyclic antidepressants (TCAs) primarily mediate their therapeutic effect by inhibiting the reuptake of both serotonin and norepinephrine, leaving more neurotransmitter in the synaptic cleft stimulating the neuron. Because tricyclics can also block different receptors (H1 histamine, alpha 1 α1-adrenergic, and muscarinic receptors), side effects are common. As such, more specific selective serotonin reuptake inhibitors (SSRIs) have largely replaced the use of them. However, TCAs still have an important use in specific types of depression and other conditions.

Amitriptyline is metabolized mainly via CYP2C19 and CYP2D6 pathways. Metabolism by CYP2C19 results in active metabolites, including nortriptyline, which is also a tricyclic antidepressant. Metabolism catalyzed by CYP2D6 results in the formation of the less active 10-hydroxy metabolite. Individuals who are “CYP2D6 ultrarapid metabolizers” carry more than two normal function alleles (i.e., multiple copies) (Table 1, 2), whereas “CYP2C19 ultrarapid metabolizers” carry two increased function alleles (Table 3, 4). Individuals who are CYP2D6 or CYP2C19 “poor metabolizers” carry two no function alleles for CYP2D6 or CYP2C19, respectively.

The FDA-approved drug label for amitriptyline states that CYP2D6 poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants when given usual doses. The FDA recommendations also include monitoring tricyclic antidepressant plasma levels whenever a tricyclic antidepressant is going to be co-administered with another drug known to be an inhibitor of CYP2D6 (1).

In 2016, the Clinical Pharmacogenetics Implementation Consortium (CPIC) made dosing recommendations for tricyclic antidepressants based on CYP2C19 and CYP2D6 genotypes (2). For CYP2D6 ultrarapid metabolizers, CPIC recommends avoiding the use of a tricyclic due to the potential lack of efficacy, and to consider an alternative drug not
metabolized by CYP2D6. If a TCA is still warranted, CPIC recommends considering titrating the TCA to a higher target dose (compared to normal metabolizers) and using therapeutic drug monitoring to guide dose adjustments. For CYP2D6 intermediate metabolizers, CPIC recommends considering a 25% reduction of the starting dose, and for CYP2D6 poor metabolizers, to avoid the use of tricyclics because of the potential for side effects. If a tricyclic is still warranted for CYP2D6 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations to avoid side effects.

For CYP2C19 ultrarapid metabolizers, CPIC recommends avoiding the use of tertiary amines (e.g., amitriptyline) due to the potential for a sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19, such as the secondary amines nortriptyline or desipramine. For CYP2C19 poor metabolizers, CPIC recommends avoiding tertiary amine use due to the potential for sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19. If a tertiary amine is still warranted for CYP2C19 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations while monitoring plasma concentrations to avoid side effects (2).

**Drug Class: Tricyclic Antidepressants**

Tricyclic antidepressants (TCAs) are mixed serotonin-norepinephrine reuptake inhibitors. They increase the amount of neurotransmitter in the synaptic cleft, thought to mediate their antidepressant effects.

From the 1960s to the 1980s, tricyclics were the first-line treatment for depression, until the introduction of SSRIs, which have fewer side effects and are safer. The common side effects of tricyclics include anticholinergic side effects (e.g., blurred vision, dry mouth, constipation, and sedation), cardiac effects, and orthostatic hypotension.

Today, the main therapeutic use of tricyclics is chronic pain management, such as neuropathic pain. However, tricyclics are still used in the treatment of depression as well as other psychiatric disorders, including obsessive–compulsive disorder, panic attacks, generalized anxiety disorder, post-traumatic stress disorder, bulimia nervosa, smoking cessation, and enuresis (bedwetting).

Tricyclics are named after their chemical structure of three central rings and a side chain important for its function and activity. Its structure determines whether a drug is classified a tertiary amine (amitriptyline, clomipramine, doxepin, imipramine, and trimipramine) or secondary amine (desipramine and nortriptyline).

Whereas tertiary amines are generally more potent in blocking reuptake of serotonin, the secondary amines are more potent in blocking the reuptake of norepinephrine. Secondary amines are better tolerated and are also associated with fewer anticholinergic side effects.

The CYP2C19 enzyme metabolizes tertiary amines to active metabolites, which include desipramine (the active metabolite of imipramine) and nortriptyline (the active
metabolite of amitriptyline). Both the tertiary and secondary amines are metabolized by CYP2D6 to less active metabolites.

The effectiveness and tolerability of tricyclics are affected by CYP2D6 metabolism and partially by CYP2C19 metabolism. Individuals who carry CYP2D6 or CYP2C19 variants that influence enzyme activity may be at an increased risk of treatment failure (if plasma drug levels are decreased) or drug toxicity (if plasma drug levels are increased).

**Drug: Amitriptyline**

Amitriptyline is used to relieve the symptoms of depression, with endogenous depression being more likely to respond to treatment than other depressive states (e.g., reactive depression) (1). Off-label uses of amitriptyline include migraine prevention, and the treatment of neuropathic pain, fibromyalgia, and enuresis (bedwetting).

Amitriptyline blocks the uptake of both serotonin and norepinephrine, but more potently blocks the reuptake of serotonin. Amitriptyline also has strong affinities for histamine (H1), alpha-1 adrenergic, and muscarinic (M1) receptors, which account for its side effects, including sedation, weight gain, blurred vision, dry mouth, and constipation. The intensity of these side effects tends to be greater for amitriptyline compared to other tricyclics (3).

Amitriptyline is metabolized by CYP2C19 to the active metabolite, nortriptyline, which is also a tricyclic antidepressant thought to be approximately twice as potent as other TCAs. In contrast to amitriptyline, nortriptyline blocks the reuptake of norepinephrine more potently than serotonin (3).

Because both the parent drug (amitriptyline) and the CYP2C19 metabolite (nortriptyline) are pharmacologically active compounds, the plasma levels of both drugs should monitored (4). The sum of amitriptyline plus nortriptyline plasma levels may correlate with an individual’s response to amitriptyline therapy (5).

The optimal therapeutic range for amitriptyline has been well-defined (6). Most individuals display an optimal response to amitriptyline when combined serum levels of amitriptyline and nortriptyline are between 80 and 200 ng/mL. Higher levels are associated with an increased risk of adverse events. At levels greater than 300 ng/mL, cardiac toxicity occurs. This is characterized by ECG changes (widening of QRS), which may lead to potentially fatal ventricular tachycardia. In some individuals, cardiac toxicity may occur at lower concentrations or even when they are within the recommended therapeutic range (7, 8).

Nortriptyline is metabolized by CYP2D6 to hydroxyl metabolites, which have been associated with cardiac toxicity. Safe levels of hydroxyl metabolites have not yet been defined (4).

Individuals who are carriers of certain CYP2D6 and/or CYP2C19 variants may have drug levels that are outside the therapeutic range after treated with standard doses of
amitriptyline. As a result, they may have an increased risk of toxicity (if the level of amitriptyline and its active metabolites are too high) or treatment failure (if drug levels are too low).

**Gene: CYP2D6**

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antipsychotics, analgesics, beta-blockers, and TCAs such as amitriptyline.

*CYP2D6* is highly polymorphic, with over 100 star (*) alleles described and currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (9).

*CYP2D6* is a particularly complex gene that is difficult to genotype, partly because of the large number of variants, but also because of the presence of gene deletions, duplications, and its neighboring pseudogenes. The complexity of genetic variation at this locus complicates the ability to interrogate *CYP2D6*.

There is substantial variation in *CYP2D6* allele frequencies among different populations (10). *CYP2D6*^*1* is the wild-type allele and is associated with normal enzyme activity and the “normal metabolizer” phenotype. The *CYP2D6* alleles *2*, *33*, and *35* are also considered to have normal activity.

Other alleles include no function variants that produce a non-functioning enzyme (e.g., *3*, *4*, *5*, *6*, *7*, *8*, and *12*) or an enzyme with decreased activity (e.g., *10*, *17*, *29*, and *41*) (see Table 1) (11). There are large inter-ethnic differences in the frequency of these alleles, with *3*, *4*, *5*, and *41* being more common in the Caucasian population, *17* more common in Africans, and *10* more common in Asians (12).
Table 1: 2016 Assignment of CYP2D6 phenotypes by CPIC

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Activity Score</th>
<th>Genotypes</th>
<th>Examples of diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6 ultrarapid metabolizer</td>
<td>Greater than 2.0</td>
<td>An individual carrying duplications of functional alleles</td>
<td>((^*/1))(\times N) ((*1/*2))(\times N) ((*2/*2))(\times N)(\text{b})</td>
</tr>
<tr>
<td>(approximately 1–20% of patients)(\text{a})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6 normal metabolizer</td>
<td>1.0 – 2.0(\text{c})</td>
<td>An individual carrying two normal function alleles or two decreased function alleles or one normal and no function allele or one normal function and decreased function allele or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0</td>
<td>(*1/*1) (*1/*2) (*2/*2) (*1/*9) (*1/*41) (*41/*41) (*1/*5) (*1/*4)</td>
</tr>
<tr>
<td>(approximately 72–88% of patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6 intermediate metabolizer</td>
<td>0.5</td>
<td>An individual carrying one decreased function and one no function allele</td>
<td>(*4/*41) (*5/*9) (*4/*10)</td>
</tr>
<tr>
<td>(approximately 1–13% of patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6 poor metabolizer</td>
<td>0</td>
<td>An individual carrying two no function alleles</td>
<td>(*4/*4) (*4/*4\times N) (*3/*4) (*5/*5) (*5/*6)</td>
</tr>
<tr>
<td>(approximately 1–10% of patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\text{a}\) For population-specific allele and phenotype frequencies, please see (2).

\(\text{b}\) Where \(xN\) represents the number of CYP2D6 gene copies (\(N\) is 2 or more).

\(\text{c}\) Patients with an activity score of 1.0 may be classified as intermediate metabolizers by some reference laboratories.

For more information about activity scores, please see the Genetic Testing section.


Individuals who are intermediate or poor metabolizers carry copies of decreased or no function CYP2D6 alleles, respectively (Table 1). Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. In these populations, only half of CYPD6 alleles are fully functional, with the reduced function *10 variant being very common (~40%, compared to ~2% in Caucasians) (13). As a result, Asians are more likely to be intermediate metabolizers than Caucasians (14). Similarly, in Africans and African Americans, only half of CYPD6 alleles are functional. However, a wider range of variants account for the remaining alleles (14-16).
Approximately 6–10% of European Caucasians and their descendants are poor metabolizers, mainly due to no function *4 and *5 alleles (14). Notably, less than 40% are homozygous normal metabolizers (carrying two copies of *1 allele) (17-19).

Individuals who are CYP2D6 poor metabolizers have higher plasma levels of amitriptyline, compared to normal metabolizers, after standard doses of amitriptyline (20). Individuals who carry at least one non-functional CYP2D6 variant have been found to be at medium to high risk of developing side effects (21).

Because standard doses of amitriptyline may lead to an increased risk of adverse events in individuals who are CYP2D6 poor metabolizers, CPIC recommends avoiding the use of amitriptyline or other tricyclic antidepressants, and to consider using an alternative drug that is not metabolized by CYP2D6. If a tricyclic is warranted, CPIC recommends considering a 50% reduction of the recommended starting dose, and they strongly recommend therapeutic drug monitoring to guide dose adjustments (4).

Individuals who have more than two copies of normal function CYP2D6 alleles are CYP2D6 ultrarapid metabolizers. The increased rate of metabolism of amitriptyline leads to less active drug being available and a poor therapeutic response. Because of the potential lack of efficacy, CPIC recommends considering an alternative drug to amitriptyline that is not metabolized by CYP2D6. If a tricyclic is warranted, CPIC recommends increasing the starting dose and using therapeutic drug monitoring to guide dose adjustments (4) (Table 2).

### Table 2. 2016 CPIC Dosing recommendations for tricyclic antidepressants based on CYP2D6 phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implication</th>
<th>Therapeutic recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6 ultrarapid metabolizer</td>
<td>Increased metabolism of TCAs to less active compounds compared to normal metabolizers</td>
<td>Avoid tricyclic use due to potential lack of efficacy. Consider alternative drug not metabolized by CYP2D6</td>
</tr>
<tr>
<td></td>
<td>Lower plasma concentrations of active drugs will increase probability of pharmacotherapy failure</td>
<td>If a TCA is warranted, consider titrating to a higher target dose (compared to normal metabolizers)(^a).</td>
</tr>
</tbody>
</table>

TCAs: Tricyclic Antidepressants
Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.

The therapeutic recommendations for amitriptyline are classified as "moderate" for intermediate CYP2D6 metabolizers and "strong" for ultrarapid, normal, and poor CYP2D6 metabolizers.

\(^a\) Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.

\(^b\) Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.


Table 2. continues on next page...
### Table 2. continued from previous page.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implication</th>
<th>Therapeutic recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6 normal metabolizer</td>
<td>Normal metabolism of TCAs</td>
<td>Utilize therapeutic drug monitoring to guide dose adjustments.</td>
</tr>
<tr>
<td>CYP2D6 intermediate metabolizer</td>
<td>Reduced metabolism of TCAs to less active compounds compared to normal metabolizers</td>
<td>Initiate therapy with recommended starting dose(^b).</td>
</tr>
<tr>
<td></td>
<td>Higher plasma concentrations of active drug will increase the probability of side effects</td>
<td>Utilize therapeutic drug monitoring to guide dose adjustments(^a).</td>
</tr>
<tr>
<td>CYP2D6 poor metabolizer</td>
<td>Greatly reduced metabolism of TCAs to less active compounds compared to normal metabolizers</td>
<td>Avoid tricyclic use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6</td>
</tr>
<tr>
<td></td>
<td>Higher plasma concentrations will increase the probability of side effects</td>
<td>If a TCA is warranted, consider a 50% reduction of recommended starting dose(^b). Utilize therapeutic drug monitoring to guide dose adjustments(^a).</td>
</tr>
</tbody>
</table>

**TCAs: Tricyclic Antidepressants**

Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.

The therapeutic recommendations for amitriptyline are classified as “moderate” for intermediate CYP2D6 metabolizers and “strong” for ultrarapid, normal, and poor CYP2D6 metabolizers.

\(^a\) Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.

\(^b\) Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.


One issue with increasing the dose of amitriptyline dose for CYP2D6 metabolizers is increasing the level of hydroxyl-metabolites, which have been associated with cardiotoxicity (22, 23). Currently, the safe range of hydroxy-metabolite plasma concentrations is not known. In addition, there are few studies on how the combination of CYP2D6 and CYP2C19 phenotypes influences an individual’s response to amitriptyline (4).

**Gene: CYP2C19**

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as several proton pump inhibitors, clopidogrel, benzodiazepines, and several tricyclic antidepressants, including amitriptyline.
The CYP2C19 gene is highly polymorphic as 35 variant star (\(*)\) alleles are currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database: (http://www.cypalleles.ki.se/cyp2c19.htm).

The CYP2C19\(^{\*1}\) wild-type allele is associated with normal enzyme activity and the “normal metabolizer” phenotype, whereas the CYP2C19\(^{\*17}\) allele is associated with increased enzyme activity and the “rapid” and “ultrarapid” metabolizer phenotypes (24).

The most common no function variant is CYP2C19\(^{\*2}\), which is characterized by c. 681G>A in exon 5 that results in an aberrant splice site and the production of a truncated and non-functioning protein. The CYP2C19\(^{\*2}\) allele frequencies are \(~15\%\) in Caucasians and Africans, and \(~29–35\%\) in Asians (24, 25).

Another commonly tested no function variant is CYP2C19\(^{\*3}\), which is characterized by c. 636G>A in exon 4 that causes a premature stop codon. The CYP2C19\(^{\*3}\) allele frequencies are \(~2–9\%\) in Asian populations, but rare in other racial groups. Other no function variants occur in less than \(1\%\) of the general population, and include CYP2C19\(^{\*4-\*8}\) (24, 25).

“CYP2C19 intermediate metabolizers” carry one copy of an allele that encodes decreased or no function (e.g. \(*1/\*2\)\)), whereas “poor metabolizers” are homozygous or compound heterozygous for two no function alleles (e.g., \(*2/\*2\, *2/\*3\) (Table 3).

### Table 3: Assignment of CYP2C19 phenotypes by CPIC

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotypes</th>
<th>Examples of diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19 ultrarapid metabolizer (approximately 2–35% of patients)(^a)</td>
<td>An individual carrying two increased function alleles</td>
<td>(^*17/*17)</td>
</tr>
<tr>
<td>CYP2C19 rapid metabolizer (approximately 2–30% of patients)</td>
<td>An individual carrying one normal function allele and one increased function allele</td>
<td>(^*1/*17)</td>
</tr>
<tr>
<td>CYP2C19 normal metabolizer (approximately 35–50% of patients)</td>
<td>An individual carrying two normal function alleles</td>
<td>(^*1/*1)</td>
</tr>
<tr>
<td>CYP2C19 Intermediate metabolizer (approximately 18–45% of patients)</td>
<td>An individual carrying one normal function and one no function allele or one no function allele and one increased function allele</td>
<td>(^*1/*2))))(b) (^*1/*3)))(b) (^*2/*17)))(b)</td>
</tr>
<tr>
<td>CYP2C19 Poor metabolizer (approximately 2–15% of patients)</td>
<td>An individual carrying two no function alleles</td>
<td>(^*2/*2))))(b) (^*2/*3)))(b) (^*3/*3)))(b)</td>
</tr>
</tbody>
</table>

\(^a\) For population-specific allele and phenotype frequencies, please see (2).

\(^b\) The predicted metabolizer phenotype for the \(^*2/\*17\) genotype is a provisional classification.

For population-specific allele and phenotype frequencies, please see (2). The predicted metabolizer phenotype for the \(^*2/\*17\) genotype is a provisional classification.

Individuals who are CYP2C19 poor metabolizers have a reduced rate of metabolism of amitriptyline compared to normal metabolizers. As a result, standard doses of amitriptyline lead to higher plasma levels of amitriptyline, lower levels of nortriptyline, and may increase the risk of side effects (20, 26-28). Therefore, for CYP2C19 poor metabolizers, CPIC recommends considering a 50% reduction of the recommended starting dose, and to use therapeutic drug monitoring to guide dose adjustments (4).

Individuals who are ultrarapid metabolizers may be at an increased risk of treatment failure and/or metabolites adverse effects. Being a carrier of the increased activity allele CYP2C19*17 is not associated with an increased level of the sum of amitriptyline plus nortriptyline levels, but the ratio is altered. A higher level of nortriptyline is seen, which may be linked to increased side effects. Therefore, for ultrarapid metabolizers, CPIC have an optional recommendation of considering using an alternative drug to amitriptyline that is not metabolized by CYP2C19, or if a tricyclic is warranted, to use therapeutic drug monitoring to guide dose adjustments (4, 26) (Table 4, Table 5).

**Table 4.** 2016 CPIC Dosing recommendations for amitriptyline based on CYP2C19 phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implication</th>
<th>Therapeutic recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19 ultrarapid metabolizer and CYP2C19 rapid metabolizer</td>
<td>Increased metabolism of tertiary amines as compared to normal metabolizers  Greater conversion of tertiary amines to secondary amines may affect response or side effects</td>
<td>Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine.  If a tertiary amine is warranted, utilize therapeutic drug monitoring to guide dose adjustments(^a).</td>
</tr>
<tr>
<td>CYP2C19 normal metabolizer</td>
<td>Normal metabolism of tertiary amines</td>
<td>Initiate therapy with recommended starting dose(^b).</td>
</tr>
<tr>
<td>CYP2C19 intermediate metabolizer</td>
<td>Reduced metabolism of tertiary amines compared to normal metabolizers</td>
<td>Initiate therapy with recommended starting dose(^b).</td>
</tr>
</tbody>
</table>

Dosing recommendations apply only to higher initial doses of amitriptyline for treatment of conditions such as depression. The therapeutic recommendations for amitriptyline are classified as “strong” for normal and intermediate CYP2C19 metabolizers, “moderate” for poor metabolizers, and “optional” for ultrarapid metabolizers.

\(^a\) Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.

\(^b\) Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implication</th>
<th>Therapeutic recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19 poor metabolizer</td>
<td>Greatly reduced metabolism of tertiary amines compared to normal metabolizers</td>
<td>Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine. For tertiary amines, consider a 50% reduction of recommended starting dose(^b). Utilize therapeutic drug monitoring to guide dose adjustments(^a).</td>
</tr>
<tr>
<td></td>
<td>Decreased conversion of tertiary amines to secondary amines may affect response or side effects</td>
<td></td>
</tr>
</tbody>
</table>

Dosing recommendations apply only to higher initial doses of amitriptyline for treatment of conditions such as depression. The therapeutic recommendations for amitriptyline are classified as “strong” for normal and intermediate CYP2C19 metabolizers, “moderate” for poor metabolizers, and “optional” for ultrarapid metabolizers.

\(^a\) Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.

\(^b\) Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.

Table 5. 2016 CPIC Dosing recommendations for amitriptyline based on both CYP2D6 and CYP2C19 phenotypes \(^a,b\)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>CYP2D6 Ultrarapid metabolizer</th>
<th>CYP2D6 Normal metabolizer</th>
<th>CYP2D6 Intermediate metabolizer</th>
<th>CYP2D6 Poor metabolizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19 ultrarapid or rapid metabolizer</td>
<td>Avoid amitriptyline use(^c) Classification of recommendation(^d): Optional</td>
<td>Consider alternative drug not metabolized by CYP2C19(^c,e) Classification of recommendation(^d): Optional</td>
<td>Consider alternative drug not metabolized by CYP2C19(^c,e) Classification of recommendation(^d): Optional</td>
<td>Avoid amitriptyline use(^c) Classification of recommendation(^d): Optional</td>
</tr>
<tr>
<td>CYP2C19 normal metabolizer</td>
<td>Avoid amitriptyline use. If amitriptyline is warranted, consider titrating to a higher target dose (compared to normal metabolizers)(^f,g) Classification of recommendation(^d): Strong</td>
<td>Initiate therapy with recommended starting dose(^h) Classification of recommendation(^d): Strong</td>
<td>Consider a 25% reduction of recommended starting dose(^f,h) Classification of recommendation(^d): Moderate</td>
<td>Avoid amitriptyline use. If amitriptyline is warranted, consider a 50% reduction of recommended starting dose(^f,h) Classification of recommendation(^d): Strong</td>
</tr>
<tr>
<td>CYP2C19 intermediate metabolizer</td>
<td>Avoid amitriptyline use(^c) Classification of</td>
<td>Initiate therapy with recommended starting dose(^h)</td>
<td>Consider a 25% reduction of recommended</td>
<td>Avoid amitriptyline use. If amitriptyline is warranted,</td>
</tr>
</tbody>
</table>

\(^a\) Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.

\(^b\) The dosing recommendations are based on studies focusing on amitriptyline. Because tricyclic antidepressants have comparable pharmacokinetic properties, it may be reasonable to apply these guidelines to other tertiary amines including clomipramine, doxepin, imipramine and trimipramine (the classification of this recommendation is optional).

\(^c\) If amitriptyline is warranted, utilize therapeutic drug monitoring to guide dose adjustment.

\(^d\) The rating scheme for the recommendation classification is described in Supplementary Data (2). See CYP2D6 and CYP2C19 combined dosing recommendations for explanation of classification of recommendations for this table.

\(^e\) TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine.

\(^f\) Utilizing therapeutic drug monitoring if a tricyclic is prescribed to a patient with CYP2D6 ultrarapid, intermediate or poor metabolism in combination with CYP2C19 ultrarapid, intermediate or poor metabolism is strongly recommended.

\(^g\) Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.

\(^h\) Patients may receive an initial low dose of TCAs, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.

Table 5. continued from previous page.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>CYP2D6 Ultrarapid metabolizer</th>
<th>CYP2D6 Normal metabolizer</th>
<th>CYP2D6 Intermediate metabolizer</th>
<th>CYP2D6 Poor metabolizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>recommendation</td>
<td>Optional</td>
<td>Classification of</td>
<td>starting dose</td>
<td>consider a 50% reduction</td>
</tr>
<tr>
<td>d</td>
<td></td>
<td>recommendation</td>
<td>of recommended starting dose</td>
<td>of recommended starting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d</td>
<td>d</td>
<td>dose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Strong</td>
<td>Optional</td>
<td></td>
</tr>
<tr>
<td>CYP2C19 poor metabolizer</td>
<td>Avoid amitriptyline use</td>
<td>Avoid amitriptyline use</td>
<td>Avoid amitriptyline use</td>
<td>Avoid amitriptyline use</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>Optional</td>
<td>Classification of</td>
<td>Classification of</td>
<td>Classification of</td>
</tr>
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<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>Optional</td>
<td>Optional</td>
<td>Optional</td>
<td>Optional</td>
</tr>
</tbody>
</table>

a Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.

b The dosing recommendations are based on studies focusing on amitriptyline. Because tricyclic antidepressants have comparable pharmacokinetic properties, it may be reasonable to apply these guidelines to other tertiary amines including clomipramine, doxepin, imipramine and trimipramine (the classification of this recommendation is optional).

c If amitriptyline is warranted, utilize therapeutic drug monitoring to guide dose adjustment.

d The rating scheme for the recommendation classification is described in Supplementary Data (2). See CYP2D6 and CYP2C19 combined dosing recommendations for explanation of classification of recommendations for this table.

e TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine.

f Utilizing therapeutic drug monitoring if a tricyclic is prescribed to a patient with CYP2D6 ultrarapid, intermediate or poor metabolism in combination with CYP2C19 ultrarapid, intermediate or poor metabolism is strongly recommended.

g Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.

h Patients may receive an initial low dose of TCAs, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.


Genetic Testing

Clinical genotyping tests are available for many CYP2D6 and CYP2C19 alleles. The NIH's Genetic Testing Registry (GTR) provides a list of test providers for “amitriptyline response,” and the CYP2D6 and CYP2C19 genes.
Results are typically reported as a diplotype, such as CYP2D6 *1/*1. A result for copy number, if available, is also important when interpreting CYP2D6 results (29). However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (30).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- A normal (previously referred to as “extensive”) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2 (2, 31)

**Therapeutic Recommendations based on Genotype**

This section contains excerpted\(^1\) information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**2016 Statement from the US Food and Drug Administration (FDA):** The biochemical activity of the drug metabolizing isozyme cytochrome P450 2D6 (debrisoquin hydroxylase) is reduced in a subset of the caucasian population (about 7 to 10% of Caucasians are so called “poor metabolizers”); reliable estimates of the prevalence of reduced P450 2D6 isozyme activity among Asian, African and other populations are not yet available. Poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants (TCAs) when given usual doses. Depending on the fraction of drug metabolized by P450 2D6, the increase in plasma concentration may be small, or quite large (8 fold increase in plasma AUC of the TCA).

In addition, certain drugs inhibit the activity of this isozyme and make normal metabolizers resemble poor metabolizers. An individual who is stable on a given dose of TCA may become abruptly toxic when given one of these inhibiting drugs as concomitant therapy. The drugs that inhibit cytochrome P450 2D6 include some that are not metabolized by the enzyme (quinidine; cimetidine) and many that are substrates for P450 2D6 (many other antidepressants, phenothiazines, and the Type 1C antiarrythmics propafenone and flecainide). While all the selective serotonin reuptake inhibitors (SSRIs), e.g., fluoxetine, sertraline, and paroxetine, inhibit P450 2D6, they may vary in the extent

\(^1\) The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
of inhibition. The extent to which SSRI-TCA interactions may pose clinical problems will depend on the degree of inhibition and the pharmacokinetics of the SSRI involved. Nevertheless, caution is indicated in the coadministration of TCAs with any of the SSRIs and also in switching from one class to the other. Of particular importance, sufficient time must elapse before initiating TCA treatment in a patient being withdrawn from fluoxetine, given the long half-life of the parent and active metabolite (at least 5 weeks may be necessary).

Concomitant use of tricyclic antidepressants with drugs that can inhibit cytochrome P450 2D6 may require lower doses than usually prescribed for either the tricyclic antidepressant or the other drug. Furthermore, whenever one of these other drugs is withdrawn from co-therapy, an increased dose of tricyclic antidepressant may be required. It is desirable to monitor TCA plasma levels whenever a TCA is going to be coadministered with another drug known to be an inhibitor of P450 2D6.

Please review the complete therapeutic recommendations that are located here: (1).

2016 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC):

**CYP2D6 dosing recommendations.**

[…] The recommended starting dose of amitriptyline or nortriptyline does not need adjustment for those with genotypes predictive of CYP2D6 normal metabolism. A 25% reduction of the recommended dose may be considered for CYP2D6 intermediate metabolizers. The strength of this recommendation is classified as "moderate" because patients with a CYP2D6 activity score of 1.0 are inconsistently categorized as intermediate or normal metabolizers in the literature, making these studies difficult to evaluate.

CYP2D6 ultrarapid metabolizers have a higher probability of failing amitriptyline or nortripsyline pharmacotherapy due to subtherapeutic plasma concentrations, and alternate agents are preferred. There are documented cases of CYP2D6 ultrarapid metabolizers receiving large doses of nortriptyline in order to achieve therapeutic concentrations. However, very high plasma concentrations of the nortriptyline hydroxy-metabolite were present, which may increase the risk for cardiotoxicity. If a tricyclic is warranted, there are insufficient data in the literature to calculate a starting dose for a patient with CYP2D6 ultrarapid metabolizer status, and therapeutic drug monitoring is strongly recommended. Adverse effects are more likely in CYP2D6 poor metabolizers due to elevated tricyclic plasma concentrations; therefore, alternate agents are preferred. If a tricyclic is warranted, consider a 50% reduction of the usual dose, and therapeutic drug monitoring is strongly recommended.

**CYP2C19 dosing recommendations.**

[…] The usual starting dose of amitriptyline may be used in CYP2C19 normal and intermediate metabolizers. Although CYP2C19 intermediate metabolizers would be expected to have a modest increase in the ratio of amitriptyline to nortripsyline plasma
concentrations, the evidence does not indicate that CYP2C19 intermediate metabolizers should receive an alternate dose.

Patients taking amitriptyline who are CYP2C19 rapid or ultrarapid metabolizers may be at risk for having low plasma concentrations and an imbalance between parent drug and metabolites causing treatment failure and/or adverse events. Although the CYP2C19*17 allele did not alter the sum of amitriptyline plus nortriptyline plasma concentrations, it was associated with higher nortriptyline plasma concentrations, possibly increasing the risk of adverse events. For patients taking amitriptyline, extrapolated pharmacokinetic data suggest that CYP2C19 rapid or ultrarapid metabolizers may need a dose increase. Due to the need for further studies investigating the clinical importance of CYP2C19*17 regarding tricyclic metabolism and the possibility of altered concentrations, we recommend to consider an alternative tricyclic or other drug not affected by CYP2C19. This recommendation is classified as optional due to limited available data. If amitriptyline is administered to a CYP2C19 rapid or ultrarapid metabolizer, therapeutic drug monitoring is recommended.

CYP2C19 poor metabolizers are expected to have a greater ratio of amitriptyline to nortriptyline plasma concentrations. The elevated amitriptyline plasma concentrations may increase the chance of a patient experiencing side effects. Use an alternative agent not metabolized by CYP2C19 (e.g., nortriptyline and desipramine) or consider a 50% reduction of the usual amitriptyline starting dose along with therapeutic drug monitoring.

Please review the complete therapeutic recommendations that are located here: (2).

2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

For CYP2D6 ultrarapid metabolizers:

The genetic polymorphism leads to increased metabolic capacity of CYP2D6, which may cause a decrease in the plasma concentrations of amitriptyline and its active metabolite nortriptyline and increased plasma concentrations of the active metabolites E-10-OH-amitriptyline and E-10-OH-nortriptyline.

Recommendation:

1. Choose an alternative if possible. Antidepressants that are not metabolized via CYP2D6 - or to a lesser extent - include, for example, citalopram and sertraline.
2. If an alternative is not an option: increase the dose to 1.25 times the standard dose, monitor the plasma concentrations and be alert to potential therapy failure due to decreased amitriptyline plus nortriptyline plasma concentrations and to increased plasma concentrations of the potentially cardiotoxic, active hydroxy metabolites.

For CYP2D6 intermediate metabolizers:

The genetic polymorphism leads to decreased metabolic capacity of CYP2D6, which may cause an increase in the plasma concentrations of amitriptyline and its active metabolite
nortriptyline and decreased plasma concentrations of the active metabolites E-10-OH-
amitriptyline and E-10-OH- nortriptyline.

Recommendation:

1. Choose an alternative if possible. Antidepressants that are not metabolized via CYP2D6 - or to a lesser extent - include, for example, citalopram and sertraline.
2. If an alternative is not an option: use 60% of the standard dose and monitor the plasma concentrations of amitriptyline and nortriptyline.

As side effects are related to nortriptyline plasma concentrations and the efficacy to amitriptyline plus nortriptyline plasma concentrations, which are influenced to a lesser extent by CYP2D6, it is not known whether it is possible to reduce the dose to such an extent that the side effects disappear, but the efficacy is maintained.

For CYP2D6 poor metabolizers:

The genetic polymorphism leads to decreased metabolic capacity of CYP2D6, which may cause an increase in the plasma concentrations of amitriptyline and its active metabolite nortriptyline and decreased plasma concentrations of the active metabolites E-10-OH-
amitriptyline and E-10-OH- nortriptyline.

Recommendation:

1. Choose an alternative if possible. Antidepressants that are not metabolized via CYP2D6 - or to a lesser extent - include, for example, citalopram and sertraline.
2. If an alternative is not an option: use 50% of the standard dose and monitor the plasma concentrations of amitriptyline and nortriptyline.

As side effects are related to nortriptyline plasma concentrations and the efficacy to amitriptyline plus nortriptyline plasma concentrations, which are influenced to a lesser extent by CYP2D6, it is not known whether it is possible to reduce the dose to such an extent that the side effects disappear, but the efficacy is maintained.

Please review the complete therapeutic recommendations that are located here: (32).

Nomenclature

Nomenclature for selected CYP2D6 alleles

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Coding</td>
<td>Protein</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>1846G&gt;A</td>
<td>NM_000106.5:c.506-1G&gt;A</td>
<td>Not applicable - variant occurs in a non-coding region</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td></td>
<td>Not applicable - variant results in a whole gene deletion</td>
<td></td>
</tr>
</tbody>
</table>

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
### Nomenclature for selected continued from previous page

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*6</td>
<td>1707 del T Trp152Gly</td>
<td>NM_000106.5:c.454delT</td>
<td>rs5030655</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>100C&gt;T Pro34Ser</td>
<td>NM_000106.5:c.100C&gt;T</td>
<td>rs1065852</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>Includes at least two functional variants*: 1023C&gt;T (Thr107Ile) 2850C&gt;T (Cys296Arg)</td>
<td>NM_000106.5:c.320C&gt;T NM_000106.5:c.886T&gt;C</td>
<td>rs28371706 rs16947</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>2988G&gt;A</td>
<td>NM_000106.5:c.985+39G&gt;A</td>
<td>rs28371725</td>
</tr>
</tbody>
</table>

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

### Nomenclature for selected CYP2C19 alleles

<table>
<thead>
<tr>
<th>Common allele name</th>
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<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
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</thead>
<tbody>
<tr>
<td>CYP2C19*2</td>
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<td>NM_000769.1:c.681G&gt;A</td>
<td>rs4244285</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>636G&gt;A Trp212Ter</td>
<td>NM_000769.1:c.636G&gt;A</td>
<td>rs4986893</td>
</tr>
<tr>
<td>CYP2C19*17</td>
<td>-806C&gt;T</td>
<td>NM_000769.2:c.-806C&gt;T</td>
<td>rs12248560</td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

### Acknowledgments

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American University, Lebanon; Stuart Scott, Assistant Professor of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai; Chakradhara Rao S Uppugunduri, Maître-assistant at the CANSEARCH Laboratory, University of Geneva, Switzerland; and Mandy van Rhenen, secretary of the Dutch Pharmacogenetics Working Group (DPWG), Centre for Information on Medicines, Royal Dutch Pharmacists Association (KNMP).

References

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype


**Related Summaries by Gene**

Aripiprazole Therapy and *CYP2D6* Genotype

Atomoxetine Therapy and *CYP2D6* Genotype

Carisoprodol Therapy and *CYP2C19* Genotype

Clopidogrel Therapy and *CYP2C19* Genotype

Clozapine Therapy and *CYP2D6, CYP1A2, and CYP3A4* Genotypes

Codeine Therapy and *CYP2D6* Genotype

Diazepam Therapy and *CYP2C19* Genotype

Esomepazole Therapy and *CYP2C19* Genotype

Imipramine Therapy and *CYP2D6* and *CYP2C19* Genotype

Metoprolol Therapy and *CYP2D6* Genotype
Omeprazole Therapy and CYP2C19 Genotype
Prasugrel Therapy and CYP Genotype
Propafenone Therapy and CYP2D6 Genotype
Risperidone Therapy and CYP2D6 Genotype
Tamoxifen Therapy and CYP2D6 Genotype
Thioridazine Therapy and CYP2D6 Genotypes
Tramadol Therapy and CYP2D6 Genotype
Venlafaxine Therapy and CYP2D6 Genotype

**Related Summaries by Drug Class**
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype

**Tests in GTR by Condition**
Amitriptyline response

**Tests in GTR by Gene**
CYP2C19 gene
CYP2D6 gene
Aripiprazole Therapy and CYP2D6 Genotype

Laura Dean, MD
Created: September 22, 2016.

Introduction

Aripiprazole is an atypical antipsychotic used in the management of schizophrenia, bipolar disorder, major depressive disorder, irritability associated with autistic disorder, and treatment of Tourette's disorder.

The metabolism and elimination of aripiprazole is mainly mediated through two enzymes, CYP2D6 and CYP3A4. Approximately 8% of Caucasians, 3–8% of Black/African Americans and up to 2% of Asians cannot metabolize CYP2D6 substrates and are classified as “poor metabolizers” (1).

The FDA-approved drug label for aripiprazole states that in CYP2D6 poor metabolizers, half of the usual dose should be administered. In CYP2D6 poor metabolizers who are taking concomitant strong CYP3A4 inhibitors (e.g., itraconazole, clarithromycin), a quarter of the usual dose should be used (Table 1) (2).

Table 1. The FDA-recommended dose adjustments for aripiprazole in patients who are known CYP2D6 poor metabolizers and patients taking concomitant CYP2D6 inhibitors, CYP3A4 inhibitors, and/or CYP3A4 inducers (2016)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Dosage Adjustments for ABILIFY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known CYP2D6 Poor Metabolizers</td>
<td>Administer half of usual dose</td>
</tr>
<tr>
<td>Known CYP2D6 Poor Metabolizers taking concomitant strong CYP3A4 inhibitors (e.g., itraconazole, clarithromycin)</td>
<td>Administer a quarter of usual dose</td>
</tr>
<tr>
<td>Strong CYP2D6 (e.g., quinidine, fluoxetine, paroxetine) or CYP3A4 inhibitors (e.g., itraconazole, clarithromycin)</td>
<td>Administer half of usual dose</td>
</tr>
<tr>
<td>Strong CYP2D6 and CYP3A4 inhibitors</td>
<td>Administer a quarter of usual dose</td>
</tr>
<tr>
<td>Strong CYP3A4 inducers (e.g., carbamazepine, rifampin)</td>
<td>Double usual dose over 1 to 2 weeks</td>
</tr>
</tbody>
</table>

Table is adapted from a FDA-approved drug label for aripiprazole (2).

Drug: Aripiprazole

Aripiprazole is an atypical antipsychotic primarily used in the treatment of schizophrenia and bipolar disorder. Aripiprazole may also be used as part of the management of major depressive disorder, irritability associated with autism, and treatment of Tourette's disorder (2).

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine. Known as “first-generation” or “typical” antipsychotics, these drugs are used to treat psychosis (regardless of the cause), chronic psychotic disorders (e.g., schizophrenia), and other psychiatric conditions. However, prominent adverse effects included extrapyramidal side effects such as tardive dyskinesia, muscle rigidity, tremors, and Parkinsonian-like symptoms.

Newer antipsychotics, known as “second generation” or “atypical” antipsychotics, have a lower risk of extrapyramidal side effects such as tardive dyskinesia. However, many have serious metabolic effects. Aripiprazole is an atypical antipsychotic that is noted for having fewer metabolic side effects than other atypicals, such as clozapine, olanzapine, risperidone, and quetiapine. Other atypicals currently approved by the FDA include asenapine, brexpiprazole, cariprazine, lurasidone, paliperidone, and ziprasidone. The main action of both first-generation and second-generation antipsychotics is thought to be the post-synaptic blockade of D2 dopamine receptors. All antipsychotics, with the exception of aripiprazole, are D2 antagonists.

Aripiprazole is a partial D2 agonist. Aripiprazole binds to the D2 receptor with a high affinity similar to dopamine. However, because it has low intrinsic activity, it causes much lower activation of the receptor compared to dopamine.

The combination of a high affinity for the D2 receptor and its partial agonist activity may result in aripiprazole reducing the high-frequency firing of dopamine neurons in the brain's mesolimbic system. Overactivity in this region is thought to underlie psychosis and other positive symptoms of schizophrenia. In addition, the preservation of some D2 receptor activity in other dopamine-rich pathways in the brain (mesocortical and nigrostriatal areas) may provide more protection against extrapyramidal side effects (3, 4).

Aripiprazole also has a high affinity for the serotonin 5-HT2A receptors, where it acts as an antagonist and it moderately blocks the alpha 1 adrenergic and histamine H1 receptors, which may account for the lower incidence of orthostatic hypotension and sedation compared to other antipsychotics (5).

Adverse events to aripiprazole include increased mortality in elderly patients with psychosis caused by dementia, suicidal thoughts and behavior in children and young adults, neuroleptic malignant syndrome, and tardive dyskinesia (2).

Aripiprazole is extensively metabolized in the liver by CYP450 enzymes, mainly CYP2D6 and CYP3A4. Aripiprazole activity is thought to be primarily due to the parent drug, and to a lesser extent its major metabolite, dehydro-aripiprazole. The mean elimination half-life is about 75 hours for aripiprazole, but in individuals who have no appreciable CYP2D6 activity (poor metabolizers), the mean elimination half-life for aripiprazole is about 146 hours.

Genetic variations in the CYP2D6 gene have been found to impact serum levels of aripiprazole (6, 7). Because standard doses of aripiprazole lead to higher plasma levels of
Aripiprazole and dehydro-aripiprazole, the dose of aripiprazole should be adjusted in subjects carrying two nofunction alleles causing poor metabolizer status.

The FDA recommends that patients who are known to be CYP2D6 poor metabolizers should receive half the standard dose of aripiprazole, or a quarter of the standard dose if they are also taking medicines that strongly inhibit CYP3A4 (e.g., itraconazole, clarithromycin) (See Table 1).

A recent study substantiates the FDA recommendations by concluding that poor metabolizers should receive a reduced dose of aripiprazole (30–50% reduction). This study also suggested that individuals with increased CYP2D6 activity (ultrarapid metabolizers) may need to take an alternative antipsychotic not metabolized by CYP2D6 because of reduced drug levels (8).

The cytochrome P450 superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are highly polymorphic and can result in no, decreased, normal or increased enzyme activity.

Gene: CYP2D6

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described (9). CYP2D6*1 is the reference (or wild-type) allele encoding enzyme with normal activity. The CYP2D6*2, *33, and *35 alleles are also considered to confer normal activity (Table 2).

<table>
<thead>
<tr>
<th>Allele type</th>
<th>CYP2D6 Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td>*1xN, *2xN (xN denoting gene duplication or multiplication)</td>
</tr>
<tr>
<td>Normal</td>
<td>*1, *2, *35</td>
</tr>
</tbody>
</table>

For a detailed list of CYP2D6 alleles, please see (9).

Individuals who have more than two normal function copies of the CYP2D6 gene are “ultrarapid metabolizers,” whereas individuals who carry two normal or one normal and one decreased function allele are classified as “normal metabolizers.” Subjects with one normal and one no function allele or two decreased function alleles are categorized as “normal metabolizers” by CPIC guidelines, but have also been categorized as “intermediate metabolizers” in the literature. Subjects with one decreased and one no function allele are predicted to be intermediate metabolizers and those with two no function alleles, as mentioned above, are poor metabolizers.
The most common no function alleles include CYP2D6*3, *4, *5, and *6 (10, 11), and the most common decreased function alleles include CYP2D6*9, *10, *17, *29 and *41 (Table 2). There are large inter-ethnic differences in the frequency of these alleles. For example, CYP2D6*4 is the most common no function allele in Caucasians, but less abundant in subjects with African ancestry, and rare in Asians. In contrast, the decreased function allele CYP2D6*10 is the most common allele in Asians, and CYP2D6*17 is almost exclusively found in individuals with African ancestry (1). Consequently, the phenotype frequencies also vary substantially among the major ethnicities and may vary among populations. Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function CYP2D6*4 and *5 alleles (12, 13).

**Gene: CYP3A4**

In contrast to CYP2D6, genetic variation cannot explain CYP3A4 variability. Although 26 allelic variants are currently described, the majority have not been shown to alter CYP3A4 activity (14, 15). To date, only three no function CYP3A4 alleles, all being rare, have been identified (CYP3A4*6, CYP3A4*20 and CYP3A4*26) (16, 17). The CYP3A4*20 allele, for example, has been reported to have a frequency of about 0.2% in European Americans and 0.05% in African Americans, while it was observed at a frequency of 1.2% in Spain; notably, it reached up to 3.8% in specific Spanish regions (16). Although a decreased function allele, CYP3A4*22, has been associated with tacrolimus dose requirements (18), its clinical utility warrants further investigation.

**Genetic Testing**

Genetic testing for CYP2D6 and CYP3A4 is available. Test panels may include tests for additional genes involved in drug metabolism including aripiprazole. For tests available to predict CYP2D6 activity to optimize aripiprazole therapy (i.e., adjust dosage or opt for an alternative drug) please see the Genetic Testing Registry.

Results are typically reported as a diplotype, such as CYP2D6 *1/*1. A result for copy number, if available, is also important when interpreting CYP2D6 results (19).

**Therapeutic Recommendations based on Genotype**

*This section contains excerpted\(^1\) information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.*

\(^1\) The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
2016 Statement from the US Food and Drug Administration (FDA): Dosage adjustments are recommended in patients who are known CYP2D6 poor metabolizers and in patients taking concomitant CYP3A4 inhibitors or CYP2D6 inhibitors or strong CYP3A4 inducers (see Table 1). When the coadministered drug is withdrawn from the combination therapy, aripiprazole dosage should then be adjusted to its original level. When the coadministered CYP3A4 inducer is withdrawn, aripiprazole dosage should be reduced to the original level over 1 to 2 weeks. Patients who may be receiving a combination of strong, moderate, and weak inhibitors of CYP3A4 and CYP2D6 (e.g., a strong CYP3A4 inhibitor and a moderate CYP2D6 inhibitor or a moderate CYP3A4 inhibitor with a moderate CYP2D6 inhibitor), the dosing may be reduced to one-quarter (25%) of the usual dose initially and then adjusted to achieve a favorable clinical response.

Please review the complete therapeutic recommendations that are located here: (2).

2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): In CYP2D6 poor metabolizers, reduce the maximum dose of aripiprazole to 10 mg/day (67% of the maximum recommended daily dose).

Please review the complete therapeutic recommendations that are located here: (20).

Nomenclature

Nomenclature of selected CYP2D6 alleles

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*4</td>
<td>1846G&gt;A</td>
<td>NM_000106.5:c.506-1G&gt;A</td>
<td>rs3892097</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variant occurs in a non-coding region (splice variant causes a frameshift)</td>
<td></td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>1707 del T</td>
<td>NM_000106.5:c.454delT</td>
<td>rs5030655</td>
</tr>
<tr>
<td></td>
<td>Trp152Gly</td>
<td>NP_000097.3:p.Trp152Glyfs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• CYP2D6T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>100C&gt;T</td>
<td>NM_000106.5:c.100C&gt;T</td>
<td>rs1065852</td>
</tr>
<tr>
<td></td>
<td>(Pro34Ser)</td>
<td>NP_000097.3:p.Pro34Ser</td>
<td></td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>1023C&gt;T[1]</td>
<td>NM_000106.5:c.320C&gt;T</td>
<td>rs28371706</td>
</tr>
<tr>
<td></td>
<td>(Thr107Ile)</td>
<td>NP_000097.3:p.Thr107Ile</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Cys296Arg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[1] In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

[2] In the literature, 2850C>T is also referred to as 2938C>T.
Nomenclature of selected continued from previous page.

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*41</td>
<td>2850C&gt;T</td>
<td>NM_000106.5:c.886T&gt;C</td>
<td>NP_000097.3:p.Cys296Arg rs16947</td>
</tr>
<tr>
<td></td>
<td>2988G&gt;A</td>
<td>NM_000106.5:c.985+39G&gt;</td>
<td>Variant occurs in a non-coding region (impacts slicing). rs28371725</td>
</tr>
</tbody>
</table>

[1] In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.  
[2] In the literature, 2850C>T is also referred to as 2938C>T.

Nomenclature of selected CYP3A4 alleles

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4*6</td>
<td>17661_17662insA</td>
<td>NM_017460.5:c.830_831insA</td>
<td>NP_059488.2:p.Asp277Glufs rs4646438</td>
</tr>
<tr>
<td>CYP3A4*20</td>
<td>1461_1462insA</td>
<td>NM_017460.5:c.1461_1462insA</td>
<td>NP_001189784.1:p.Pro487Thrfs rs67666821</td>
</tr>
<tr>
<td>CYP3A4*26</td>
<td>17633C&gt;T</td>
<td>R268Stop</td>
<td></td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

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References


Related Summaries by Gene

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Aripiprazole response

Tests in GTR by Gene
CYP2D6 gene
CYP3A4 gene
Atomoxetine Therapy and CYP2D6 Genotype

Laura Dean, MD
Created: September 10, 2015.

Introduction

Atomoxetine was the first non-stimulant drug to be used in the treatment of attention-deficit hyperactivity disorder (ADHD). Atomoxetine is a selective noradrenaline reuptake inhibitor, and is part of a treatment plan for ADHD that may include other measures such as psychological, educational, and social support.

The CYP2D6 enzyme metabolizes a quarter of all prescribed drugs, including atomoxetine. Individuals who carry two nonfunctional copies of the CYP2D6 gene are known as poor metabolizers and have higher plasma concentrations of atomoxetine compared with individuals who have two copies of normal activity alleles.

The FDA states that the dose of atomoxetine may need to be adjusted in patients known to be CYP2D6 poor metabolizers (1). A guideline from The Dutch Pharmacogenetics Working Group includes the recommendation that poor metabolizers can be given the standard dose of atomoxetine, but physicians should be aware of adverse drug events. They also state that for individuals who have more than two functional gene copies of CYP2D6, i.e., individuals with so-called ultrarapid metabolizer status, physicians should either be alert to reduced efficacy with the standard dose of atomoxetine, or they should prescribe an alternative drug, such as methylphenidate or clonidine (Table 1) (2).

Table 1. CYP2D6 phenotypes and the therapeutic recommendations for atomoxetine therapy

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Recommendations for atomoxetine therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>Three or more functional gene copies</td>
<td>Insufficient data to allow calculation of dose adjustment. Be alert to reduced efficacy or select alternative drug (e.g., methylphenidate, clonidine).</td>
</tr>
<tr>
<td>Extensive metabolizer</td>
<td>Two functional gene copies</td>
<td>No recommendations.</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>One active allele and one inactive allele, or two decreased activity alleles, or one decreased activity allele and one inactive allele</td>
<td>No recommendations.</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>Two inactive alleles</td>
<td>Standard dose. Dose increase probably not necessary; be alert to adverse drug events.</td>
</tr>
</tbody>
</table>

1 NCBI; Email: dean@ncbi.nlm.nih.gov.

Table 2. Activity status of selected CYP2D6 alleles

<table>
<thead>
<tr>
<th>Allele type</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal function</td>
<td>*1, *2, *33, *35</td>
</tr>
</tbody>
</table>

For a more detailed list of CYP2D6 alleles, please see (3).

Drug: Atomoxetine

Atomoxetine is used in the treatment of attention-deficit hyperactivity disorder (ADHD), which is one of the most common childhood disorders. Symptoms include difficulty focusing and paying attention, difficulty controlling behavior, and hyperactivity. Symptoms may continue into adulthood. Atomoxetine may be used alone or in combination with behavioral treatment, as an adjunct to psychological, educational, social, and other remedial measures.

Atomoxetine was the first non-stimulant drug to be approved for use in ADHD. Atomoxetine is a selective norepinephrine reuptake inhibitor and it is thought to exert its therapeutic effect by increasing the concentration of synaptic norepinephrine. Because it is a non-stimulant, atomoxetine has the advantages of having less potential for abuse, and it is not scheduled as a controlled substance (4).

Atomoxetine is primarily metabolized through the CYP2D6 enzymatic pathway. The main metabolite, 4-hydroxyatomoxetine, is equipotent to atomoxetine as an inhibitor of the norepinephrine transport, but is found at much lower levels in the plasma (5). In individuals who lack CYP2D6 activity (poor metabolizers), 4-hydroxyatomoxetine is formed by other CYP enzymes, but at a much slower rate (1).

CYP2C19, along other CYP enzymes, forms the metabolite N-Desmethylatomoxetine. Although this metabolite has substantially less pharmacological activity compared to atomoxetine, and is present at much lower plasma concentrations, one study found that genetic polymorphisms of the CYP2C19 gene also influenced the pharmacokinetics of atomoxetine (6).

Atomoxetine has a wide therapeutic window, but the risk of adverse effects may be increased by the presence of CYP2D6 genetic variants (7-9). Common adverse effects of atomoxetine therapy include weight loss, headache, and irritability. Psychiatric side effects
may also occur; these include anxiety, depression, and the development of suicidal thoughts.

The FDA-approved drug label for atomoxetine includes a boxed warning and additional warning statements regarding the increased risk of suicidal thinking in children and adolescents treated with atomoxetine. The warning includes: “Children and teenagers sometimes think about suicide, and many report trying to kill themselves. Results from atomoxetine clinical studies with over 2200 child or teenage ADHD patients suggest that some children and teenagers may have a higher chance of having suicidal thoughts or actions. Although no suicides occurred in these studies, 4 out of every 1000 patients developed suicidal thoughts.”(1)

**Gene: CYP2D6**

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are often polymorphic and can result in no decreased or increased activity impacting drug metabolism.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. The CYP2D6 gene is highly polymorphic—one more than 100 alleles have been described (10).

CYP2D6*1 is the wild-type allele and is associated with normal enzyme activity and the normal “extensive metabolizer” phenotype. The CYP2D6 alleles *2, *33, and *35, among others, are also considered to have normal activity (11, 12).

Individuals who have multiple functional copies of the CYP2D6 gene are known as “ultrarapid metabolizers” (UM) (Table 1). Because each CYP2D6 allele contributes to the metabolism and inactivation of atomoxetine, atomoxetine may have decreased efficacy in UM individuals (2). The UM phenotype is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; up to 10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (13).

The Dutch Pharmacogenetics Working Group recommendations state that for ultrarapid metabolizers, there are insufficient data to allow for an adjusted dose to be calculated, and therefore, the physician should be alert to reduced efficacy of a standard dose of atomoxetine, or prescribe an alternative drug, such as methylphenidate or clonidine.

The most common non-functional and reduced function CYP2D6 alleles include CYP2D6*3, *4, *5, and *6 (2, 10, 11, 13-16) and CYP2D6*10, *17 and *41 (4, 12, 17-19) (Table 2). There are large inter-ethnic differences in the frequency of these alleles, with *3, *4, *5, *6, and *41 being more common in Caucasians, *17 more common in Africans, and *10 more common in Asians (20).

Individuals who are intermediate or poor metabolizers carry copies of reduced-activity or non-functioning CYP2D6 alleles (see Table 1 and 2). In these individuals, the metabolic
capacity of CYP2D6 is decreased which may result in higher levels of atomoxetine. The FDA-approved drug label for atomoxetine states that poor metabolizers of CYP2D6 have a higher exposure to atomoxetine (10-fold higher area under the cover and a 5 fold-higher peak concentration) compared to extensive metabolizers who received the same dose. The label also states that in individuals who are known to be poor metabolizers, the dose of atomoxetine should be adjusted—treatment should be initiated at 0.5mg/kg/day and only increased to the usual target dose of 1.2 mg/kg/day if symptoms fail to improve after 4 weeks and the initial dose is well tolerated (see Therapeutic Recommendations) (1). However, the Dutch Pharmacogenetics Working Group recommendations state that for poor metabolizers, “a standard dose of atomoxetine is recommended. An increase in dose is probably not necessary, but the physician should be alert to adverse drug events. (2)”

One small study of 100 children with ADHD receiving atomoxetine therapy found that the presence of at least one nonfunctional or reduced function CYP2D6 allele led to an increase in adverse effects, such as gastrointestinal problems and sleep disorders, and a late response to treatment (longer than 9 weeks). The study concluded that CYP2D6 genotyping before atomoxetine treatment may be beneficial in preventing overdosing or early cessation of treatment because of initial adverse effects (21). However, another study found genotyping to be unnecessary, because during the routine clinical management of ADHD, investigators were able to adjust the dose of atomoxetine in children and adolescents who had normal or reduced CYP2D6 activity—so that their treatment was comparable in safety and efficacy—without knowing what their CYP2D6 genotype was (22).

Poor metabolizers are commonly found in European Caucasians and their descendants (6-10%). The most common alleles in this population are the functional CYP2D6*1 and *2 alleles (70%); the remaining alleles include CYP2D6*10 and *41 conveying decreased function and the nonfunctional CYP2D6*3, *4, *5 and *6 variants that largely account for the poor metabolizer phenotype in these populations (12). About 2-5% of African Americans are poor metabolizers, due to the presence of CYP2D6*4 and *5 and a number of other nonfunctional alleles (1, 11, 15, 18, 20).

Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. In these populations, 40-60% of individuals carry CYP2D6*10, a decreased function variant (only ~2-3% of Caucasians have this allele) (23, 24). As a result, Asians are more likely to have decreased CYP2D6 activity compared to Caucasians (12). Neither the FDA-approved drug label of the Dutch Pharmacogenetic Working Group gives dosing recommendations for subjects with decreased function alleles, often classified as intermediate metabolizers.

**Genetic Testing**

CYP2D6 genetic testing is available. Usually a patient’s result is reported as a diplotype, such as CYP2D6*1/*1 or *2/*4. A result for copy number is also important when interpreting results for this gene. However, it needs to be noted that the number of
variants tested varies substantially among laboratories and there is no standardized way to report results (25).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- An extensive (normal) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2 (3, 19, 26).

**Therapeutic Recommendations based on Genotype**

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**Statement from the US Food and Drug Administration (FDA):**

Dosing adjustment for use with a strong CYP2D6 inhibitor or in patients who are known to be CYP2D6 PMs — In children and adolescents up to 70 kg body weight administered strong CYP2D6 inhibitors, e.g., paroxetine, fluoxetine, and quinidine, or in patients who are known to be CYP2D6 PMs, atomoxetine should be initiated at 0.5 mg/kg/day and only increased to the usual target dose of 1.2 mg/kg/day if symptoms fail to improve after 4 weeks and the initial dose is well tolerated.

In children and adolescents over 70 kg body weight and adults administered strong CYP2D6 inhibitors, e.g., paroxetine, fluoxetine, and quinidine, atomoxetine should be initiated at 40 mg/day and only increased to the usual target dose of 80 mg/day if symptoms fail to improve after 4 weeks and the initial dose is well tolerated.

Please review the complete therapeutic recommendations that are located here: (1)

**Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):** For individuals who are poor metabolizers, a standard dose of atomoxetine is recommended. An increase in dose is probably not necessary, but the physician should be alert to adverse drug events. For individuals who are ultrarapid metabolizers, there are insufficient data to allow for an adjusted dose to be calculated. The physician should be alert to reduced efficacy of a

---

1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

2 PMs: Poor metabolizers
standard dose of atomoxetine, or prescribe an alternative drug, such as methylphenidate or clonidine.

Please review the complete therapeutic recommendations that are located here: (2)

Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*4</td>
<td>1846G&gt;A</td>
<td>NM_000106.4:c.506-1G&gt;A</td>
<td>Not applicable—variant occurs in a non-coding region</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td>CYP2D6,DEL</td>
<td>NC_000022.10:g. (42534124_42531353)_(42521970_42519196)del</td>
<td>Not applicable—variant results in a whole gene deletion</td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>1707 del T Trp152Gly</td>
<td>NM_000106.4:c.454delT</td>
<td>NP_000097.2:p.Trp152Glyfs</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>100C&gt;T Pro34Ser</td>
<td>NM_000106.4:c.100C&gt;T</td>
<td>NP_000097.2:p.Pro34Ser</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>Includes at least two functional variants*: 1023C&gt;T (Thr107Ile) 2850C&gt;T (Cys296Arg)</td>
<td>NM_000106.4:c.320C&gt;T NM_000106.4:c.886T&gt;C</td>
<td>NP_000097.2:p.Thr107Ile NP_000097.2:p.Cys296Arg</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>2988G&gt;A</td>
<td>NM_000106.4:c.985+39G&gt;A</td>
<td>Not applicable—variant occurs in a non-coding region</td>
</tr>
</tbody>
</table>

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

Acknowledgments

The author would like to thank Andrea Gaedigk, MS, PhD, Children’s Mercy Kansas City, Director, Pharmacogenetics Core Laboratory, Division of Clinical Pharmacology, Toxicology & Therapeutic Innovation, Kansas City, Professor, School of Medicine, University of Missouri-Kansas City; and Mia Wadelius, Senior Lecturer, Uppsala University; for reviewing this summary.
References


Related Summaries by Gene
Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Aripiprazole Therapy and CYP2D6 Genotype
Clozapine Therapy and CYP2D6, CYP1A2, and CYP3A4 Genotypes
Codeine Therapy and CYP2D6 Genotype
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Metoprolol Therapy and CYP2D6 Genotype
Propafenone Therapy and CYP2D6 Genotype
Risperidone Therapy and CYP2D6 Genotype
Tamoxifen Therapy and CYP2D6 Genotype
Thioridazine Therapy and CYP2D6 Genotypes
Tramadol Therapy and CYP2D6 Genotype
Venlafaxine Therapy and CYP2D6 Genotype

Tests in GTR by Condition
Atomoxetine response

Tests in GTR by Gene
CYP2D6 gene
Azathioprine Therapy and $TPMT$ Genotype

Laura Dean, MD


Introduction

Azathioprine is an immunosuppressant that belongs to the drug class of thiopurines. It is used in combination with other drugs to prevent kidney transplant rejection and in the management of rheumatoid arthritis when other treatments have not been effective (1). In addition, off-label uses include the treatment of inflammatory bowel disease (2).

Azathioprine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), the major active metabolites. Thiopurine S-methyltransferase ($TPMT$) inactivates azathioprine, leaving less parent drug available to form TGNs.

An adverse effect of azathioprine therapy is bone marrow suppression, which can occur in any patient, is dose-dependent, and may be reversed by reducing the dose of azathioprine. However, patients who carry two nonfunctional $TPMT$ alleles universally experience life-threatening myelosuppression when treated with azathioprine, due to high levels of TGNs. Patients who carry one nonfunctional $TPMT$ allele may also be unable to tolerate conventional doses of azathioprine (3, 4).

The FDA recommends $TPMT$ genotyping or phenotyping before starting treatment with azathioprine. This allows patients who are at increased risk for toxicity to be identified and for the starting dose of azathioprine to be reduced, or for an alternative therapy to be used (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published recommendations for $TPMT$ genotype-based azathioprine dosing. These recommendations include:

- Consider an alternate agent or extreme dose reduction of azathioprine for patients with low or deficient TPMT activity. Start at 30-70% of target dose for patients with intermediate enzyme activity (see Table 1) (2-4).

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
Table 1. *TPMT* phenotypes and the therapeutic recommendations for azathioprine therapy, adapted from CPIC

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Phenotype details</th>
<th><em>TPMT</em> Genotype</th>
<th>Examples of diplotypes</th>
<th>Therapeutic recommendations for azathioprine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous wild-type (&quot;normal&quot;)</td>
<td>High enzyme activity. Found in ~86–97% of patients.</td>
<td>Two or more functional <em>TPMT</em> alleles</td>
<td>*1/*1</td>
<td>Start with normal starting dose (e.g., 2–3 mg/kg/d) and adjust doses of azathioprine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment.</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>Intermediate enzyme activity. Found in ~3–14% of patients.</td>
<td>One functional <em>TPMT</em> allele plus one nonfunctional <em>TPMT</em> allele</td>
<td>*1/*2 *1/*3A *1/*3B *1/*3C *1/*4</td>
<td>If disease treatment normally starts at the “full dose”, consider starting at 30–70% of target dose (e.g., 1–1.5 mg/kg/d), and titrate based on tolerance. Allow 2–4 weeks to reach steady state after each dose adjustment.</td>
</tr>
<tr>
<td>Homozygous variant</td>
<td>Low or deficient enzyme activity. Found in ~1 in 178 to 1–3736 patients.</td>
<td>Two nonfunctional <em>TPMT</em> alleles</td>
<td>*3A/*3A *2/*3A *3C/*3A *3C/*4 *3C/*2 *3A/*4</td>
<td>Consider alternative agents. If using azathioprine start with drastically reduced doses (reduce daily dose by 10-fold and dose thrice weekly instead of daily) and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. Azathioprine is the likely cause of myelosuppression.</td>
</tr>
</tbody>
</table>

The strength of therapeutic recommendations is “strong” for all phenotypes. Table is adapted from Relling M.V. et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clinical pharmacology and therapeutics. 2011;89(3):387–91 (3, 4).

**Drug Class: Thiopurines**

Thiopurines are used as anticancer agents and as immunosuppressants in inflammatory bowel disease, rheumatoid arthritis, and other autoimmune conditions. Three thiopurines are used clinically: thioguanine, mercaptopurine, and azathioprine (a prodrug for mercaptopurine). All three agents have similar effects but are typically used for different indications. Thioguanine is most commonly used in the treatment of myeloid leukemias,
mercaptopurine is used for lymphoid malignancies, and mercaptopurine and azathioprine are used for immune conditions.

Thiopurines are either activated to form TGNs (the major active metabolite) or deactivated by TPMT. Individuals who carry two non-functional TPMT alleles (“TPMT homozygotes”) universally experience life-threatening bone marrow suppression because of high levels of TGNs when treated with conventional doses. Individuals who carry one non-functional TPMT allele (“TPMT heterozygotes”) may also be unable to tolerate conventional doses of thiopurines due to increased levels of TGNs.

**Drug: Azathioprine**

Azathioprine is an immunosuppressive agent that is used in combination with other drugs to prevent the rejection of kidney transplants. It is also used in the management of active rheumatoid arthritis (1).

An off-label use of azathioprine is in the treatment of inflammatory bowel disease (IBD). Along with the closely related drug mercaptopurine (azathioprine is metabolized to mercaptopurine), azathioprine is used as an “immunomodulator” and as a “steroid-sparing agent” in the treatment of Crohn’s disease and ulcerative colitis (2).

Azathioprine is a slow-acting drug and for IBD, it typically takes at least three months of therapy before a therapeutic effect is observed. Therefore, azathioprine is used for the induction and maintenance of IBD remission rather than as a monotherapy for acute relapses (5). Because the discontinuation of azathioprine is associated with a high rate of relapse of IBD, azathioprine is usually continued long-term if there are no adverse effects (6, 7).

The use of azathioprine or the related drug mercaptourine has been associated with a 4-fold increased risk of developing lymphoma, which does not persist after discontinuation of therapy (8, 9).

The increased risk of malignancy led to the following boxed label on the FDA-approved drug label for azathioprine:

**Malignancy:** Patients receiving immunosuppressants, including azathioprine, are at increased risk of developing lymphoma and other malignancies, particularly of the skin. Physicians should inform patients of the risk of malignancy with azathioprine. As usual for patients with increased risk for skin cancer, exposure to sunlight and ultraviolet light should be limited by wearing protective clothing and using a sunscreen with a high protection factor (1).

Like all thiopurines, azathioprine is a purine analogue, and acts as an antimetabolite by interfering with nucleic acid synthesis and inhibiting purine metabolism. Azathioprine is first metabolized to mercaptopurine, which is then activated via HPRT1 (hypoxanthine phosphoribosyltransferase). This is followed by a series of reactions to form TGNs. The cytotoxicity of azathioprine is due, in part, to the incorporation of TGNs into DNA.
Inactivation of azathioprine occurs via two different pathways, via methylation (by TPMT) or via oxidation (by xanthine oxidase). TPMT activity is highly variable in patients because of genetic polymorphism in the TPMT gene.

One of the most frequent adverse reactions to azathioprine is myelosuppression, which can occur in any patient, and can usually be reversed by decreasing the dose of azathioprine. However, all patients who carry two nonfunctional TPMT alleles (approximately 0.3%) experience life-threatening myelosuppression after starting treatment with conventional doses of azathioprine due to high levels of TGNs.

Individuals who are heterozygous for nonfunctional TPMT alleles (approximately 10%) are at a significantly higher risk for toxicity than individuals with two functional alleles. However, some of these individuals, approximately 40–70%, can tolerate the full dose of azathioprine. This may be because heterozygous-deficient individuals have lower concentrations of less active metabolites, such as MeMPN (methylmercaptopurine nucleotides), than homozygous-deficient individuals (3, 4).

Approximately 90% of individuals have normal TPMT activity with two functional alleles; however, all individuals receiving azathioprine require close monitoring (3, 4, 10, 11). One study reports that in patients with IBD receiving thiopurine therapy, TPMT polymorphisms are associated with the overall incidence of adverse reactions and with bone marrow toxicity, but not with other adverse reactions, such as liver damage and pancreatitis. Therefore, although determining TPMT genotype is helpful before initiating therapy, regular blood tests to monitor for side effects are needed during therapy (12, 13).

The other azathioprine inactivation pathway is via oxidation, which is catalyzed by xanthine oxidase. If this pathway is inhibited, for example, in patients taking allopurinol (an inhibitor of xanthine oxidase), the decreased break down of azathioprine can lead to azathioprine toxicity (13). However, some studies have found that the co-administration of allopurinol, with a reduced dose of azathioprine (or mercaptopurine), can help optimize the treatment response in patients with IBD (14, 15).

Gene: TPMT

The TPMT gene encodes one of the important enzymes of phase II metabolism, thiopurine S-methyltransferase. TPMT is one of the main enzymes involved in the metabolism of thiopurines, such as azathioprine. TPMT activity is inherited as a co-dominant trait, as the TPMT gene is highly polymorphic with over 40 reported variant alleles (16-19).

The wild-type TPMT*1 allele is associated with normal enzyme activity. Individuals who are homozygous for TPMT*1 (TPMT normal metabolizers) are more likely to have a typical response to azathioprine and a lower risk of myelosuppression. This accounts for the majority of patients (~86–97%) (3, 4).

Individuals who are TPMT poor (approximately 0.3%) or intermediate (approximately 3–14%) metabolizers carry variant TPMT alleles that encode reduced or absent enzyme
activity. Three variant TPMT alleles account for over 90% of the reduced or absent activity TPMT alleles (16, 17):

- TPMT*2 (c.238G>C)
- TPMT*3A (c.460G>A and c.719A>G)
- TPMT*3B (c.460G>A)
- TPMT*3C (c.719A>G)

The frequency of TPMT alleles varies among different populations. In the United States, the most common low-activity allele in the Caucasian population is TPMT*3A (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently (18, 19).

In East Asian, African-American, and some African populations, the most common variant is TPMT*3C (~2%), although TPMT*8 may be more common in African populations than previously thought (~2%). In general, TPMT*2 occurs much less commonly, and TPMT*3B occurs rarely (18, 20).

Genetic Testing

Genetic testing is available for several TPMT variant alleles, which most commonly includes TPMT*2, *3A, and *3C as they account for >90% of inactivating alleles. Of note, rare and/or previously undiscovered variants will not be detected by variant-specific genotyping methods (3, 4, 21-24).

TPMT phenotype enzyme activity testing is also available by measuring TPMT activity in red blood cells directly. However, the results will not be accurate in patients who have received recent blood transfusions (13) and TPMT activity will also be falsely low in patients with leukemia, because of atypical hematopoiesis (25).

One study reported that TPMT genotyping was more reliable than phenotyping in identifying patients at risk of adverse reactions from thiopurine treatment (26). In addition, several studies report that the TPMT genotype is a better indicator than TPMT activity for predicting TGN accumulation or treatment outcome (11, 27-29).

Therapeutic Recommendations based on Genotype

This section contains excerpted¹ information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

¹ The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.
2015 Statement from the US Food and Drug Administration (FDA): TPMT TESTING CANNOT SUBSTITUTE FOR COMPLETE BLOOD COUNT (CBC) MONITORING IN PATIENTS RECEIVING AZATHIOPRINE. TPMT genotyping or phenotyping can be used to identify patients with absent or reduced TPMT activity. Patients with low or absent TPMT activity are at an increased risk of developing severe, life threatening myelotoxicity from azathioprine if conventional doses are given. Physicians may consider alternative therapies for patients who have low or absent TPMT activity (homozygous for non-functional alleles). Azathioprine should be administered with caution to patients having one non-functional allele (heterozygous) who are at risk for reduced TPMT activity that may lead to toxicity if conventional doses are given. Dosage reduction is recommended in patients with reduced TPMT activity. Early drug discontinuation may be considered in patients with abnormal CBC results that do not respond to dose reduction.

Please review the complete therapeutic recommendations that are located here: (1).

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Testing for TPMT status is recommended prior to starting azathioprine therapy so that the starting dosages can be adjusted accordingly—see Table 1 for dosing recommendations. In homozygous variant individuals, either an alternative agent should be used, or the doses of azathioprine should be drastically reduced. In heterozygous individuals, depending on the disease being treated, starting doses should be reduced. In both patient groups, a longer period of time should be left after each dose adjustment to allow for a steady state to be reached.

Please review the complete therapeutic recommendations that are located here: (3, 4).

Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
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</thead>
<tbody>
<tr>
<td>TPMT*2</td>
<td>238G&gt;C Ala80Pro</td>
<td>NM_000367.2:c.238G&gt;C</td>
<td>NP_000358.1:p.Ala80Pro rs1800462</td>
</tr>
<tr>
<td>TPMT*3A</td>
<td>This allele contains two variants in cis: c.460G&gt;A and c.719A&gt;G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPMT*3B</td>
<td>460G&gt;A Ala154Thr</td>
<td>NM_000367.2:c.460G&gt;A</td>
<td>NP_000358.1:p.Ala154Thr rs1800460</td>
</tr>
<tr>
<td>TPMT*3C</td>
<td>719A&gt;G Tyr240Cys</td>
<td>NM_000367.2:c.719A&gt;G</td>
<td>NP_000358.1:p.Tyr240Cys rs1142345</td>
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</tbody>
</table>

The TPMT Nomenclature Committee defines the nomenclature and numbering of novel TPMT variants: http://www.imh.liu.se/tpmtalleles

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines
Acknowledgments

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The Clinical Pharmacogenetics Implementation Consortium: http://www.pharmgkb.org/page/cpic

Version History

To view an earlier version of this summary (Update: March 18, 2013), please click here.

References


Related Summaries by Gene
Mercaptopurine Therapy and TPMT Genotype
Thioguanine Therapy and TPMT Genotype

Related Summaries by Drug Class
Mercaptopurine Therapy and TPMT Genotype
Thioguanine Therapy and TPMT Genotype

Tests in GTR by Condition
Azathiprine response

Tests in GTR by Gene
TPMT gene
Capecitabine Therapy and \textit{DPYD} Genotype

Laura Dean, MD\textsuperscript{1}

Created: September 15, 2016.

**Introduction**

Capecitabine is a chemotherapy agent that belongs to the drug class of fluoropyrimidines. It is widely used in the treatment of colon cancer, metastatic colorectal cancer, and metastatic breast cancer. Capecitabine is a prodrug that is enzymatically converted to its active form, fluorouracil, which acts as an antimetabolite to slow tumor growth.

The \textit{DPYD} gene encodes dihydropyrimidine dehydrogenase (DPD), an enzyme that catalyzes the rate-limiting step in fluorouracil metabolism. Individuals who are carriers of non-functional \textit{DPYD} variants, such as \textit{DPYD*2A}, may not be able to metabolize capecitabine at normal rates, and are at risk of potentially life-threatening capecitabine toxicity, such as bone marrow suppression and neurotoxicity. The prevalence of DPD deficiency in Caucasians is approximately 3\%-5\%.

The FDA-approved drug label for capecitabine states that no capecitabine dose has been proven safe in patients with absent DPD activity, and that there is insufficient data to recommend a specific dose in patients with partial DPD activity as measured by any specific test (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published dosing recommendations for fluoropyrimidines (capecitabine, fluorouracil, and tegafur) based on \textit{DPYD} genotype (2) (Table 1). CPIC recommends using an alternative drug for patients who are “poor metabolizers”. These individuals carry two copies of non-functional \textit{DPYD} variants and typically have complete DPD deficiency. CPIC also recommends considering a 50\% reduction in starting dose for “intermediate metabolizers”. These individuals carry a combination of a normal-function and a non-functional variant and typically have reduced DPD activity (approximately 50\% reduced) (2, 3).

**Drug Class: Fluoropyrimidines**

Fluoropyrimidines are a class of antimetabolite drugs that are widely used in the treatment of cancer. Currently, there are three types of fluoropyrimidines in clinical use: capecitabine, fluorouracil, and tegafur. Capecitabine and tegafur are both prodrugs of fluorouracil.

Fluoropyrimidines are thought to exert their chemotherapeutic effects in a number of ways, through several active metabolites. The main mechanism of action is thought to be

\textsuperscript{1} NCBI; Email: dean@ncbi.nlm.nih.gov.
the inhibition of thymidylate synthase, which plays an important part in the folate-homocysteine cycle, and purine and pyrimidine synthesis pathways. Also, active metabolites can be incorporated into RNA and DNA, ultimately leading to cell death (4).

Approximately 10-40% of patients develop severe and potentially life-threatening toxicity early during treatment with fluoropyrimidines (5). This typically leads to an interruption or discontinuation of potentially effective anticancer therapy, and often requires hospitalization (6).

The inter-individual variation in the occurrence and severity of adverse events in patients receiving fluoropyrimidines can be partly explained by clinical factors, such as age and sex. However, much of the variability in adverse events remains unexplained (7).

Of the genetic factors thought to contribute to fluoropyrimidine intolerance, the DPYD gene has been the most studied. This gene encodes the primary enzyme involved in breaking down fluoropyrimidines to inactive metabolites. Individuals who have a deficiency of the DPD enzyme have a significantly increased risk of suffering from severe fluoropyrimidine toxicity, and the stratification of patients on the basis of the DPYD genotype may help to prevent such adverse events (8-13)

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published genetics-based dosing recommendations for fluoropyrimidines based on DPYD genotype (Table 1).

Table 1. 2013 Recommended dosing of Fluoropyrimidines by DPD phenotype, from Clinical Pharmacogenetics Implementation Consortium (CPIC)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implications for phenotypic measures</th>
<th>Dosing recommendations</th>
<th>Classification of recommendationsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal metabolizer</td>
<td>Normal DPD activity and “normal” risk for fluoropyrimidine toxicity</td>
<td>Use label-recommended dosage and administration</td>
<td>Moderate</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>Decreased DPD activity (leukocyte DPD activity at 30–70% that of the normal population) and increased risk for severe or even fatal</td>
<td>Start with at least a 50% reduction in starting dose, followed by titration of dose based on toxicityb or</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Fluoropyrimidines: 5-fluorouracil, capecitabine, and tegafur.
DPD, dihydropyrimidine dehydrogenase.

a Rating scheme is described here (2)

b Increase the dose in patients experiencing no or clinically tolerable toxicity to maintain efficacy; decrease the dose in patients who do not tolerate the starting dose to minimize toxicities.

Table is adapted from Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical pharmacology and therapeutics.2013:94(6):640-5 (2)

Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, “Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)” (14).

Table 1. continues on next page...
Table 1. continued from previous page.

<table>
<thead>
<tr>
<th>Phenotype</th>
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<tbody>
<tr>
<td>Poor metabolizer</td>
<td>drug toxicity when treated with fluoropyrimidine drugs</td>
<td>pharmacokinetic test (if available)</td>
<td>Strong</td>
</tr>
</tbody>
</table>

Fluoropyrimidines: 5-fluorouracil, capecitabine, and tegafur.

DPD, dihydropyrimidine dehydrogenase.

<sup>a</sup> Rating scheme is described here (2)

<sup>b</sup> Increase the dose in patients experiencing no or clinically tolerable toxicity to maintain efficacy; decrease the dose in patients who do not tolerate the starting dose to minimize toxicities.


Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, “Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)” (14).

**Drug: Capecitabine**

Capecitabine is a form of chemotherapy used as an adjunct treatment for colon cancer, and as either monotherapy or part of combination therapy for metastatic colorectal cancer and metastatic breast cancer (1).

Capecitabine is an orally administered prodrug—it is converted to its active form, fluorouracil, by thymidine phosphorylase—an enzyme that tends to be found in higher concentrations in tumors compared to normal tissue and plasma. Fluorouracil is structurally similar to pyrimidines, and the enzyme that catalyzes the rate-limiting step in the breakdown of pyrimidines (DPD, dihydropyrimidine dehydrogenase) also catalyzes the rate-limiting step in 5-fluorouracil catabolism. DPD catalyzes the conversion of fluorouracil to the non-cytotoxic dihydrofluorouracil (DHFU) (15).

Symptomatic DPD deficiency is a rare autosomal recessive disorder with a wide range of symptoms, ranging from no symptoms or signs, to severe neurological problems. In affected individuals, the absent or greatly reduced DPD activity results in uracil and thymine accumulating in the blood, urine, and cerebrospinal fluid. Neurological symptoms typically manifest in early childhood and include seizures, small head size, and delayed cognitive and motor development (16).

Symptomatic DPD deficiency is typically caused by homozygous inactivation of *DPYD*; whereas individuals who are heterozygotes tend to be asymptomatic. However, all patients with less than 70% DPD activity are considered at risk for the development of severe drug toxicity when treated with fluoropyrimidine drugs.
toxicity when treated with fluoropyrimidines (17). Signs of capecitabine toxicity include severe diarrhea, severe mucositis, neutropenia, hand-foot syndrome, and neurotoxicity (1).

Approximately 3-5% of Caucasians have partial DPD deficiency and 0.2% have complete DPD deficiency (18). Currently, most patients are not screened for DPD deficiency before starting capecitabine therapy (19).

**Gene: DPYD**

The *DPYD* gene encodes the enzyme dihydropyrimidine dehydrogenase (DPD), which catalyzes the first and the rate-limiting step in the breakdown of the pyrimidine nucleotides thymine and uracil. DPD also catalyzes the rate-limiting step in the breakdown of fluoropyrimidines.

Many *DPYD* variants have been described, although only a few have been demonstrated to influence DPD enzyme activity. *DPYD*1 is the wild-type allele and is associated with normal enzyme activity. Individuals who carry two copies of *DPYD* alleles with normal activity are known as “normal metabolizers” and have fully functional DPD enzyme activity (Table 2 and Table 3). Next to *DPYD*1, the *DPYD* alleles *4, *5, *6, and *9A are also considered to have normal activity (20).

**Table 2. Activity status of selected DPYD Alleles**

<table>
<thead>
<tr>
<th>Allele type</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonfunctional</td>
<td>*2A, *13, rs67376798</td>
</tr>
</tbody>
</table>

Table is adapted from (12, 15) For the nomenclature of human DPYD alleles, please see (21)

The nonfunctional *DPYD* variants which have been associated with low DPD activity and an increased risk of toxicity with fluoropyrimidines include *2A, *13, and rs67376798 (15). The most well studied variant is *DPYD*2A, in which a single nucleotide substitution at the invariant splice donor site of intron 14 leads to translation skipping exon 14, resulting in the production of a truncated protein with virtually no enzyme activity.

Individuals who carry combinations of normal function, decreased function, and/or no function *DPYD* alleles are known as “intermediate metabolizers”. They have partial DPD deficiency and are at increased risk of capecitabine toxicity. And individuals who carry a combination of nonfunctional *DPYD* alleles and/or decreased function *DPYD* alleles are known as “poor metabolizers”. They have complete DPD deficiency and are at an even higher risk of capecitabine toxicity. Overall, the prevalence of individuals who are heterozygous for nonfunctional variant *DPYD* alleles (partially DPD deficient) that place them at risk of severe drug reactions is estimated to be as high as 3-5%, but this varies in different populations (5, 17, 22-25). For example, in the Dutch population, the *DPYD*2A had an allele frequency of 0.91% in Caucasians (17).
Table 3 Assignment of likely phenotype based on DPYD genotypes

<table>
<thead>
<tr>
<th>Likely phenotype</th>
<th>Functional definition</th>
<th>Genetic definition</th>
<th>Example diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal metabolizer</td>
<td>Fully functional DPD enzyme activity</td>
<td>Combinations of normal function and decreased function alleles</td>
<td>DPYD*1/*1</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>Decreased DPD enzyme activity (activity between normal and poor metabolizer)</td>
<td>Combinations of normal function, decreased function, and/or no function alleles</td>
<td>*1/*2A; *1/*13; or *1/rs67376798</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>Little to no DPD enzyme activity</td>
<td>Combination of no function alleles and/or decreased function alleles</td>
<td>*2A/*2A; 13/*13; *2/*13; or rs67376798/rs67376798</td>
</tr>
</tbody>
</table>

Table is adapted from Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical pharmacology and therapeutics. 2013:94(6):640-5 (2)

Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in the 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (14).

A recent study proposed distinguishing between the various DPYD alleles and their functionality by assigning gene activity scores. The use of such scores could result in differentiated individualized dosing advice for fluororpyrimidines, which is essential for reducing toxic side effects while maintaining efficacy (12).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the DPYD gene and the capecitabine drug response. The DPYD*2A variant is the most commonly tested.

Biochemical genetic tests may also be used, which assess the level of activity of the DPD enzyme. These tests include biochemical assays such as analyte testing (e.g., measuring the amount of thymine and uracil in the urine or blood) or an enzyme assay (e.g., directly measuring the activity of DPD using RNA extracted from blood cells and measuring the DPD mRNA copy number) (2, 26, 27).

GTR provides a list of biochemical tests that assess the levels of thymine and uracil analytes, and the activity of the enzyme dihydropyrimidine dehydrogenase.

Therapeutic Recommendations based on Genotype

This section contains excerpted¹ information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Based on postmarketing reports, patients with certain homozygous or certain compound
heterozygous mutations in the $DPD^2$ gene that result in complete or near complete absence of DPD activity are at increased risk for acute early-onset of toxicity and severe, life-threatening, or fatal adverse reactions caused by capecitabine (e.g., mucositis, diarrhea, neutropenia, and neurotoxicity). Patients with partial DPD activity may also have increased risk of severe, life-threatening, or fatal adverse reactions caused by capecitabine.

Withhold or permanently discontinue capecitabine based on clinical assessment of the onset, duration and severity of the observed toxicities in patients with evidence of acute early-onset or unusually severe toxicity, which may indicate near complete or total absence of DPD activity. No capecitabine dose has been proven safe for patients with complete absence of DPD activity. There is insufficient data to recommend a specific dose in patients with partial DPD activity as measured by any specific test.

Please review the complete therapeutic recommendations that are located here: (1).

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): [...] Furthermore, patients who are heterozygous for the nonfunctional $DPYD$ variants mostly demonstrate partial DPD deficiency (leukocyte DPD activity at 30–70% that of the normal population). Thus, our recommendation is to start with at least a 50% reduction of the starting dose; followed by an increase in dose in patients experiencing no or clinically tolerable toxicity, to maintain efficacy; and a decrease in dose in patients who do not tolerate the starting dose, to minimize toxicities. An alternative is pharmacokinetic-guided dose adjustment (if available). Patients who are homozygous for $DPYD^*2A$, $^*13$, or rs67376798 may demonstrate complete DPD deficiency, and the use of 5-fluouracil or capecitabine is not recommended in these patients. Because capecitabine and tegafur are converted to 5-fluorouracil and then metabolized by DPD, the clearance of and exposure to 5-fluorouracil, in addition to its toxic effects, are similar in patients with these variants.

Please review the complete therapeutic recommendations that are located here: (2).

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1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

2 Note: the official gene symbol is DYPD. DPD is an alternate gene symbol.
Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPYD*2A</td>
<td>IVS14+1G&gt;A</td>
<td>NM_000110.3:c.1905+1G&gt;A</td>
<td>rs3918290</td>
</tr>
<tr>
<td></td>
<td>c.1905+1G&gt;A</td>
<td>NP_000101.2:p.Ile560Ser</td>
<td></td>
</tr>
<tr>
<td>DPYD*13</td>
<td>1679T&gt;G</td>
<td>NM_000110.3:c.1679T&gt;G</td>
<td>rs55886062</td>
</tr>
<tr>
<td>rs67376798</td>
<td>2846A&gt;T</td>
<td>NP_000101.2:p.Asp949Val</td>
<td>rs67376798</td>
</tr>
<tr>
<td></td>
<td>Asp949Val</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Acknowledgments

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18. Morel A., Boisdron-Celle M., Fey L., Soulie P., et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-


Related Summaries by Gene

Fluorouracil Therapy and DPYD Genotype

Related Summaries by Drug Class

Fluorouracil Therapy and DPYD Genotype

Tests in GTR by Condition

Capecitabine response
Tests in GTR by Gene

DPYD gene
Carbamazepine Therapy and HLA Genotypes

Laura Dean, MD
Created: October 14, 2015.

Introduction

Carbamazepine is an antiseizure drug used in the treatment of epilepsy. It is also used to relieve pain in trigeminal neuralgia and is used to treat bipolar disorder (1, 2).

The human leukocyte antigens A and B (HLA-A and HLA-B) play an important role in how the immune system recognizes and responds to pathogens. HLA-A and -B belong to a class of molecules that are found on the surface of most cells. These molecules are responsible for presenting peptides to immune cells. Peptides derived from normal human proteins are recognized as such, whereas foreign peptides derived from pathogens trigger an immune response whose goal is to dispose of the pathogen or foreign body.

The genes encoding HLA-A and -B are among the most polymorphic genes in the human genome, and certain variant alleles can influence an individual’s response to medication. HLA-B*15:02 is a variant allele that occurs most commonly in individuals of Southeast Asian descent. Carriers of HLA-B*15:02 are at a high risk of developing Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), a severe, and sometimes fatal, cutaneous hypersensitivity reaction, while taking carbamazepine (Table 1).

Individuals most likely to carry HLA-B*15:02 are those of Han Chinese descent, followed by those in Vietnam, Cambodia, the Reunion Islands, Thailand, India (specifically Hindus), Malaysia, and Hong Kong (3). Another HLA variant, HLA-A*31:01, which is present more globally, may also be a risk factor for other carbamazepine-induced hypersensitivity reactions, such as drug-induced hypersensitivity syndrome (HSS) or maculopapular exanthema (MPE) (2).

The FDA recommends that patients with ancestry in genetically at-risk populations should be screened for the presence of HLA-B*15:02 prior to initiating treatment with carbamazepine. Patients testing positive for the allele should not be treated with carbamazepine unless the benefit clearly outweighs the risk (1). The Clinical Pharmacogenetics Implementation Consortium (CPIC) cautions that many people may be unaware of, or fail to disclose, more distant Asian ancestry in their families, a fact that the healthcare professional needs to be aware of. CPIC also points out that both children and adults are at risk (3).

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
### Table 1. HLA-B genotype and the therapeutic recommendations for carbamazepine therapy

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotypic implications</th>
<th>Therapeutic recommendations</th>
<th>Classification of recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncarrier of HLA-B*15:02</td>
<td>Normal or reduced risk of carbamazepine-induced SJS/TEN</td>
<td>Use carbamazepine per standard dosing guidelines</td>
<td>Strong</td>
</tr>
<tr>
<td>Carrier of HLA-B*15:02</td>
<td>Increased risk of carbamazepine-induced SJS/TEN</td>
<td>If patient is carbamazepine-naive, do not use carbamazepine*</td>
<td>Strong</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If patient has previously used carbamazepine for longer than 3 months without incidence of cutaneous adverse reactions, cautiously consider use of carbamazepine</td>
<td>Optional</td>
</tr>
</tbody>
</table>

Noncarrier of HLA-B*15:02: No *1502 alleles reported, often reported as “negative” on a genotyping test. Carrier of HLA-B*15:02: One or two *1502 alleles, often reported as “positive” on a genotyping test. SJS/TEN: Stevens–Johnson syndrome/toxic epidermal necrolysis. * Alternative medications such as phenytoin, fosphenytoin, oxcarbazepine, eslicarbazepine acetate, and lamotrigine have some evidence linking SJS/TEN with the HLA-B*15:02 allele, and thus caution should be used in choosing alternatives to carbamazepine.


### Drug: Carbamazepine

Carbamazepine is an antiseizure drug used in the treatment of epilepsy. Carbamazepine is also used as analgesic in trigeminal neuralgia, and may be used in the treatment of bipolar disorder (2, 4).

Epilepsy is characterized by spontaneous recurrent epileptic seizures, which may be classified as focal or generalized. The symptoms of focal seizures depend upon where the focus of the seizure originates in the brain e.g., jerking of a limb indicates a focus in the contralateral motor cortex. In contrast, generalized seizures appear to originate in all regions of the cortex simultaneously and include absence seizures (sudden impaired consciousness and staring) and general tonic-clonic seizures (loss of consciousness, stiffening of limbs in the tonic phase, and twitching or jerking muscles in the clonic phase).

Recent guidelines for the treatment of epilepsy recommend carbamazepine as one of the first-line treatments for focal seizures in adults, adolescents, and children; and also as drug for consideration in the treatment of general tonic-clonic seizures (2, 5).
Carbamazepine is a tricyclic compound that belongs to the class of antiseizure drugs that act by blocking voltage-dependent sodium channels present on neuronal cell membranes. Carbamazepine stabilizes the sodium channel in the inactivated state, leaving fewer of the channels available to open. This prolonged inactivated phase of the channel inhibits the rapid and repetitive generation of action potentials in the epileptic focus (3, 6).

Carbamazepine is metabolized in the liver by the cytochrome P-450 (CYP) system. The major metabolite is carbamazepine-epoxide, which has an anticonvulsant activity of uncertain significance. CYP3A4 is the main enzyme involved in the metabolism of carbamazepine; a lesser role is played by CYP2C8 and possibly CYP3A5. Minor metabolic pathways include multiple CYP enzymes, such as CYP2B6.

Carbamazepine stimulates transcriptional upregulation of CYP3A4 and other genes involved in its own metabolism. In addition, there are many drug-drug interactions with carbamazepine, because numerous drugs have been shown to induce or inhibit CYP3A4, or are metabolized by CYP3A4. Therefore, when carbamazepine is given with drugs that can decrease or increase carbamazepine levels, close monitoring of carbamazepine levels is indicated and dosage adjustment may be required (7, 8).

**Carbamazepine-induced Adverse Drug Reactions**

In general, there are two categories of adverse drug reactions. Type A reactions account for up to 85-90% of all adverse drug reactions. They are predictable based on the known properties of the drug, and they can affect any individual, if their exposure to the drug is high enough. For carbamazepine, type A adverse effects include sedation, CNS depression, and vestibular symptoms such as nystagmus and ataxia.

Type B reactions account for the remaining 10-15% of adverse drug reactions. These include hypersensitivity reactions that occur in susceptible individuals. Such idiosyncratic hypersensitivity reactions can occur at any dose and develop through a mechanism that is unrelated to the mechanism of action of the drug. For this reason, it is difficult to predict in whom a drug-induced hypersensitivity reaction is likely to occur. For carbamazepine, however, carriers of specific HLA variants are known to be susceptible to carbamazepine-induced hypersensitivity reactions, and HLA testing of patients can identify those at-risk individuals so that an alternative drug can be used.

Carbamazepine-induced hypersensitivity reactions frequently involve the skin. Cutaneous adverse drug reactions (cADR) are experienced by approximately 5-10% of patients taking carbamazepine. Most of these carbamazepine-induced cutaneous reactions are considered to be mild, such as maculopapular exanthema (MPE) and erythema multiforme. Nevertheless, these cutaneous reactions can cause considerable discomfort to the patient and often lead to the discontinuation of carbamazepine therapy (2, 9, 10). Due to their structural similarity, up to 80% of patients who have an unexpected adverse reaction to carbamazepine will also have an adverse reaction to other anticonvulsants, thereby restricting treatment options (11).
More rarely, the use of carbamazepine can trigger serious hypersensitivity reactions, such as Stevens-Johnson syndrome (SJS) and the more severe form, toxic epidermal necrolysis (TEN) (12). SJS /TEN are life-threatening conditions that are primarily characterized by lesions of the skin (detachment of the epidermis) and mucus membranes (severe erosions) (12). SJS/TEN occurs in approximately 1-10 per 10,000 patients taking carbamazepine. Onset is delayed and may occur several weeks after the initiation of carbamazepine therapy. The mortality rate is high—up to 10% for SJS, and 50% for TEN (12-14).

Other severe and potentially life-threatening carbamazepine-induced hypersensitivity reactions include drug-induced hypersensitivity syndrome (HSS), also known as drug reaction with eosinophilia and systemic symptoms (DRESS); and acute generalized exanthematous pustulosis (AGEP).

The mechanisms underlying these hypersensitivity reactions are largely unknown, but they are thought to involve the drug, or a molecule derived from the drug, interacting with the major histocompatibility complex (MHC) expressed on the surface of cells, resulting in a stimulation of the immune system, particularly T cells and eosinophils (2, 14).

**HLA gene family**

The human leukocyte antigen (HLA) genes are members of the MHC gene family, which includes more than 200 genes. The MHC family has been subdivided into 3 subgroups based on the structure and function of the encoded proteins: Class I, Class II, and Class III.

The class I region contains the genes encoding the HLA molecules HLA-A, HLA-B, and HLA-C. These molecules are expressed on the surfaces of almost all cells and play an important role in processing and presenting of antigens. The class I gene region also contains a variety of other genes, many of which are not known to be involved in immune function.

An important role of HLA class I molecules is to present peptide fragments to immune cells (CD8+ T cells). Most of these peptides originate from the breakdown of normal cellular proteins (“self”). However, if foreign peptide fragments are presented, e.g., from a pathogen, CD8+ T cells will recognize the peptides as “non-self” and will be activated to release inflammatory cytokines and launch an immune response to dispose of the pathogen (or foreign body).

Because HLA molecules need to present such a wide variety of “self” and “non-self” peptides, the HLA genes are both numerous and highly polymorphic. More than 1,500 HLA-B alleles have been identified (15). Each HLA allele has a name that is prefixed by HLA, followed by the gene name, an asterisk and a two-digit number that corresponds to antigen specificity, and the assigned allele number (16). For example, for the allele HLA-DRB1*13:01 is composed of:

- HLA: the HLA prefix (the HLA region on chromosome 6)
• DRB1: the DRB1 gene (a particular HLA gene in this region)
• 13: the allele group (historically determined by serotyping, i.e., a group of alleles that share the same serotype)
• 01: the specific HLA allele (a specific protein sequence; determined by genetic analysis).

Additional digits have recently been added to the nomenclature to discriminate alleles that do not differ in the protein amino acid sequence, but differ in their genetic sequence (i.e., due to synonymous and noncoding genetic variants).

Variation in the HLA genes plays an important role in the susceptibility to autoimmune disease and infections and they are also critical in the context of transplant surgery where better outcomes are observed if the donor and recipient are HLA-compatible. More recently, HLA variants have been associated with the susceptibility to Type B adverse drug reactions, including carbamazepine hypersensitivity reactions. Specifically, two HLA variants have been found to be associated with carbamazepine-induced hypersensitivity reactions: HLA-B*15:02 and HLA-A*31:01.

**HLA-B*15:02**

The association between the HLA-B*15:02 allele and carbamazepine-induced SJS/TEN was first reported in the Han Chinese. In the initial study, every patient who had carbamazepine-induced SJS/TEN was found to be a carrier of the HLA-B*15:02 allele (44/44, 100%), whereas the allele was much less common in carbamazepine-tolerant patients (3/101, 3%) (17). In subsequent studies, this strong association was replicated, with a HLA-B*15:02 carrier frequency of between 70 and 100% among cases of carbamazepine-induced SJS/TEN (2).

The HLA-B*15:02 allele frequency is highest in Southeast Asia, and the prevalence of carbamazepine-induced SJS/TEN is higher in populations where HLA-B*15:02 is common. The HLA*15:02 allele is strongly associated with carbamazepine-induced SJS/TEN in Taiwanese, Chinese, Indians, Malay, and Chinese-Americans, but not in Caucasians or Japanese individuals (17-24).

In Hong Kong, Thailand, Malaysia, Vietnam, and parts of the Philippines, the allele frequency is over 15%; it is slightly lower (around 10-13%) in Taiwan and Singapore, and around 4% in North China. South Asians, including Indians, appear to have intermediate prevalence of HLA-B*15:02, averaging 2 to 4%, with higher frequencies in some subpopulations (1-3, 25-28).

The HLA-B*15:02 allele is rare (carrier frequency of less than 1%) in East Asia (Japan and Korea) and in individuals who are not of Asian descent. For example, the variant is very rare in Europeans, Hispanics, Africans, African Americans, and Native Americans (2, 25). The absence of this variant in these population explains the lack of association of this variant with carbamazepine-induced SJS/TEN in Caucasians and Japanese individuals.
Current data suggest that *HLA-B*15:02 is a risk factor specific to SJS/TEN since it does not appear to increase the risk of other carbamazepine-induced cutaneous reactions, such as maculopapular exanthema or the carbamazepine-induced hypersensitivity syndrome (2).

**HLA-A*31:01**

The *HLA-A*31:01 allele has been consistently associated with carbamazepine-induced hypersensitivity syndrome and maculopapular exanthema (MPE) in Europeans, Han Chinese, Japanese, and North Americans of mixed ancestries (13, 18, 29-31). This variant may also be associated with SJS/TEN but so far the association has been inconsistent (2).

Whereas the *HLA-B*15:02 allele is mainly found in individuals of Asian descent, the *HLA-A*31:01 variant is common globally with carrier frequencies of at least 3% in many populations (2-5% in Northern Europeans, 2% in Han Chinese, 9% in Japanese populations) (2, 13, 30).

**Genetic Testing**

Genetic testing is available for *HLA-B*15:02 and *HLA-A*31:01. The genotype results for an HLA allele such as *HLA-B*15:02 can either be "positive" (the HLA allele being present in one or both copies of the gene) or "negative" (no copies of HLA allele are present). There are no intermediate phenotypes because the HLA genes are expressed in a codominant manner (15).

**Therapeutic Recommendations based on Genotype**

This section contains excerpted¹ information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**Statement from the US Food and Drug Administration (FDA):** Serious and sometimes fatal dermatologic reactions, including toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS), have been reported during treatment with carbamazepine. These reactions are estimated to occur in 1 to 6 per 10,000 new users in countries with mainly Caucasian populations, but the risk in some Asian countries is estimated to be about 10 times higher. Studies in patients of Chinese ancestry have found a strong association between the risk of developing SJS/TEN and the presence of *HLA-B*1502, an inherited allelic variant of the HLA-B gene. *HLA-B*1502 is found almost exclusively in patients with ancestry across broad areas of Asia. Patients with ancestry in genetically at-risk populations should be screened for the presence of *HLA-B*1502 prior to initiating

¹ The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.
treatment with carbamazepine. Patients testing positive for the allele should not be treated with carbamazepine unless the benefit clearly outweighs the risk.

Please review the complete therapeutic recommendations from the FDA that are located here: (1).

Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Currently, the Food and Drug Administration recommends that “patients with ancestry in at-risk populations should be screened for the presence of HLA-B*1502 allele prior to starting carbamazepine”. Individuals at highest risk are those of Han Chinese descent, followed by those in Vietnam, Cambodia, the Reunion Islands, Thailand, India (specifically Hindus), Malaysia, and Hong Kong. The frequency of HLA-B*15:02 is very low in other populations. However, it is important that the prescribing physician bear in mind that many people may be unaware of or fail to disclose more distant Asian ancestry in their families. In addition, much of the evidence linking HLA-B*15:02 to SJS/TEN was generated in both children and adults. Therefore, regardless of ancestry or age of the individual, if the genetic testing results are “positive” for the presence of at least one copy of the HLA-B*15:02 allele, it is recommended that a different agent be used depending on the underlying disease, unless the benefits clearly outweigh the risk (Table 1).

Carbamazepine-induced SJS/TEN usually develops within the first 3 months of therapy; therefore, patients who have been taking carbamazepine for longer than 3 months without developing cutaneous reactions are at low risk (but not zero) of carbamazepine-induced adverse events in the future, regardless of HLA-B*15:02 status.

Please review the complete therapeutic recommendations that are located here: (3).

Recommendations from the Canadian Pharmacogenomics Network for Drug Safety (CPNDS):

Recommendation 1.1: Genetic testing for HLA- B*15:02 is recommended for all CBZ-naive patients before initiation of CBZ therapy (Level A – strong in patients originating from populations where HLA- B*15:02 is common, its frequency unknown or whose origin is unknown; Level C – optional in patients originating from populations where HLA-B*15:02 is rare). Genetic testing for HLA-A*31:01 is recommended for all CBZ-naive patients before initiation of CBZ therapy (Level B – moderate in all patients; Table 2).

Recommendation 1.2: In patients who have previously taken CBZ for > 3 months without any adverse effects, and in whom reinitiation of CBZ is considered, genetic testing is NOT recommended (B). In patients who have previously taken CBZ for a shorter period, genetic testing should be considered (B).

Recommendation 1.3: In patients who have previously experienced a HSR potentially related to CBZ, genetic testing is recommended as part of the differential diagnosis and for the direction of future therapy (B).
Recommendation 1.4: In patients for whom no alternative treatment options are available, genetic testing is recommended to ensure increased alertness to hypersensitivity symptoms in positive patients (B).

Recommendation 2.1: Genetic testing for *HLA-B*15:02 is most beneficial in patients originating from a population where *HLA-B*15:02 is common (e.g., Chinese, Thai, Indian, Malay, Filipino, Indonesian; A). Nevertheless, genotyping for *HLA-B*15:02 should be considered in ALL patients, irrespective of their ancestry, as the safest option (C).

Recommendation 2.2: *HLA-A*31:01 is common in most populations studied so far. Therefore, genetic testing for this variant is recommended in patients of all ancestries (B).

Recommendation 3.1: In patients who are positive for *HLA-B*15:02 or *HLA-A*31:01, alternative medications should be used as first-line therapy (A). Consideration in the choice of alternative medications should be given to the possibility of cross-reactivity with structurally similar AEDs (oxcarbazepine, lamotrigine, phenytoin, phenobarbital, primidone).

Recommendation 3.2: In patients who are negative for *HLA-B*15:02 and *HLA-A*31:01, CBZ can be used as first-line therapy (A). However, the occurrence of a HSR cannot be excluded based on a negative genetic test result.

**Table 2.** Grading scheme used for clinical practice recommendations

<table>
<thead>
<tr>
<th>Level</th>
<th>Strength</th>
<th>Evidence basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Strong</td>
<td>Based on strong scientific evidence; benefits clearly outweigh risks</td>
</tr>
<tr>
<td>B</td>
<td>Moderate</td>
<td>Based on reduced confidence scientific evidence and expert opinion; benefits likely to outweigh risks</td>
</tr>
<tr>
<td>C</td>
<td>Optional</td>
<td>Based mainly on expert opinion, for use with evidence development in a research context</td>
</tr>
</tbody>
</table>


Please review the complete therapeutic recommendations that are located here: (2)
Nomenclature

<table>
<thead>
<tr>
<th>Allele name</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-B*15:02</td>
<td>rs2844682 and rs3909184**</td>
</tr>
<tr>
<td>HLA-A*31:01</td>
<td>rs1061235 and rs16333021**</td>
</tr>
</tbody>
</table>

* For the MHC region, variations in genes such as HLA-B occur across the whole sequence of the gene, not a single locus. Therefore, the HLA-B*15:02 allele is defined by its sequence rather than single coding or protein variations. If there is strong linkage disequilibrium between one or more SNPs and a specific HLA allele, the presence of these SNPs (tag SNPs) may be used for HLA typing (32).

** Because of the extreme diversity at the HLA locus, different tag SNPs may be associated with different HLA variants in different populations. For HLA-B*15:02, rs2844682 and rs3909184 are the tag SNPs (33). For HLA-A*31:01, rs1061235 is a tag SNP in Europeans (13) and rs16333021 is a tag SNP in Japanese (29). A study involving North American children of various ancestries showed that rs1061235 is not a suitable tag SNP in non-Caucasian individuals (30).

Guidelines on nomenclature of the HLA system are available from HLA Nomenclature: http://hla.alleles.org/

Acknowledgments

The author would like to thank Michael A. Rogawski, Professor of Neurology, University of California, Davis; and Ursula Amstutz, Research Group Leader at the Institute of Clinical Chemistry, Inselspital University Hospital, University of Bern; and an investigator of the Canadian Institutes of Health Research Drug Safety and Effectiveness Network and the Canadian Pharmacogenomics Network for Drug Safety, for reviewing this summary.

References


Related Summaries by Gene
Abacavir Therapy and $HLA-B^*57:01$ Genotype
Allopurinol Therapy and $HLA-B^*58:01$ Genotype
Phenytoin Therapy and $HLA-B^*15:02$ and $CYP2C9$ Genotypes

Related Summaries by Drug Class
Phenytoin Therapy and $HLA-B^*15:02$ and $CYP2C9$ Genotypes

Tests in GTR by Condition
Carbamazepine response
Carbamazepine hypersensitivity

Tests in GTR by Gene
HLA-B gene
HLA-A gene
Carisoprodol Therapy and CYP2C19 Genotype

Laura Dean, MD
Created: April 4, 2017.

Introduction

Carisoprodol is a centrally acting muscle relaxant used to relieve acute back pain. Due to the risk of dependence and abuse, carisoprodol should only be used for treatment periods of up to two or three weeks. Carisoprodol is a Schedule IV controlled substance and carisoprodol overdose can lead to CNS respiratory depression, seizures, and death.

Carisoprodol is metabolized by CYP2C19 to meprobamate, a sedative used to treat anxiety disorders. In individuals who have little or no CYP2C19 activity (“CYP2C19 poor metabolizers”), standard doses of carisoprodol can lead to a 4-fold increase in exposure to carisoprodol and a concomitant 50% reduced exposure to meprobamate compared to normal metabolizers. Approximately 3–5% of Caucasians and Africans, and 15–20% of Asians, are CYP2C19 poor metabolizers (1).

The FDA-approved drug label for carisoprodol states that caution should be used when administering carisoprodol to patients with reduced CYP2C19 activity and when co-administering drugs that inhibit or induce CYP2C19 (1). There are no data on the use of carisoprodol in pregnancy, and the efficacy, safety, and pharmacokinetics of carisoprodol have not been established in pediatric patients (less than 16 years of age).

Drug: Carisoprodol

Carisoprodol is a centrally acting muscle relaxant used to treat acute musculoskeletal pain. It is often used to treat acute low back pain, providing pain relief and helping patients mobilize. However, its clinical use is limited by the risk of abuse (it is a Schedule IV controlled substance) and its toxic effects in overdose, which may be fatal.

The mechanism of action of carisoprodol is not well understood, but it is an indirect agonist of the GABA receptor associated with altered neuronal communication at the reticular formation in the brainstem and at the spinal cord. In addition to its skeletal muscle relaxing effects, carisoprodol also has weak anticholinergic, antipyretic, and analgesic properties. Adverse effects include sedation, tachycardia, shortness of breath, and dizziness (2, 3).

Carisoprodol is metabolized by CYP2C19 into meprobamate—an active metabolite that has similar potency to carisoprodol. Meprobamate is used to treat anxiety. Again, its
mechanism of action is not well understood, but it has barbiturate-like properties and is toxic in overdose (4).

Individuals who have reduced or absent activity of CYP2C19 have higher plasma levels of carisoprodol, and a higher ratio of carisoprodol:meprobamate, compared to individuals who have normal levels of CYP2C19 activity. Carisoprodol's narrow therapeutic index implies there may be increased risk of toxicity in CYP2C19 poor metabolizers. However, data are limited. Small studies have found no evidence to support an association between CYP2C19 genotype status and the mortality risk of carisoprodol or adverse effects after a single dose of carisoprodol (4-6).

The FDA-approved drug label for carisoprodol states that caution should be used when administering carisoprodol to patients with reduced CYP2C19 activity. The label also states that the co-administration of CYP2C19 inhibitors, such as omeprazole or fluvoxamine, could result in increased exposure of carisoprodol and decreased exposure of meprobamate, and the co-administration of CYP2C19 inducers, such as rifampin or St. John's Wort, could result in decreased exposure of carisoprodol and increased exposure of meprobamate. Low dose aspirin also showed induction effect on CYP2C19. The label states that the full pharmacological impact of these potential alterations of exposures in terms of either efficacy or safety of carisoprodol is unknown (1).

**Gene: CYP2C19**

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as several proton pump inhibitors, clopidogrel, benzodiazepines, and several tricyclic antidepressants, including imipramine.

The CYP2C19 gene is highly polymorphic—35 variant star (*) alleles are catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/cyp2c19.htm](http://www.cypalleles.ki.se/cyp2c19.htm).

The CYP2C19*1 wild-type allele is associated with normal enzyme activity and the “normal metabolizer” phenotype, whereas the CYP2C19*17 allele is associated with increased enzyme activity and the “rapid” and “ultrarapid” metabolizer phenotypes (7).

The most common loss-of-function variant is CYP2C19*2, which contains a c.681G>A variant in exon 5 that results in an aberrant splice site. This leads to the production of a truncated and non-functioning protein. The CYP2C19*2 allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (7, 8).

Another commonly tested loss-of-function variant is CYP2C19*3, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The CYP2C19*3 allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other loss-of-function variants occur in less than 1% of the general population and include CYP2C19*4-*8 (7, 8).
**CYP2C19** intermediate metabolizers carry one copy of an allele that encodes reduced or absent function (e.g. *1/*2), whereas “poor metabolizers” are homozygous or compound heterozygous for two loss-of-function alleles (e.g., *2/*2, *2/*3) (table 1).

**Table 1. CYP2C19 functional status and phenotypes**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Examples of diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19 Ultrarapid metabolizer</td>
<td>An individual carrying two increased function alleles.</td>
<td>*17/*17</td>
</tr>
<tr>
<td>(~2–5% of patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19 Rapid metabolizer</td>
<td>An individual carrying one normal function allele and one increased function allele.</td>
<td>*1/*17</td>
</tr>
<tr>
<td>(~2–30% of patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19 Normal metabolizer</td>
<td>An individual carrying two normal function alleles.</td>
<td>*1/*1</td>
</tr>
<tr>
<td>(~35–50% of patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19 Intermediate metabolizer</td>
<td>An individual carrying one normal function allele and one no function allele or one no function allele and one increased function allele.</td>
<td>*1/*2, *1/*3, *2/*17b</td>
</tr>
<tr>
<td>(~18–45% of patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19 Poor metabolizer</td>
<td>An individual carrying two no function alleles.</td>
<td>*2/*2, *2/*3, *3/*3</td>
</tr>
<tr>
<td>(~2–15% of patients)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the CYP2C19 Frequency Tables for population-specific allele and phenotype frequencies (9).

*b* The predicted metabolizer phenotype for the *2/*17 genotype is a provisional classification. The currently available evidence indicates that the CYP2C19*17* increased function allele is unable to completely compensate for the CYP2C19*2* no function allele.


Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, “Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)” (10).

**Genetic Testing**

Clinical genotyping tests are available for several CYP2C19 alleles. The NIH’s Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for carisoprodol response, CYP2C19-related poor drug metabolism, and the CYP2C19 gene.

**Therapeutic Recommendations based on Genotype**

This section contains excerpted1 information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.
2016 Statement from the US Food and Drug Administration (FDA):

Carisoprodol Tablets are metabolized in the liver by CYP2C19 to form meprobamate. Co-administration of CYP2C19 inhibitors, such as omeprazole or fluvoxamine, with Carisoprodol Tablets could result in increased exposure of carisoprodol and decreased exposure of meprobamate. Co-administration of CYP2C19 inducers, such as rifampin or St. John's Wort, with Carisoprodol Tablets could result in decreased exposure of carisoprodol and increased exposure of meprobamate. Low dose aspirin also showed induction effect on CYP2C19.

The full pharmacological impact of these potential alterations of exposures in terms of either efficacy or safety of Carisoprodol Tablets is unknown.

[...]

*Patients with Reduced CYP2C19 Activity:* Carisoprodol Tablets should be used with caution in patients with reduced CYP2C19 activity. Published studies indicate that patients who are poor CYP2C19 metabolizers have a 4-fold increase in exposure to carisoprodol, and concomitant 50% reduced exposure to meprobamate compared to normal CYP2C19 metabolizers. The prevalence of poor metabolizers in Caucasians and African Americans is approximately 3-5% and in Asians is approximately 15-20%.

Please review the complete therapeutic recommendations that are located here: (1).

### Nomenclature for selected CYP2C19 alleles

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19*2</td>
<td>681G&gt;A Pro227Pro</td>
<td>NM_000769.1:c.681G&gt;A</td>
<td>rs4244285</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>636G&gt;A Trp212Ter</td>
<td>NM_000769.1:c.636G&gt;A</td>
<td>rs4986893</td>
</tr>
<tr>
<td>CYP2C19*17</td>
<td>-806C&gt;T</td>
<td>NM_000769.2:c.-806C&gt;T</td>
<td>rs12248560</td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

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1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
Acknowledgments

The author would like to thank JT Callaghan, M.D., Ph.D., Associate Dean of Veterans Affairs Research, Associate Professor of Medicine, and Pharmacology and Toxicology, Department of Veterans Affairs and Indiana University School of Medicine; Ben Kong, PharmD, BCPS, Clinical Pharmacist, Oregon Health & Science University, Oregon; Gouri Mukerjee, Scientific Officer at Geneyouin Inc., Toronto, Canada; and Mohamed Nagy, Clinical Pharmacist, Head of the Personalised Medication Management Unit, Department of Pharmaceutical Services, Children's Cancer Hospital, Egypt; for reviewing this summary.

References


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Clopidogrel Therapy and CYP2C19 Genotype
Diazepam Therapy and CYP2C19 Genotype
Esomeprazole Therapy and CYP2C19 Genotype
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Omeprazole Therapy and CYP2C19 Genotype
Prasugrel Therapy and CYP Genotype

Tests in GTR by Condition

Carisoprodol response

Tests in GTR by Gene

CYP2C19 gene
Celecoxib Therapy and CYP2C9 Genotype

Laura Dean, MD¹
Created: August 18, 2016.

Introduction

Celecoxib is a nonsteroidal anti-inflammatory drug (NSAID) that is used in the management of osteoarthritis, rheumatoid arthritis, menstrual symptoms, and acute pain. Most NSAIDs inhibit both types of cyclooxygenase, COX-1 and COX-2. These enzymes catalyze pathways that play a key role in the generation of the inflammatory response; however, celecoxib, selectively inhibits COX-2.

The CYP2C9 gene encodes an enzyme involved in the metabolism of many drugs, and is one of the main enzymes that metabolizes and inactivates celecoxib. Two common variants, CYP2C9*2 and CYP2C9*3, are associated with significantly reduced CYP2C9 enzyme activity. Individuals who carry two copies of these variants (or other loss-of-function variant CYP2C9 alleles) are considered CYP2C9 “poor metabolizers” and may be exposed to high drug levels after standard celecoxib doses.

The FDA-approved drug label for celecoxib states: “patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin, phenytoin) should be administered celecoxib with caution. Consider starting treatment at half the lowest recommended dose in poor metabolizers (i.e., CYP2C9*3/*3). Consider using alternative management in juvenile rheumatoid arthritis (JRA) patients who are poor metabolizers” (1).

Drug: Celecoxib

Celecoxib is a NSAID that is used to treat osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, painful menstruation, and acute pain (1). It is also used to reduce the number of colon and rectum polyps in patients with familial adenomatous polyposis.

Worldwide, it is estimated that more than 30 million people receive NSAIDs daily (2). They are one of the most commonly used classes of medicine. Several NSAIDs (aspirin, ibuprofen, and naproxen) are available over-the-counter, but stronger doses and other types of NSAIDs, such as celecoxib, are only available via prescription. It is thought that approximately 25% of the population has experienced NSAID-related side effects that require medical care (3).

Most NSAIDs are non-selective COX inhibitors that reduce the production of pro-inflammatory prostaglandins by inhibiting both COX-1 and COX-2. COX is the central
enzyme in the synthesis of prostaglandins and thromboxane $A_2$ from arachidonic acid. Prostaglandins can be protective (e.g., protect the gastric mucosal lining and aids platelet aggregation) or inflammatory (e.g., recruiting inflammatory white blood cells).

Celecoxib is a selective COX-2 inhibitor, which promotes the production of the gastric mucosal lining. Although celecoxib may be more gastroprotective than non-selective NSAIDs (4-7), the use of celecoxib still increases the risks of gastrointestinal adverse events. The FDA-approved label for celecoxib includes the warning that:

NSAIDs, including CELECOXIB, cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. These events can occur at any time during use and without warning symptoms (1).

In the US, acute gastrointestinal bleeding associated with the use of NSAIDs may cause more than 30,000 hospitalizations per year (8). Several risk factors for NSAID-related bleeding have been identified, including old age, a history of peptic ulcer disease, high dosages of NSAIDs, concomitant use of different NSAIDs (9), and \textit{CYP2C9} genotype.

\textbf{Gene: CYP2C9}

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity. CYP2C9 metabolizes approximately 15% of clinically used drugs, and atypical metabolic activity caused by genetic variants in the \textit{CYP2C9} gene can play a major role in adverse drug reactions (10, 11).

At least 16 different NSAIDS are metabolized, in part, by CYP2C9 (12). Celecoxib is extensively metabolized by CYP2C9, with minor contributions from CYP3A4, CYP2C8 and CYP2C19 (3).

\textit{CYP2C9*1} is the wild-type allele and is associated with normal enzyme activity and the normal metabolizer phenotype. Two common variants, \textit{CYP2C9*2} (p.Arg144Cys) and \textit{CYP2C9*3} (p.Ile359Leu), are associated with significantly reduced enzyme activity. Carriers of these variants have altered pharmacokinetics of several NSAIDs: celecoxib, flurbiprofen, ibuprofen, and tenoxicam (12, 13). This could potentially lead to dose recommendations based upon \textit{CYP2C9} genotype, and be used to identify individuals who are at increased risk of adverse events. However, pharmacogenetic testing has been limited to retrospective studies to identify the causes of an atypical response to NSAID (11).

Studies have found that \textit{CYP2C9*3} is associated with an increased risk of bleeding associated with NSAID use (9, 14). In contrast, \textit{CYP2C9*3} was found to be beneficial in a trial where celecoxib was given to prevent colorectal adenomas. High dose celecoxib had greater efficacy in preventing new adenomas than low-dose celecoxib, but only among individuals who were carriers of \textit{CYP2C9*3} (15, 16).
The frequencies of variant CYP2C9 alleles vary between different ethnic groups (17-19). The *2 allele is more common in Caucasian and Middle Eastern populations (10-20%), than in Asian or African populations (0-6%) (19-21). The *3 allele is less common (<10% in most populations) and extremely rare in African populations (19, 22).

The influence of other variant alleles, such as CYP2C9*8 and CYP2C9*11, on celecoxib levels in the plasma has not yet been evaluated.

**Genetic Testing**

Clinical genotyping tests are available for several CYP2C9 alleles, and a list of tests is available at the Genetic Testing Registry (GTR) of the National Institutes of Health: [http://www.ncbi.nlm.nih.gov/gtr/tests/?term=1559[geneid]].

The variants that are most commonly tested for are CYP2C9*2 and CYP2C9*3. Test results are typically reported as a diplotype (e.g., CYP2C9 *3/*3), and may also include an interpretation of the patient's predicted metabolizer phenotype: ultrarapid, normal (extensive), intermediate, or poor.

**Therapeutic Recommendations based on Genotype**

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): Poor Metabolizers of CYP2C9 Substrates: Patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin, phenytoin) should be administered celecoxib with caution. Consider starting treatment at half the lowest recommended dose in poor metabolizers (i.e., CYP2C9*3/*3). Consider using alternative management in junior rheumatoid arthritis (JRA) patients who are poor metabolizers.

Please review the complete therapeutic recommendations that are located here: (1).

---

1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*2</td>
<td>430C&gt;T Arg144Cys</td>
<td>NM_000771.3:c.430C&gt;T</td>
<td>rs1799853</td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>1075A&gt;C Ile359Leu</td>
<td>NM_000771.3:c.1075A&gt;C</td>
<td>rs1057910</td>
</tr>
</tbody>
</table>

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References


Related Summaries by Gene

Phenytoin Therapy and HLA-B*15:02 and CYP2C9 Genotypes

Prasugrel Therapy and CYP Genotype

Warfarin Therapy and the Genotypes CYP2C9 and VKORC1

Tests in GTR by Condition

Celecoxib response

Tests in GTR by Gene

CYP2C9 gene
Introductions

Clopidogrel is an antiplatelet agent used to reduce the risk of myocardial infarction (MI) and stroke among high-risk patients. It is also approved for secondary prevention of atherosclerotic events for patients who have recently had an MI or a stroke and those with established peripheral arterial disease, and administered with aspirin as dual antiplatelet therapy (DAPT) for patients with acute coronary syndrome (ACS) caused by an MI or unstable angina.

CYP2C19 is one of the principal enzymes involved in the hepatic bioactivation of clopidogrel. Of note, the recommended doses of clopidogrel are less effective in patients with loss-of-function variant alleles in the CYP2C19 gene. Approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese carry two loss-of-function variant alleles and are classified as “poor metabolizers” due to having little or no CYP2C19 enzyme activity (1).

The FDA-approved drug label for clopidogrel contains a boxed warning, stating that clopidogrel has diminished effectiveness among CYP2C19 poor metabolizers. It advises that tests are available to identify a patient's CYP2C19 genotype, they can be used as an aid in determining therapeutic strategy, and that alternative treatment strategies should be considered in patients identified as CYP2C19 poor metabolizers.

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has made antiplatelet therapy recommendations based on CYP2C19 genotype for patients with ACS who are undergoing percutaneous coronary interventions (PCI), such as the placement of a stent. Given the reduced efficacy reported for both CYP2C19 intermediate and poor metabolizers, CPIC recommends using an alternative antiplatelet agent (e.g., prasugrel or ticagrelor) when not contraindicated (Table 1) (2).
Table 1. Antiplatelet therapy recommendations based on CYP2C19 status when considering clopidogrel for ACS/PCI patients

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Phenotype details</th>
<th>Examples of diplotypes</th>
<th>Therapeutic recommendations for clopidogrel in ACS/PCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>Normal or increased enzyme activity. Found in ~5–30% of patients.</td>
<td>*1/*17 *17/*17</td>
<td>Dose recommended by drugs label</td>
</tr>
<tr>
<td>Extensive metabolizer</td>
<td>Normal enzyme activity (homozygous wild-type). Found in ~35-50% of patients.</td>
<td>*1/*1</td>
<td>Dose recommended by drugs label</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>Intermediate enzyme activity. Found in ~18-45% of patients.</td>
<td>*1/*2 *1/*3 *2/*17</td>
<td>Alternative antiplatelet therapy recommended if no contraindication, e.g., prasugrel, ticagrelor</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>Low or absent enzyme activity. Found in ~2-15% of patients.</td>
<td>*2/*2 *2/*3 *3/*3</td>
<td>Alternative antiplatelet therapy recommended if no contraindication, e.g., prasugrel, ticagrelor</td>
</tr>
</tbody>
</table>

The strength of therapeutic recommendations is “moderate” for intermediate metabolizers and “strong” for all other metabolizers.

ACS, acute coronary syndrome
PCI, percutaneous coronary intervention

Drug: Clopidogrel

Clopidogrel is a second-generation thienopyridine antiplatelet agent, which binds irreversibly to the P2RY12 receptor and inhibits ADP-mediated platelet activation and aggregation. Other currently approved antiplatelet agents include prasugrel and ticagrelor.

As an antiplatelet agent, clopidogrel is used to inhibit the formation of blood clots in the coronary, peripheral, and cerebrovascular arteries. It is typically given in addition to daily aspirin and other standard treatments, and is used in patients with ACS, and in patients who have either had a recent MI or stroke, or have established peripheral arterial disease (1).

ACS reflects a decreased blood flow in the coronary arteries, and comprises the entities’ unstable angina, “STEMI” and “NSTEMI”. Unstable angina, in contrast to stable angina, occurs suddenly, often at rest or with minimal exertion. It may be new in onset, or it may occur with less exertion than previously. An MI may be classified in two types, depending on what is shown on the EKG. If the EKG findings include an elevation of the ST-segment, this is known as an “ST segment elevation MI” (STEMI), and if there is no elevation but
an increase in myocardial biomarkers such as troponin I or T, this is known as a “non-ST segment elevation MI” (NSTEMI).

In patients who have ACS caused by STEMI, clopidogrel has been found to reduce the rate of death from any cause (3). In patients who have ACS caused by NSTEMI or unstable angina, clopidogrel has been found to decrease the rate of a combined endpoint of cardiovascular death, MI, stroke, or refractory ischemia (4).

In patients with atherosclerotic vascular disease, as indicated by a recent MI, a recent ischemic stroke, or symptomatic peripheral arterial disease, the long-term administration of clopidogrel reduces the combined risk of a new ischemic stroke or MI, and other vascular death (5).

Clopidogrel is a potent antithrombotic drug that inhibits ADP-induced platelet aggregation by selectively binding to the platelet purinergic receptor, P2RY12 (6). Because clopidogrel is a pro-drug, it first requires conversion into an active metabolite before it can act as an antiplatelet agent.

Clopidogrel activation takes place via two sequential oxidation reactions that are catalyzed by the cytochrome P450 (CYP450) system: the first involving CYP1A2, CYP2B6, and CYP2C19, and the second involving CYP2B6, CYP2C9, CYP2C19, CYP3A4, and CYP3A5. Less than 15% of the clopidogrel pro-drug is activated, the remaining 85% is hydrolyzed by esterases to inactive forms and excreted (6-9). Of note, one small study suggested that a non P450 (CYP) enzyme, paraoxonase 1 (PON1), may also be involved in clopidogrel activation (10); however, this finding has not been replicated in dedicated studies (11-13) or in meta-analysis (14).

The active clopidogrel metabolite contains a reactive thiol group, which forms a disulfide bridge with a free cysteine residue on the P2RY12 receptor. Once bound, the receptor is unable to bind ADP, and platelet activation via this pathway is prevented for the rest of its lifespan (~10 days) (6).

Despite the general efficacy of clopidogrel to inhibit platelet aggregation, a substantial variability in response to clopidogrel is frequently observed—resistance and treatment failure can occur in some patients. It has been estimated that between 16-50% of patients treated with clopidogrel have “high on-treatment platelet reactivity” (HTPR), which indicates that a major portion of P2RY12 receptors are not blocked, despite treatment with clopidogrel (15).

An individual’s response to clopidogrel can be influenced by many factors, such as age, comorbidities, and drug-drug interactions. In addition, genetic susceptibility to clopidogrel response has been reported, including variant alleles in the ABCB1 gene, which influence the absorption of clopidogrel from the gut (16, 17), and variant alleles in the CYP2C19 gene, which influence the generation of the active clopidogrel metabolite (18, 19).
CYP2C19 is the principal hepatic enzyme involved in converting clopidogrel to its active metabolite, and CYP2C19 loss-of-function alleles result in reduced active clopidogrel metabolites and HTPR, as well as an increased risk for both major adverse cardiovascular events (MACE) and stent thrombosis compared to CYP2C19 wild-type patients with ACS/PCI (20-22).

**Gene: CYP2C19**

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, benzodiazepines, some proton pump inhibitors, and the antiplatelet agent clopidogrel.

The variability of clopidogrel metabolism and treatment outcomes between individuals is partly determined by variant alleles of the CYP2C19 gene. CYP2C19 is highly polymorphic, as more than 25 variant star (*) alleles are currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2c19.htm). The CYP2C19*1 wild-type allele and is associated with normal enzyme activity and the “extensive metabolizer” phenotype.

The CYP2C19*17 allele is associated with increased enzyme activity due to increased gene transcription and has allele frequencies range from 3 to 21% across different populations (2). Individuals with one or two copies of the *17 allele are typically classified as “ultrarapid metabolizers”.

Individuals who carry one and two reduced-activity or non-functioning CYP2C19 alleles are “intermediate” and “poor metabolizers”, respectively. Given that the efficacy of clopidogrel is, in part, dependent upon it being metabolized by CYP2C19 to an active metabolite, intermediate and poor metabolizers can have reduced antiplatelet responses when treated with clopidogrel. However, only 6 to 12% of the observed variability in antiplatelet effect of clopidogrel is thought to be attributed to carriage of CYP2C19*2 allele(s) (23). Approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese are CYP2C19 poor metabolizers (1).

The most common loss-of-function variant is CYP2C19*2, which contains a c.681G>A variant in exon 5 that results in an aberrant splice site. This leads to the production of a truncated and non-functioning protein. The CYP2C19*2 allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (2).

Another commonly tested loss-of-function variant is CYP2C19*3, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The CYP2C19*3 allele frequencies are ~2-9% in Asian populations, but rare in other racial groups. Other loss-of-
function variants occur in less than 1% of the general population, and include \( CYP2C19^{*4-8} \) (2).

As noted above, ACS/PCI patients that are \( CYP2C19 \) intermediate or poor metabolizers and who are treated with clopidogrel have increased risks for major cardiovascular events including stent thrombosis (1) (17, 21).

**Genetic Testing**

Clinical genotyping tests are available for several \( CYP2C19 \) alleles, and a list of test providers is available at the Genetic Testing Registry (GTR) of the National Institutes of Health: [http://www.ncbi.nlm.nih.gov/gtr/tests/?term=1557[geneid]].

Usually a patient's result is reported as a diplotype, such as \( CYP2C19 \) *1/*1, and may also include an interpretation of the patient's predicted metabolizer phenotype (ultrarapid, extensive, intermediate, or poor). Table 1 summarizes common \( CYP2C19 \) phenotypes with antiplatelet therapy recommendations developed by CPIC.

The association between \( CYP2C19^{*2} \) and \( *3 \) and clopidogrel response has been extensively studied; however, the less common loss-of-function alleles (e.g., \( CYP2C19^{*4-8} \)) also likely influence clopidogrel response similar to \( *2 \) and \( *3 \). Therefore, when other loss-of-function alleles are identified in patients with ACS/PCI, these alleles should be considered to reduce the effectiveness of clopidogrel therapy in a similar manner to the more common \( CYP2C19^{*2} \) allele (2, 24).

**Therapeutic Recommendations based on Genotype**

This section contains excerpted\(^1\) information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**Statement from the US Food and Drug Administration (FDA):** WARNING: DIMINISHED EFFECTIVENESS IN POOR METABOLIZERS. The effectiveness of clopidogrel is dependent on its activation to an active metabolite by the cytochrome P450 (CYP) system, principally \( CYP2C19 \). Clopidogrel at recommended doses forms less of that metabolite and has a smaller effect on platelet function in patients who are \( CYP2C19 \) poor metabolizers. Poor metabolizers with acute coronary syndrome or undergoing percutaneous coronary intervention treated with clopidogrel at recommended doses exhibit higher cardiovascular event rates than do patients with normal \( CYP2C19 \) function. Tests are available to identify a patient's \( CYP2C19 \) genotype; these tests can be used as an aid in determining therapeutic strategy. Consider alternative treatment or

\(^1\) The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
treatment strategies in patients identified as CYP2C19 poor metabolizers.  
Please review the complete therapeutic recommendations that are located here: (1).

Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Standard dosing of clopidogrel, as recommended in the product insert, is warranted among ACS/PCI patients with a predicted CYP2C19 extensive metabolizer or ultrarapid metabolizer phenotype (i.e., *1/*1, *1/*17, and *17/*17). If genotyping from a Clinical Laboratory Improvement Amendments–certified laboratory identifies a patient as a CYP2C19 PM (i.e., *2/*2), current literature supports the use of an alternative antiplatelet agent (e.g., prasugrel or ticagrelor) when not contraindicated clinically.

The most challenging patient population to address is the CYP2C19 IM phenotype (e.g., *1/*2, *1/*3, and *2/*17). IMs have higher on-treatment residual platelet activity on average as compared with extensive metabolizers, and ACS/PCI CYP2C19*2 heterozygotes treated with clopidogrel have increased risks for serious adverse CV outcomes, including stent thrombosis. Consequently, these data support switching to an alternative antiplatelet agent for IMs when not contraindicated. However, given the wide interindividual variability in residual platelet activity observed among clopidogrel-treated IMs, clinical judgment also taking into account other factors that may place an IM at increased risk of a CV event (or adverse bleeding event) must be considered to most effectively individualize therapy.  
Please review the complete therapeutic recommendations that are located here: (2).

Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): There is an increased risk for a reduced response to clopidogrel in CYP2C19 intermediate and poor metabolizers. Consider using an alternative drug. Prasugrel is not or to a much smaller extent metabolized by CYP2C19 but is associated with an increased bleeding risk compared to clopidogrel (Table 2).

Please review the complete therapeutic recommendations that are located here: (25).

**Table 2.** CYP2C19 phenotypes and the therapeutic recommendations for clopidogrel therapy

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Therapeutic (dose) recommendation for clopidogrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>More than two copies of functional alleles</td>
<td>No</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>One active allele and one inactive allele, or two decreased activity alleles, or</td>
<td>Increased risk for reduced response to clopidogrel. Consider alternative drug. Prasugrel is not or to a much smaller extent metabolized by CYP2C19 but is associated with an increased bleeding risk compared to clopidogrel</td>
</tr>
</tbody>
</table>

Table 2. continued from previous page.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Therapeutic (dose) recommendation for clopidogrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>One decreased activity allele and one inactive allele</td>
<td>Poor metabolizer</td>
<td>Increased risk for reduced response to clopidogrel. Consider alternative drug. Prasugrel is not or to a much smaller extent metabolized by CYP2C19 but is associated with an increased bleeding risk compared to clopidogrel</td>
</tr>
</tbody>
</table>


**Nomenclature**

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence Coding</th>
<th>Protein</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19*2</td>
<td>681G&gt;A Pro227Pro</td>
<td>NM_000769.1:c.681G&gt;A</td>
<td>NP_000760.1:p.Pro227=</td>
<td>rs4244285</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>636G&gt;A Trp212Ter</td>
<td>NM_000769.1:c.636G&gt;A</td>
<td>NP_000760.1:p.Trp212Ter</td>
<td>rs4986893</td>
</tr>
<tr>
<td>CYP2C19*17</td>
<td>-806C&gt;T</td>
<td>NM_000769.1:c.-806C&gt;T</td>
<td>Not applicable - variant occurs in a non-coding region</td>
<td>rs12248560</td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

**Acknowledgments**

The author would like to thank Stuart Scott, Assistant Professor of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai; and Dietmar Trenk, Head of Clinical Pharmacology at the University Heart Center, Bad Krozingen and Professor at the Albert Ludwig University of Freiburg, Germany; for reviewing this summary.

**References**


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype

Carisoprodol Therapy and CYP2C19 Genotype

Diazepam Therapy and CYP2C19 Genotype

Esomeprazole Therapy and CYP2C19 Genotype

Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Omeprazole Therapy and *CYP2C19* Genotype
Prasugrel Therapy and *CYP* Genotype

**Related Summaries by Drug Class**
Prasugrel Therapy and *CYP* Genotype

**Tests in GTR by Condition**
Clopidogrel response

**Tests in GTR by Gene**
*CYP2C19* gene
Clozapine Therapy and CYP2D6, CYP1A2, and CYP3A4 Genotypes

Laura Dean, MD
Created: June 8, 2016.

Introduction

Clozapine is one of the most effective antipsychotics available in the treatment of schizophrenia and the only antipsychotic found to be effective in treatment-resistant schizophrenia. Clozapine is also used to reduce the risk of recurrent suicidal behavior in patients with schizophrenia or schizoaffective disorder (1, 2).

Compared to typical antipsychotics, clozapine is far less likely to cause movement disorders, known as extrapyramidal side effects, which include dystonia, akathisia, parkinsonism, and tardive dyskinesia. However, there are significant risks associated with clozapine therapy that limits its use to only the most severely ill patients who have not responded adequately to standard drug therapy. Most notably, because of the risk of clozapine-induced agranulocytosis, clozapine treatment requires monitoring of white blood counts and absolute neutrophil counts, and in the US, the FDA requires that patients receiving clozapine be enrolled in a computer-based registry (3).

Clozapine is metabolized in the liver by the cytochrome P450 (CYP) system of enzymes. CYP1A2 is the main CYP isoform in clozapine metabolism and CYP1A2 activity is an important determinant of clozapine dose (4). Other CYP enzymes involved in clozapine metabolism include CYP2D6 and CYP3A4.

Approximately 6-10% of Caucasians have reduced activity of CYP2D6 (“poor metabolizers”). These individuals may develop higher than expected plasma concentrations of clozapine with usual doses. The FDA-approved drug label for clozapine states that a dose reduction may be necessary in patients who are CYP2D6 poor metabolizers (1).

Drug: Clozapine

Clozapine is an antipsychotic used in the treatment of schizophrenia. Schizophrenia is a severe neurodevelopmental disorder with a worldwide prevalence of around 1%. The etiology of schizophrenia is unknown, but it is thought to result from a combination of complex genetic and environmental factors. Before the discovery of the first antipsychotics in the 1950s, the management of schizophrenia relied heavily upon sedation, electroconvulsive therapy, and institutionalization.
The symptoms of schizophrenia fall into three main categories: positive, negative, and cognitive. Positive symptoms are generally not found in healthy individuals, but may come and go or persist in individuals with schizophrenia. Positive symptoms include reality distortion (e.g., delusions, hallucinations), and thought disorders. These symptoms often respond well to treatment.

Negative symptoms are deficits in normal emotions and behavior, and may be mistaken for depression. Symptoms divide into reduced expression of emotion (e.g., speaking without moving or with a monotonous voice), and avolition (a lack of motivation to start or continue with a task). No treatment has established efficacy for these pathologies.

Cognitive symptoms may also be difficult to recognize. They include poor executive functioning (understanding information and using it to make decisions) and trouble focusing or paying attention. And again, no treatment has established efficacy.

Clozapine is unique among the antipsychotics because it effectively treats positive symptoms, and appears to be more effective in treating negative symptoms, and some cognitive symptoms when compared with other antipsychotics that cause negative symptoms or impair cognition (5-7).

Clozapine has also been shown to reduce aggression and reduce the risk of suicide, and is the only antipsychotic found to be effective in treatment-resistant schizophrenia (2, 8-10). More than one third of patients are thought to have schizophrenia that only partially responds or is resistant to standard drugs; these patients may then be treated with clozapine (2, 10, 11).

The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine. Known as “first generation” or “typical” antipsychotics, these drugs are used to treat psychosis (regardless of the cause), chronic psychotic disorders (e.g., schizophrenia), and other psychiatric conditions. However, prominent adverse effects included extrapyramidal side effects such as tardive dyskinesia, muscle rigidity, tremors, and Parkinsonian-like symptoms.

Newer antipsychotics, known as “second generation” or “atypical” antipsychotics, have a lower risk of extrapyramidal side effects such as tardive dyskinesia. However, many have serious metabolic effects. These antipsychotics include aripiprazole, clozapine, iloperidone, olanzapine, and risperidone.

Clozapine was introduced in 1971 as the first atypical antipsychotic, but the manufacturer (Novartis, formerly Sandoz) voluntarily withdrew the drug in 1975 because of safety concerns (7). One of the most dangerous risks reported was that of clozapine-induced neutropenia—a severely low level of neutrophils (a type of white blood cell), which places patients at high risk of infection. However, because it was later shown that clozapine was the most effective antipsychotic in the management of treatment-resistant schizophrenia, in 1989 the FDA reapproved clozapine for that use (5, 7, 9).
The main action of both first-generation and second-generation antipsychotics appears to be the post-synaptic blockade of D2 dopamine receptors in the brain. (An exception is aripiprazole, which is a D2 partial agonist.) Blockade of the D2 receptor in the brain's limbic system are thought to improve the “positive” symptoms of schizophrenia (12).

However, because the first-generation antipsychotics also block dopamine receptors in the nigrostriatal pathway, they cause movement disorders known as extrapyramidal side effects. These disorders include akathisia (motor restlessness), dystonia (abnormal muscle tone), and tardive dyskinesia (involuntary and repetitive movements).

Clozapine only transiently occupies D2 receptors and then rapidly dissociates to allow normal dopamine neurotransmission. It is thought that because clozapine has a relatively low affinity for the D2 receptor and binds “loosely,” extrapyramidal side effects are less likely (11, 13).

In addition to binding the D2 receptor, clozapine has a high affinity for the serotonin 5-HT₂A receptors. Blockade of 5-HT₂A in the mesocortical tract may also provide some protection against extrapyramidal side effects by increasing amounts of dopamine. Clozapine and its major metabolite (N-desmethylclozapine) have been shown to indirectly activate NMDA receptors, and may also modulate GABA and cholinergic pathways. However, despite these findings, it remains unclear what gives clozapine its superior efficacy to other antipsychotics (7).

One of the most prominent side effects of clozapine therapy is weight gain. The most severe side effects are included in five boxed warnings on the drug label: 1) severe neutropenia, 2) seizures (more likely at higher doses), 3) myocarditis (inflammation of the heart muscle induced by clozapine, that can be fatal), 4) increased mortality in elderly patients with dementia-related psychosis, and 5) an increased risk of orthostatic hypotension, bradycardia, and syncope (1).

Because of the risk of neutropenia, clozapine can only be prescribed according to a schedule that monitors the patient's white blood cell count (WBC) and absolute neutrophil count (ANC). Neutropenia, defined as an ANC of less than 500/mm³, is estimated to occur in around 1% of patients, and could prove fatal if not detected early by regular monitoring (14).

Genetic risk factors for clozapine-induced neutropenia have been identified, consisting of two independent amino acid changes in HLA-DQB1 (126Q) and HLA-B (158T). HLA-DQB1 is associated with autoimmune disease and HLA-B is an important component of severe drug reactions, including carbamazepine-induced Stevens-Johnson syndrome and abacavir hypersensitivity. Despite this genetic insight, a genetic test based solely on HLA-DQB1 and HLA-B would not be able to adequately identify if all the patients are truly at low risk of clozapine-induced neutropenia (15).
The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Clozapine is extensively metabolized in the liver by CYP450 enzymes, especially by CYP1A2, CYP3A4, and CYP2D6. Most of the metabolites are inactive, but N-desmethylclozapine has been found to have limited activity (7, 16).

The dose of clozapine may need to be adjusted when clozapine is given with medications that inhibit or induce the enzymes responsible for metabolizing clozapine. Inhibitors of CYP enzymes include the antibiotic ciprofloxacin (CYP1A2 inhibitor) and the antidepressant fluvoxamine (CYP3A4 and CYP2D6 inhibitor). Inducers include the antiseizure drug carbamazepine (strong CYP3A4 inducer). In addition, other agents can influence CYP enzymes—caffeine and oral contraceptives are weak or moderate CYP1A2 inhibitors, and tobacco smoke is a moderate inducer of CYP1A2 (and smoking is common among patients with schizophrenia).

Gene: CYP2D6

CYP2D6 is highly polymorphic, with more than 100 star (*) alleles described (17). CYP2D6*1 is the wild-type allele and is associated with normal enzyme activity and the “extensive metabolizer” phenotype. The CYP2D6 alleles *2, *33, and *35 are also considered to have near-normal activity (Table 1).

<table>
<thead>
<tr>
<th>Allele type</th>
<th>CYP2D6 Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>*1, *2, *33, *35</td>
</tr>
</tbody>
</table>

For a detailed list of CYP2D6 alleles, please see (18).

Individuals who have multiple functional copies of the CYP2D6 gene are known as “ultrarapid metabolizers,” whereas individuals who carry one or two copies of reduced-activity or non-functioning CYP2D6 alleles are known as “intermediate” or “poor metabolizers.”

The most common non-functional alleles include *3, *4, *5, and *6 (19-22), and the most common reduced activity alleles include *10, *17, and *41 (23-25). There are large inter-ethnic differences in the frequency of these alleles, with *3, *4, *5, *6, and *41 being more common in Caucasians, *17 more common in Africans, and *10 more common in Asians (26-29).
Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to the more prevalent nonfunctional *4 and *5 alleles (26, 30). These individuals may develop higher than expected plasma concentrations of clozapine when given in usual doses. Therefore, the FDA-approved drug label for clozapine states that in poor metabolizers, a lower dose of clozapine may be necessary (1).

However, although in theory poor metabolizers may require lower doses of clozapine to achieve the desired therapeutic effects, evidence for this is lacking. Several studies investigating the association between CYP2D6 genotypes and response to antipsychotic therapy did not report significant findings (31, 32).

**Gene: CYP1A2**

CYP1A2 alleles influence the treatment response of several antipsychotics (4). However, understanding the pharmacogenomic effects of CYP1A2 variation is still at an early stage compared with that of other CYP2D6 and other CYP enzymes (33).

CYP1A2 comprises around 13% of all CYP protein in the liver, whereas CYP2D6 comprises around 2%. Approximately 25 variant alleles of CYP1A2 have been reported, some of which have been shown to alter the activity of CYP1A2. For example, the *1C allele is associated with decreased enzyme activity (by altering the binding site of an unknown transcription factor in the gene promoter), and the *1F allele is associated with increased enzyme activity (by increasing the induction of expression) (33, 34).

CYP1A2 is the main CYP isoform in clozapine metabolism (35). Case studies have found that patients with one or more copies of CYP1A2*1F (ultrarapid metabolizers) respond poorly to clozapine therapy. However, the treatment response is improved by increasing the dose of clozapine, and also co-administering fluvoxamine, a CYP1A2 inhibitor (36, 37).

The frequency of CYP1A2*1F (defined by a C > A polymorphism in intron 1) exists at similar frequencies in all populations (starting at around 0.29) with the highest frequency among Africans (up to 0.51) (38). Environmental factors also strongly influence CYP1A2 activity, such as oral contraceptive use (inhibition) and smoking (induction). Indeed, the sudden cessation of smoking during clozapine therapy may trigger side effects, because of sudden increase in drug levels (39).

**Gene: CYP3A4**

In contrast to CYP2D6, CYP1A2, and other genes that encode drug-metabolizing enzymes, CYP3A4 shows little genetic variation. Although around 40 variant alleles of CYP3A4 have been reported, most have not been shown to alter the activity of CYP3A4 (40, 41). To date, only three loss-of-function CYP3A4 alleles have been identified (CYP3A4*6, CYP3A4*20 and CYP3A4*26) (42, 43).
The CYP3A4*20 allele contains a premature stop codon which results in a loss-of-function of CYP3A. It appears to be the most common CYP3A4-defective allele but is still relatively rare, with about 0.2% of European Americans and 0.05% African Americans being carriers. However in Spain, the CYP3A4*20 allele is present in 1.2% of the population, and up to 3.8% in specific Spanish regions (42).

**Genetic Testing**

Genetic testing is available for common CYP2D6, CYP3A4, and CYP1A2 alleles. Often a panel of tests is performed. These panels test for variants in multiple genes, which are involved in the metabolism of many drugs, including clozapine. For examples of the tests available for the clozapine drug response, please see the Genetic Testing Registry.

Results are typically reported as a diplotype, such as CYP2D6 *1/*1. A result for copy number, if available, is also important when interpreting CYP2D6 results (44).

If the test results include an interpretation of the patient’s predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- An extensive (normal) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2

**Therapeutic Recommendations based on Genotype**

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2014 Statement from the US Food and Drug Administration (FDA): Dose reduction may be necessary in patients who are CYP2D6 poor metabolizers. Clozapine concentrations may be increased in these patients, because clozapine is almost completely metabolized and then excreted. Please review the complete therapeutic recommendations that are located here: (1).

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1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
## Nomenclature

### CYP2D6 Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
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<tr>
<td></td>
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<td>Coding</td>
<td>Protein</td>
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<td><strong>CYP2D6*4</strong></td>
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<td><strong>NM_000106.5:c.506-1G&gt;A</strong></td>
<td>Not applicable - variant occurs in a non-coding region</td>
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<td><strong>CYP2D6*6</strong></td>
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<td><strong>NP_000097.3:p.Trp152Glyfs</strong></td>
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<tr>
<td><strong>CYP2D6*10</strong></td>
<td>100C&gt;T Pro34Ser</td>
<td><strong>NM_000106.5:c.100C&gt;T</strong></td>
<td><strong>NP_000097.3:p.Pro34Ser</strong></td>
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<tr>
<td><strong>CYP2D6*17</strong></td>
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<td><strong>CYP2D6*41</strong></td>
<td>2988G&gt;A</td>
<td><strong>NM_000106.5:c.985+39G&gt;A</strong></td>
<td>Not applicable – variant occurs in a non-coding region</td>
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</table>

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

### CYP1A2 Nomenclature

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<thead>
<tr>
<th>Common allele name</th>
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<th>dbSNP reference identifier for allele location</th>
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<tbody>
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<td><strong>CYP1A2*1F</strong></td>
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<td>Not applicable—variant occurs in a non-coding region</td>
</tr>
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### CYP3A4 Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
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<tbody>
<tr>
<td><strong>CYP3A4*6</strong></td>
<td>17661_17662insA 277Frameshift</td>
<td><strong>NM_017460.5:c.830_831insA</strong></td>
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</table>

*CYP3A4 Nomenclature continues on next page...*
CYP3A4 Nomenclature continued from previous page.

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence Coding</th>
<th>Protein</th>
<th>dbSNP reference identifier for allele location</th>
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<td>CYP3A4*20</td>
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<td>17633C&gt;T</td>
<td>R268Stop</td>
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</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

**Acknowledgments**

The author would like to thank Anil K. Malhotra, MD, Director, Division of Psychiatry Research, The Zucker Hillside Hospital and Vice Chair of Research, Department of Psychiatry, Hofstra Northwell School of Medicine; William T. Carpenter Jr., MD, Professor of Psychiatry and Pharmacology, Maryland Psychiatric Research Center, University of Maryland School of Medicine; and Daniel J. Müller, Head, Pharmacogenetics Research Clinic, Centre for Addiction and Mental Health, and Associate Professor, Department of Psychiatry, University of Toronto, for reviewing this summary.

**References**


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34. The Human Cytochrome P450 (CYP) Allele Nomenclature Database [Internet]. CYP1A2 allele nomenclature [Cited 30 October 2015]. Available from: http://www.cypalleles.ki.se/cyp1a2.htm


40. The Human Cytochrome P450 (CYP) Allele Nomenclature Database [Internet]. CYP3A4 allele nomenclature [Cited 30 October 2015]. Available from: http://www.cypalleles.ki.se/cyp3a4.htm


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Aripiprazole Therapy and CYP2D6 Genotype
Atomoxetine Therapy and CYP2D6 Genotype
Codeine Therapy and CYP2D6 Genotype
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Metoprolol Therapy and CYP2D6 Genotype
Propafenone Therapy and CYP2D6 Genotype
Risperidone Therapy and CYP2D6 Genotype
Tamoxifen Therapy and CYP2D6 Genotype
Thioridazine Therapy and CYP2D6 Genotypes
Tramadol Therapy and CYP2D6 Genotype
Venlafaxine Therapy and CYP2D6 Genotype

Related Summaries by Drug Class
Aripiprazole Therapy and CYP2D6 Genotype
Risperidone Therapy and CYP2D6 Genotype

Tests in GTR by Condition
Clozapine response

Tests in GTR by Gene
CYP2D6 gene
CYP1A2 gene
CYP3A4 gene
Codeine Therapy and CYP2D6 Genotype

Laura Dean, MD

Created: September 20, 2012; Updated: March 16, 2017.

Introduction

Codeine is used to relieve mild to moderately severe pain, and it belongs to the drug class of opioid analgesics.

The hepatic CYP2D6 enzyme metabolizes a quarter of all prescribed drugs, including codeine. CYP2D6 converts codeine into its active metabolite, morphine, which provides its analgesic effect. However, pain relief may be inadequate in individuals who carry two inactive copies of CYP2D6 (“poor metabolizers”), because of reduced morphine levels.

In contrast, individuals who carry more than two normal function copies of the CYP2D6 gene (“ultrarapid metabolizers”) are able to metabolize codeine to morphine more rapidly and more completely. As a result, even with normal doses of codeine, these individuals may experience the symptoms of morphine overdose, which include extreme sleepiness, confusion, and shallow breathing. Nursing mothers may also produce breast milk containing higher than expected levels of morphine that can lead to severe adverse events in their infants (1).

The FDA drug label for codeine states that even at labeled dosage regimens, individuals who are ultra-rapid metabolizers may have life-threatening or fatal respiratory depression or experience signs of overdose. The label also contains a boxed warning, which states that respiratory depression and death have occurred in children who received codeine following tonsillectomy and/or adenoidectomy and had evidence of being ultra-rapid metabolizers of codeine due to a CYP2D6 polymorphism (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends that for a patient identified as a CYP2D6 ultrarapid metabolizer, another analgesic should be used to avoid the risk of severe toxicity with a “normal” dose of codeine. CPIC also recommends avoiding codeine in patients identified as CYP2D6 poor metabolizers due to the possibility of lack of effect (see Table 1) (2).

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
Table 1. 2014 Codeine therapy recommendations based on cytochrome P4502D6 (CYP2D6) phenotype, adapted from CPIC

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Activity score</th>
<th>Phenotype details</th>
<th>Genotype</th>
<th>Examples of diplotypes</th>
<th>Recommendations for codeine therapy</th>
<th>Considerations for alternative opioids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer (approximately 1–2% of patients)</td>
<td>Greater than 2.0</td>
<td>Increased enzyme activity. Increased formation of morphine following codeine administration, leading to higher risk of toxicity.</td>
<td>More than two copies of normal function alleles</td>
<td>*1/*1xN *1/*2xN</td>
<td>Avoid codeine use due to potential for toxicity.</td>
<td>Alternatives that are not affected by this CYP2D6 phenotype include morphine and non-opioid analgesics. Tramadol and, to a lesser extent, hydrocodone and oxycodone are not good alternatives because their metabolism is affected by CYP2D6 activity.</td>
</tr>
<tr>
<td>Normal metabolizer (approximately 77–92% of patients)</td>
<td>1.0-2.0*</td>
<td>Normal enzyme activity. Normal morphine formation.</td>
<td>Two normal function alleles, or two decreased function alleles, or one normal function allele and one decreased or no function allele, or</td>
<td>*1/*1 *1/*2 *2/*2 *1/*41 *1/*4 *2/*5 *1/*10</td>
<td>Use label-recommended age- or weight-specific dosing.</td>
<td></td>
</tr>
</tbody>
</table>

* Activity scores are based on the formation of morphine from codeine. Other investigators may define normal metabolizers with a score of 1.5-2.0, and intermediate metabolizers with a score of 0.5-1.0.

1 The strength of therapeutic recommendations is “moderate” for intermediate metabolizers, and “strong” for all other metabolizers.

Table 1. continued from previous page.

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<thead>
<tr>
<th>Phenotype</th>
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<th>Recommendations for codeine therapy</th>
<th>Considerations for alternative opioids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate metabolizer</td>
<td>0.5*</td>
<td>Low or absent enzyme activity. Greatly reduced morphine formation following codeine administration, leading to insufficient pain relief.</td>
<td>*4/*10 *5/*41</td>
<td>Use label-recommended age- or weight-specific dosing. If no response, consider alternative analgesics such as morphine or a nonopioid.</td>
<td>Monitor tramadol use for response.</td>
<td></td>
</tr>
<tr>
<td>Poor metabolizer (approximately 5–10% of patients)</td>
<td>0</td>
<td>Low or absent enzyme activity. Greatly reduced morphine formation following codeine administration, leading to insufficient pain relief.</td>
<td>*4/*4 *4/*5 *5/*5 *4/*6</td>
<td>Avoid codeine use due to lack of efficacy.</td>
<td>Alternatives that are not affected by this CYP2D6 phenotype include morphine and non-opioid analgesics. Tramadol and, to a lesser extent, hydrocodone and oxycodone are not good alternatives because their metabolism is affected by...</td>
<td></td>
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* Activity scores are based on the formation of morphine from codeine. Other investigators may define normal metabolizers with a score of 1.5-2.0, and intermediate metabolizers with a score of 0.5-1.0.

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1 The strength of therapeutic recommendations is “moderate” for intermediate metabolizers, and “strong” for all other metabolizers.


**Drug: Codeine**

Codeine is an opioid analgesic. It exerts its effects via the opioid receptors found throughout the body including the central nervous system and the gastrointestinal system. Codeine is a prodrug that only weakly binds the mu opioid receptor. Its analgesic properties depend upon its conversion to morphine that binds to the mu opioid receptor with 200-fold greater affinity than codeine.

Codeine is indicated for the relief of mild to moderately severe pain, where the use of an opioid analgesic is appropriate. Codeine is a Schedule II controlled substance, and there is a risk of misuse and abuse. As with any opioid drug, the dosing regimen should be adjusted for each individual patient. When the patient no longer requires codeine, the doses should be tapered gradually to prevent withdrawal symptoms in patients who have become physically dependent (1).

For codeine to exert its opioid activity, it must first undergo o-demethylation by CYP2D6 to morphine. Only about 5–10% of codeine is metabolized in this pathway, with about 80% of an administered dose of codeine being converted to inactive metabolites and excreted. However, the percentage of codeine converted to morphine can be much higher in individuals who have 3 or more active copies of CYP2D6 (“ultrarapid metabolizers”) (2). In contrast, individuals who lack active copies of CYP2D6 (“poor metabolizers”) have lower levels of morphine.

Morphine is further metabolized to morphine-6-glucuronide, which also has analgesic properties. Other metabolites are thought to be mostly inactive; they include codeine-6-glucuronide (~60%) and norcodeine (~5–10%), both of which share with codeine a similarly weak affinity for the mu opioid receptor (4).
To avoid treatment complications in patients who are either ultrarapid or poor metabolizers, opioids that are not metabolized by CYP2D6 may be used (e.g., morphine, oxymorphone, buprenorphine, fentanyl, methadone, hydromorphone), alongside non-opioids, depending upon the type of pain being treated (2, 5-7).

The most common adverse reactions to codeine include drowsiness, lightheadedness, dizziness, sedation, shortness of breath, nausea, vomiting, and sweating. One of the main serious adverse reactions associated with codeine is respiratory depression. The FDA-drug label for codeine now includes a boxed warning that states “Warning: Death related to ultra-rapid metabolism of codeine to morphine. Respiratory depression and death have occurred in children who received codeine following tonsillectomy and/or adenoidec-tomy and had evidence of being ultra-rapid metabolizers of codeine due to a CYP2D6 polymorphism” (1, 8, 9).

**Gene: CYP2D6**

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in decreased, absent, or increased enzyme activity.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. The CYP2D6 gene is highly polymorphic, with more than 100 star (*) alleles described (10).

CYP2D6*1 is the wild-type allele and is associated with normal enzyme activity and the “normal metabolizer” phenotype. The CYP2D6 alleles *2, *33, and *35 are also considered to have near-normal activity.

About 77–92% of individuals have at least one copy of a normal function allele (*1 or *2), or two partially functioning alleles. These individuals are also “normal metabolizers” and are most likely to have a phenotypically normal response to codeine. However, there is a large amount of variability in codeine response within patients genotyped as normal metabolizers, and the causes of this variation, among individuals with the same diplotype, are unknown (2).

Other CYP2D6 alleles include variants that produce a non-functioning enzyme (e.g., *3, *4, *5, and *6) (4, 11-13) or an enzyme with decreased activity (e.g., *10, *17, and *41) (14-16) (see Table 2). There are large inter-ethnic differences in the frequency of these alleles, with *3, *4, *5, *6, and *41 being more common in Caucasians, *17 more common in Africans, and *10 more common in Asians (17).

About 2–11% of patients are intermediate metabolizers—they carry either two decreased function alleles or one decreased function and one no function allele (18). These individuals may not respond as well to codeine because the metabolism of codeine to morphine is reduced.
In Asians and in individuals of Asian descent, only about 50% of CYPD6 alleles are normal function, and the frequency of CYP2D6 allele duplications is as high as 45% (19). Common no function variants are CYP2D6*36 (the most commonly duplicated CYP2D6 allele in the Asian population) and CYP2D6*10. Both these variants contain the SNP “100C>T” (see Nomenclature table) (17, 19-21). In Africans and African Americans, again, only about 50% of CYPD6 alleles are normal function (11, 16, 17, 22).

About 5–10% of patients are poor metabolizers—they carry two no function alleles (18). In these individuals, codeine will provide little or no pain relief. Poor metabolizers are more commonly found in European Caucasians and their descendants. The majority allele in this population is the normal function CYP2D6*1 (70%), but the remaining alleles include the no function CYP2D6*4 and CYP2D6*5 variants that largely account for the poor metabolizer phenotype in these populations (12, 15, 23).

Patients who are ultrarapid metabolizers carry at least 3 copies of the CYP2D6 gene. The ultrarapid metabolizer phenotype has been estimated to be present in 1–2% of patients, but the prevalence varies widely in different populations. It is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; up to 10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (1, 18).

Each normal function CYP2D6 allele increases the rate of codeine metabolism, increasing the risk of an initial morphine "overdose", with more side effects and a shorter duration of pain control (24). Even low codeine doses can result in toxic levels of morphine in patients with more than 2 normal function alleles (2). Several case reports have recorded the severe or life-threatening adverse effects that have occurred in patients who were ultrarapid metabolizers and were treated with standard doses of codeine (25, 26).

**Genetic Testing**

Genetic testing is available for many (~30) of the variant CYP2D6 alleles. Usually a patient's result is reported as a diplotype, which includes one maternal and one paternal allele, e.g., CYP2D6*1/*2. When patients have more than two copies of the CYP2D6, the copies of the allele are denoted by an “xN”, e.g., CYP2D6*2x2.

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and calculating the CYP2D6 activity score. Each allele is assigned an activity value: 0 for no function, 0.5 for decreased function, and 1 for each copy of a normal function allele. The total CYP2D6 activity score is the sum of the values assigned to each allele—patients with a score of 1.0, 1.5, or 2.0 represent a range of normal metabolizers with normal enzyme activity. Poor metabolizers have an activity score of 0, patients with a score of 0.5 are intermediate metabolizers, and patients with a score of greater than 2.0 are ultrarapid metabolizers (see Table 1) (2).
Variants in other genes, such as COMT, ABCB1, UGT2B7 and OPRM1, may also influence an individual’s response to codeine. However, evidence is lacking on whether genetic testing for these variants will aid optimum codeine dosing (7, 27-29).

**Therapeutic Recommendations based on Genotype**

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**2015 Statement from the US Food and Drug Administration (FDA):** Respiratory depression and death have occurred in children who received codeine in the post-operative period following tonsillectomy and/or adenoidectomy and had evidence of being ultra-rapid metabolizers of codeine (i.e., multiple copies of the gene for cytochrome P450 isoenzyme 2D6 [CYP2D6] or high morphine concentrations). Deaths have also occurred in nursing infants who were exposed to high levels of morphine in breast milk because their mothers were ultra-rapid metabolizers of codeine.

Some individuals may be ultra-rapid metabolizers because of a specific CYP2D6 genotype (gene duplications denoted as *1/*1xN or *1/*2xN). The prevalence of this CYP2D6 phenotype varies widely and has been estimated at 0.5 to 1% in Chinese and Japanese, 0.5 to 1% in Hispanics, 1 to 10% in Caucasians, 3% in African Americans, and 16 to 28% in North Africans, Ethiopians, and Arabs. Data are not available for other ethnic groups. These individuals convert codeine into its active metabolite, morphine, more rapidly and completely than other people. This rapid conversion results in higher than expected serum morphine levels. Even at labeled dosage regimens, individuals who are ultra-rapid metabolizers may have life-threatening or fatal respiratory depression or experience signs of overdose (such as extreme sleepiness, confusion, or shallow breathing).

Please review the complete therapeutic recommendations that are located here: (1)

**2014 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC):** A standard starting dose of codeine, as recommended in the product label, is warranted in patients with an extensive metabolizer phenotype (i.e., a CYP2D6 activity score of 1.0–2.0). Likewise, a standard starting dose of codeine is warranted in patients

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1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

with an intermediate metabolizer phenotype (i.e., activity score of 0.5); these patients should be monitored closely for less-than-optimal response and should be offered an alternative analgesic if warranted. If the CYP2D6 substrate tramadol is selected as alternative therapy in intermediate metabolizers, therapy should be monitored closely due to the possibility of poor response.

If clinical genotyping identifies a patient as a CYP2D6 poor metabolizer (i.e., activity score of 0), current evidence supports the avoidance of codeine and the use of an alternative analgesic due to the possibility of lack of effect. Use of an analgesic other than the CYP2D6 substrates tramadol, hydrocodone, or oxycodone in poor metabolizers may be preferable. There is insufficient evidence in the literature to recommend a higher dose of codeine in poor metabolizers, especially considering the evidence that select adverse effects do not differ between poor and extensive metabolizers. In a patient identified as a CYP2D6 ultrarapid metabolizer (i.e., activity score of >2.0), the choice of another analgesic should be made to avoid the risk of severe toxicity with a “normal” dose of codeine.

Please review the complete therapeutic recommendations that are located here: (2).

2013 Clinical practice Guideline from the “Canadian Pharmacogenomics Network for Drug Safety (CPNDS) Clinical Recommendations Group: CYP2D6 genotyping for safe and efficacious codeine therapy”:

1. Who should be tested and when?
   - Young children about to receive codeine for pain management and women about to receive codeine for postpartum pain while breastfeeding should be tested for CYP2D6 (Grade A – strong recommendation).
   - Children and adults who continue to have pain despite high doses of codeine should be tested for CYP2D6 (Grade B – moderate recommendation).
   - Genetic testing for CYP2D6 should be considered before administering codeine for the first time in all children and adults in order to rule out non-responders and to identify individuals who may be susceptible to adverse effects from codeine (Grade C – optional recommendation).

2. What gene variants should be tested?

Given the numerous polymorphisms in CYP2D6 and the diversity of the Canadian population, a full-scale analysis of both common and rare CYP2D6 variants is advised (Grade B- moderate recommendation)

- \textit{CYP2D6} alleles with decreased or no function: \textit{CYP2D6} *3- 12, 14-15, 17, 19-20, 29, 40-42, 44, 49, 50, 54-56, 59; *4XN, *10XN
- \textit{CYP2D6} alleles with normal or increased function: \textit{CYP2D6} *2 (normal), *1XN (increased), *2XN (increased), *17XN, *35XN (increased), *41XN, in addition to \textit{CYP2D6} copy number determination.

Recommendations: Genotype-Specific Treatment Options
• Poor metabolizers of CYP2D6 should not receive codeine for pain relief (Grade A–strong recommendation).
• Ultrarapid metabolizers of CYP2D6 should avoid codeine for pain relief and receive alternative analgesics that do not have potent CYP2D6 metabolites (Grade B–moderate recommendation).
• Certain populations, especially opioid naïve breastfed neonates of mothers with functional CYP2D6 gene duplications taking codeine and young children may be particularly susceptible to codeine-induced central nervous system depression. Breastfeeding mothers and young children who are ultrarapid metabolizers of CYP2D6 should avoid codeine (Grade A – strong recommendation).
• In individuals with IM or EM CYP2D6 genotypes, codeine can be used as per standard of care. Existing evidence suggests that caution is still warranted in CYP2D6 EMs receiving codeine if they are receiving maximal therapeutic doses of codeine and have additional risk factors for toxicity.

Please review the complete therapeutic recommendations that are located here: (30)

Nomenclature

Nomenclature of selected CYP2D6 alleles

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*4</td>
<td>1846G&gt;A</td>
<td>NM_000106.5:c.506-1G&gt;A</td>
<td>rs3892097</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>1707 del T Trp152Gly CYP2D6T</td>
<td>NM_000106.5:c.454delT</td>
<td>NP_000097.3:p.Trp152Glyfs rs5030655</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>100C&gt;T (Pro34Ser)</td>
<td>NM_000106.5:c.100C&gt;T</td>
<td>NP_000097.3:p.Pro34Ser rs1065852</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>1023C&gt;T[1] (Thr107Ile)</td>
<td>NM_000106.5:c.320C&gt;T</td>
<td>NP_000097.3:p.Thr107Ile rs28371706</td>
</tr>
<tr>
<td></td>
<td>2850C&gt;T[2] (Cys296Arg)</td>
<td>NM_000106.5:c.886T&gt;C</td>
<td>NP_000097.3:p.Cys296Arg rs16947</td>
</tr>
<tr>
<td></td>
<td>2988G&gt;A</td>
<td>NM_000106.5:c.985+39G&gt;A</td>
<td>Variant occurs in a non-coding region (impacts slicing). rs28371725</td>
</tr>
</tbody>
</table>

[1] In the literature, 1023C>T is also referred to as 1111C>T
[2] In the literature, 2850C>T is also referred to as 2938C>T
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

**Acknowledgments**

The author would like to thank Todd Skaar, Associate Professor of Medicine, Indiana University; and Kristine R. Crews, Director, Translational Research Laboratory, and Director, PGY2 Pharmacogenetics Residency Program, St. Jude Children's Research Hospital, Memphis, TN, for reviewing this summary.

**First edition:**

The Pharmacogenomics Knowledgebase: [http://www.pharmgkb.org](http://www.pharmgkb.org)


**Version History**

To view an earlier version of this summary, please see:

Update 08 March 2016

Update 18 March 2013

**References**


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Aripiprazole Therapy and CYP2D6 Genotype
Atomoxetine Therapy and CYP2D6 Genotype
Clozapine Therapy and CYP2D6, CYP1A2, and CYP3A4 Genotypes
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**Tests in GTR by Condition**
Codeine response

**Tests in GTR by Gene**
CYP2D6 gene gene
Dabrafenib Therapy and *BRAF* and *G6PD* Genotype

Laura Dean, MD

Created: August 15, 2017.

**Introduction**

Dabrafenib is a kinase inhibitor used in the treatment of patients with unresectable or metastatic melanoma with specific *BRAF* variants. Dabrafenib can be used as a single agent to treat melanoma with the *BRAF* V600E variant, or in combination with the MEK inhibitor trametinib to treat melanoma with *BRAF* V600E or V600K variants.

*BRAF* is an intracellular kinase in the mitogen-activated protein kinases (MAPK) pathway. *BRAF* is involved in regulating important cell functions such as cell growth, division, differentiation, and apoptosis. *BRAF* is also a proto-oncogene—when mutated it has the ability to transform normal cells into cancerous cells.

Variation in the kinase domain of *BRAF* have been associated with various cancers. The most common *BRAF* variant, V600E, constitutively activates the kinase, and causes cell proliferation in the absence of growth factors that would normally be required. The V600E variant is detected in approximately 50% of melanomas (1, 2).

The FDA-approved label for dabrafenib states that the presence of *BRAF* mutation in tumor specimens (V600E for dabrafenib monotherapy; V600E or V600K for dabrafenib plus trametinib) should be confirmed, using an FDA-approved test, before starting treatment with dabrafenib. Dabrafenib is not indicated for treatment of patients with wild-type *BRAF* melanoma.

The label also states that patients who have glucose-6-phosphate dehydrogenase (G6PD) deficiency should be monitored for signs of hemolytic anemia while taking dabrafenib (3).

**Drug: Dabrafenib**

Dabrafenib is a *BRAF* kinase inhibitor indicated for the treatment of patients with unresectable or metastatic melanoma. It acts by decreasing signaling through the MAPK pathway, leading to the reduced transcription of genes involved in various cellular responses.

Dabrafenib can be used as a single agent to treat melanoma with *BRAF* V600E variant, or in combination with trametinib to treat melanoma with *BRAF* V600E or V600K variants (3). Dabrafenib and other *BRAF* inhibitors have also demonstrated responses in patients

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1 NCBI; Email: dean@ncbi.nlm.nih.gov.
with rare \textit{BRAF} V600 variants (V600R, V600D) (4). These agents appear to be less active in pre-clinical studies of melanomas with atypical (non-V600) variants (e.g. L597, K601) (5).

Skin cancer is the most common of all cancers. Although melanoma is the least common type of skin cancer, accounting for approximately 1% of cases, it is responsible for the majority of deaths from skin cancer. In the US, the lifetime risk of melanoma is approximately 2.5% for whites, 0.5% for Hispanics, and 0.1% for blacks (6).

Most cases of malignant melanoma are diagnosed at an early stage, when the tumor is localized and surgical excision can be curative. However, the 5-year survival rate drops from 98% for localized disease, to only 16% for patients with metastatic disease.

For patients with advanced metastatic or unresectable malignant melanoma, treatment options typically include immunotherapy and targeted therapy. Although chemotherapy was once widely used, it does not increase survival and therefore its use is now limited to patients who are not candidates for further treatment with either immunotherapy or targeted therapy, and for whom there is no appropriate clinical trial.

High-dose interleukin 2 (IL2) therapy may be successful in a minority of cases, but can only be used in select patients with good organ function because of the risk of severe toxicity. Immunotherapy drugs include antibodies that target programmed cell death protein 1 (PD-1), e.g., nivolumab and pembrolizumab (7); and ipilimumab, a monoclonal antibody that targets cytotoxic T-lymphocyte-associated protein 4 (CTLA4). Oncolytic virus therapy with T-VEC (talimogene laherparepvec) is one of the newer immunotherapy drugs approved for melanoma.

Targeted therapies are designed to inhibit components of the MAPK signaling pathway, primarily when it is constitutively activated in melanomas with the activating \textit{BRAF} variant, V600E. Drugs in this category include vemurafenib and dabrafenib, which inhibit \textit{BRAF}, and trametinib and cobimetinib, which target downstream kinases MEK1 and MEK2, respectively.

Dabrafenib is a potent inhibitor of the kinase domain of the variant \textit{BRAF} V600E. It acts by decreasing signaling through the MAPK pathway, leading to the reduced transcription of genes involved in various cellular responses. Combining dabrafenib with MEK inhibitors has been shown to extend survival (8, 9), and dabrafenib is often used in combination with a MEK inhibitor, e.g., trametinib.

Dabrafenib increased progression-free survival, compared to cytotoxic chemotherapy (e.g., dacarbazine), in patients with advanced melanoma with the \textit{BRAF} V600E variant (10, 11). However, at this time there are no randomized trials that compare targeted therapies such as dabrafenib, with immunotherapy, and there are no data regarding the appropriate combinations and sequencing of these therapies for patients with a V600E variant.

A recent phase 3 trial for patients with melanoma with a V600E variant was stopped early because of positive results. The study found that the combination of dabrafenib plus
trametinib led to a higher 3-year overall survival rate, compared to vemurafenib monotherapy (25% versus 11%). In addition, the incidence of cutaneous squamous cell carcinoma was decreased in patients taking the combination of dabrafenib plus trametinib (12).

The drug label advises that a dermatological evaluation should be carried out prior to initiating dabrafenib therapy, and every 2 months during therapy. The most common adverse events associated with dabrafenib are skin lesions (benign and malignant). Other side effects include fever, arthralgia, fatigue, alopecia, and palmar-plantar erythrodysesthesia syndrome (“hand-foot syndrome”).

In vitro experiments with BRAF inhibitors, such as dabrafenib, have been found to cause a paradoxical activation of signaling pathways and proliferation in BRAF wild-type cells. Therefore, dabrafenib should only be used after the presence of BRAF V600E variant in tumor specimens has been confirmed using an FDA-approved test (3). The FDA also recommends to permanently discontinue dabrafenib use in patients who develop RAS mutation-positive non-cutaneous malignancies.

**Gene: BRAF**

RAF is a family of intracellular kinases within the MAPK signaling pathway. The RAF family has three members, ARAF, BRAF, and CRAF (13). RAF, along with RAS (see below), are proto-oncogenes.

Proto-oncogenes are genes that, when mutated or expressed at abnormally high levels, can transform normal cells into cancerous cells. Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. The increased production of oncogenic proteins can lead to the proliferation of poorly differentiated cancer cells (14).

Germline mutations in BRAF, as well as other components of the MAPK signaling pathway, are associated with birth defects, such as cardiofaciocutaneous syndrome, characterized by heart defects, mental retardation, and a distinctive facial dysmorphology. Somatic BRAF mutations are also associated with several malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, colorectal carcinoma, and malignant melanoma.

Variations in BRAF are detectable in approximately 50% of malignant melanomas, and drive progression of the disease (1, 2). The BRAF variant V600E accounts for approximately 90% of variants. This variant is a substitution of adenine for thymine at position 1799 and results in the substitution of valine for glutamate at codon 600. The variant BRAF protein kinase is constitutively active and a highly potent oncogene, with an increase in kinase activity by as much as 500-fold compared to the wild-type (15). The second most common BRAF variant is V600K. Substitutions at other sites are rarer (16, 17).
Several drugs are being developed to target BRAF variants, and so far, two drugs have been FDA-approved: vemurafenib and dabrafenib. Unfortunately, less progress has been made in developing targeted therapies for melanoma with wild-type BRAF. There are fewer treatment options available, but these include immunotherapy and MEK inhibitors (7, 18).

**Gene: G6PD**

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an inherited X-linked recessive disorder that results from genetic variation in the G6PD gene. The G6PD gene is located on the X chromosome and G6PD deficiency occurs almost exclusively in males, who have only one X chromosome. G6PD deficiency mainly affects red blood cells, which carry oxygen from the lungs to tissues throughout the body.

G6PD deficiency affects 400 million people worldwide (19), and is common among African Americans, affecting approximately 12% (20). G6PD deficiency appears to be protective against malaria infection (21).

G6PD catalyzes the initial step in the hexose monophosphate (HMP) pathway. In mature red blood cells, the HMP pathway is the only source of NADPH, a coenzyme essential for protection against oxidative stress and repair of oxidative damage.

Red blood cells that are G6PD deficient are more susceptible to oxidative stress caused by exposure to drugs (e.g. sulfamethoxazole, primaquine, and dabrafenib), infections, diabetic ketoacidosis, or following ingestion of fresh fava beans (favism). Because of the oxidative stress, the red blood cells become rigid, become trapped, and are subsequently destroyed by macrophages in the spleen, bone marrow, and liver. Premature and/or fast destruction of red blood cells is called hemolysis and can result in hemolytic anemia.

Most affected individuals are asymptomatic; however, those with symptoms may suffer from episodes of acute hemolytic anemia or chronic hemolytic anemia. The management of hemolytic episodes depends on the severity of hemolysis. More severe cases may require a transfusion of packed red blood cells. Folic acid may be given to prevent the worsening of anemia in individuals with folate deficiency.

The normal (wild-type) copy of the G6PD gene is known as G6PD A+ (p.Asn126Asp), and is found in up to 30% of blacks from Africa (22). More than 400 genetic variants of the G6PD gene have been identified so far, and most are missense point mutations (23). Common variants include:

- G6PD A- (p.Asn126Asp and p.Val68Met) which is associated with mild to moderate hemolysis, and is found in up to 15% of African-Americans (24)
- G6PD Mediterranean (p.Ser218Phe) which can cause severe hemolysis, and is the most common variant in Caucasians (25)
- G6PD Canton (p.Arg489Leu) which can cause severe hemolysis, and is found in Asians (26)
All individuals with G6PD deficiency should avoid oxidizing agents when possible, including specific drugs and chemicals. Dabrafenib can cause hemolytic anemia. The FDA-approved drug label for dabrafenib warns that “dabrafenib, which contains a sulfonamide moiety, confers a potential risk of hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Monitor patients with G6PD deficiency for signs of hemolytic anemia while taking dabrafenib” (3).

No cases of hemolytic anemia associated with dabrafenib have been published, although it is unclear whether individuals with G6PD deficiency have received dabrafenib.

**Genetic Testing**

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the genes *BRAF* and *G6PD*.

The FDA-approved label for dabrafenib states that the presence of *BRAF* mutation in tumor specimens (V600E for dabrafenib monotherapy; V600E or V600K for dabrafenib plus trametinib) should be confirmed, using an FDA-approved test, before starting treatment with dabrafenib. The label also states that dabrafenib is not indicated for treatment of patients with wild-type *BRAF* melanoma.

G6PD deficiency is typically diagnosed by screening tests that measure the activity of G6PD in red blood cells. A false positive may result immediately after an episode of hemolysis, so the test should be repeated at a later date. Molecular genetic testing can be used to confirm the diagnosis of G6PD, and may also be used to screen females with a family history of G6PD to see if they are carriers (27).

Screening for G6PD deficiency is recommended so that affected individuals can avoid agents that can cause oxidative stress and trigger hemolysis. G6PD deficiency is inherited in an X-linked recessive pattern. A heterozygous mother has a 50% chance of passing G6PD deficiency to a son and a 50% chance of passing the carrier trait to a daughter. Affected fathers pass the variant *G6PD* to their daughters, but not to their sons.

**Therapeutic Recommendations based on Genotype**

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**2016 Statement from the US Food and Drug Administration (FDA):**

*BRAF* V600E Mutation-Positive Unresectable or Metastatic Melanoma: Dabrafenib is indicated as a single agent for the treatment of patients with unresectable or metastatic melanoma with *BRAF* V600E mutation as detected by an FDA-approved test.

*BRAF* V600E or V600K Mutation-Positive Unresectable or Metastatic Melanoma: Dabrafenib is indicated, in combination with trametinib, for the treatment of patients...
with unresectable or metastatic melanoma with BRAF V600E or V600K mutations, as detected by an FDA-approved test.

Limitation of Use: Dabrafenib is not indicated for treatment of patients with wild-type BRAF melanoma.

Patient Selection: Confirm the presence of BRAF V600E mutation in tumor specimens prior to initiation of treatment with dabrafenib as a single agent. Confirm the presence of BRAF V600E or V600K mutation in tumor specimens prior to initiation of treatment with dabrafenib and trametinib. Information on FDA-approved tests for the detection of BRAF V600 mutations in melanoma is available at: http://www.fda.gov/CompanionDiagnostics.

[...]

Dabrafenib, which contains a sulfonamide moiety, confers a potential risk of hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Monitor patients with G6PD deficiency for signs of hemolytic anemia while taking dabrafenib.

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature

Selected BRAF variants

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>Coding</th>
<th>Protein</th>
</tr>
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<td>NP_004324.2:p.Val600Glu</td>
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<td>V600K</td>
<td>p.Val600Lys</td>
<td>NM_004333.4:c.1798_1799delGTinsAA</td>
<td>NP_004324.2:p.Val600Lys</td>
<td>rs121913227</td>
</tr>
<tr>
<td>V600R</td>
<td>p.Val600Arg</td>
<td>NM_004333.4:c.1798_1799delGTinsAG</td>
<td>NP_004324.2:p.Val600Arg</td>
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</tr>
<tr>
<td>V600D</td>
<td>p.Val600Asp</td>
<td>NM_004333.4:c.1799_1800delTGinsAT</td>
<td>NP_004324.2:p.Val600Asp</td>
<td>rs121913377</td>
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Selected G6PD variants

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<th>Protein</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>NM_000402.4:c.292G&gt;A</td>
<td>NP_001035810.1:p.Val68Met</td>
<td></td>
</tr>
</tbody>
</table>

Table continues on next page...
Table continued from previous page.

<table>
<thead>
<tr>
<th>Common allele name / condition</th>
<th>Alternative names / condition</th>
<th>HGVS reference sequence Coding</th>
<th>HGVS reference sequence Protein</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD Mediterranean</td>
<td>p.Ser218Phe</td>
<td>NM_000402.4(G6PD):c.653C&gt;T</td>
<td>NP_000393.4:p.Ser218Phe</td>
<td>rs5030868</td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

**Acknowledgments**

The author would like to thank Matthew Hardison, PhD, FACMG, Director of BioPharma Laboratory, Aegis Sciences Corporation, Nashville, TN; Douglas B. Johnson, MD, Assistant Professor of Medicine, Clinical Director of Melanoma Research Program, and Medical Oncologist at Vanderbilt University Medical Center, Nashville, Tennessee, Avadhut Joshi, PhD, Clinical Pharmacogenomics Lead, Translational Software, Bellevue, Washington; and Pamala A. Pawloski, PharmD, Research Investigator, HealthPartners Institute, Bloomington, MN; for reviewing this summary.

**References**


Related Summaries by Gene

Vemurafenib Therapy and BRAF and NRAS Genotype

Related Summaries by Drug Class

Vemurafenib Therapy and BRAF and NRAS Genotype

Tests in GTR by Gene

BRAF gene

G6PD gene
Diazepam Therapy and CYP2C19 Genotype
Laura Dean, MD

Introduction
Diazepam is a benzodiazepine with several clinical uses, including the management of anxiety, insomnia, muscle spasms, seizures, and alcohol withdrawal. The clinical response to benzodiazepines, such as diazepam, varies widely between individuals (1, 2).

Diazepam is primarily metabolized by CY2C19 and CYP3A4 to the major active metabolite, desmethyldiazepam. Approximately 3% of Caucasians and 15 to 20% of Asians have reduced or absent CYP2C19 enzyme activity (“poor metabolizers”). In these individuals, standard doses of diazepam may lead to a higher exposure to diazepam.

The FDA-approved drug label for diazepam states that “The marked inter-individual variability in the clearance of diazepam reported in the literature is probably attributable to variability of CYP2C19 (which is known to exhibit genetic polymorphism; about 3-5% of Caucasians have little or no activity and are “poor metabolizers”) and CYP3A4” (1).

Drug: Diazepam
Diazepam is used in the management of anxiety disorders or for the short-term relief of the symptoms of anxiety. In acute alcohol withdrawal, diazepam may provide symptomatic relief from agitation, tremor, delirium tremens, and hallucinations. Diazepam is also useful as an adjunct treatment for the relief of acute skeletal muscle spasms, as well as spasticity caused by upper motor neuron disorders (3).

There are currently 16 benzodiazepines licensed by the FDA. Diazepam was the second benzodiazepine to be used clinically (after chlordiazepoxide), after being approved for use in 1963. It remains a commonly used drug today, and is included in the World Health Organization’s core list of essential medicines needed for a basic healthcare system (4).

The use of benzodiazepines has replaced the use of barbiturates. Although these drug classes share similar therapeutic effects, barbiturates have a narrower therapeutic index, they are more sedative at therapeutic doses, and a barbiturate overdose is more likely to be fatal (5).

Like all benzodiazepines, diazepam is a controlled substance. Chronic use, either at standard therapeutic doses or through recreational abuse, can lead to tolerance and physical dependence. If diazepam treatment is abruptly discontinued, withdrawal

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
symptoms can arise which can be severe and include seizures. Therefore, a gradual tapering of dose is recommended after chronic therapy.

Diazepam has several therapeutic effects—it is a sedative, anxiolytic, anticonvulsant muscle relaxant, and has amnestic effects. Diazepam is thought to exert these effects through an interaction with GABA A-type receptors (GABA_A). GABA is the major inhibitory neurotransmitter in the central nervous system. When GABA binds to the GABA_A receptor, the receptor opens, allowing the influx of chloride ions into neurons. This reduces the ability of neurons to depolarize and produce action potentials (excessive action potentials are implicated in seizures). It is thought that diazepam enhances the effects of GABA by increasing the affinity between GABA and its receptor, causing GABA to bind more tightly to the GABA_A receptor (1).

Diazepam is primarily metabolized via CYP2C19 and CYP3A4 to the major active metabolite (desmethyldiazepam), which is found in the plasma at concentrations equivalent to diazepam. Two minor active metabolites include temazepam and oxazaepam, which are usually not detectable. Other CYP enzymes involved in diazepam metabolism include CYP2C9, CYP2B, and CYP3A5 (2).

It is well documented that wide inter-individual variation in the metabolism of benzodiazepines occurs, which includes diazepam metabolism. This can result in marked differences in drug levels when standard dosing is used, and may potentially influence both therapeutic and adverse effects. It is thought that the variability in clearance of many benzodiazepines, including diazepam, is due to the variability in CYP2C19 and CYP3A4 genotypes (2, 3, 6, 7).

**Gene: CYP2C19**

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, several proton pump inhibitors, clopidogrel, and benzodiazepines, including diazepam.

The CYP2C19 gene is highly polymorphic, as 35 variant star (*) alleles are currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2c19.htm). The CYP2C19*1 wild-type allele is associated with normal enzyme activity and the “normal metabolizer” phenotype, whereas the CYP2C19*17 allele is associated with increased enzyme activity and the “ultrarapid metabolizer” phenotype (8).

The most common loss-of-function variant is CYP2C19*2, which contains a c.681G>A variant in exon 5 that results in an aberrant splice site. This leads to the production of a
truncated and non-functioning protein. The CYP2C19*2 allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (8, 9).

Another commonly tested loss-of-function variant is CYP2C19*3, which contains a c. 636G>A variant in exon 4 that causes a premature stop codon. The CYP2C19*3 allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other loss-of-function variants occur in less than 1% of the general population, and include CYP2C19*4-*8 (8, 9).

“Intermediate CYP2C19 metabolizers” carry one copy of an allele that encodes reduced or absent function (e.g., *1/*2), whereas “poor metabolizers” are homozygous or compound heterozygous for two loss-of-function alleles (e.g., *2/*2, *2/*3) (table 1).

**Table 1: CYP2C19 phenotypes**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Phenotype Definition</th>
<th>Genetic Definition</th>
<th>Diploptide Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19 Ultrarapid metabolizer</td>
<td>Increased enzyme activity compared to rapid metabolizers</td>
<td>Two increased function alleles</td>
<td>*17/*17</td>
</tr>
<tr>
<td>CYP2C19 Rapid metabolizer</td>
<td>Increased enzyme activity compared to normal metabolizers, but less than ultrarapid metabolizers</td>
<td>Combinations of normal function and increased function alleles</td>
<td>*1/*17</td>
</tr>
<tr>
<td>CYP2C19 Normal metabolizer</td>
<td>Fully functional enzyme activity</td>
<td>Two normal function alleles</td>
<td>*1/*1</td>
</tr>
</tbody>
</table>
| CYP2C19 Intermediate metabolizer   | Decreased enzyme activity (activity between normal and poor metabolizer) | Combinations of normal function, decreased function, and/or no function alleles | *1/*2,
|                                    |                                                           |                                                         | *1/*3,
|                                    |                                                           |                                                         | *2/*17,
|                                    |                                                           |                                                         | *3/*17            |
| CYP2C19 Poor metabolizer           | Little or no enzyme activity                              | Combination of no function alleles, and/or decreased function alleles | *2/*2,
|                                    |                                                           |                                                         | *2/*3,
|                                    |                                                           |                                                         | *3/*3              |

Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)” (10).

Studies have found that individuals who are poor metabolizers have a lower plasma clearance of diazepam compared to normal metabolizers, and that diazepam had a longer plasma half-life (7, 11-13). However, currently, the FDA does not recommend a reduced dose of diazepam in CYP2C19 poor metabolizers.

One common use of diazepam is to relieve preoperative anxiety in patients. One study found that CYP2C19 poor metabolizers took a longer period of time to emerge from general anesthesia than normal metabolizers. This study also found that the “slow emergers” had lower levels of CYP3A4 mRNA (14).
Although CYP3A4 is also involved in diazepam metabolism, there have been conflicting results from studies of the impact of CYP3A4 and CYP3A5 variants on benzodiazepine metabolism (15-18).

**Genetic Testing**

Clinical genotyping tests are available for several CYP2C19 alleles, and a list of test providers is available at the Genetic Testing Registry (GTR) of the National Institutes of Health.

Usually a patient’s result is reported as a diplotype, such as CYP2C19 *1/*1, and may also include an interpretation of the patient’s predicted metabolizer phenotype: ultrarapid, rapid, normal, intermediate, or poor (see table 1).

**Therapeutic Recommendations based on Genotype**

This section contains excerpted\(^1\) information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**Statement from the US Food and Drug Administration (FDA):** It has been reported in the literature that diazepam is extensively metabolized to one major active metabolite (desmethyldiazepam) and two minor active metabolites, 3- hydroxydiazepam (temazepam) and 3-hydroxy-N-diazepam (oxazepam) in plasma. At therapeutic doses, desmethyldiazepam is found in plasma at concentrations equivalent to those of diazepam while oxazepam and temazepam are not usually detectable. The metabolism of diazepam is primarily hepatic and involves demethylation (involving primarily CYP2C19 and CYP3A4) and 3-hydroxylation (involving primarily CYP3A4), followed by glucuronidation. The marked inter-individual variability in the clearance of diazepam reported in the literature is probably attributable to variability of CYP2C19 (which is known to exhibit genetic polymorphism; about 3-5% of Caucasians have little or no activity and are “poor metabolizers”) and CYP3A4. No inhibition was demonstrated in the presence of inhibitors selective for CYP2A6, CYP2C9, CYP2D6, CYP2E1, or CYP1A2, indicating that these enzymes are not significantly involved in metabolism of diazepam.

Please review the complete therapeutic recommendations that are located here: (1).

\(^1\) The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
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<td>CYP2C19*2</td>
<td>681G&gt;A Pro227Pro</td>
<td>NM_000769.2:c.681G&gt;A</td>
<td>NP_000760.1:p.Pro227= rs4244285</td>
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<tr>
<td>CYP2C19*3</td>
<td>636G&gt;A Trp212Ter</td>
<td>NM_000769.2:c.636G&gt;A</td>
<td>NP_000760.1:p.Trp212Ter rs4986893</td>
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<tr>
<td>CYP2C19*17</td>
<td>-806C&gt;T</td>
<td>NM_000769.2:c.-806C&gt;T</td>
<td>Not applicable—variant occurs in a non-coding region rs12248560</td>
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Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

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References


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Carisoprodol Therapy and CYP2C19 Genotype
Clopidogrel Therapy and CYP2C19 Genotype
Esomeprazole Therapy and CYP2C19 Genotype
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Omeprazole Therapy and CYP2C19 Genotype
Prasugrel Therapy and CYP Genotype

Tests in GTR by Condition

Diazepam response

Tests in GTR by Gene

CYP2C19 gene
Esomeprazole Therapy and CYP2C19 Genotype

Laura Dean, MD
Created: October 1, 2012; Updated: March 8, 2016.

Introduction

Esomeprazole blocks the secretion of gastric acid and belongs to the drug class of proton pump inhibitors. It is used to treat gastroesophageal reflux disease (GERD) and to reduce the risk of gastric ulcers associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs). Esomeprazole is also used in the treatment of hypersecretory conditions, such as Zollinger-Ellison syndrome, and is used in combination with antibiotics to eradicate Helicobacter pylori (H. pylori) infection (1).

Esomeprazole is metabolized primarily by the CYP2C19 enzyme. Approximately 3% of Caucasians and 15 to 20% of Asians have reduced or absent CYP2C19 enzyme activity (“poor metabolizers”). In these individuals, standard doses of esomeprazole leads to higher exposure of the drug (2). In contrast, individuals with increased CYP2C19 activity (“ultrarapid metabolizers”) may be exposed to lower levels of esomeprazole and have an insufficient response to treatment.

The FDA-approved drug label for esomeprazole states that poor metabolizers are exposed to approximately twice the level of drug compared to the rest of the population (“extensive metabolizers”), but the label does not require dose changes for poor metabolizers (1). However, the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) has published dose alterations based on CYP2C19 genotype. For CYP2C19 poor metabolizers, they do not recommend altering the dose; however for ultrarapid metabolizers, they recommend being extra alert to an insufficient response to treatment. For the eradication of H. pylori in ultrarapid metabolizers, KNMP recommends increasing the dose of omeprazole by 50–100%, and to consider the same dose increase for other conditions (see Table 1) (3, 4).
Table 1. CYP2C19 phenotypes and the therapeutic recommendations for esomeprazole therapy, adapted from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP).

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Phenotype details</th>
<th>Examples of diplotypes</th>
<th>Therapeutic recommendations for esomeprazole</th>
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</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>Normal or increased CYP2C19 activity</td>
<td>*17/*17</td>
<td>Be extra alert to insufficient response. For the eradication of <em>H. pylori</em>, increase dose by 50–100%. For other conditions, consider dose increase by 50–100%.</td>
</tr>
<tr>
<td>Extensive metabolizer</td>
<td>Normal CYP2C19 activity</td>
<td>*1/*1</td>
<td>No recommendations</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>Decreased CYP2C19 activity</td>
<td>*1/*2, *1/*3, *2/*17, *3/*17</td>
<td>No recommendations</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>Markedly reduced or absent CYP2C19 activity</td>
<td>*2/*2, *2/*3, *3/*3</td>
<td>No recommendations</td>
</tr>
</tbody>
</table>

Good quality evidence supports the dose recommendations for poor and intermediate metabolizers; data are lacking for ultrarapid metabolizers.


**Drug class: Proton Pump Inhibitors**

Proton pump inhibitors (PPIs) are inhibitors of gastric acid secretion that are used in the treatment of stomach-acid related disorders. PPIs are also used to prevent and treat ulcers associated with nonsteroidal anti-inflammatory drugs (NSAIDs), and can be used in combination with antibiotics to eradicate *H. pylori* infection.

Six PPIs are currently FDA-approved for clinical use: esomeprazole, dexlansoprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole. All PPIs are similarly potent at inhibiting gastric acid secretion and are thought to be similarly efficacious (5, 6).

PPIs are metabolized and inactivated by a number of CYP enzymes, including CYP2C19, which has a principal role in the metabolism of omeprazole. The increased function *CYP2C19*+17 variant allele may enhance PPI clearance (7) resulting in less active PPI available to inhibit gastric acid secretion. In contrast, the *CYP2C19*+2 loss-of-function allele is associated with decreased PPI clearance, resulting in more active PPI available and enhanced treatment. For several PPIs, including omeprazole and lansoprazole, higher drug levels in patients with low or absent CYP2C19 activity have been associated with increased drug efficacy and improved treatment outcomes (2, 8).
Drug: Esomeprazole

Esomeprazole is used in the treatment of GERD in both adults and children. In adults, esomeprazole is also used to reduce the risk of developing a gastric ulcer associated with NSAID use, and in the treatment of pathological hypersecretory conditions such as Zollinger-Ellison syndrome (1).

The human stomach contains approximately one billion parietal cells that secrete hydrochloric acid (HCl) into the gastric lumen. Gastric acid aids digestion by hydrolyzing dietary protein and facilitating the absorption of calcium, iron, and vitamin B. Gastric acid also helps maintain a sterile environment by suppressing the growth of bacteria (9).

Hydrogen ions (H+) are actively secreted in to the gastric lumen in exchange for potassium ions (K+) via an H⁺/K⁺-ATPase, which is also known as a “proton pump”. Located on the surface of gastric parietal cells, the proton pump controls the last step in acid secretion, and by targeting this step, esomeprazole and the other PPIs are able to potently inhibit gastric acid secretion.

Esomeprazole is metabolized in the liver by the cytochrome P450 system to inactive metabolites. CYP2C19 metabolizes esomeprazole to hydroxy and desmethyl metabolites, and the remaining drug is metabolized by CYP3A4 to sulphone metabolites (1).

Individuals with reduced CYP2C19 enzyme activity may experience twice the exposure to esomeprazole compared to individuals with normal enzyme function. For other PPIs, such as omeprazole and lansoprazole, increased drug levels are associated with improved clinical outcomes (10, 11). For example, one study reported that when using omeprazole as part of the treatment to eradicate H. pylori, success was achieved in all patients who had little or no CYP2C19 activity, but in only 29% of patients who had “normal” CYP2C19 activity. Similar results were found in another study that evaluated lansoprazole in the treatment of GERD: the cure rate was 85% for patients with little or no CYP2C19 activity, compared to 16% for patients with normal CYP219 activity (12-14).

However, the efficacy of esomeprazole appears to be less influenced by CYP2C19 genotype, at least for the treatment of GERD (15-17). This may be because of the shift towards CYP3A4-mediated metabolism and elimination of the drug (9).

The FDA-approved drug label for esomeprazole does not comment on dose adjustments based on CYP2C19 status. However, guidelines from KNMP recommend that patients with increased CYP2C19 activity (“ultrarapid metabolizers”) should receive an increased dose of esomeprazole for the eradication of H. pylori, and that an increased dose should be considered for other indications (Table 1).

The long-term use of PPIs has been associated with several adverse effects. Daily treatment with any PPI for longer than three years may lead to malabsorption of vitamin B12, caused by hypochlorhydria. Because prolonged hypochlorhydria also increases the risk of Clostridium difficile infection, and may increase the risk for osteoporosis-related
fractures, the FDA recommends that patients should use the lowest dose and shortest duration of PPI therapy appropriate to the condition being treated (1).

**Gene: **CYP2C19

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, benzodiazepines, and some of the PPIs, including esomeprazole. CYP2C19 is highly polymorphic, as 35 variant star (*) alleles are currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2c19.htm). The CYP2C19*1 wild-type allele is associated with normal enzyme activity and the “extensive metabolizer” phenotype (18).

The most common loss-of-function variant is CYP2C19*2, which contains a c.681G>A variant in exon 5 that results in an aberrant splice site. This leads to the production of a truncated and non-functioning protein. The CYP2C19*2 allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (19). “Intermediate metabolizers” carry one copy of an allele that encodes reduced or absent function (e.g. *1/*2), whereas “poor metabolizers” are homozygous or compound heterozygous for two loss-of-function alleles (e.g., *2/*2, *2/*3).

Another commonly tested loss-of-function variant is CYP2C19*3, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The CYP2C19*3 allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other loss-of-function variants occur in less than 1% of the general population, and include CYP2C19*4-8 (19, 20).

In contrast to non-functional alleles, the CYP2C19*17 allele (c.-806C>T) is associated with increased enzyme activity. Allele frequencies range from 3 to 21% across different populations (21). Individuals who are homozygous for the *17 allele are known as “ultrarapid metabolizers”, and it is this patient group who may benefit from an increased dose of omeprazole. However, not all studies have identified a significant effect of CYP2C19*17 on the metabolism of PPIs and treatment outcomes (14, 22, 23).

**Genetic Testing**

Currently, the FDA does not provide recommendations about the use of CYP2C19 genetic testing for esomeprazole treatment (1).
Clinical genotyping tests are available for several CYP2C19 alleles, and a list of some test providers is available at the Genetic Testing Registry (GTR) of the National Institutes of Health: [http://www.ncbi.nlm.nih.gov/gtr/tests/?term=1557](http://www.ncbi.nlm.nih.gov/gtr/tests/?term=1557).

Usually a patient’s result is reported as a diplotype, such as CYP2C19 *1/*1, and may also include an interpretation of the patient’s predicted metabolizer phenotype (ultrarapid, extensive, intermediate, or poor).

Table 1 summarizes common CYP2C19 phenotypes with recommendations developed by the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP).

### Therapeutic Recommendations based on Genotype

This section contains excerpted¹ information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**2015 Statement from the US Food and Drug Administration (FDA):** Esomeprazole is extensively metabolized in the liver by the cytochrome P450 (CYP) enzyme system. The metabolites of esomeprazole lack antisecretory activity. The major part of esomeprazole’s metabolism is dependent upon the CYP2C19 isoenzyme, which forms the hydroxy and desmethyl metabolites. The remaining amount is dependent on CYP3A4 which forms the sulphone metabolite. CYP2C19 isoenzyme exhibits polymorphism in the metabolism of esomeprazole, since some 3% of Caucasians and 15 to 20% of Asians lack CYP 2C19 and are termed Poor Metabolizers. At steady state, the ratio of AUC in Poor Metabolizers to AUC in the rest of the population (Extensive Metabolizers) is approximately 2.

Please review the [complete therapeutic recommendations that are located here](#): (1).

**2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):** For individuals who are ultrarapid metabolizers, physicians should be vigilant to an insufficient response to esomeprazole therapy. For the eradication of *H. pylori*, the dose of esomeprazole should be increased by 50–100%.

Please review the [complete therapeutic recommendations that are located here](#): (4).

---

¹ The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.
Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence Coding</th>
<th>Protein</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19*2</td>
<td>681G&gt;A Pro227Pro</td>
<td>NM_000769.1:c.681G&gt;A</td>
<td>NP_000760.1:p.Pro227=</td>
<td>rs4244285</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>636G&gt;A Trp212Ter</td>
<td>NM_000769.1:c.636G&gt;A</td>
<td>NP_000760.1:p.Trp212Ter</td>
<td>rs4986893</td>
</tr>
<tr>
<td>CYP2C19*17</td>
<td>-806C&gt;T</td>
<td>NM_000769.2:c.-806C&gt;T</td>
<td>Not applicable—variant occurs in a non-coding region</td>
<td>rs12248560</td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

Acknowledgments

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Version History

To view an earlier version of this summary (update: 18 March 2013), please click here.

References


Related Summaries by Gene
Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Carisoprodol Therapy and CYP2C19 Genotype
Clopidogrel Therapy and CYP2C19 Genotype
Diazepam Therapy and CYP2C19 Genotype
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Omeprazole Therapy and CYP2C19 Genotype
Prasugrel Therapy and CYP Genotype

Related Summaries by Drug Class
Omeprazole Therapy and CYP2C19 Genotype

Tests in GTR by Condition
Esomeprazole response
Tests in GTR by Gene

CYP2C19 gene
Fluorouracil Therapy and *DPYD* Genotype

Laura Dean, MD

Created: November 3, 2016.

**Introduction**

Fluorouracil is a chemotherapy agent that belongs to the drug class of fluoropyrimidines. When given as an IV solution, fluorouracil is used in the palliative management of carcinoma of the colon, rectum, breast, stomach, and pancreas (1). When prescribed as a cream for topical use, fluorouracil is used to treat multiple actinic or solar keratoses of the face and scalp (2).

The *DPYD* gene encodes dihydropyrimidine dehydrogenase (DPD), an enzyme that catalyzes the rate-limiting step in fluorouracil metabolism. Individuals who carry at least one copy of no function *DPYD* variants, such as *DPYD*2A, may not be able to metabolize fluorouracil at normal rates, and are at risk of potentially life-threatening fluorouracil toxicity, such as bone marrow suppression and neurotoxicity. The prevalence of DPD deficiency in Caucasians is approximately 3%-5%.

The FDA-approved drug label for fluorouracil states that “rarely, unexpected, severe toxicity (e.g., stomatitis, diarrhea, neutropenia and neurotoxicity) associated with 5-fluorouracil has been attributed to deficiency of dipyrimidine dehydrogenase activity” (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published dosing recommendations for fluoropyrimidines (capecitabine, fluorouracil, and tegafur) based on *DPYD* genotype (3) (Table 1). CPIC recommends using an alternative drug for patients who are “poor metabolizers.” These individuals carry two copies of no function *DPYD* variants and typically have complete DPD deficiency. CPIC also recommends considering a 50% reduction in starting dose for “intermediate metabolizers.” These individuals carry a combination of a normal function and a no function variant and typically have reduced DPD activity (approximately 50% reduced) (3, 4).

**Drug Class: Fluoropyrimidines**

Fluoropyrimidines are a class of antimetabolite drugs that are widely used in the treatment of cancer. Currently, there are three types of fluoropyrimidines in clinical use: capecitabine, fluorouracil, and tegafur. Capecitabine and tegafur are both active precursors of fluorouracil.

Fluoropyrimidines are thought to exert their chemotherapeutic effects in a number of ways, through several active metabolites. The main mechanism of action is thought to be
the inhibition of thymidylate synthase, which plays an important part in the folate-homocysteine cycle, and purine and pyrimidine synthesis pathways. Also, active metabolites can be incorporated into RNA and DNA, ultimately leading to cell death (5).

Approximately 10-40% of patients develop severe and potentially life-threatening toxicity early during treatment with fluoropyrimidines (6). This toxicity typically leads to an interruption or discontinuation of potentially effective anticancer therapy, and often requires hospitalization (7).

The inter-individual variation in the occurrence and severity of adverse events in patients receiving fluoropyrimidines can be partly explained by clinical factors, such as age and sex. However, much of the variability in adverse events remains unexplained (8).

Of the genetic factors thought to contribute to fluoropyrimidine intolerance, the DPYD gene has been the most studied. This gene encodes the primary enzyme involved in breaking down fluoropyrimidines to inactive metabolites. Individuals who have a deficiency of the DPD enzyme have a significantly increased risk of suffering from severe fluoropyrimidine toxicity, and the stratification of patients on the basis of the DPYD genotype may help to prevent such adverse events (9-14).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published genetics-based dosing recommendations for fluoropyrimidines based on DPYD genotype (Table 1).

### Table 1. 2013 Recommended dosing of Fluoropyrimidines by DPD phenotype, from Clinical Pharmacogenetics Implementation Consortium (CPIC)

| Phenotype               | Implications for phenotypic measures                                      | Dosing recommendations                                      | Classification of recommendations
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal metabolizer</td>
<td>Normal DPD activity and “normal” risk for fluoropyrimidine toxicity</td>
<td>Use label-recommended dosage and administration</td>
<td>Moderate</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>Decreased DPD activity (leukocyte DPD activity at 30–70% that of the normal population) and increased risk for severe or even fatal</td>
<td>Start with at least a 50% reduction in starting dose, followed by titration of dose based on toxicity or increase the dose in patients experiencing no or clinically tolerable toxicity to maintain efficacy; decrease the dose in patients who do not tolerate the starting dose to minimize toxicities.</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Fluoropyrimidines: 5-fluorouracil, capecitabine, and tegafur.
DPD, dihydropyrimidine dehydrogenase.

a Rating scheme is described here (3)
b Increase the dose in patients experiencing no or clinically tolerable toxicity to maintain efficacy; decrease the dose in patients who do not tolerate the starting dose to minimize toxicities.

Table is adapted from Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical pharmacology and therapeutics.2013:94(6):640-5 (3)

Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, “Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)” (15).
Fluorouracil Therapy and *DPYD* Genotype

Table 1. continued from previous page.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implications for phenotypic measures</th>
<th>Dosing recommendations</th>
<th>Classification of recommendations&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor metabolizer</td>
<td>Complete DPD deficiency and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs</td>
<td>Select alternative drug</td>
<td>Strong</td>
</tr>
</tbody>
</table>

Fluoropyrimidines: 5-fluorouracil, capcitabine, and tegafur.

<sup>a</sup> Rating scheme is described here (3).

<sup>b</sup> Increase the dose in patients experiencing no or clinically tolerable toxicity to maintain efficacy; decrease the dose in patients who do not tolerate the starting dose to minimize toxicities.


Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (15).

**Drug: Fluorouracil**

Fluorouracil is a form of chemotherapy that when given as an IV solution, is used in the palliative management of carcinoma of the colon, rectum, breast, stomach, and pancreas. Fluorouracil may also be used topically as a cream, for the treatment of multiple actinic or solar keratoses of the face and anterior scalp.

Fluorouracil is structurally similar to pyrimidines, and the enzyme that catalyzes the rate-limiting step in the breakdown of pyrimidines (DPD, dihydropyrimidine dehydrogenase) also catalyzes the rate-limiting step in 5-fluorouracil metabolism. DPD catalyzes the conversion of fluorouracil to the non-cytotoxic dihydrofluorouracil (DHFU) (16).

Fluorouracil is a highly toxic drug with a narrow margin of safety. The FDA-approved label contains the following boxed warning: "It is recommended that Fluorouracil Injection, USP be given only by or under the supervision of a qualified physician who is experienced in cancer chemotherapy and who is well versed in the use of potent antimetabolites. Because of the possibility of severe toxic reactions, it is recommended that patients be hospitalized at least during the initial course of therapy. These instructions should be thoroughly reviewed before administration of Fluorouracil Injection, USP."

The FDA also states that fluorouracil therapy should be discontinued promptly whenever one of the following signs of toxicity appears:

- Stomatitis or esophageal pharyngitis, at the first visible sign
• Leukopenia (WBC under 3500) or a rapidly falling white blood count
• Vomiting, intractable
• Diarrhea, frequent bowel movements, or watery stools
• Gastrointestinal ulceration and bleeding
• Thrombocytopenia (platelets under 100,000)
• Hemorrhage from any site

Symptomatic DPD deficiency is a rare autosomal recessive disorder with a wide range of symptoms, ranging from no symptoms or signs, to severe neurological problems. In affected individuals, the absent or greatly reduced DPD activity results in uracil and thymine accumulating in the blood, urine, and cerebrospinal fluid. Neurological symptoms typically manifest in early childhood and include seizures, small head size, and delayed cognitive and motor development (17).

Symptomatic DPD deficiency is typically caused by homozygous inactivation of DPYD; whereas individuals who are heterozygotes tend to be asymptomatic. However, all patients with less than 70% DPD activity are considered at risk for the development of severe drug toxicity when treated with fluoropyrimidines (18). Signs of fluorouracil toxicity include severe diarrhea, severe mucositis, neutropenia, neurotoxicity, and hand-foot syndrome (redness, swelling, and blisters on the palms of the hands and soles of the feet) (1).

Approximately 3-5% of Caucasians have partial DPD deficiency and 0.2% have complete DPD deficiency (19). Currently, most patients are not screened for DPD deficiency before starting capecitabine therapy (20).

**Gene: DPYD**

The DPYD gene encodes the enzyme dihydropyrimidine dehydrogenase (DPD), which catalyzes the first and the rate-limiting step in the breakdown of the pyrimidine nucleotides thymine and uracil. DPD also catalyzes the rate-limiting step in the breakdown of fluoropyrimidines.

Many DPYD variants have been described, although only a few have been demonstrated to influence DPD enzyme activity. DPYD*1 is the wild-type allele and is associated with normal enzyme activity. Individuals who carry two copies of DPYD alleles with normal activity are known as “normal metabolizers” and have fully functional DPD enzyme activity (Table 2 and Table 3). Next to DPYD*1, the DPYD alleles *4, *5, *6, and *9A are also considered to have normal activity (21).

**Table 2. Activity Status of Selected DPYD Alleles**

<table>
<thead>
<tr>
<th>Allele type</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>No function</td>
<td>*2A, *13, rs67376798</td>
</tr>
</tbody>
</table>

Table is adapted from (13, 16) For the nomenclature of human DPYD alleles, please see (22)
The no function *DPYD* variants which have been associated with low DPD activity and an increased risk of toxicity with fluoropyrimidines include *2A, *13, and rs67376798 (16). The most well studied variant is *DPYD*2A, in which a single nucleotide substitution at the invariant splice donor site of intron 14 leads to translation skipping exon 14, resulting in the production of a truncated protein with virtually no enzyme activity.

Individuals who carry combinations of normal function, decreased function, and/or no function *DPYD* alleles are known as “intermediate metabolizers.” They have partial DPD deficiency and are at increased risk of capecitabine toxicity. And individuals who carry a combination of no function *DPYD* alleles and/or decreased function *DPYD* alleles are known as “poor metabolizers.” They have complete DPD deficiency and are at an even higher risk of capecitabine toxicity. Overall, the prevalence of individuals who are heterozygous for no function variant *DPYD* alleles (partially DPD deficient) that place them at risk of severe drug reactions is estimated to be as high as 3-5%, but this varies in different populations (6, 23-27). For example, in the Dutch population, the *DPYD*2A had an allele frequency of 0.91% in Caucasians (18).

**Table 3** Assignment of likely phenotype based on *DPYD* genotypes

<table>
<thead>
<tr>
<th>Likely phenotype</th>
<th>Functional definition</th>
<th>Genetic definition</th>
<th>Example diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal metabolizer</td>
<td>Fully functional DPD enzyme activity</td>
<td>Combinations of normal function and decreased function alleles</td>
<td><em>DPYD</em>1/*1</td>
</tr>
<tr>
<td>Intermediate metabolizer (~3–5% of patients)</td>
<td>Decreased DPD enzyme activity (activity between normal and poor metabolizer)</td>
<td>Combinations of normal function, decreased function, and/or no function alleles</td>
<td>*1/*2A; *1/*13; or *1/rs67376798</td>
</tr>
<tr>
<td>Poor metabolizer (~0.2% of patients)</td>
<td>Little to no DPD enzyme activity</td>
<td>Combination of no function alleles and/or decreased function alleles</td>
<td>*2A/*2A; 13/*13; *2/*13; or rs67376798/rs67376798</td>
</tr>
</tbody>
</table>

Table is adapted from Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical pharmacology and therapeutics.2013:94(6):640-5 (3)

Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in the 2016 paper, “Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)” (15).

A recent study proposed distinguishing between the various *DPYD* alleles and their functionality by assigning gene activity scores. The use of such scores could result in differentiated individualized dosing advice for fluoropyrimidines, which is essential for reducing toxic side effects while maintaining efficacy (13).

**Genetic Testing**

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the *DPYD* gene and the fluorouracil drug response. The *DPYD*2A variant is the most commonly tested.
Biochemical genetic tests may also be used, which assess the level of activity of the DPD enzyme. These tests include biochemical assays such as analyte testing (e.g., measuring the amount of thymine and uracil in the urine or blood) or an enzyme assay (e.g., directly measuring the activity of DPD using RNA extracted from blood cells and measuring the DPD mRNA copy number) (3, 28, 29).

GTR provides a list of biochemical tests that assess the levels of thymine and uracil analytes, and the activity of the enzyme dihydropyrimidine dehydrogenase.

**Therapeutic Recommendations based on Genotype**

This section contains excerpted\(^1\) information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Rarely, unexpected, severe toxicity (e.g., stomatitis, diarrhea, neutropenia and neurotoxicity) associated with 5-fluorouracil has been attributed to deficiency of dipyrimidine dehydrogenase activity. A few patients have been rechallenged with 5-fluorouracil and despite 5-fluorouracil dose lowering, toxicity recurred and progressed with worse morbidity. Absence of this catabolic enzyme appears to result in prolonged clearance of 5-fluorouracil.

Please review the complete therapeutic recommendations that are located here: (1).

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): [...] Furthermore, patients who are heterozygous for the nonfunctional *DPYD* variants mostly demonstrate partial DPD deficiency (leukocyte DPD activity at 30–70% that of the normal population). Thus, our recommendation is to start with at least a 50% reduction of the starting dose; followed by an increase in dose in patients experiencing no or clinically tolerable toxicity, to maintain efficacy; and a decrease in dose in patients who do not tolerate the starting dose, to minimize toxicities. An alternative is pharmacokinetic-guided dose adjustment (if available). Patients who are homozygous for *DPYD*\(^{*2A, *13, or rs67376798}\) may demonstrate complete DPD deficiency, and the use of 5-fluouracil or capecitabine is not recommended in these patients. Because capecitabine and tegafur are converted to 5-fluorouracil and then metabolized by DPD, the clearance of and exposure to 5-fluorouracil, in addition to its toxic effects, are similar in patients with these variants.

Please review the complete therapeutic recommendations that are located here: (3).

---

\(^1\) The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>Coding</th>
<th>Protein</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>DPYD</em>^2A^</td>
<td>IVS14+1G&gt;A c.1905+1G&gt;A</td>
<td>NM_000110.3:c.1905+1G&gt;A</td>
<td>Not applicable—deletion of exon 14 leads to the production of a truncated protein</td>
<td>rs3918290</td>
<td></td>
</tr>
<tr>
<td><em>DPYD</em>^13^</td>
<td>1679T&gt;G Ile560Ser</td>
<td>NM_000110.3:c.1679T&gt;G</td>
<td>NP_000101.2:p.Ile560Ser</td>
<td>rs55886062</td>
<td></td>
</tr>
<tr>
<td><em>rs67376798</em></td>
<td>2846A&gt;T Asp949Val</td>
<td>NM_000110.3:c.2846A&gt;T</td>
<td>NP_000101.2:p.Asp949Val</td>
<td>rs67376798</td>
<td></td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Acknowledgments

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References


Related Summaries by Gene
Capecitabine Therapy and DPYD Genotype

Related Summaries by Drug Class
Capecitabine Therapy and DPYD Genotype

Tests in GTR by Condition
Fluorouracil response

Tests in GTR by Gene
DPYD gene
Gentamicin Therapy and *MT-RNR1* Genotype

Laura Dean, MD

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**Introduction**

Gentamicin is an aminoglycoside antibiotic that is active against gram-negative bacteria. It is administered by injection to treat serious infections caused by susceptible strains of the following microorganisms: *Pseudomonas aeruginosa*, *Proteus* species, *Escherichia coli*, *Klebsiella-Enterobacter-Serratia* species, *Citrobacter* species and *Staphylococcus* species (1). Gentamicin may also be used topically to treat ophthalmic and dermatological infections.

There are reports that a single injection of gentamicin may cause hearing loss in individuals who have a variant in the mitochondrial gene *MT-RNR1*, known as m.1555A>G. Hearing loss is bilateral, usually moderate to profound, and irreversible. Importantly, this occurs in genetically susceptible individuals even in cases where drug levels remain within the therapeutic range. Note that this effect is distinct from “dose-dependent ototoxicity” (damage to the inner ear), which can affect any individual, typically occurring after 5-7 days of aminoglycoside therapy (2).

Currently, the FDA-approved drug label for gentamicin does not include a statement about m.1555A>G. However, an American College of Medical Genetics and Genomics (ACMG) guideline includes the following recommendation: “Single-gene testing may be warranted in cases in which the medical or family history, or presentation of the hearing loss, suggests a specific etiology. For example, testing for mitochondrial DNA mutations associated with aminoglycoside ototoxicity may be considered for individuals with a history of use of aminoglycoside antibiotics” (3, 4).

**Drug: Gentamicin**

Aminoglycosides such as gentamicin are among the earliest formulations of antibiotics (5). They act by inhibiting protein synthesis in susceptible microorganisms through ribosome binding and are effective against most Gram-positive and -negative aerobic bacteria but are inactive against anaerobes. They may be used in combination with another antibiotic, such as a beta-lactam antibiotic or a cephalosporin, to increase coverage (1).

Six aminoglycoside drugs currently are approved for use by the FDA: amikacin, gentamicin, neomycin, paromomycin, streptomycin, and tobramycin. The ending of these drug names, -mycin or -micin, reflects from which genus of bacteria the aminoglycoside was derived, respectively *Streptomyces* or *Micromonospora* (6).
Aminoglycosides exert antibacterial effects by binding to bacterial ribosomes and inhibiting bacterial protein synthesis. They bind to the 30s ribosomal subunit, which interferes with the decoding site—this is where the ribosome has to accurately select tRNA in accordance with the appropriate mRNA codon. Errors here lead to inappropriate translation of the mRNA codons so that incorrect amino acids are inserted into the polypeptide chain, which can disrupt elongation of the peptide chain (7, 8).

Like all aminoglycosides, gentamicin is poorly absorbed from the gut so is not administered orally. It is either given by injection, with regular blood tests to monitor drug levels, or given topically in the form of drops, cream, or ointment, to treat infections of the eye or skin.

The toxicity of aminoglycosides, along with the discovery of equally potent but less toxic antibiotics, has meant that the use of aminoglycoside injections is reserved for serious infections that are proven, or strongly suspected, to be caused by susceptible microorganisms. They are most commonly used in the treatment of neonatal septicemia, especially in premature babies. They are used as surgical prophylaxis in patients who are allergic to penicillin and for febrile neutropenia, septic shock, and drug-resistant tuberculosis (5).

The main toxicities of aminoglycoside injections are kidney damage (nephrotoxicity) and damage to the inner ear (ototoxicity) (9). Nephrotoxicity primarily involves the proximal tubules and is generally reversible (10). In contrast, aminoglycoside-induced otoxicity is usually irreversible. Damage may occur to the cochlea, resulting in sensorineural hearing loss, and/or to the vestibular system, causing problems with balance, vertigo, ataxia, nausea, and vomiting. Gentamicin is considered to be more toxic to the vestibular system, and for this reason is used for vestibular ablation to treat Ménière's disease. Amikacin and neomycin are examples of aminoglycosides that are more toxic to the cochlea (9, 11).

Rarely, neuromuscular blockade can occur after aminoglycoside therapy. The boxed warning on the FDA-approved drug label recommends that aminoglycosides “be used with caution in patients with neuromuscular disorders, such as myasthenia gravis or parkinsonism, because they may aggravate muscle weakness (1)”; whereas the British National Formulary states that aminoglycosides should not be given to patients with myasthenia gravis (12).

Gene: **MT-RNR1**

Mitochondria are the principal source of energy in most cells—they use oxygen and sugars and fats to create energy in the form of ATP, in a process known as oxidative phosphorylation. Mitochondria have their own genome, which is small and circular, resembling the bacterial prokaryotes from which they evolved. The genome is passed down from mother to child (maternal inheritance) and contains 37 genes, one of which is the **MT-RNR1** gene (mitochondrially encoded 12S RNA).
The *MT-RNR1* gene may have variants associated with both aminoglycoside-induced and nonsyndromic hearing loss (see Nomenclature). The rRNA encoded by *MT-RNR1* is found only within mitochondria, and it is essential in the synthesis of proteins that carry out oxidative phosphorylation.

Consistent with their bacterial origin, mitochondrial rRNA resembles bacterial rRNA more closely than human rRNA found in the cell cytoplasm. However, at the highly conserved decoding region in the 12S RNA gene, the sequence in humans is different to the corresponding site in bacterial ribosomes. Thus, aminoglycosides that bind to bacterial ribosomes do not normally bind to human ribosomes (7).

However, sequence variants in the ribosomal decoding region make mitochondrial RNA more similar to bacterial rRNA, thereby facilitating the binding of aminoglycosides. Although the mechanism is unclear, aminoglycosides damage the sensory hair cells in the cochlea that mediate hearing, which may be mediated by the generation of free radicals (13-15).

The most common *MT-RNR1* variant is a single nucleotide substitution of a guanine at position 1555 for an adenine (m.1555A>G). Individuals with this variant are exquisitely sensitive to aminoglycoside-induced hearing loss, which is moderate to profound, bilateral, irreversible, and may have a rapid onset. This presentation occurs in the setting of receiving standard doses of aminoglycosides with monitoring to ensure the drug levels are within therapeutic range. Even a single dose can be sufficient to cause ototoxicity (2, 16).

The m.1555A>G variant is nearly always homoplasmic (present in all mitochondria), and the penetrance of hearing loss after exposure to aminoglycosides is high (16). Susceptible individuals who are not exposed to aminoglycosides may nonetheless develop hearing loss, referred to as “non-syndromic mitochondrial hearing loss.” The course of hearing loss may be affected by the presence of additional genetic factors as well as environmental factors, such as exposure to loud noise. However, normal hearing is usually preserved until at least 44 years of age (2). In cases with m.1555A>G heteroplasmy (variant is present in some but not all mitochondria), the proportion of variant mitochondria generally correlates with the degree of hearing loss (17).

The prevalence of the m.1555A>G variant varies among different populations but frequency data are limited. In the US, the population prevalence is estimated to be 0.09%, and in the UK, 0.20% (4, 18, 19). In hearing impaired populations, the prevalence is much greater, but the estimates vary widely based on study differences such as the age of onset of hearing loss and whether there has been exposure to aminoglycosides. Estimates include a prevalence of 3.5% among the hearing impaired population in Japan (20), 5% among deaf individuals in Indonesia (21), and 6% of individuals with post-lingual hearing loss from the UK and Southern Italy (22). Additionally, a prevalence of 15% has been reported in “ethnically diverse patients in the United States with hearing loss after aminoglycoside exposure” (23), and in 15-20% of individuals from Spain with hearing loss (24).
Interestingly, although m.1555A>G is present in mitochondria in all tissues of affected individuals, it appears that only the cochlea of the inner ear—but not the vestibular system—is extremely susceptible to aminoglycosides. In contrast, “dose-dependent ototoxicity” can occur after aminoglycoside therapy in any individual, including those with wild-type MT-RNR1, and the toxicity involves both the cochlea and vestibular system (see above) (9).

Several studies have highlighted the complex issues regarding genetic testing for m.1555A>G, with the aim of preventing avoidable hearing loss in carriers by administering an alternative antibiotic whenever possible. These issues include the costs and benefits of universal screening, for example, as part of the newborn screening program, given that the prevalence of m.1555A>G is thought to be 1 in 385 Caucasians (2, 25, 26), versus limiting genetic testing to a case-by-case basis (e.g., patients with tuberculosis, children with leukemia, individuals with cystic fibrosis, and surgical patients allergic to beta-lactam antibiotics)(4).

Genetic screening may provide more benefit in countries where aminoglycosides are more commonly used, or when their use becomes more widespread because of growing resistance to other antibiotics (27). In the US, aminoglycoside use is most common in the neonatal intensive care unit where acute, life-threatening situations may dictate that aminoglycosides are given before the results of genetic testing are available (28). One potential alternative would be to screen all pregnant women, because mitochondrial variants are maternally inherited and m.1555A>G is almost always homoplasmic (4).

A report from the World Health Organization’s Essential Medicines and Pharmaceutical Policies comments that “pre-treatment screening is an important consideration to prevent aminoglycoside related hearing loss but given cost and access issues, asking about a maternal family history of deafness may be more practical” (29). In countries where the use of aminoglycosides is more common, a quarter of people with aminoglycoside-induced hearing loss have maternal relatives who also have drug-related hearing loss (30, 31).

**Genetic Testing**

As with all mitochondrial variants, variants of the MT-RNR1 gene are either maternally inherited, or occur sporadically.

Genetic testing is available for the MT-RNR1 gene. Targeted mutation panels vary among testing laboratories but laboratories typically include m.1555A>G at a minimum.

MT-RNR1 variants are associated with both extreme idiosyncratic aminoglycoside hypersensitivity, resulting in post-exposure deafness, and nonsyndromic mitochondrial hearing loss, which tends to develop gradually over time. While the presence of an MT-RNR1 variant indicates a predisposition to aminoglycoside hypersensitivity, the test results do not predict the age of onset or severity of nonsyndromic mitochondrial hearing loss (16).
**Therapeutic Recommendations based on Genotype**

**Excerpt from the American College of Medical Genetics and Genomics (ACMG) Guideline for the Clinical Evaluation and Etiologic Diagnosis of Hearing Loss:**

For individuals lacking physical findings suggestive of a known syndrome and having medical and birth histories that do not suggest an environmental cause of hearing loss, a tiered diagnostic approach should be implemented.

Pretest genetic counseling should be provided, and, with patient’s informed consent, genetic testing should be ordered.

Single-gene testing may be warranted in cases in which the medical or family history, or presentation of the hearing loss, suggests a specific etiology. For example, testing for mitochondrial DNA mutations associated with aminoglycoside ototoxicity may be considered for individuals with a history of use of aminoglycoside antibiotics.

Please review the complete therapeutic recommendations that are located here: (3).

**Nomenclature**

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>m.1555A&gt;G</td>
<td>A1555G</td>
<td>NA</td>
<td>rs267606617</td>
</tr>
<tr>
<td></td>
<td>MTRNR1</td>
<td>NA (encodes ribosomal RNA)</td>
<td></td>
</tr>
</tbody>
</table>

**Acknowledgments**

The author would like to thank Stuart Scott, Assistant Professor of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai; Shamima Rahman, FRCP, PhD, Professor of Paediatric Metabolic Medicine at University College London and Honorary Consultant in Paediatric Metabolic Medicine at Great Ormond Street Hospital; and Maria Bitner-Glindzicz, FRCP, PhD, Professor of Clinical Molecular Genetics at University College London and Honorary Consultant in Clinical Genetics at Great Ormond Street Hospital.

**References**


**Tests in GTR by Gene**

**MT-RNR1 gene**
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Laura Dean, MD

Introduction

Imipramine is a tricyclic antidepressant used in the treatment of several psychiatric disorders including major depression, obsessive-compulsive disorder, generalized anxiety disorder, post-traumatic stress disorder, and bulimia. Imipramine may also be useful as an adjunctive treatment in the management of panic attacks, neuropathic pain, attention-deficit disorder, and childhood enuresis (bedwetting) (1).

Tricyclic antidepressants (TCAs) primarily mediate their therapeutic effect by inhibiting the reuptake of both serotonin and norepinephrine, leaving more neurotransmitter in the synaptic cleft stimulating the neuron. Because tricyclics can also block different receptors (histamine H1, α1-adrenergic, and muscarinic receptors), side effects are common. As such, more specific selective serotonin reuptake inhibitors (SSRIs) have largely replaced the use of them. However, TCAs still have an important use in specific types of depression and other conditions.

Imipramine is primarily metabolized via CYP2C19 to active metabolites, including desipramine, also a tricyclic antidepressant. Further metabolism is catalyzed by CYP2D6. Individuals who are “CYP2D6 ultrarapid metabolizers” carry more than two normal function alleles (i.e., multiple copies) (Table 1, 2), whereas individuals who are “CYP2C19 ultrarapid metabolizers” carry two increased function alleles (Table 3, 4). Individuals who are CYP2D6 or CYP2C19 “poor metabolizers” carry two no function alleles for CYP2D6 or CYP2C19, respectively.

The FDA-approved drug label for imipramine states that CYP2D6 poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants when given usual doses. Their recommendations include monitoring tricyclic antidepressant plasma levels whenever a tricyclic antidepressant is going to be co-administered with another drug known to be an inhibitor of CYP2D6 (1).

In 2016, the Clinical Pharmacogenetics Implementation Consortium (CPIC) made dosing recommendations for tricyclic antidepressants based on CYP2C19 and CYP2D6 genotypes. Amitriptyline and nortriptyline were used as model drugs for this guideline because the majority of pharmacogenomic studies have focused on these two drugs. According to the CPIC guideline, because TCAs have comparable pharmacokinetic

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
properties, it may be reasonable to apply the recommendations to other tricyclics, including imipramine (2).

For CYP2D6 ultrarapid metabolizers, CPIC recommends avoiding the use of a tricyclic due to the potential lack of efficacy, and to consider an alternative drug not metabolized by CYP2D6. If a TCA is still warranted, CPIC recommends considering titrating the TCA to a higher target dose (compared to normal metabolizers) and using therapeutic drug monitoring to guide dose adjustments. For CYP2D6 intermediate metabolizers, CPIC recommends considering a 25% reduction of the starting dose, and for CYP2D6 poor metabolizers, to avoid the use of tricyclics because of the potential for side effects. If a tricyclic is still warranted for CYP2D6 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations to avoid side effects.

For CYP2C19 ultrarapid metabolizers, CPIC recommends avoiding the use of tertiary amines (e.g., imipramine) due to the potential for a sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19, such as the secondary amines nortriptyline or desipramine. For CYP2C19 poor metabolizers, CPIC recommends avoiding tertiary amine use due to the potential for sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19. If a tertiary amine is still warranted for CYP2C19 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations to avoid side effects (2).

**Drug Class: Tricyclic Antidepressants**

Tricyclic antidepressants (TCAs) are mixed serotonin-norepinephrine reuptake inhibitors. They increase the amount of neurotransmitter in the synaptic cleft, thought to mediate their antidepressant effects.

From the 1960s to the 1980s, tricyclics were the first-line treatment for depression, until the introduction of SSRIs, which have fewer side effects and are safer. The common side effects of tricyclics include anticholinergic side effects (e.g., blurred vision, dry mouth, constipation, and sedation), cardiac effects, and orthostatic hypotension.

Today, the main therapeutic use of tricyclics is chronic pain management, such as neuropathic pain. However, tricyclics are still used in the treatment of depression as well as other psychiatric disorders including obsessive-compulsive disorder, panic attacks, generalized anxiety disorder, post-traumatic stress disorder, bulimia nervosa, smoking cessation, and enuresis (bedwetting).

Tricyclics are named after their chemical structure of three central rings and a side chain important for their function and activity. Its structure determines whether a drug is classified a tertiary amine (amitriptyline, clomipramine, doxepin, imipramine, and trimipramine) or secondary amine (desipramine and nortriptyline).
Whereas tertiary amines are generally more potent in blocking reuptake of serotonin, the secondary amines are more potent in blocking the reuptake of norepinephrine. Secondary amines are better tolerated and are also associated with fewer anticholinergic side effects.

The CYP2C19 enzyme metabolizes tertiary amines to active metabolites, which include desipramine (the active metabolite of imipramine) and nortriptyline (the active metabolite of amitriptyline). Both the tertiary and secondary amines are metabolized by CYP2D6 to less active metabolites.

The effectiveness and tolerability of tricyclics are affected by CYP2D6 metabolism and partially by CYP2C19 metabolism. Individuals who carry CYP2D6 or CYP2C19 variants that influence enzyme activity may be at an increased risk of treatment failure (if plasma drug levels are decreased) or drug toxicity (if plasma drug levels are increased).

**Drug: Imipramine**

Imipramine was the first tricyclic used in the treatment of depression in the late 1950s. Imipramine is still used to relieve the symptoms of major depressive disorder, and it may be useful too as temporary adjunctive therapy in reducing enuresis (bedwetting) in children aged 6 years and older. Off-label uses of imipramine also include the treatment of neuropathic pain and attention deficit disorder.

Imipramine is a tertiary amine and is similar in structure to amitriptyline, another tertiary amine. Both drugs potently block the reuptake of serotonin and to a lesser degree norepinephrine. Imipramine has also strong affinities for alpha-1 adrenergic, histamine H1, and muscarinic M1 receptors, which account for its side effects of orthostatic hypotension, sedation, weight gain, and anticholinergic effects. However, the intensity of these side effects is generally less than it is for amitriptyline (3).

Imipramine is metabolized by CYP2C19 to desipramine, which is also a tricyclic antidepressant with distinct clinical features that differ from the imipramine. Desipramine is then metabolized by CYP2D6 to the less active hydroxy-imipramine. For therapeutic drug monitoring, the levels of imipramine and hydroxy-imipramine should be monitored (4).

The optimal therapeutic range for imipramine is well-defined (5). Most individuals display an optimal response to imipramine when combined serum levels of imipramine and desipramine are between 175 and 300 ng/mL (6). However, individuals who are carriers of certain CYP2D6 and/or CYP2C19 variants may have drug levels that are outside this range even after being treated with standard doses of imipramine. As a result, they may have an increased risk of side effects (if the level of imipramine and its active metabolites are too high) or treatment failure (if drug levels are too low).
**Gene: CYP2D6**

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antipsychotics, analgesics, beta-blockers, and TCAs such as imipramine.

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described and currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (7).

CYP2D6 is a particularly complex gene that is difficult to genotype, partly because of the large number of variants, but also because of the presence of gene deletions, duplications, and its neighboring pseudogenes. The complexity of genetic variation at this locus complicates the ability to interrogate CYP2D6.

There is substantial variation in CYP2D6 allele frequencies among different populations (8). CYP2D6*1 is the wild-type allele and is associated with normal enzyme activity and the “normal metabolizer” phenotype. The CYP2D6 alleles *2, *33, and *35 are also considered to have normal activity.

Other alleles include no function variants that produce a non-functioning enzyme (e.g., *3, *4, *5, *6, *7, *8, and *12) or an enzyme with decreased activity (e.g., *10, *17, *29, and *41) (see Table 1) (9). There are large inter-ethnic differences in the frequency of these alleles, with *3, *4, *5, *6, and *41 being more common in the Caucasian population, *17 more common in Africans, and *10 more common in Asians (10).

**Table 1:** 2016 Assignment of CYP2D6 phenotypes by CPIC

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Activity Score</th>
<th>Genotypes</th>
<th>Examples of diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6 ultrarapid metabolizer (approximately 1–20% of patients)²</td>
<td>Greater than 2.0</td>
<td>An individual carrying duplications of functional alleles</td>
<td>(*1/*1)xN (1/1/2)xN (2/2)xN²</td>
</tr>
</tbody>
</table>

a For population-specific allele and phenotype frequencies, please see (2).
b Where xN represents the number of CYP2D6 gene copies (N is 2 or more).
c Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.

For more information about activity scores, please see the Genetic Testing section.


*Table 1 continues on next page...*
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<th>Phenotype</th>
<th>Activity Score</th>
<th>Genotypes</th>
<th>Examples of diplotypes</th>
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</thead>
</table>
| CYP2D6 normal metabolizer         | 1.0 - 2.0<sup>c</sup> | An individual carrying two normal function alleles or two decreased function alleles or one normal and no function allele or one normal function and decreased function allele or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0 | *1/*1  
*1/*2  
*2/*2  
*1/*9  
*1/*41  
*41/*41  
*1/*5  
*1/*4 |
| CYP2D6 intermediate metabolizer   | 0.5            | An individual carrying one decreased function and one no function allele   | *4/*41  
*5/*9  
*4/*10 |
| CYP2D6 poor metabolizer           | 0              | An individual carrying two no function alleles                            | *4/*4  
*4/*4xN  
*3/*4  
*5/*5  
*5/*6 |

<sup>a</sup> For population-specific allele and phenotype frequencies, please see (2).

<sup>b</sup> Where xN represents the number of CYP2D6 gene copies (N is 2 or more).

<sup>c</sup> Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.

For more information about activity scores, please see the Genetic Testing section.


Individuals who are intermediate or poor metabolizers carry copies of reduced-activity or no function CYP2D6 alleles, respectively (Table 1). Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. In these populations, only half of CYP2D6 alleles are fully functional, with the reduced function *10 variant being very common (~40%, compared to ~2% in Caucasians) (11). As a result, Asians are more likely to be intermediate metabolizers than Caucasians (12). Similarly, in Africans and African Americans, only half of CYPD6 alleles are functional; however, a wider range of variants account for the remaining alleles (12-14).

Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to no function *4 and *5 alleles (12). Notably, less than 40% are homozygous normal metabolizers (carrying two copies of *1 allele) (15-17).
Individuals who are CYP2D6 poor metabolizers require a lower dose of imipramine to be in therapeutic range than CYP2D6 normal metabolizers (18). When treated with standard doses of imipramine, individuals who are CYP2D6 poor metabolizers will also have higher plasma concentrations of imipramine and desipramine compared to CYP2D6 normal metabolizers (19).

Because adverse effects are more likely due to elevated tricyclic plasma concentrations, CPIC recommends alternative agents for individuals who are CYP2D6 poor metabolizers. If a tricyclic is warranted, CPIC recommends considering a 50% reduction of the usual starting dose, and they strongly recommend therapeutic drug monitoring (4).

Individuals who have more than two copies of normal function CYP2D6 alleles are CYP2D6 ultrarapid metabolizers. These individuals require higher doses of imipramine to be within therapeutic range compared to normal metabolizers (18). However, increasing the dose of imipramine can lead to high plasma concentrations of desipramine, which may increase the risk for cardiotoxicity. Therefore, CPIC recommends that an alternative agent be used for CYP2D6 ultrarapid metabolizers. However, if a tricyclic is warranted, there is insufficient evidence to calculate a starting dose, and so therapeutic drug monitoring is strongly recommended (4) (Table 2).

**Table 2.** 2016 CPIC Dosing recommendations for tricyclic antidepressants based on CYP2D6 phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implication</th>
<th>Therapeutic recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6 ultrarapid metabolizer</td>
<td>Increased metabolism of TCAs to less active compounds compared to normal metabolizers</td>
<td>Avoid tricyclic use due to potential lack of efficacy. Consider alternative drug not metabolized by CYP2D6</td>
</tr>
<tr>
<td></td>
<td>Lower plasma concentrations of active drugs will increase probability of pharmacotherapy failure</td>
<td>If a TCA is warranted, consider titrating to a higher target dose (compared to normal metabolizers)(^a). Utilize therapeutic drug monitoring to guide dose adjustments.</td>
</tr>
</tbody>
</table>

TCAs: Tricyclic Antidepressants

Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.

The therapeutic recommendations for amitriptyline and nortriptyline are classified as “moderate” for intermediate CYP2D6 metabolizers, and “strong” for ultrarapid, normal, and poor CYP2D6 metabolizers. CPIC state that it may be reasonable to apply these recommendations to other TCAs also metabolized by CYP2D6, including clomipramine, desipramine, doxepin, imipramine, and trimipramine.

\(^a\) Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.

\(^b\) Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.

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<thead>
<tr>
<th>Phenotype</th>
<th>Implication</th>
<th>Therapeutic recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6 normal metabolizer</td>
<td>Normal metabolism of TCAs</td>
<td>Initiate therapy with recommended starting dose$^b$</td>
</tr>
<tr>
<td>CYP2D6 intermediate metabolizer</td>
<td>Reduced metabolism of TCAs to less active compounds compared to normal metabolizers</td>
<td>Consider a 25% reduction of recommended starting dose$^b$. Utilize therapeutic drug monitoring to guide dose adjustments$^a$.</td>
</tr>
<tr>
<td></td>
<td>Higher plasma concentrations of active drug will increase the probability of side effects</td>
<td></td>
</tr>
<tr>
<td>CYP2D6 poor metabolizer</td>
<td>Greatly reduced metabolism of TCAs to less active compounds compared to normal metabolizers</td>
<td>Avoid tricyclic use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6</td>
</tr>
<tr>
<td></td>
<td>Higher plasma concentrations will increase the probability of side effects</td>
<td>If a TCA is warranted, consider a 50% reduction of recommended starting dose$^b$. Utilize therapeutic drug monitoring to guide dose adjustments$^a$.</td>
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TCAs: Tricyclic Antidepressants

Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.

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$^a$ Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.

$^b$ Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.


**Gene: CYP2C19**

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as several proton pump inhibitors, clopidogrel, benzodiazepines, and several tricyclic antidepressants, including imipramine.

The CYP2C19 gene is highly polymorphic as 35 variant star (*) alleles are currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database: (http://www.cypalleles.ki.se/cyp2c19.htm).
The CYP2C19*1 wild-type allele is associated with normal enzyme activity and the “normal metabolizer” phenotype, whereas the CYP2C19*17 allele is associated with increased enzyme activity and the “rapid” and “ultrarapid” metabolizer phenotypes (20).

The most common no function variant is CYP2C19*2, which is characterized by c. 681G>A in exon 5 that results in an aberrant splice site and the production of a truncated and non-functioning protein. The CYP2C19*2 allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (20, 21).

Another commonly tested no function variant is CYP2C19*3, which is characterized by c. 636G>A in exon 4 that causes a premature stop codon. The CYP2C19*3 allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other no function variants occur in less than 1% of the general population, and include CYP2C19*4-8 (20, 21).

“CYP2C19 intermediate metabolizers” carry one copy of a no function allele (e.g. *1/*2), whereas “poor metabolizers” are homozygous or compound heterozygous for two no function alleles (e.g., *2/*2, *2/*3) (Table 3).

**Table 3: 2016 Assignment of CYP2C19 phenotypes by CPIC**

<table>
<thead>
<tr>
<th>Phenotype</th>
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<tbody>
<tr>
<td>CYP2C19 ultrarapid metabolizer (approximately 2–35% of patients)</td>
<td>An individual carrying two increased function alleles</td>
<td>*17/*17</td>
</tr>
<tr>
<td>CYP2C19 rapid metabolizer (approximately 2–30% of patients)</td>
<td>An individual carrying one normal function allele and one increased function allele</td>
<td>*1/*17</td>
</tr>
<tr>
<td>CYP2C19 normal metabolizer (approximately 35–50% of patients)</td>
<td>An individual carrying two normal function alleles</td>
<td>*1/*1</td>
</tr>
<tr>
<td>CYP2C19 intermediate metabolizer (approximately 18–45% of patients)</td>
<td>An individual carrying one normal function and one no function allele or one no function allele and one increased function allele</td>
<td>*1/*2, *1/*3, *2/*17</td>
</tr>
<tr>
<td>CYP2C19 poor metabolizer (approximately 2–15% of patients)</td>
<td>An individual carrying two no function alleles</td>
<td>*2/*2, *2/*3, *3/*3</td>
</tr>
</tbody>
</table>

* For population-specific allele and phenotype frequencies, please see (2).
* The predicted metabolizer phenotype for the *2/*17 genotype is a provisional classification.


Studies have found that individuals who are CYP2C19 poor metabolizers have a lower plasma clearance of imipramine compared to normal metabolizers. When given standard doses of imipramine, CYP2C19 poor metabolizers have greater concentrations of imipramine and its active metabolite desipramine (22-24). Increased drug levels could
potentially lead to an increased risk of adverse events. CPIC recommends considering a 50% reduction in the starting dose of tricyclics for CYP2C19 poor metabolizers (4).

Individuals who are CYP2C19 ultrarapid metabolizers may require an increased dose of tricyclics (25).

One study found that the imipramine plasma concentration was significantly lower in ultrarapid metabolizers (i.e., CYP2C19*17/*17) when compared to normal metabolizers (i.e., CYP2C19*1/*1) patients. However, the imipramine + desipramine plasma concentrations were not significantly different between CYP2C19 genotypes (26). Because of the possibility of altered tricyclic plasma concentrations, CPIC recommends an alternative tricyclic or other drug for ultrarapid metabolizers (4) (Table 4).

Table 4. 2016 CPIC Dosing recommendations for tertiary amines based on CYP2C19 phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implication</th>
<th>Therapeutic recommendation</th>
</tr>
</thead>
</table>
| CYP2C19 ultrarapid metabolizer and CYP2C19 rapid metabolizer | Increased metabolism of tertiary amines as compared to normal metabolizers Greater conversion of tertiary amines to secondary amines may affect response or side effects | Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine. If a tertiary amine is warranted, utilize therapeutic drug monitoring to guide dose adjustments.
| CYP2C19 normal metabolizer | Normal metabolism of tertiary amines | Initiate therapy with recommended starting dose.
| CYP2C19 intermediate metabolizer | Reduced metabolism of tertiary amines compared to normal metabolizers | Initiate therapy with recommended starting dose.
| CYP2C19 poor metabolizer | Greatly reduced metabolism of tertiary amines compared to normal metabolizers | Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without |

Dosing recommendations apply only to higher initial doses of amitriptyline for treatment of conditions such as depression. The therapeutic recommendations for amitriptyline are classified as "strong" for normal and intermediate CYP2C19 metabolizers, "moderate" for poor metabolizers, and "optional" for ultrarapid metabolizers. CPIC state that it may be reasonable to apply these recommendations to other TCAs also metabolized by CYP2C19, including clomipramine, doxepin, imipramine, and trimipramine.

a Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.
b Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.

Table 4. continued from previous page.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implication</th>
<th>Therapeutic recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased conversion of tertiary amines to secondary amines may affect response or side effects</td>
<td>major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine. For tertiary amines, consider a 50% reduction of recommended starting dose(^b). Utilize therapeutic drug monitoring to guide dose adjustments(^a).</td>
<td></td>
</tr>
</tbody>
</table>

Dosing recommendations apply only to higher initial doses of amitriptyline for treatment of conditions such as depression. The therapeutic recommendations for amitriptyline are classified as “strong” for normal and intermediate CYP2C19 metabolizers, “moderate” for poor metabolizers, and “optional” for ultrarapid metabolizers. CPIC state that it may be reasonable to apply these recommendations to other TCAs also metabolized by CYP2C19, including clomipramine, doxepin, imipramine, and trimipramine.

\(^a\) Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects. \(^b\) Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.


**Genetic Testing**

Clinical genotyping tests are available for many CYP2D6 and CYP2C19 alleles. The NIH’s Genetic Testing Registry (GTR) provides a list of test providers for “imipramine response,” and the CYP2D6 and CYP2C19 genes.

Results are typically reported as a diplotype, such as CYP2D6 \(*1/^1\). A result for copy number, if available, is also important when interpreting CYP2D6 results (27). However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (28).

If the test results include an interpretation of the patient’s predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- A normal (previously referred to as “extensive”) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2 (2, 29)
Therapeutic Recommendations based on Genotype

This section contains excerpted\(^1\) information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): The biochemical activity of the drug metabolizing isozyme cytochrome P450 2D6 (debrisoquin hydroxylase) is reduced in a subset of the Caucasian population (about 7% to 10% of Caucasians are so-called “poor metabolizers”); reliable estimates of the prevalence of reduced P450 2D6 isozyme activity among Asian, African, and other populations are not yet available. Poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants (TCAs) when given usual doses. Depending on the fraction of drug metabolized by P450 2D6, the increase in plasma concentration may be small, or quite large (8-fold increase in plasma AUC of the TCA).

In addition, certain drugs inhibit the activity of this isozyme and make normal metabolizers resemble poor metabolizers. An individual who is stable on a given dose of TCA may become abruptly toxic when given one of these inhibiting drugs as concomitant therapy. The drugs that inhibit cytochrome P450 2D6 include some that are not metabolized by the enzyme (quinidine; cimetidine) and many that are substrates for P450 2D6 (many other antidepressants, phenothiazines, and the Type 1C antiarrhythmics propafenone and flecaïnide). While all the selective serotonin reuptake inhibitors (SSRIs), e.g., fluoxetine, sertraline, and paroxetine, inhibit P450 2D6, they may vary in the extent of inhibition. The extent to which SSRI-TCA interaction may pose clinical problems will depend on the degree of inhibition and the pharmacokinetics of the SSRI involved. Nevertheless, caution is indicated in the co-administration of TCAs with any of the SSRIs and also in switching from one class to the other. Of particular importance, sufficient time must elapse before initiating TCA treatment in a patient being withdrawn from fluoxetine, given the long half-life of the parent and active metabolite (at least 5 weeks may be necessary).

Concomitant use of tricyclic antidepressants with drugs that can inhibit cytochrome P450 2D6 may require lower doses than usually prescribed for either the tricyclic antidepressant or the other drug. Furthermore, whenever one of these other drugs is withdrawn from co-therapy, an increased dose of tricyclic antidepressant may be required. It is desirable to monitor TCA plasma levels whenever a TCA is going to be co-administered with another drug known to be an inhibitor of P450 2D6.

Please review the complete therapeutic recommendations that are located here: \(^1\).

\(^1\) The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
2016 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC):

Because the TCAs have comparable pharmacokinetic properties, it may be reasonable to extrapolate this guideline to other TCAs including clomipramine, desipramine, doxepin, imipramine, and trimipramine, with the acknowledgement that there are fewer data supporting dose adjustments for these drugs than for amitriptyline or nortriptyline. […] CYP2D6 dosing recommendations.

[...]. The recommended starting dose of amitriptyline or nortriptyline does not need adjustment for those with genotypes predictive of CYP2D6 normal metabolism. A 25% reduction of the recommended dose may be considered for CYP2D6 intermediate metabolizers. The strength of this recommendation is classified as “moderate” because patients with a CYP2D6 activity score of 1.0 are inconsistently categorized as intermediate or normal metabolizers in the literature, making these studies difficult to evaluate. CYP2D6 ultrarapid metabolizers have a higher probability of failing amitriptyline or nortriptyline pharmacotherapy due to subtherapeutic plasma concentrations, and alternate agents are preferred. There are documented cases of CYP2D6 ultrarapid metabolizers receiving large doses of nortriptyline in order to achieve therapeutic concentrations. However, very high plasma concentrations of the nortriptyline hydroxy-metabolite were present, which may increase the risk for cardiotoxicity. If a tricyclic is warranted, there are insufficient data in the literature to calculate a starting dose for a patient with CYP2D6 ultrarapid metabolizer status, and therapeutic drug monitoring is strongly recommended. Adverse effects are more likely in CYP2D6 poor metabolizers due to elevated tricyclic plasma concentrations; therefore, alternate agents are preferred. If a tricyclic is warranted, consider a 50% reduction of the usual dose, and therapeutic drug monitoring is strongly recommended.

CYP2C19 dosing recommendations.

[...]. The usual starting dose of amitriptyline may be used in CYP2C19 normal and intermediate metabolizers. Although CYP2C19 intermediate metabolizers would be expected to have a modest increase in the ratio of amitriptyline to nortriptyline plasma concentrations, the evidence does not indicate that CYP2C19 intermediate metabolizers should receive an alternate dose.

Patients taking amitriptyline who are CYP2C19 rapid or ultrarapid metabolizers may be at risk for having low plasma concentrations and an imbalance between parent drug and metabolites causing treatment failure and/or adverse events. Although the CYP2C19*17 allele did not alter the sum of amitriptyline plus nortriptyline plasma concentrations, it was associated with higher nortriptyline plasma concentrations, possibly increasing the risk of adverse events. For patients taking amitriptyline, extrapolated pharmacokinetic data suggest that CYP2C19 rapid or ultrarapid metabolizers may need a dose increase. Due to the need for further studies investigating the clinical importance of CYP2C19*17 regarding tricyclic metabolism and the possibility of altered concentrations, we
recommend to consider an alternative tricyclic or other drug not affected by CYP2C19. This recommendation is classified as optional due to limited available data. If amitriptyline is administered to a CYP2C19 rapid or ultrarapid metabolizer, therapeutic drug monitoring is recommended.

CYP2C19 poor metabolizers are expected to have a greater ratio of amitriptyline to nortriptyline plasma concentrations. The elevated amitriptyline plasma concentrations may increase the chance of a patient experiencing side effects. Use an alternative agent not metabolized by CYP2C19 (e.g., nortriptyline and desipramine) or consider a 50% reduction of the usual amitriptyline starting dose along with therapeutic drug monitoring.

Please review the complete therapeutic recommendations that are located here: (2).

2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):

For CYP2D6 poor metabolizers, defined as patients carrying two inactive alleles, reduce the dose of imipramine by 70% and monitor imipramine and desipramine plasma concentrations.

For CYP2D6 intermediate metabolizers, defined as patients carrying two decreased-activity alleles or one active/decreased-activity allele and one inactive allele, reduce the dose of imipramine by 30% and monitor imipramine and desipramine plasma concentrations.

For CYP2D6 ultrarapid metabolizers, defined as patients carrying a gene duplication in the absence of inactive or decreased-activity alleles, select an alternative drug (e.g., citalopram, sertraline) or increase dose by 70% and monitor imipramine and desipramine plasma concentration (Table 5).

For CYP2C19 poor metabolizers, reduce the dose of imipramine by 30% and monitor plasma concentration of imipramine and desipramine or select an alternative drug (e.g., fluvoxamine, mirtazapine).

For CYP2C19 intermediate metabolizers, there is insufficient data to allow calculation of dose adjustment for imipramine, select an alternative drug (e.g., fluvoxamine, mirtazapine).

There are no data for dose recommendations for CYP2C19 ultrarapid metabolizers (Table 6).
Table 5. CYP2D6 phenotypes and the therapeutic recommendations for imipramine therapy, from The Dutch Pharmacogenetics Working Group (2011)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Recommendations for imipramine therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>Select alternative drug (e.g., citalopram, sertraline) or increase dose by 70% and monitor imipramine and desipramine plasma concentration</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>Reduce dose by 30% and monitor imipramine and desipramine plasma concentrations</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>Reduce dose by 70% and monitor imipramine and desipramine plasma concentrations</td>
</tr>
</tbody>
</table>

The level of evidence for the therapeutic (dose) recommendations is 4/4 ("good quality") for all metabolizer types. There are no data for ultrarapid metabolizers. The Table is adapted from Swen J.J., Nijenhuis M., de Boer A., Grandia L. et al. Pharmacogenetics: from bench to byte - an update of guidelines. Clinical pharmacology and therapeutics. 2011;89(5):662–73 (30).

Table 6. CYP2C19 phenotypes and the therapeutic recommendations for imipramine therapy, from The Dutch Pharmacogenetics Working Group (2011)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Recommendations for imipramine therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>No dose recommendations</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>No dose recommendations</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>Reduce dose by 70% and monitor plasma concentration of imipramine and desipramine or select alternative drug (e.g., fluvoxamine, mirtazapine)</td>
</tr>
</tbody>
</table>

The level of evidence for the therapeutic (dose) recommendations is 4/4 ("good quality") for all metabolizer types. The table is adapted from (31)

**Please review the complete therapeutic recommendations that are located here:** (30, 31).

**Nomenclature**

**Nomenclature for selected CYP2D6 alleles**

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*4</td>
<td>1846G&gt;A</td>
<td>NM_000106.5:c.506-1G&gt;A</td>
<td>Not applicable - variant occurs in a non-coding region</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td></td>
<td></td>
<td>Not applicable - variant results in a whole gene deletion</td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>1707 del T Trp152Gly</td>
<td>NM_000106.5:c.454delT</td>
<td>NP_000097.3:p.Trp152Glyfs</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>100C&gt;T Pro34Ser</td>
<td>NM_000106.5:c.100C&gt;T</td>
<td>NP_000097.3:p.Pro34Ser</td>
</tr>
</tbody>
</table>

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
### Nomenclature for selected continued from previous page

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence Coding</th>
<th>Protein</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*17</td>
<td>Includes at least two functional variants*: 1023C&gt;T (Thr107Ile) 2850C&gt;T (Cys296Arg)</td>
<td>NM_000106.5:c.320C&gt;T&lt;br&gt;NM_000106.5:c.886T&gt;C</td>
<td>NP_000097.3:p.Thr107Ile&lt;br&gt;NP_000097.3:p.Cys296Arg</td>
<td>rs28371706&lt;br&gt;rs16947</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>2988G&gt;A</td>
<td>NM_000106.5:c.985+39G&gt;A</td>
<td>Not applicable – variant occurs in a non-coding region</td>
<td>rs28371725</td>
</tr>
</tbody>
</table>

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

### Nomenclature for selected CYP2C19 alleles

<table>
<thead>
<tr>
<th>Common allele name</th>
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<th>HGVS reference sequence Coding</th>
<th>Protein</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19*2</td>
<td>681G&gt;A Pro227Pro</td>
<td>NM_000769.1:c.681G&gt;A</td>
<td>NP_000760.1:p.Pro227=</td>
<td>rs4244285</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>636G&gt;A Trp212Ter</td>
<td>NM_000769.1:c.636G&gt;A</td>
<td>NP_000760.1:p.Trp212Ter</td>
<td>rs4986893</td>
</tr>
<tr>
<td>CYP2C19*17</td>
<td>-806C&gt;T</td>
<td>NM_000769.2:c.-806C&gt;T</td>
<td>Not applicable—variant occurs in a non-coding region</td>
<td>rs12248560</td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

### Acknowledgments

The author would like to thank the following individuals for reviewing this summary:

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Chakradhara Rao S Uppugunduri, Maître-assistant at the CANSEARCH Laboratory, University of Geneva, Switzerland; and Mandy van Rhenen, secretary of the Dutch Pharmacogenetics Working Group (DPWG), Centre for Information on Medicines, Royal Dutch Pharmacists Association (KNMP).

References


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Aripiprazole Therapy and CYP2D6 Genotype
Atomoxetine Therapy and CYP2D6 Genotype
Carisoprodol Therapy and CYP2D6 Genotype
Clopidogrel Therapy and CYP2C19 Genotype
Clozapine Therapy and CYP2D6, CYP1A2, and CYP3A4 Genotypes
Codeine Therapy and CYP2D6 Genotype
Diazepam Therapy and CYP2C19 Genotype
Esomeprazole Therapy and \textit{CYP2C19} Genotype
Metoprolol Therapy and \textit{CYP2D6} Genotype
Omeprazole Therapy and \textit{CYP2C19} Genotype
Prasugrel Therapy and \textit{CYP} Genotype
Propafenone Therapy and \textit{CYP2D6} Genotype
Risperidone Therapy and \textit{CYP2D6} Genotype
Tamoxifen Therapy and \textit{CYP2D6} Genotype
Thioridazine Therapy and \textit{CYP2D6} Genotypes
Tramadol Therapy and \textit{CYP2D6} Genotype
Venlafaxine Therapy and \textit{CYP2D6} Genotype

\textbf{Related Summaries by Drug Class}

\textbf{Amitriptyline Therapy and \textit{CYP2D6} and \textit{CYP2C19} Genotype}

\textbf{Tests in GTR by Condition}

Imipramine response

\textbf{Tests in GTR by Gene}

\textit{CYP2C19} gene
\textit{CYP2D6} gene
Irinotecan Therapy and UGT1A1 Genotype

Laura Dean, MD

Created: May 27, 2015; Updated: June 3, 2015.

Introduction

Irinotecan is a topoisomerase inhibitor that is widely used in the treatment of cancer. It is often used in combination with other drugs to treat metastatic colorectal cancer. However, irinotecan therapy is associated with a high incidence of toxicity, including severe neutropenia and diarrhea (1, 2).

Irinotecan is metabolized and inactivated by an UDP-glucuronosyltransferase enzyme encoded by the gene UGT1A1. UDP-glucuronosyltransferase enzymes are part of the glucuronidation pathway that transforms small lipophilic molecules, such as certain drugs like irinotecan, into water-soluble, excretable metabolites. Variants of this gene, such as UGT1A1*28, are associated with reduced enzyme activity and an increased risk of irinotecan toxicity. Approximately 10% of North Americans are homozygous for the UGT1A1*28 allele and are more likely to develop neutropenia following irinotecan therapy (3).

The FDA-approved drug label for irinotecan states that “when administered as a single-agent, a reduction in the starting dose by at least one level of irinotecan hydrochloride injection should be considered for patients known to be homozygous for the UGT1A1*28 allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment” (3). A guideline from the Dutch Pharmacogenetics Working Group (KNMP) mentions “although results are not consistent, there is sufficient evidence that a reduction in the initial dose by 30% is required for regimens containing >250 mg/m² of irinotecan prescribed to homozygous carriers of the UGT1A1*28 allele. This is in agreement with the Food and Drug Administration–mandated label change. No dose reduction is recommended for heterozygous carriers of the UGT1A1*28 allele because dose reduction might result in under treatment” (Table 1) (4). A guideline from the Evaluation of Genomic Applications in Practice and Prevention (EGAPP™) Working Group (published in 2009, prior to the FDA statement or KNMP guideline) states that “the evidence is currently insufficient to recommend for or against the routine use of UGT1A1 genotyping in patients with metastatic colorectal cancer who are to be treated with irinotecan, with the intent of modifying the dose as a way to avoid adverse drug reactions (severe neutropenia)” (5).
Table 1. UGT1A1 phenotypes and the therapeutic recommendations for Irinotecan therapy

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Genotype details</th>
<th>Therapeutic (dose) recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1/*28</td>
<td>One active allele and one reduced activity allele</td>
<td>No dose adjustment.</td>
</tr>
<tr>
<td>*28/*28</td>
<td>Two reduced activity alleles</td>
<td>Dose more than 250 mg/m$^2$: reduce initial dose by 30%. Increase dose in response to neutrophil count. Dose less than or equal to 250 mg/m$^2$: no dose adjustment.</td>
</tr>
</tbody>
</table>


**Drug: Irinotecan**

Irinotecan is used to treat colorectal cancer, which is the third most common cancer worldwide (6). It is often used in combination with other drugs to treat patients with metastatic colorectal cancer when the cancer has recurred or has progressed following initial treatment. A common irinotecan-based combination therapy is referred to as FOLFIRI (FOLinic acid, Fluorouracil (also known as leucovorin), IRInotecan).

Irinotecan is a semisynthetic derivative of the antineoplastic agent camptothecin, which takes its name from the tree where it was first isolated (Camptotheca). Like camptothecin, irinotecan inhibits the nuclear enzyme, topoisomerase I. This enzyme catalyzes a number of nuclear processes, such as regulating DNA supercoiling, replication, recombination, and repair.

Topoisomerase I decreases the torsional strain in the helical strands of DNA by making single strand breaks in the DNA. Single strands of DNA pass through the breaks and they bind to the topoisomerase to form a cleavable complex. Once the DNA is sufficiently relaxed and the passage of strands has been completed, the topoisomerase re-ligates the broken DNA strands and allows for transcription to proceed (7, 8).

Irinotecan is a pro-drug. After it is administered by intravenous injection, it is metabolized to its active form, SN-38, which is 100–1000 times more potent that its parent drug (9). It is inactivated by undergoing phase II metabolism (glucuronidation) in the liver. The resulting conjugated SN-38 glucuronide is water soluble, and is mainly excreted through the bile, with about 30% excreted by the kidneys (10).

SN-38 exerts its cytotoxic effects by binding to the cleavable complex to form a ternary complex (drug-topoisomerase-DNA complex). This complex is thought to prevent the re-ligation of the single strand breaks, which interrupts the moving DNA replication fork. The arrest of replication and the interaction between replication enzymes and the ternary complex introduces lethal double-stranded breaks in DNA. Because the DNA damage cannot be repaired, the cells undergo apoptosis (11, 12).
Irinotecan-based combination therapy has been found to be superior in overall response and survival when compared to the use of 5-fluorouracil/leucovorin therapy alone (2). However, the use of irinotecan is limited by a high incidence of unpredictable and severe toxicity, including severe neutropenia, fever, and diarrhea. Approximately 7% of patients who present with severe neutropenia and fever following treatment with irinotecan will die from these complications (2, 13-16).

**Gene: UGT1A1**

The uridine diphosphate glucuronosyltransferase (UDP-glucuronosyltransferase, or UGT) enzymes are a superfamily of enzymes that metabolize a wide range of molecules such as bilirubin, steroids, toxins, and drugs—including irinotecan. These enzymes are responsible for glucuronidation, which is a phase II metabolic pathway during which glucuronic acid is conjugated to specific targets to convert them to water-soluble metabolites that can then be eliminated from the body.

The UGT genes are often polymorphic, and genomic processes, such as copy-number variations, variant splicing, and epigenetic factors, are likely to contribute to their diversity. As a result, the metabolic pathways the UGT enzymes catalyze are particularly variable (17).

The UGT superfamily contains 117 enzymes that are divided into four families, and UGT1A is one of these families (18). The UGT1A gene locus, located on chromosome 2q37, is complex—it encodes multiple genes and pseudogenes, and alternatively spliced isoforms also exist (19).

The UGT1A locus contains multiple alternative first coding exons, each of which has its own promoter site, enabling the transcription of nine unique UGT1A enzymes (20). One of these transcripts is UGT1A1, which encodes the bilirubin-UGT enzyme (bilirubin uridine diphosphate glucuronosyl transferase enzyme). Whereas many UGT enzymes overlap in the substrates they glucuronidate, UGT1A1 is the only enzyme that glucuronidates bilirubin (21).

Bilirubin is a yellow waste product produced during the catabolism of heme, a constituent of hemoglobin. When old or damaged red blood cells are broken down in the spleen, their hemoglobin is broken down to heme, which is then converted into bilirubin. The UGT1A1 enzyme converts this toxic, insoluble form of bilirubin (unconjugated bilirubin) to its nontoxic form (conjugated bilirubin). Because conjugated bilirubin is water-soluble, it can be dissolved in bile and eliminated with solid waste. If bilirubin is not eliminated and instead, accumulates to high levels (hyperbilirubinemia) it can cause a yellowish discoloration of the skin and eyes, a condition known as jaundice.

Variants of the UGT1A1 gene that decrease UGT1A1 enzyme activity can lead to jaundice. The jaundice may be mild, as seen in Gilbert’s syndrome, or severe, as seen in Crigler-Najjar syndrome. Crigler-Najjar syndrome is divided into two types. Type 1 is the extremely severe form where affected individuals can die in childhood due to kernicterus
Type 2 is less severe; the affected individuals are less likely to develop kernicterus and most survive into adulthood.

Currently, over 113 genetic variants of UGT1A1 have been reported (21). UGT1A1*1 is the wild-type allele and is associated with normal enzyme activity. The most common variant allele is UGT1A1*28, which is commonly found in African-Americans (0.42 – 0.45 allele frequency) and Caucasians (0.26–0.31), and is less common in Asian populations (0.09–0.16) (22, 23).

The *28 variant contains an extra thymine-adenine (TA) repeat within the TATA box promoter region (seven TA repeats compared to six in the wild-type allele). This extra (TA) repeat decreases the rate of transcription initiation of the UGT1A1 gene, leading to decreased enzyme activity and decreased glucuronidation of bilirubin to about 30% of wild-type levels (24). A different allele, UGT1A1*37, has eight TA repeats at this site, and results in reduced promoter activity to levels lower than that of promoters with the UGT1A1*28 allele. In contrast, the allele UGT1A1*36 has only five repeats, and is associated with increased promoter activity of the gene and a reduced risk of neonatal hyperbilirubinemia, a common and typically benign condition. Both UGT1A1*36 and UGT1A1*37 occur almost exclusively in populations of African origin, with estimated allele frequencies of 0.03–0.10 and 0.02–0.07, respectively.

Within Caucasian and African American populations, the UGT1A1*28 variant is a common cause of Gilbert syndrome, and is also a cause of Crigler-Najjar syndrome types 1 and 2 (17, 22). The UGT1A1*28 variant is also associated with drug toxicity.

Approximately 10% of the North American population is homozygous for the *28 allele (*28/*28 genotype, also known as UGT1A1 7/7 genotype) and are at an increased risk of neutropenia following injections of irinotecan treatment (23). The rate of severe neutropenia in *28/*28 homozygous patients is as high as 36%, and is strongly associated with a higher hospitalization rate (25-27).

There is less evidence to support a link between UGT1A1 genotype and irinotecan treatment-related diarrhea, and there is conflicting data on whether an individual's UGT1A1 genotype influences their response to irinotecan therapy (5, 28).

Another variant allele, UGT1A1*6, is more prevalent in Asian populations, with an allele frequency of around 0.13% in Chinese, Korean, and Japanese populations (29). In this variant, there is a switch of amino acids, from a glycine to an arginine at position 71 within a coding region (Arg71Gly). Individuals who are homozygous for this allele have reduced UGT1A1 enzyme activity, which can cause Gilbert syndrome and prolonged neonatal jaundice (30-33). This variant also appears to be an important predictor of severe toxicity to irinotecan therapy in Northeastern Asian populations (34).

Emerging data suggests that other variant alleles may have a protective effect. The newly discovered marker rs11563250, located in the 3′-flanking region of UGT1, has a major A allele (rs11563250A) and a relatively common variant G allele (rs11563250G, found in
12% of the population). Carriers of the G allele have a lower risk of irinotecan-induced neutropenia. They also tend to have lower total plasma bilirubin levels, suggesting that this variant is associated with an enhanced capacity for glucuronidation. Evidence suggests that carriers of rs11563250G could tolerate a higher dose of irinotecan, especially if they have the UGT1A1*1/*1 genotype (35).

**Genetic Testing**

Genetic testing to determine the UGT1A1 status of patients is available (36). Genotyping is used to optimize irinotecan dosing to prevent side effects when treating patients with metastatic colorectal cancer, and may also be used as part of the management of Gilbert syndrome (36). Routine genotyping usually tests for UGT1A1 6/6, 6/7, and 7/7 genotypes (*1/*1, *1/*28, and *28/*28 respectively).

Because the UGT1A1*28 variant allele is associated with severe neutropenia following irinotecan therapy, the use of genotyping in selective cases may make the following patient choices possible:

- If the patient prefers aggressive treatment: genotyping might allow higher dosing for *1/*1 and *1/*28 genotypes.
- If the patient prefers maximizing quality of life: genotyping might allow lower dosing for *28/*28 genotype (25-27).

The common *1 and *28 UGT1A1 alleles comprise 98–99% of the genotypes found in the U.S. Caucasian population. However, routine genotyping of UGT1A1 does not rule out other UGT1A1 polymorphisms that might be more common in other populations (27). In addition, currently routine screening does not identify patients who would tolerate an even higher irinotecan.

**Therapeutic Recommendations based on Genotype**

This section contains excerpted¹ information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**Statement from the US Food and Drug Administration (FDA):** Individuals who are homozygous for the UGT1A1*28 allele (UGT1A1 7/7 genotype) are at increased risk for neutropenia following initiation of irinotecan hydrochloride injection treatment.

In a study of 66 patients who received single-agent irinotecan hydrochloride injection (350 mg/m² once-every-3-weeks), the incidence of grade 4 neutropenia in patients homozygous for the UGT1A1*28 allele was 50%, and in patients heterozygous for this

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¹ The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
allele (UGT1A1 6/7 genotype) the incidence was 12.5%. No grade 4 neutropenia was observed in patients homozygous for the wild-type allele (UGT1A1 6/6 genotype).

When administered as a single-agent, a reduction in the starting dose by at least one level of irinotecan hydrochloride injection should be considered for patients known to be homozygous for the UGT1A1*28 allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment.

Please review the complete therapeutic recommendations that are located here: (3).

Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): The UGT1A1*28 allele is associated with irinotecan toxicity. Although results are not consistent, there is sufficient evidence that a reduction in the initial dose by 30% is required for regimens containing >250 mg/m2 of irinotecan prescribed to homozygous carriers of the UGT1A1*28 allele. This is in agreement with the Food and Drug Administration–mandated label change. No dose reduction is recommended for heterozygous carriers of the UGT1A1*28 allele because dose reduction might result in undertreatment (Table 1).

Please review the complete therapeutic recommendations that are located here: (4).

Summary of Findings on UGT1A1 Genotyping to Predict Response to Irinotecan, from the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group:

In 2009, the independent Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group evaluated the use of UGT1A1 genotyping to determine the best dose of irinotecan to prevent side effects when treating patients with metastatic colorectal cancer. The Working Group determined that there was not enough evidence to conclude whether UGT1A1 genotyping should be used for this purpose. The balance of benefits and harms of UGT1A1 genotyping to guide irinotecan use could not be determined from the available evidence.

(Note that the EGAPP recommendation statement was published prior to the FDA statement or KNMP guidelines and may not have considered evidence available to those groups.)

The EGAPP recommendation statement was based on the following key points from the evidence review:

- UGT1A1 genotyping results appear accurate for the common variants.
- Observational studies identified associations between UGT1A1 genotype results and the occurrence of certain side effects, as well as a potential impact on treatment effectiveness.
- The EGAPP Working Group (EWG) found no evidence that demonstrated that targeted dosing of irinotecan based on UGT1A1 genotyping leads to improved patient outcomes.
Even if targeted dosing were shown to be highly effective, it is not clear that benefits (reduced side effects) would outweigh harms (unresponsive tumors) (27).

Please review the complete therapeutic recommendations that are located here: (5).

Nomenclature

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<th>Common allele name</th>
<th>Alternative names</th>
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<th>dbSNP reference identifier for allele location</th>
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</table>

For an overview of the haplotypes for UGT1A1, please see the PharmGKB's haplotype translation table.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines).

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References


Tests in GTR by Condition

Irinotecan response

Tests in GTR by Gene

UGT1A1 gene
Introduction

Maraviroc is a chemokine receptor antagonist that is used in combination with other antiretroviral agents to treat human immunodeficiency virus type 1 (HIV-1) infection. Maraviroc exerts its therapeutic activity by blocking entry of the HIV-1 virus into immune cells—specifically the CD4-expressing T-helper cells, which play a major role in protecting the body from infection—precursor cells, and dendritic cells.

HIV-1 infection is classified in two major forms according to the co-receptor it employs to gain entry into the cell, namely the chemokine receptor 5 (CCR5) or the CXC chemokine receptor 4 (CXCR4). These co-receptors are expressed on different types of cells, and HIV tropism refers to the types of cells and tissues in which the virus infects and replicates. A tropism assay is conducted to determine which co-receptor the HIV-1 virus uses, i.e., whether the virus is CCR5-tropic, CXCR4-tropic, dual tropic (i.e., HIV-1 virus that is able to use both receptors), or mixed tropic (i.e., a mixture of HIV-1 viruses, some of which use CCR5 and others that use CXCR4).

Maraviroc is only indicated for treatment of adults with CCR5 tropic HIV-1 and is not recommended when the CXCR4-tropic virus has been detected. The FDA-approved drug label for maraviroc states that “prior to initiation of maraviroc, test all patients for CCR5 tropism using a highly sensitive tropism assay” (1).

Drug: Maraviroc

Maraviroc is the first FDA-approved drug in a class of HIV drugs called entry and fusion inhibitors. Maraviroc blocks the interaction between HIV-1 and CCR5 in healthy immune cells, preventing certain strains (CCR5-tropic) of HIV from entering and infecting the cell. Maraviroc must be taken twice daily and must always be used with other HIV drugs. Taken in combination with these drugs, maraviroc may lower the HIV virus load in the blood.

Currently, maraviroc is the only CCR5 co-receptor inhibitor that has been approved for clinical use (2). It is used to treat HIV-1-infected patients who have a virus that uses CCR5 for entry, and either never received antiretroviral treatment before, or have experienced therapeutic failure following traditional antiretroviral therapies (3). Among other CCR5 antagonists currently under investigation is cenicriviroc, which is in Phase II trials and appears to block the CCR2 receptor (4, 5).

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
Maraviroc treatment regimens may be used less often than other regimens. Possible reasons include the requirement to test for tropism, which is time-consuming and expensive (see Genetic Testing). Furthermore, there is a large selection of potent and tolerable treatment regimens currently available that do not require genotyping prior to use. These treatment regimens may be based on nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse-transcriptase inhibitors (NNRTI), boosted protease inhibitors (PI), and integrase inhibitors (2, 6).

The entry of HIV-1 into a host cell is a complex process, which begins when the viral envelope glycoprotein, gp120, binds to the cellular protein, CD4. Binding induces conformational changes in gp120 resulting in the exposure of gp4, another viral envelope protein that helps mediate the interaction between the virus and cellular co-receptors, and the fusion of viral and cellular membranes.

The CD4 count is often used to determine the stages of HIV disease. CD4 is a glycoprotein found on the surface of T helper immune cells. HIV-1 infection leads to a progressive reduction in the number of T cells that express CD4, and a CD4 count of less than 200 cells/mm$^3$ is one of the qualifications for a diagnosis of AIDS (7, 8).

Measurement of the CD4 count is useful before HIV treatment is started because the CD4 count provides information on the overall immune function of the patient. In the United States, antiretroviral therapy (ART) is now recommended for all HIV-infected patients, regardless of their CD4 count or viral load (9), to keep viral loads at undetectable levels for as long as possible. In adults receiving optimized background treatment for infection with CCR5-tropic HIV-1, the addition of maraviroc leads to a greater increase in CD4 counts compared to the addition of placebo (1).

HIV-1 most commonly uses either the CCR5 or CXCR4 co-receptors to enter its target cells (10). Maraviroc is an effective antiretroviral agent in individuals who only harbor the CCR5-tropic HIV-1 virus. It is incapable of inhibiting infection against viruses that do not use CCR5 (i.e., CXCR-using virus or dual/mixed virus) (1).

Maraviroc is metabolized by the cytochrome P450 system, mainly CYP3A, in the liver to inactive metabolites (11, 12). As noted above, maraviroc must be used in combination with other antiretroviral medications; the recommended dosage of maraviroc depends on whether the co-medications are inhibitors or inducers of CYP3A (1).

Gene: CCR5

The chemokine (CC motif) receptor 5 (CCR5) is primarily expressed on the surface of white blood cells. Chemokines are a type of cytokine—they are small, secreted proteins that have a crucial role in the inflammatory response by helping immune cells migrate to areas of tissue damage. Other functions of chemokines include influencing the maturation of various immune cells and promoting the growth of new blood vessels.

Most chemokines have four characteristic cysteine residues in a conserved location, and they are classified into four families by the location of the first two cysteine residues: CXC,
CC, C, and CX3C. For example, members of the “CC” cytokine family have two adjacent cysteine residues near their amino terminus.

The receptors for chemokines are G-protein coupled, seven-transmembrane domain receptors. Two of these receptors, CCR5 (binds CC chemokines) and CXCR4 (binds CXC chemokines), are also co-receptors used by HIV to enter human white blood cells. CCR5 is expressed on fewer cells (e.g., specific T cells, precursor cells (or macrophages) and dendritic cells) than CXCR4 (e.g., most immune cells, vascular endothelial cells, and neurons).

HIV-1 virus that uses the CCR5 co-receptor (CCR5-tropic) is more commonly found in the early stages of infection. It is also more common among individuals who have yet to receive treatment, and at least half of all infected individuals harbor only CCR5-tropic viruses throughout the course of infection. The CXCR4-tropic virus is more commonly found during later stages of disease and among individuals who have received HIV treatment. The presence of CXCR4-tropic virus is a predictor of lower CD4 count, a higher viral load, and a more rapid progression to AIDS (7).

A variant of CCR5, CCR5-Δ32 (NM_000579.3:c.554_585del32), contains a 32 bp deletion and codes a nonfunctional receptor that hinders the entry of CCR5-tropic virus into cells. Individuals who have two copies of this allele are highly resistant to HIV infection, and although individuals who have one copy of the allele remain susceptible to HIV infection, the progression of HIV infection to AIDS is delayed (13).

The CCR5-Δ32 allele occurs at high frequency in European Caucasians (5%–14%) but is rare among African, Native American, and East Asian populations, suggesting that the allele may have conferred an evolutionary survival advantage (14). Possible causes of a positive selection pressure include protection against the bubonic plague (Yersinia pestis) or smallpox (Variola virus) during the Middle Ages. However, other studies have found that the CCR5-Δ32 allele arose long before this time and underwent neutral evolution (15).

**Genetic Testing**

Testing of the HIV-1 virus (i.e., the virus, not the patient) should be carried out prior to initiation of treatment with maraviroc. A tropism assay is needed to identify individuals with CCR5-tropic HIV-1. The assay must be highly sensitive to detect low levels of CXCR4-tropic viruses. Maraviroc should not be prescribed if non-CCR5 variants (CXCR4-tropic or dual/mixed-tropic) are detected (1, 11). HIV tropism can be determined by phenotype or genotype testing. Phenotypic assays can be performed using plasma RNA (if viral load is greater than 1000 copies/ml) or cell-associated DNA (if viral load is less than 1000 copies/ml). Phenotypic assays use replication-defective laboratory viruses that carry the complete cloned viral envelope proteins gp120 and gp41 derived from the patient. Phenotypic assays measure the ability of these pseudoviruses to infect CD4+ target cells that express either CCR5 or CXCR4 (9).
Genotyping methods are used to predict which co-receptors on the cell are used by the virus rather than directly assessing tropism. Genotyping methods involve sequencing the third variable region (V3) of gp120 and using algorithms to predict co-receptor usage.

While phenotypic assays are still considered to be the gold standard, the use of genotyping to determine patient eligibility for maraviroc is increasing due to low cost, greater accessibility, and faster turnaround time for the results as compared to the other methods (16, 17). Although there can be discrepancies between the results from phenotypic and genotypic assays, the correlation between genotypic assays and the clinical efficacy of maraviroc is improving (18).

The NIH’s Genetic Testing Registry (GTR) displays genetic testing information for human genes and conditions, including tests for maraviroc response. These tests investigate the human genes that contribute to the pharmacokinetics of maraviroc, as opposed to the FDA-recommended genetic tests, which are tests for viral genes.

**Therapeutic Recommendations based on Genotype**

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Prior to initiation of maraviroc, test all patients for CCR5 tropism using a highly sensitive tropism assay. Maraviroc is recommended for patients with only CCR5-tropic HIV-1 infection. Outgrowth of pre-existing low-level CXCR4- or dual/mixed-tropic HIV-1 not detected by tropism testing at screening has been associated with virologic failure while on maraviroc.

Please review the complete therapeutic recommendations that are located here: (1).

**Nomenclature**

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</table>

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1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.
Acknowledgments

The author would like to thank Aniwaa Owusu Obeng, PharmD, Assistant Professor, The Charles Bronfman Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai; and Victoria M. Pratt, Ph.D., FACMG, Director, Pharmacogenomics Laboratory, Department of Medical and Molecular Genetics, Indiana University School of Medicine; for reviewing this summary.

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Version History

To view an earlier version of this summary (18 March 2015), please click here.

References


Related Summaries by Drug Class

Abacavir Therapy and HLA-B*57:01 Genotype

Tests in GTR by Gene

CCR5 gene
Mercaptopurine Therapy and *TPMT* Genotype

Laura Dean, MD


**Introduction**

Mercaptopurine is an immunosuppressant and antineoplastic agent that belongs to the drug class of thiopurines. It is used in combination with other drugs to treat acute lymphoblastic leukemia, which is the most common form of cancer in children (1). In addition, off-label uses include the treatment of inflammatory bowel disease (IBD).

Mercaptopurine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), the major active metabolites. Thiopurine S-methyltransferase (TPMT) inactivates mercaptopurine, leaving less parent drug available to form TGNs.

An adverse effect of mercaptopurine therapy is bone marrow suppression, which can occur in any patient, is dose-dependent, and may be reversed by reducing the dose of mercaptopurine. However, patients who carry two nonfunctional *TPMT* alleles universally experience life-threatening myelosuppression when treated with mercaptopurine, due to high levels of TGNs. Patients who carry one nonfunctional *TPMT* allele may also be unable to tolerate conventional doses of mercaptopurine (2, 3).

The FDA-approved drug label for mercaptopurine states that heterozygous patients with low or intermediate TPMT activity accumulate higher concentrations of active TGNs than people with normal TPMT activity and are more likely to experience mercaptopurine toxicity; and that TPMT genotyping or phenotyping (red blood cell TPMT activity) can identify patients who are homozygous deficient or have low or intermediate TPMT activity (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published dosing recommendations for *TPMT* genotype-based mercaptopurine dosing. These recommendations include:

- Start with reduced doses of mercaptopurine for patients with one nonfunctional *TPMT* allele, or drastically reduced doses for patients with malignancy and two nonfunctional alleles; adjust dose based on degree of myelosuppression and disease-specific guidelines.
- Consider alternative nonthiopurine immunosuppressant therapy for patients with nonmalignant conditions and two nonfunctional alleles (see Table 1) (2-4).

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1 NCBI; Email: dean@ncbi.nlm.nih.gov.
<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Phenotype details</th>
<th>TPMT Genotype</th>
<th>Examples of diplotypes</th>
<th>Therapeutic recommendations for mercaptopurine (MP)</th>
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**MP: Mercaptopurine**

The strength of therapeutic recommendations is "strong" for all phenotypes. Table is adapted from Relling M.V. et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clinical pharmacology and therapeutics. 2011;89(3):387–91 (2, 3).
<table>
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MP: Mercaptopurine

The strength of therapeutic recommendations is "strong" for all phenotypes.

Table is adapted from Relling M.V. et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clinical pharmacology and therapeutics. 2011;89(3):387–91 (2, 3).

**Drug Class: Thiopurines**

Thiopurines are used as anticancer agents and as immunosuppressants in inflammatory bowel disease, rheumatoid arthritis, and other autoimmune conditions. Three thiopurines are used clinically: thioguanine, mercaptopurine, and azathioprine (a prodrug for mercaptopurine). All three agents have similar effects but are typically used for different indications. Thioguanine is most commonly used in the treatment of myeloid leukemias, mercaptopurine is used for lymphoid malignancies, and mercaptopurine and azathioprine are used for immune conditions.

Thiopurines are either activated to form TGNs (the major active metabolite) or deactivated by TPMT. Individuals who carry two non-functional TPMT alleles ("TPMT homozygotes") universally experience life-threatening bone marrow suppression because of high levels of TGNs when treated with conventional doses. Individuals who carry one non-functional TPMT allele ("TPMT heterozygotes") may also be unable to tolerate conventional doses of thiopurines due to increased levels of TGNs.

**Drug: Mercaptopurine**

Mercaptopurine is a neoplastic agent and an immunosuppressive agent that is used in the treatment of acute lymphoblastic leukemia (ALL) as part of a combination regimen. ALL is the most common form of cancer in children, accounting for approximately 30% of childhood malignancies with a peak incidence occurring at 3 to 5 years of age (5).

An off-label use of mercaptopurine is in the treatment of inflammatory bowel disease (IBD). Along with the closely related azathioprine (which is metabolized to mercaptopurine), mercaptopurine is used as an “immunomodulator” and as a “steroid-sparing agent” in the treatment of Crohn’s disease and ulcerative colitis.

Mercaptopurine is a slow-acting drug and for IBD, it typically takes at least three months of therapy before a therapeutic effect is observed. Therefore, mercaptopurine is used for the induction and maintenance of IBD remission rather than as a monotherapy for acute relapses (6). Because the discontinuation of mercaptopurine is associated with a high rate
of relapse of IBD, mercaptopurine is usually continued long-term if there are no adverse effects (7, 8).

The use of mercaptopurine or the related drug azathioprine, has been associated with a 4-fold increased risk of developing lymphoma, which does not persist after discontinuation of therapy (9, 10).

Like all thiopurines, mercaptopurine is a purine analogue, and acts as an antimetabolite by interfering with nucleic acid synthesis and inhibiting purine metabolism. Activation of mercaptopurine occurs via HPRT1 (hypoxanthine phosphoribosyltransferase) followed by a series of reactions to form TGNs. The cytotoxicity of mercaptopurine is due, in part, to the incorporation of TGNs into DNA.

Inactivation of mercaptopurine occurs via two different pathways, via methylation (by TPMT) or via oxidation (by xanthine oxidase). TPMT activity is highly variable in patients because of genetic polymorphism in the TPMT gene.

One of the most frequent adverse reactions to mercaptopurine is myelosuppression, which can occur in any patient, and can usually be reversed by decreasing the dose of mercaptopurine. However, all patients who carry two nonfunctional TPMT alleles (approximately 0.3%) experience life-threatening myelosuppression after starting treatment with conventional doses of mercaptopurine, due to high levels of TGNs.

Individuals who are heterozygous for nonfunctional TPMT alleles (approximately 10%) are at a significantly higher risk for toxicity than individuals with two functional alleles. However, some of these individuals, approximately 40–70%, can tolerate the full dose of mercaptopurine. This may be because heterozygous-deficient individuals have lower concentrations of less active metabolites, such as MeMPN (methylmercaptopurine nucleotides), than homozygous-deficient individuals (2, 3).

Approximately 90% of individuals have normal TPMT activity with two functional alleles; however, all individuals receiving mercaptopurine require close monitoring (2, 3, 11, 12). One study reports that in patients with IBD receiving thiopurine therapy, TPMT polymorphisms are associated with the overall incidence of adverse reactions and with bone marrow toxicity, but not with other adverse reactions, such as liver damage and pancreatitis. Therefore, although determining TPMT genotype is helpful before initiating therapy, regular blood tests to monitor for side effects are needed during therapy (1, 13).

The other mercaptopurine inactivation pathway is via oxidation, which is catalyzed by xanthine oxidase. If this pathway is inhibited, for example, in patients taking allopurinol (an inhibitor of xanthine oxidase), the decreased break down of mercaptopurine can lead to mercaptopurine toxicity (1). However, some studies have found that the co-administration of allopurinol, with a reduced dose of mercaptopurine (or azathioprine), can help optimize the treatment response in patients with IBD (14, 15).
Gene: **TPMT**

The *TPMT* gene encodes one of the important enzymes of phase II metabolism, thiopurine S-methyltransferase. TPMT is one of the main enzymes involved in the metabolism of thiopurines, such as mercaptopurine. TPMT activity is inherited as a co-dominant trait, as the *TPMT* gene is highly polymorphic with over 40 reported variant alleles (16-19).

The wild-type *TPMT*/*1* allele is associated with normal enzyme activity. Individuals who are homozygous for *TPMT*/*1* (TPMT normal metabolizers) are more likely to have a typical response to mercaptopurine and a lower risk of myelosuppression. This accounts for the majority of patients (~86–97%) (2, 3).

Individuals who are TPMT poor (approximately 0.3%) or intermediate (approximately 3–14%) metabolizers carry variant *TPMT* alleles that encode reduced or absent enzyme activity. Three variant *TPMT* alleles account for over 90% of the reduced or absent activity *TPMT* alleles (20, 21):

- *TPMT*/*2* (c.238G>C)
- *TPMT*/*3A* (c.460G>A and c.719A>G)
- *TPMT*/*3B* (c.460G>A)
- *TPMT*/*3C* (c.719A>G)

The frequency of *TPMT* alleles varies among different populations. In the United States, the most common low-activity allele in the Caucasian population is *TPMT*/*3A* (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently (16, 20).

In East Asian, African-American, and some African populations, the most common variant is *TPMT*/*3C* (~2%), although *TPMT*/*8* may be more common in African populations than previously thought (~2%). In general, *TPMT*/*2* occurs much less commonly, and *TPMT*/*3B* occurs rarely (16, 22).

**Genetic Testing**

Genetic testing is available for several *TPMT* variant alleles, which most commonly includes *TPMT*/*2*, *3A*, and *3C* as they account for >90% of inactivating alleles. Of note, rare and/or previously undiscovered variants will not be detected by variant-specific genotyping methods (2, 3, 23-26).

TPMT phenotype enzyme activity testing is also available by measuring TPMT activity in red blood cells directly (11). In adult patients taking mercaptopurine as an immunosuppressive agent, there is strong evidence of a near 100% concordance between phenotype and genotype testing. Inflammatory disease processes do not interfere with the accuracy of TPMT activity measurements if the blood sample is taken under standard conditions (e.g., not within two months of a blood transfusion).
However, in patients with leukemia, the concordance between TPMT phenotype and genotype is poor (27). By the time of diagnosis, red cell TPMT activity is typically greatly reduced because of atypical hematopoiesis. Therefore, phenotype testing may wrongly identify an individual as having a TPMT deficiency, e.g., a patient who has two functional copies of the TPMT gene (homozygous wild-type) may be determined as having only one functional copy and one nonfunctional variant (TPMT heterozygous); and a patient who is TPMT heterozygous may be wrongly determined to be TPMT homozygous (two copies of nonfunctional TPMT variants). In addition, during the course of chemotherapy, TPMT phenotype testing may reveal excessively high TPMT activity. This is thought to be due to an excess of young red blood cells with their associated higher level of TPMT enzyme activity. Therefore, to avoid an incorrect TPMT status, genotype testing is recommended for patients with leukemia (27).

Finally, one study reported that TPMT genotyping was more reliable than phenotyping in identifying patients at risk of adverse reactions from thiopurine treatment (28), and several studies reported that the TPMT genotype is a better indicator than TPMT activity for predicting TGN accumulation or treatment outcome (12, 29-31).

Therapeutic Recommendations based on Genotype

This section contains excerpted1 information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): Individuals who are homozygous for an inherited defect in the TPMT (thiopurine-S-methyltransferase) gene are unusually sensitive to the myelosuppressive effects of mercaptopurine and prone to developing rapid bone marrow suppression following the initiation of treatment. Laboratory tests are available, both genotypic and phenotypic, to determine the TPMT status. Substantial dose reductions are generally required for homozygous-TPMT deficient patients (two non-functional alleles) to avoid the development of life threatening bone marrow suppression. Although heterozygous patients with intermediate TPMT activity may have increased mercaptopurine toxicity, this is variable, and the majority of patients tolerate normal doses of mercaptopurine. If a patient has clinical or laboratory evidence of severe toxicity, particularly myelosuppression, TPMT testing should be considered. In patients who exhibit excessive myelosuppression due to 6-mercaptopurine, it may be possible to adjust the mercaptopurine dose and administer the usual dosage of other myelosuppressive chemotherapy as required for treatment.

Please review the complete therapeutic recommendations that are located here: (1).

1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.
2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Testing for TPMT status is recommended prior to starting mercaptopurine therapy so that the starting dosages can be adjusted accordingly—see Table 1 for dosing recommendations. In homozygous variant individuals, consider an alternative agent for nonmalignant conditions and drastically reduce doses in malignant conditions. In heterozygous individuals, depending on the disease being treated, starting doses should be reduced. In both patient groups, a longer period of time should be left after each dose adjustment to allow for a steady state to be reached.

Please review the complete therapeutic recommendations that are located here: (2, 3).

**Nomenclature**

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>Protein</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT*2</td>
<td>238G&gt;C Ala80Pro</td>
<td>NM_000367.2:c.238G&gt;C</td>
<td>NP_000358.1:p.Ala80Pro</td>
<td>rs1800462</td>
</tr>
<tr>
<td>TPMT*3A</td>
<td>This allele contains two variants in cis: c.460G&gt;A and c.719A&gt;G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPMT*3B</td>
<td>460G&gt;A Ala154Thr</td>
<td>NM_000367.2:c.460G&gt;A</td>
<td>NP_000358.1:p.Ala154Thr</td>
<td>rs1800460</td>
</tr>
<tr>
<td>TPMT*3C</td>
<td>719A&gt;G Tyr240Cys</td>
<td>NM_000367.2:c.719A&gt;G</td>
<td>NP_000358.1:p.Tyr240Cys</td>
<td>rs1142345</td>
</tr>
</tbody>
</table>

The TPMT Nomenclature Committee defines the nomenclature and numbering of novel TPMT variants: [http://www.imh.liu.se/tpmtalleles](http://www.imh.liu.se/tpmtalleles)

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

**Acknowledgments**

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**Version History**

To view an earlier version of this summary (Update: March 18, 2013), please click [here](http://www.pharmgkb.org/page/cpic).
References


19. TPMT Nomenclature Committee [Internet]. Sweden: Linköping University. Table of TPMT alleles. [Cited 2016 February 02]. Available from: http://www.imh.liu.se/tpmtalleles/tabell-over-tptm-alleler?l=en


Related Summaries by Gene
Azathioprine Therapy and TPMT Genotype
Thioguanine Therapy and TPMT Genotype

Related Summaries by Drug Class
Azathioprine Therapy and TPMT Genotype
Thioguanine Therapy and TPMT Genotype

Tests in GTR by Condition
Mercaptopurine response

Tests in GTR by Gene
TPMT gene
Metoprolol Therapy and CYP2D6 Genotype

Laura Dean, MD
Created: April 4, 2017.

Introduction

Metoprolol is a beta blocker used in the treatment of hypertension, angina, and heart failure. Metoprolol selectively blocks beta_1 adrenoreceptors mainly expressed in cardiac tissue. Blockade of these receptors reduces the heart rate and decreases the force of heart contractions.

Metoprolol is primarily metabolized by the CYP2D6 enzyme. Approximately 8% of Caucasians and 2% of most other populations have absent CYP2D6 activity and are known as “CYP2D6 poor metabolizers.” In addition, a number of drugs inhibit CYP2D6 activity, such as quinidine, fluoxetine, paroxetine, and propafenone.

The FDA-approved drug label for metoprolol states that CYP2D6 poor metabolizers, and normal metabolizers who concomitantly take drugs that inhibit CYP2D6, will have increased (several-fold) metoprolol blood levels, decreasing metoprolol’s cardioselectivity (1).

The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) has published metoprolol dosing recommendations based on CYP2D6 genotype. For individuals who have a CYP2D6 gene variation that reduces the conversion of metoprolol to inactive metabolites, DPWG states that the clinical consequences are limited mainly to the occurrence of asymptomatic bradycardia. For CYP2D6 poor metabolizers, if a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia, DPWG recommends increasing the dose of metoprolol in smaller steps and/or prescribing no more than 25% of the standard dose. For other cases, no action is required (2).

Please note: Beta blockers such as metoprolol have been demonstrated in several large trials to be safe and effective for treatment of patients with cardiovascular disease. As a mainstay of therapy associated with improvements in quality of life, hospitalization rates, and survival (3, 4), clinical care pathways that might lead to underutilization of beta blockers require scrutiny. FDA points out that CYP2D6 poor metabolizers will have decreased cardioselectivity for metoprolol due to increased metoprolol blood levels. Yet, it is common clinical practice to adjust the dose of metoprolol according to the patient’s heart rate. FDA does not specifically comment on the role of genetic testing for initiating therapy.

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1 NCBI; Email: dean@ncbi.nlm.nih.gov.
**Drug: Metoprolol**

Metoprolol is a commonly prescribed drug that belongs to the drug class of beta-adrenoreceptor antagonists, also known as “beta blockers.” Metoprolol is indicated to treat hypertension, angina, and heart failure (stable, symptomatic (NYHA Class II or III) heart failure). Metoprolol selectively blocks the beta\textsubscript{1} adrenoreceptor (1).

There are two main types of adrenoreceptors, alpha and beta, each of which have numbered subtypes. The beta adrenoreceptors have three subtypes, beta\textsubscript{1}, beta\textsubscript{2}, and beta\textsubscript{3}. All three subtypes are coupled to the G\textsubscript{s} protein, which in turn activates adenylate cyclase enzyme, which catalyzes the production of cyclic AMP (cAMP).

The binding of an agonist, such as the catecholamines adrenaline and noradrenaline, to beta receptors leads to a rise in the intracellular concentration of cAMP, which triggers signaling pathways. Stimulation of the beta\textsubscript{1} receptor, which is predominantly expressed in cardiac tissue, leads to an increase in heart rate and an increase in the contractility of the atria and ventricles. It also leads to the increased secretion of hormones from other tissues—renin (from the kidneys), ghrelin (from the stomach), and amylase (from the salivary glands).

In the treatment of heart failure, beta blockers such as extended-release metoprolol are thought to protect the heart from increased catecholamine stimulation. In the short term, adrenergic activation can help the heart maintain cardiac performance, but over time, continued activation can be detrimental. Harmful effects include a persistently increased heart rate, down-regulation and impaired functioning of the beta receptors, and myocyte hypertrophy and death—which leads to adverse remodeling of heart tissue (5, 6).

Metoprolol exerts its therapeutic effects by reducing the impact of catecholamine stimulation. Metoprolol reduces the heart rate, improves contractile function by stimulating the upregulation of beta-1 receptors, reduces vasoconstriction, and possibly also reduces the risk of arrhythmias (3, 5, 7, 8).

Metoprolol is a racemic mixture of R- and S-enantiomers (an equal amount of left- and right-handed enantiomers, which are molecules that are mirror images of each other, but are not superimposable on one another).

Metoprolol is primarily metabolized by CYP2D6, an enzyme which is absent in about 8% of Caucasians (poor metabolizers) and about 2% of most other populations. Individuals who lack CYP2D6 activity will have higher plasma concentrations of metoprolol, almost 5-fold higher, and may be at an increased risk of side effects (9-12).

In addition, at higher plasma concentrations, metoprolol is less cardio-selective. Metoprolol can inhibit beta\textsubscript{2} receptors, which are mainly located in the bronchial and vascular musculature.

Genetic variants of the CYP2D6 gene have been found to influence the ratio of enantiomers, the dose and dose titration of metoprolol, and to influence heart rate—
CYP2D6 poor metabolizers have an increased risk of bradycardia (13-16). However, CYP2D6 does not appear to influence the efficacy of metoprolol when used to treat hypertension (17).

Variants within the beta1 receptor have also been found to influence the treatment response to specific beta blockers. The most commonly studied is a reduced function variant, Arg389Gly, which leads to reduced levels of cAMP and diminished beta1 receptor signaling cascades (18). Individuals who are homozygous Arg389 carriers may have a more favorable response to metoprolol treatment than individuals who are homozygous for Gly389 (18), (19), (20), (21).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Gene: CYP2D6

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described (22). CYP2D6*1 is the reference (or wild-type) allele encoding enzyme with normal activity. The CYP2D6*2, *33, and *35 alleles are also considered to confer normal activity (Table 1).

<table>
<thead>
<tr>
<th>Allele type</th>
<th>CYP2D6 Allele(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal function</td>
<td>*1, *2, *33, *35</td>
</tr>
</tbody>
</table>

For a detailed list of CYP2D6 alleles, please see (22).

Individuals who have more than two normal function copies of the CYP2D6 gene are “ultrarapid metabolizers,” whereas individuals who carry two normal or one normal and one decreased function allele are classified as “normal metabolizers.”

Individuals with one normal and one no function allele or two decreased function alleles are categorized as “normal metabolizers” by recent nomenclature guidelines (23), but have also been categorized as “intermediate metabolizers” in the literature. Subjects with one decreased and one no function allele are predicted to be intermediate metabolizers and those with two no function alleles, poor metabolizers.

The most common no function alleles include CYP2D6*3, *4, *5, and *6 (24-27), and the most common decreased function alleles include CYP2D6*9, *10, *17, *29 and *41 (28-32) (Table 1).
There are large inter-ethnic differences in the frequency of these alleles. For example, \( CYP2D6*4 \) is the most common no function allele in Caucasians, but is less abundant in subjects with African ancestry, and is rare in Asians. In contrast, the decreased function allele \( CYP2D6*10 \) is the most common allele in Asians, and \( CYP2D6*17 \) is almost exclusively found in individuals with African ancestry (33).

Consequently, the phenotype frequencies also vary substantially among the major ethnicities and may vary among populations. Approximately 6-8% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function \( CYP2D6*4 \) and \( *5 \) alleles (34, 35).

**Genetic Testing**

The NIH’s Genetic Testing Registry provides examples of the genetic tests that are currently available for metoprolol response and the \( CYP2D6 \) gene.

Results are typically reported as a diplotype, such as \( CYP2D6 \ *1/*1 \). A result for copy number, if available, is also important when interpreting \( CYP2D6 \) results (36).

**Therapeutic Recommendations based on Genotype**

This section contains excerpted\(^1\) information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**2016 Statement from the US Food and Drug Administration (FDA):** Metoprolol is metabolized predominantly by \( CYP2D6 \), an enzyme that is absent in about 8% of Caucasians (poor metabolizers) and about 2% of most other populations. \( CYP2D6 \) can be inhibited by a number of drugs. Poor metabolizers and extensive metabolizers who concomitantly use \( CYP2D6 \) inhibiting drugs will have increased (several-fold) metoprolol blood levels, decreasing metoprolol’s cardioselectivity.

Please review the complete therapeutic recommendations that are located here: (1).

**2016 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):**

**CYP2D6 Poor Metabolizers:**

The gene variation reduces the conversion of metoprolol to inactive metabolites. However, the clinical consequences are limited mainly to the occurrence of asymptomatic bradycardia.

\(^1\) The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
Recommendation:

If a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia:

1. Increase the dose in smaller steps and/or prescribe no more than 25% of the standard dose

Other cases:

1. No action required

**CYP2D6 Intermediate Metabolizers:**

The gene variation reduces the conversion of metoprolol to inactive metabolites. However, the clinical consequences are limited mainly to the occurrence of asymptomatic bradycardia.

Recommendation:

If a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia:

1. Increase the dose in smaller steps and/or prescribe no more than 50% of the standard dose

Other cases:

1. No action required

**CYP2D6 Ultrarapid Metabolizers:**

The gene variation increases the conversion of metoprolol to inactive metabolites. This can increase the dose requirement. However, with a target dose of 200 mg/day, there was no effect on the blood pressure and hardly any effect on the reduction of the heart rate.

Recommendation:

1. Use the maximum dose for the relevant indication as a target dose
2. If the effectiveness is still insufficient: increase the dose based on effectiveness and side effects to 2.5 times the standard dose or select an alternative

Possible alternatives include:

- Heart failure: bisoprolol or carvedilol. Bisoprolol: advantage: not metabolised by CYP2D6; disadvantage: elimination depends on the kidney function. Carvedilol: advantage: elimination does not depend on the kidney function; disadvantage: is metabolised (to a lesser extent than metoprolol) by CYP2D6.
- Other indications: atenolol or bisoprolol. Neither is metabolised by CYP2D6.

**Please review the complete therapeutic recommendations that are located here:** (2)
# Nomenclature of selected CYP2D6 alleles

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>Protein location</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*4</td>
<td>1846G&gt;A</td>
<td>NM_000106.5:c.506-1G&gt;A</td>
<td>Variant occurs in a non-coding region (splice variant causes a frameshift)</td>
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<tr>
<td>CYP2D6*5</td>
<td>Variant results in a whole gene deletion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>1707 del T Trp152Gly CYP2D6T</td>
<td>NM_000106.5:c.454delT</td>
<td>NP_000097.3:p.Trp152Glyfs</td>
<td>rs5030655</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>100C&gt;T (Pro34Ser)</td>
<td>NM_000106.5:c.100C&gt;T</td>
<td>NP_000097.3:p.Pro34Ser</td>
<td>rs1065852</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>1023C&gt;T[1] (Thr107Ile)</td>
<td>NM_000106.5:c.320C&gt;T</td>
<td>NP_000097.3:p.Thr107Ile</td>
<td>rs28371706</td>
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<td></td>
<td>2850C&gt;T[2] (Cys296Arg)</td>
<td>NM_000106.5:c.886T&gt;C</td>
<td>NP_000097.3:p.Cys296Arg</td>
<td>rs16947</td>
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<tr>
<td>CYP2D6*41</td>
<td>2850C&gt;T[2] (Cys296Arg)</td>
<td>NM_000106.5:c.886T&gt;C</td>
<td>NP_000097.3:p.Cys296Arg</td>
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</tr>
<tr>
<td></td>
<td>2988G&gt;A</td>
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<td>Variant occurs in a non-coding region (impacts slicing)</td>
<td>rs28371725</td>
</tr>
</tbody>
</table>

[1] In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

[2] In the literature, 2850C>T is also referred to as 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

**Acknowledgments**

The author would like to thank Larisa H. Cavallari, Pharm.D., Associate Professor & Director, Center for Pharmacogenomics, Pharmacotherapy and Translational research, University of Florida; Mandy van Rhenen, secretary of the Dutch Pharmacogenetics Working Group (DPWG), Centre for Information on Medicines, Royal Dutch Pharmacists Association (KNMP); and John Wikstrand, Professor of Clinical Physiology, Wallenberg Laboratory, University of Gothenburg, Sweden, for reviewing this summary.
References


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Aripiprazole Therapy and CYP2D6 Genotype
Atomoxetine Therapy and CYP2D6 Genotype
Clozapine Therapy and CYP2D6, CYP1A2, and CYP3A4 Genotypes
Codeine Therapy and CYP2D6 Genotype
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Propafenone Therapy and CYP2D6 Genotype
Risperidone Therapy and CYP2D6 Genotype
Tamoxifen Therapy and CYP2D6 Genotype
Thioridazine Therapy and CYP2D6 Genotypes
Tramadol Therapy and CYP2D6 Genotype
Venlafaxine Therapy and \textit{CYP2D6} Genotype

\textbf{Tests in GTR by Condition}

Metoprolol response

\textbf{Tests in GTR by Gene}

\textit{CYP2D6} gene
Omeprazole Therapy and CYP2C19 Genotype

Laura Dean, MD

Created: October 1, 2012; Updated: March 8, 2016.

Introduction

Omeprazole blocks the secretion of gastric acid and belongs to the drug class of proton pump inhibitors. It is used to treat gastroesophageal reflux disease (GERD), gastric ulcers, duodenal ulcers, erosive esophagitis, and other acid-related disorders. It is also used in the treatment of hypersecretory conditions, such as Zollinger-Ellison syndrome, and is used in combination with antibiotics to eradicate Helicobacter pylori (H. pylori) infection (1).

CYP2C19 is the principal enzyme that metabolizes omeprazole to inactive metabolites. Approximately 3% of Caucasians and 15 to 20% of Asians have reduced or absent CYP2C19 enzyme activity (“poor metabolizers”). In these individuals, standard doses of omeprazole may lead to higher exposure to the drug and improved treatment outcomes (2). In contrast, individuals with increased CYP2C19 activity (“ultrarapid metabolizers”) may have an insufficient response to treatment as the active drug is inactivated at a faster rate.

The FDA-approved drug label for omeprazole states that a dose reduction should be considered in the Asian population, particularly for the maintenance of healing of erosive esophagitis (1). The Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) has published dose alterations based on CYP2C19 genotype. For CYP2C19 poor metabolizers, they do not recommend altering the dose; however for ultrarapid metabolizers, they recommend being extra alert to an insufficient response to treatment. For the eradication of H. pylori in ultrarapid metabolizers, they recommend increasing the dose of omeprazole by 100–200%, and to consider the same dose increase for other conditions (see Table 1) (3, 4).

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
Table 1. CYP2C19 phenotypes and the therapeutic recommendations for omeprazole therapy, adapted from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP).

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Phenotype details</th>
<th>Examples of diplotypes</th>
<th>Therapeutic (dose) recommendations for omeprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>Normal or increased CYP2C19 activity</td>
<td>*17/*17</td>
<td>Be extra alert to insufficient response. For the eradication of <em>H. pylori</em>, increase dose by 100–200%. For other conditions, consider dose increase by 100–200%.</td>
</tr>
<tr>
<td>Extensive metabolizer</td>
<td>Normal CYP2C19 activity</td>
<td>*1/*1</td>
<td>No recommendations</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>Decreased CYP2C19 activity</td>
<td>*1/*2, *1/*3, *2/*17, *3/*17</td>
<td>No recommendations</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>Markedly reduced or absent CYP2C19 activity</td>
<td>*2/*2, *2/*3, *3/*3</td>
<td>No recommendations</td>
</tr>
</tbody>
</table>

Good quality evidence supports the dose recommendations for poor and intermediate metabolizers; moderate quality evidence supports the dose recommendations for ultrarapid metabolizers.


Drug class: Proton Pump Inhibitors

Proton pump inhibitors (PPIs) are inhibitors of gastric acid secretion that are used in the treatment of stomach-acid related disorders. PPIs are also used to prevent and treat ulcers associated with nonsteroidal anti-inflammatory drugs (NSAIDs), and can be used in combination with antibiotics to eradicate *H. pylori* infection.

Six PPIs are currently FDA-approved for clinical use: esomeprazole, dexlansoprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole. All PPIs are similarly potent at inhibiting gastric acid secretion and are thought to be similarly efficacious (5, 6).

PPIs are metabolized and inactivated by a number of CYP enzymes, including CYP2C19, which has a principal role in the metabolism of omeprazole. The increased function *CYP2C19*+17 variant allele may enhance PPI clearance (7) resulting in less active PPI available to inhibit gastric acid secretion. In contrast, the *CYP2C19*+2 loss-of-function allele is associated with decreased PPI clearance, resulting in more active PPI available and enhanced treatment. For several PPIs, including omeprazole and lansoprazole, higher drug levels in patients with low or absent CYP2C19 activity have been associated with increased drug efficacy and improved treatment outcomes (2, 8).
**Drug: Omeprazole**

Omeprazole was the first PPI to be introduced to the US market in 1989. Today, omeprazole is one of the PPIs that are available both as prescription and over-the-counter (OTC) medications.

In adults, omeprazole is used in the treatment of ulcers (gastric and duodenal), GERD, and to maintain healing of erosive esophagitis. Omeprazole is also used in the long-term treatment of hypersecretory conditions such as Zollinger-Ellison syndrome, multiple endocrine adenomas, and systemic mastocytosis. In children, omeprazole is used in the treatment of GERD and erosive esophagitis (1).

The human stomach contains approximately one billion parietal cells that secrete hydrochloric acid (HCl) into the gastric lumen. Gastric acid aids digestion by hydrolyzing dietary protein and facilitating the absorption of calcium, iron, and vitamin B. Gastric acid also helps maintain a sterile environment by suppressing the growth of bacteria (9).

Hydrogen ions (H+) are actively secreted in to the gastric lumen in exchange for potassium ions (K+) via an H⁺/K⁺-ATPase, which is also known as a “proton pump”. Located on the surface of gastric parietal cells, the proton pump controls the last step in acid secretion, and by targeting this step, omeprazole and the other PPIs are able to potently inhibit gastric acid secretion.

Omeprazole is metabolized and inactivated in the liver by the cytochrome P450 system. CYP2C19 is the principal enzyme involved, although other enzymes such as CYP3A4 may also contribute. Omeprazole is metabolized to hydroxy and desmethyl metabolites, which have no effect on gastric acid secretion (10).

Individuals with reduced CYP2C19 enzyme activity may experience twice the exposure to omeprazole compared to individuals with normal enzyme function. This reduced enzyme activity has a positive effect on clinical outcomes, and because PPIs are generally regarded as safe drugs, especially in the short-term (less than 6 months), this can have a beneficial effect without an increased risk of omeprazole toxicity (11, 12).

One study reported that when using omeprazole as part of the treatment to eradicate *H. pylori*, success was achieved in all patients who had little or no CYP2C19 activity, but in only 29% of patients who had “normal” CYP2C19 activity. Similar results were found in another study that evaluated lansoprazole in the treatment of GERD: the cure rate was 85% for patients with little or no CYP2C19 activity, compared to 16% for patients with normal CYP219 activity (13-15).

The FDA-approved drug label for omeprazole does not comment on dose adjustments based on CYP2C19 status. However, guidelines from KNMP recommend that patients with increased CYP2C19 activity (“ultrarapid metabolizers”) should receive an increased dose of omeprazole for the eradication of *H. pylori*, and that an increased dose should be considered for other indications (Table 1).
The long-term use of PPIs has been associated with several adverse effects. Daily treatment with any PPI for longer than three years may lead to malabsorption of vitamin B12, caused by hypochlorhydria. Because prolonged hypochlorhydria also increases the risk of Clostridium difficile infection, and may increase the risk for osteoporosis-related fractures, the FDA recommends that patients should use the lowest dose and shortest duration of PPI therapy appropriate to the condition being treated (1).

**Gene: CYP2C19**

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, benzodiazepines, and some of the PPIs, including omeprazole.

*CYP2C19* is highly polymorphic, as 35 variant star (*) alleles are currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2c19.htm). The *CYP2C19* wild-type allele is associated with normal enzyme activity and the “extensive metabolizer” phenotype (16).

The most common loss-of-function variant is *CYP2C19*2, which contains a c.681G>A variant in exon 5 that results in an aberrant splice site. This leads to the production of a truncated and non-functioning protein. The *CYP2C19*2 allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (17). “Intermediate metabolizers” carry one copy of an allele that encodes reduced or absent function (e.g. *1/*2), whereas “poor metabolizers” are homozygous or compound heterozygous for two loss-of-function alleles (e.g., *2/*2, *2/*3).

Another commonly tested loss-of-function variant is *CYP2C19*3, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3 allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other loss-of-function variants occur in less than 1% of the general population, and include *CYP2C19*4-*8 (17, 18).

In contrast to non-functional alleles, the *CYP2C19*17 allele (c.-806C>T) is associated with increased enzyme activity. Allele frequencies range from 3 to 21% across different populations (19). Individuals who are homozygous for the *17 allele are known as “ultrarapid metabolizers”, and it is this patient group who may benefit from an increased dose of omeprazole. However, not all studies have identified a significant effect of *CYP2C19*17 on the metabolism of PPIs and treatment outcomes (15, 20, 21).
Genetic Testing

Currently, the FDA does not provide recommendations about the use of CYP2C19 genetic testing for omeprazole treatment (1).

Clinical genotyping tests are available for several CYP2C19 alleles, and a list of some test providers is available at the Genetic Testing Registry (GTR) of the National Institutes of Health: http://www.ncbi.nlm.nih.gov/gtr/tests/?term=1557[geneid].

Usually a patient’s result is reported as a diplotype, such as CYP2C19 *1/*1, and may also include an interpretation of the patient’s predicted metabolizer phenotype (ultrarapid, extensive, intermediate, or poor).

Table 1 summarizes common CYP2C19 phenotypes with recommendations developed by the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP).

Therapeutic Recommendations based on Genotype

This section contains excerpted¹ information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2014 Statement from the US Food and Drug Administration (FDA): Asian Population: In pharmacokinetic studies of single 20 mg omeprazole doses, an increase in AUC of approximately four-fold was noted in Asian subjects compared with Caucasians. Dose reduction, particularly where maintenance of healing of erosive esophagitis is indicated, for Asian subjects should be considered.

Please review the complete therapeutic recommendations that are located here: (1)

2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): For individuals who are ultrarapid metabolizers, an increase in the dose of omeprazole by 100–200% is recommended for the eradication of H.pylori, and the physician should be extra alert to an insufficient response. For other conditions, the physician should remain extra alert to an insufficient response, and consider a dose increase by 100–200%.

There are no therapeutic (dose) recommendations for individuals who are either poor or intermediate metabolizers.

Please review the complete therapeutic recommendations that are located here: (3).

¹ The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.
Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence Coding</th>
<th>Protein</th>
<th>dbSNP reference identifier for allele location</th>
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<tr>
<td>CYP2C19*2</td>
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<td>NM_000769.1:c.681G&gt;A</td>
<td>NP_000760.1:p.Pro227=</td>
<td>rs4244285</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>636G&gt;A Trp212Ter</td>
<td>NM_000769.1:c.636G&gt;A</td>
<td>NP_000760.1:p.Trp212Ter</td>
<td>rs4986893</td>
</tr>
<tr>
<td>CYP2C19*17</td>
<td>-806C&gt;T</td>
<td>NM_000769.2:c.-806C&gt;T</td>
<td>Not applicable—variant occurs in a non-coding region</td>
<td>rs12248560</td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

Acknowledgments

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Version History

To view an earlier version of this summary (update: 18 March 2013), please click [here](http://www.nlm.nih.gov/dailymed/lookup.cfm?setid=a1b077e6-b070-43f2-a98e-380cc635419d).

References


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype

Carisoprodol Therapy and CYP2C19 Genotype

Clopidogrel Therapy and CYP2C19 Genotype

Diazepam Therapy and CYP2C19 Genotype

Esomeprazole Therapy and CYP2C19 Genotype

Imipramine Therapy and CYP2D6 and CYP2C19 Genotype

Prasugrel Therapy and CYP Genotype

Related Summaries by Drug Class

Esomeprazole Therapy and CYP2C19 Genotype

Tests in GTR by Condition

Omeprazole response

Tests in GTR by Gene

CYP2C19 gene
Pertuzumab Therapy and \textit{ERBB2} (\textit{HER2}) Genotype

Laura Dean, MD

Created: September 10, 2015.

\textbf{Introduction}

Pertuzumab is a monoclonal antibody used in the treatment of breast cancer. It targets a receptor in the epidermal growth factor family encoded by the \textit{ERBB2} gene, which is commonly referred to as the \textit{HER2} gene.

The \textit{HER2} gene is overexpressed in 15-20\% of breast cancers and is also overexpressed in some cases of other cancer types (gastric, colon, head and neck). Overall, “\textit{HER2} positive” tumors are associated with a faster rate of growth and a poorer prognosis. The use of pertuzumab in treatment regimens for breast cancer improves outcomes, but adverse effects of therapy include cardiac toxicity.

The FDA-approved drug label for pertuzumab states that pertuzumab should only be used to treat patients with tumors which have either \textit{HER2} protein overexpression or \textit{HER2} gene amplification, as determined by an accurate and validated FDA-approved assay. This is because these are the only patients studied for whom benefit has been shown (1).

A guideline from ASCO/CAP states that oncologists must request \textit{HER2} testing on every primary invasive breast cancer (and on a metastatic site, if stage IV and if specimen available) from a patient with breast cancer to guide decision to pursue \textit{HER2}-targeted therapy. This should be especially considered for a patient who previously tested \textit{HER2} negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of \textit{HER2}-positive or triple-negative disease (2).

\textbf{Drug: Pertuzumab}

Pertuzumab (brand name, Perjeta) is a monoclonal antibody that targets \textit{ERBB2} (a tyrosine kinase receptor, also known as \textit{HER2} or \textit{HER-2/neu}). Pertuzumab is only used to treat specific tumors that overexpress \textit{ERBB2}; these tumors are known as “\textit{HER2}-positive” tumors.

Pertuzumab is used in the treatment of \textit{HER2}-positive metastatic breast cancer to increase the chance of long-term disease-free survival. Pertuzumab is used in combination with trastuzumab (another monoclonal antibody that targets \textit{ERBB2}) and docetaxel (a chemotherapy drug) (1).

\footnote{\textsuperscript{1} NCBI; Email: dean@ncbi.nlm.nih.gov.}
Recently, HER2 targeted therapy has been approved by the FDA for use in the neoadjuvant setting. Neoadjuvant therapy is given before surgical therapy in women with early stage breast cancer. In the neoadjuvant setting, pertuzumab, along with trastuzumab and docetaxel, is used to treat HER2-positive breast cancer, which may be at an early stage, locally advanced, or inflammatory (1, 3, 4).

Before treatment with pertuzumab begins, overexpression of the HER-2 protein or amplification of the HER-2 gene must first be determined. In clinical studies of pertuzumab, patients with breast cancer were required to have evidence of HER-2 overexpression defined as 3+ IHC or FISH amplification ratio of 2 or greater (see Genetic Testing) (1). The FDA recommends that testing be performed using an FDA-approved test, in a laboratory with demonstrated proficiency with the technology being used. This is because the benefits of pertuzumab have only been proven in patients with tumors that overexpress HER2. In addition, although pertuzumab is generally well tolerated, the risks of treatment include infusion reactions, and rarely pulmonary toxicity, and cardiomyopathy that can result in cardiac failure.

Pertuzumab targets the HER2 receptor by binding to a specific region in its extracellular domain. The HER2 receptor is an epidermal growth factor receptor, consisting of an intracellular tyrosine kinase domain, a single transmembrane spanning region, and an extracellular domain, comprised of four subdomains (I – IV). Pertuzumab binds to subdomain II and trastuzumab binds to subdomain IV. This binding limits the receptor’s ability to activate its intrinsic kinase, which in turn, limits the activation of numerous signaling pathways that can promote cell growth.

A number of proposed mechanisms may underlie the anti-tumor effects of pertuzumab and trastuzumab. One such mechanism is that these drugs block the HER3 receptor from binding to HER2. The HER2-HER3 dimerized receptor is thought to be highly active, triggering many signaling cascades in the absence of a “true” ligand (5–8).

Another proposed mechanism is antibody-dependent cellular cytotoxicity (ADCC). Once pertuzumab or trastuzumab have bound to a cancer cell, immune cells (typically activated natural killer cells) bind to the drug and initiate lysis of the cancer cell (9). Trastuzumab may also mediate the enhanced internalization and degradation of the HER2 receptor, inhibit angiogenesis, and inhibit HER2 shedding by preventing the cleavage of HER2 and the subsequent release of its extracellular domain (10, 11).

Unfortunately, breast cancer may start to progress again during HER2 targeted therapy. Possible mechanisms that may facilitate drug resistance and disease progression during treatment include increased signaling from the HER family of receptors, an upregulation of downstream signaling pathways, and an increased level of insulin growth factor -1 receptor (12, 13).

At the time of writing, four drugs have been approved to target HER2 (pertuzumab, trastuzumab, lapatinib, and T-DM1), with more drugs in clinical trials.
**Gene: ERBB2 (HER2)**

The human epidermal growth factor receptor (HER) family consists of four members: the epidermal growth factor receptor (EGFR), HER2, HER3, and HER4 (see Nomenclature). All four members are transmembrane tyrosine kinase receptors, and they regulate a number of important cellular processes, such as cell growth, survival, and differentiation (14).

HER2, along with EGFR, are proto-oncogenes. Proto-oncogenes are a group of genes that, when mutated or expressed at abnormally high levels, can contribute to abnormal cell growth. The mutated version of the proto-oncogene is called an oncogene. Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. All these are important biological processes. However, the increased production of these proteins, caused by oncogenes, can lead to the proliferation of poorly differentiated cancer cells (15).

The official gene symbol for HER2 is ERBB2, which is derived from a viral oncogene with which the receptor shares homology; "v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2." However, clinicians commonly refer to the ERBB2 gene as “HER2” (Human Epidermal growth factor Receptor 2) or “HER2/neu” (neu was the name given to the gene that caused cancer derived from a rodent neuro/glioblastoma). HER2 is an alternate gene symbol for ERBB2 and is more commonly used by the community in clinical care.

One unique feature of ERBB2 compared to the other receptors in the HER family is the absence of a known ligand. It is therefore thought that this receptor may permanently be in an activated state, or it may become activated during heterodimerization with one of the other members of the HER family (11). And, one unique feature of HER3 is that it has very little enzymatic activity compared to the other tyrosine kinase receptors in the HER family. It is therefore thought that an important role of HER3 is to act as a heterodimerization partner for ERBB2 (16, 17).

When a partner such as HER3 binds to ERBB2, the heterodimer undergoes activation, which stimulates the intrinsic tyrosine kinase activity of the receptor. Autophosphorylation of several key residues of the receptor triggers the downstream activation of many commonly used growth factor signaling pathways, such as the PI3K/AKT/mTOR pathway and the RAS/RAF/MEK/ERK pathway (18, 19). Impaired ERBB2 signaling is associated with the development of neurodegenerative diseases, such as multiple sclerosis and Alzheimer disease, whereas excessive ERBB2 signaling is associated with the development of cancers.

ERBB2 is overexpressed in approximately 15-20% of breast tumors, as a result of amplification of the ERBB2 gene, and tumors with increased ERBB2 usually have a higher growth rate and more aggressive clinical behavior (2, 20-22). Although gene amplification is frequently seen in cancer and other degenerative disorders, the underlying basis for amplification remain largely unknown (23). And in the case of ERBB2, although sequence
variants have been identified, it is nearly always the wildtype ERBB2 gene that is 
overexpressed in tumors (24). In about 1% of breast cancers, activating mutations in 
ERBB2 can be identified that are likely to drive tumorigenesis, without ERBB2 
amplification (25).

**Tumor Testing for ERBB2 (HER2)**

There are two main methods used for HER2 testing: testing for overexpression of the 
HER2 protein using immunohistochemistry (IHC), or testing for gene amplification using 
in-situ hybridization (ISH). Each assay type has diagnostic pitfalls that must be avoided, 
and so the pathologist who reviews the histologic findings should determine the optimal 
assay (IHC or ISH) for the determination of HER2 status (2, 22).

In an IHC assay, a slice of tumor tissue is stained, along with a control sample that 
contains high levels of HER2. The tumor sample is then examined by light microscopy to 
assess the intensity of membrane staining—the amount of staining correlates with the 
quantity of HER2 protein and is typically graded from 0 to 3+:

- IHC 0 means no visible staining and is an “HER2 negative” result
- IHC 1+ is also an “HER2 negative” result—there is a staining pattern with weak and 
incomplete staining, or weak and complete staining of very few tumor cells
- IHC 2+ is an “HER2 equivocal result”—there is a staining pattern with moderately 
intense staining, or intense staining of very few tumor cells
- IHC 3+ is an “HER2 positive result”—there is a staining pattern with intense 
membrane staining on more than 10% of tumor cells, indicating a higher than 
normal level of HER2

For an equivocal (IHC 2+) result, either a reflex test must be ordered (same specimen 
using ISH), or a new test must be ordered (using a new specimen, if available, using IHC 
or ISH) to confirm the results.

The ISH assay, or FISH assay (fluorescence in situ hybridization), measures HER2 gene 
amplification by measuring HER2 DNA—the actual number of copies of the HER2 genes 
are counted. Under the microscope, the genes appear as red signals or dots, in a blue-
stained cancer cell nucleus. The result is usually either FISH negative (normal level of 
HER2 gene) or FISH positive (at least twice as much as normal level of HER2 gene), but in 
a small number of cases the FISH result will be equivocal due to a low level of HER2 
amplification. The use of a control helps distinguish between a negative result and a non-
informative result caused by an error. Approximately 25% of patients who have an IHC 2+ 
result will have a FISH positive result (26).

For the complete algorithms for evaluation of HER2 protein expression using IHC or 
ISH, please see the American Society of Clinical Oncology (ASCO) guidelines, located 
here: (27)
Therapeutic Recommendations based on Genotype

This section contains excerpted\footnote{The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.} information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA):

Detection of HER2 protein overexpression is necessary for selection of patients appropriate for pertuzumab therapy because these are the only patients studied and for whom benefit has been shown. Patients with breast cancer were required to have evidence of HER2 overexpression defined as 3+ IHC or FISH amplification ratio ≥ 2.0 in the clinical studies. Only limited data were available for patients whose breast cancer was positive by FISH, but did not demonstrate protein overexpression by IHC.

Assessment of HER2 status should be performed by laboratories using FDA-approved tests with demonstrated proficiency in the specific technology being utilized. Improper assay performance, including use of sub-optimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results.

Please review the complete therapeutic recommendations that are located here: (1).

FDA-approved medical devices for HER2 are listed here.

Excerpted recommendations from the American Society of Clinical Oncology / College of American Pathologists 2013 clinical practice guideline update:

Key Recommendations for Oncologists

- \begin{itemize}
  \item Must request HER2 testing on every primary invasive breast cancer (and on metastatic site, if stage IV and if specimen available) from a patient with breast cancer to guide decision to pursue HER2-targeted therapy. This should be especially considered for a patient who previously tested HER2 negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of HER2-positive or triple-negative disease.
  \item Should recommend HER2-targeted therapy if HER2 test result is positive, if there is no apparent histopathologic discordance with HER2 testing and if clinically appropriate.
  \item Must delay decision to recommend HER2-targeted therapy if initial HER2 test result is equivocal. Reflex testing should be performed on the same specimen using
\end{itemize}
the alternative test if initial HER2 test result is equivocal or on an alternative specimen.

- Must not recommend HER2-targeted therapy if HER2 test result is negative and if there is no apparent histopathologic discordance with HER2 testing.

- Should delay decision to recommend HER2-targeted therapy if HER2 status cannot be confirmed as positive or negative after separate HER2 tests (HER2 test result or results equivocal). The oncologist should confer with the pathologist regarding the need for additional HER2 testing on the same or another tumor specimen.

- If the HER2 test result is ultimately deemed to be equivocal, even after reflex testing with an alternative assay (i.e., if neither test is unequivocally positive), the oncologist may consider HER2-targeted therapy. The oncologist should also consider the feasibility of testing another tumor specimen to attempt to definitely establish the tumor HER2 status and guide therapeutic decisions. A clinical decision to ultimately consider HER2-targeted therapy in such cases should be individualized on the basis of patient status (comorbidities, prognosis, and so on) and patient preferences after discussing available clinical evidence.

Please review the complete therapeutic recommendations, including Key Recommendations for Pathologists that are located here (2).

**Nomenclature**

<table>
<thead>
<tr>
<th>Common gene symbols</th>
<th>Alternative gene symbols</th>
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<tr>
<td><strong>EGFR</strong></td>
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<td><strong>ERBB</strong></td>
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**Acknowledgments**

The author would like to thank Professor Andreas Schneeweiss, Head of Division Gynecologic Oncology, National Center for Tumor Diseases, Heidelberg University Hospital, Germany; and Jo Anne Zujewski, Head of Breast Cancer Therapeutics, Clinical Investigation Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute; for reviewing this summary.
References


24. V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2; ERBB2, in OMIM.


Related Summaries by Gene
Trastuzumab (Herceptin) Therapy and $ERBB2$ ($HER2$) Genotype

Related Summaries by Drug Class
Trastuzumab (Herceptin) Therapy and $ERBB2$ ($HER2$) Genotype
Tests in GTR by Condition

Pertuzumab response

Tests in GTR by Gene

ERBB2 gene
Phenytoin Therapy and HLA-B*15:02 and CYP2C9 Genotypes

Laura Dean, MD
Created: September 22, 2016.

Introduction

Phenytoin is an antiseizure medication used for the prevention of focal seizures and generalized tonic-clonic convulsions (1).

Phenytoin has a narrow therapeutic index—patients that have toxic blood concentrations of phenytoin have increased risks of acute side effects. Dosing can be complex due to pharmacokinetic factors, including patient weight, age, sex, concomitant medications, plasma binding protein status, the presence of uremia or hyperbilirubinemia, and specific pharmacogenetic variants. As such, therapeutic drug monitoring is often used to adjust dose and maintain serum concentrations within the therapeutic range (10–20 μg/mL).

CYP2C9 is one of the main enzymes involved in the metabolism of phenytoin, and variant CYP2C9 alleles are known to influence phenytoin drug levels. Individuals who carry decreased activity CYP2C9 variants may have reduced clearance rates of phenytoin and be at greater risk for dose-related side effects (2).

An individual’s human leukocyte antigen B (HLA-B) genotype is a known risk factor for drug-induced hypersensitivity reactions. HLA-B has an important immunological role in pathogen recognition and response, as well as to non-pathogens such as drugs. Carriers of the variant HLA-B*15:02 allele are at high risk of developing potentially life-threatening phenytoin-induced Stevens-Johnson syndrome (SJS) and the related toxic epidermal necrolysis (TEN).

The HLA-B*15:02 variant is most commonly found among individuals of Southeast Asian descent, where there is a strong association between SJS/TEN and exposure to carbamazepine. Carbamazepine is an antiseizure medication used to treat the same types of seizures as phenytoin, as well as trigeminal neuralgia and bipolar disorder.

The FDA-approved drug label for phenytoin states that consideration should be given to avoiding phenytoin as an alternative for carbamazepine in patients positive for HLA-B*15:02. The label also mentions that variant CYP2C9 alleles may contribute to unusually high levels of phenytoin (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends the use of an antiseizure medication other than carbamazepine, phenytoin (or its prodrug...
fosphenytoin) for any HLA-B*15:02 carrier regardless of CYP2C9 genotype, patient ancestry or age. CPIC also recommends consideration of at least a 25% reduction in the starting maintenance dose for patients who are CYP2C9 intermediate metabolizers and HLA-B*15:02 negative, and at least a 50% reduction for CYP2C9 poor metabolizers and HLA-B*15:02 negative, with subsequent maintenance doses adjusted based on therapeutic drug monitoring and response (Table 1) (2).

Table 1. 2014 Therapeutic recommendations for phenytoin therapy based on HLA-B and CYP2C9 genotypes, adapted from Clinical Pharmacogenetics Implementation Consortium (CPIC)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>HLA-B*15:02 positive</th>
<th>HLA-B*15:02 negative</th>
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<tbody>
<tr>
<td></td>
<td>Implication</td>
<td>Therapeutic recommendation</td>
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<tr>
<td>CYP2C9 normal metabolizer</td>
<td>Increased risk of phenytoin-induced SJS/TEN</td>
<td>If patient is phenytoin naive, do not use phenytoin/fosphenytoin</td>
</tr>
<tr>
<td>CYP2C9 intermediate metabolizer</td>
<td>Increased risk of phenytoin-induced SJS/TEN</td>
<td>If patient is phenytoin naive, do not use phenytoin/fosphenytoin</td>
</tr>
<tr>
<td>CYP2C9 poor metabolizer</td>
<td>Increased risk of phenytoin-induced SJS/TEN</td>
<td>If patient is phenytoin naive, do not use phenytoin/fosphenytoin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SJS/TEN: Stevens–Johnson syndrome/toxic epidermal necrolysis. The strength of the therapeutic recommendations is classified as “strong” for all recommendations, with the exception of the recommendation for CYP2C9 intermediate metabolizers who are HLA-B*15:02 non carriers, which is classified as “moderate.

A If the patient has previously used phenytoin for longer than 3 months without incidence of cutaneous adverse reactions, reinstitute phenytoin with caution. Adjust dose based on CYP2C9 genotype if known.

B Carbamazepine should not be used as an alternative. Alternative medications such as oxcarbazepine, eslicarbazepine acetate, and lamotrigine have some evidence linking SJS/TEN with the HLA-B*15:02 allele, and thus caution should be used in choosing alternatives to phenytoin).

C Recommended maintenance dose based on patient’s clinical characteristics.


Note: The nomenclature used in this table reflects the standardized pharmacogenetic terms proposed by CPIC (3).
### Table 1. continued from previous page.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>HLA-B*15:02 positive</th>
<th>HLA-B*15:02 negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implication</td>
<td>Therapeutic</td>
<td>Implication</td>
</tr>
<tr>
<td></td>
<td>recommendation</td>
<td></td>
</tr>
<tr>
<td>increase probability of</td>
<td></td>
<td>maintenance doses</td>
</tr>
<tr>
<td>toxicities</td>
<td></td>
<td>according to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>therapeutic drug</td>
</tr>
<tr>
<td></td>
<td></td>
<td>monitoring and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>response</td>
</tr>
</tbody>
</table>


The strength of the therapeutic recommendations is classified as “strong” for all recommendations, with the exception of the recommendation for CYP2C9 intermediate metabolizers who are HLA-B*15:02 non carriers, which is classified as “moderate”.

*If the patient has previously used phenytoin for longer than 3 months without incidence of cutaneous adverse reactions, reinitiate phenytoin with caution. Adjust dose based on CYP2C9 genotype if known.

*Carbamazepine should not be used as an alternative. Alternative medications such as oxcarbazepine, eslicarbazepine acetate, and lamotrigine have some evidence linking SJS/TEN with the HLA-B*15:02 allele, and thus caution should be used in choosing alternatives to phenytoin.

*C Recommended maintenance dose based on patient's clinical characteristics.


*Note: The nomenclature used in this table reflects the standardized pharmacogenetic terms proposed by CPIC (3).

### Drug: Phenytoin

Phenytoin is a generic antiseizure drug that is rarely prescribed to newly diagnosed patients due to its propensity for long-term side effects. Nevertheless, it continues to be used by many patients who initiated treatment prior to the availability of newer medications that have fewer side effects and drug-drug interactions. Phenytoin is used for the control of partial seizures and generalized tonic-clonic convulsions. It is also used in the treatment of status epilepticus and may be used to prevent or treat seizures that occur during and following neurosurgery (1).

Phenytoin belongs to the sodium channel blockers class of antiseizure drugs, which are thought to suppress seizure activity by blocking voltage-gated sodium channels that are responsible for the upstroke of action potentials (4, 5). The block by phenytoin and other members of this class of antiseizure drugs occurs in a state-dependent fashion, with preferential binding and block of the inactivated state of the channel. This results in voltage- and frequency-dependent block in which high frequency action potential firing, which occurs during epileptic activity, is preferentially inhibited (1, 6).

The dosing of phenytoin can be complex, as treatment is typically initiated at a low starting dose, which considers patient age, weight, and the presence of concomitant medications that may influence phenytoin metabolism or protein binding. The dose is
then carefully escalated to obtain the desired therapeutic effect. There is a wide variation in how individuals respond to phenytoin (2). Therapeutic drug monitoring is often used to adjust the dose to ensure that plasma levels are within therapeutic range (10–20 μg/dl in adults). Measurement of plasma levels is useful when adding or discontinuing concomitant medications that affect phenytoin levels. Periodic measurement of plasma phenytoin concentrations may also be valuable in pregnancy, because altered phenytoin pharmacokinetics increases the risk of seizures.

Phenytoin use during pregnancy has been associated with an 11% risk in the offspring of the fetal hydantoin syndrome, in which there is dysmorphism, hypoplasia and irregular ossification of the distal phalanges. Facial dysmorphism includes epicanthal folds, hypertelorism, broad flat nasal bridges, an upturned nasal tip, wide prominent lips, and, in addition, distal digital hypoplasia, intrauterine growth retardation, and mental retardation. An additional 30% of the in utero-exposed children express fetal hydantoin effects, in which there is a more limited pattern of dysmorphic characteristics. Some studies have found significant associations between in utero exposure to phenytoin and major congenital abnormalities (mainly, cardiac malformations and cleft palate) whereas others have failed to find such associations (7, 8).

The adverse effects of phenytoin fall into two categories, types A and B. Type A adverse drug reactions account for up to 90% of reactions. They are predictable and can occur in any individual if their drug exposure is high enough. Some of these reactions occur rapidly and are reversible when the dose is reduced. These include acute central nervous system adverse effects such as sedation, nystagmus, and ataxia. Other common side effects occur with long-term exposure and include changes to the physical appearance, such as gingival hyperplasia, coarsening of the facial features, hirsuitism, and acne.

Type B adverse drug reactions include idiosyncratic hypersensitivity reactions. Such reactions can occur at any dose and develop through a mechanism that is unrelated to the mechanism of action of the drug.

A rare but life-threatening hypersensitivity reaction associated with phenytoin treatment is Stevens-Johnson syndrome (SJS) and the related toxic epidermal necrolysis (TEN). Both are severe cutaneous reactions to specific drugs, and are characterized by fever and lesions of the skin and mucous membranes, with a mortality rate of up to 30% (9).

It is difficult to predict in whom a drug-induced hypersensitivity reaction is likely to occur. For phenytoin, however, carriers of a specific HLA variant are known to be susceptible to phenytoin-induced SJS/TEN. HLA testing of patients can identify those at-risk individuals so that an alternative drug can be used.

**HLA gene family**

The human leukocyte antigen (HLA) genes are members of the Major Histocompatibility Complex (MHC) gene family, which includes more than 200 genes. The MHC family has been subdivided into three subgroups based on the structure and function of the encoded
proteins: Class I, Class II, and Class III. The class I region contains the genes encoding the HLA molecules HLA-A, HLA-B, and HLA-C. These molecules are expressed on the surfaces of almost all cells and play an important role in processing and presenting antigens. The class I gene region also contains a variety of other genes, many of which are not known to be involved in immune function.

An important role of HLA class I molecules is to present peptide fragments to immune cells (CD8+ T cells). Most of these peptides originate from the breakdown of normal cellular proteins (“self”). However, if foreign peptide fragments are presented, e.g., from a pathogen, CD8+T cells will recognize the peptides as “non-self” and will be activated to release inflammatory cytokines and launch an immune response to dispose of the pathogen (or foreign body).

Because HLA molecules need to present such a wide variety of “self” and “non-self” peptides, the HLA genes are both numerous and highly polymorphic. More than 1,500 HLA-B alleles have been identified (10). HLA allele nomenclature includes the HLA prefix, followed by the gene, an asterisk and a two digit number that corresponds to antigen specificity, and the assigned allele number (11). For example, the HLA-B*15:02 allele is composed of:

- HLA: the HLA prefix (the HLA region on chromosome 6)
- B: the B gene (a particular HLA gene in this region)
- 15: the allele group (historically determined by serotyping, i.e., a group of alleles that share the same serotype)
- 02: the specific HLA allele (a specific protein sequence; determined by genetic analysis).

Additional digits have recently been added to the nomenclature to discriminate alleles that do not differ in the protein amino acid sequence, but differ in their genetic sequence (i.e., due to synonymous and noncoding genetic variants).

Variation in HLA genes plays an important role in the susceptibility to autoimmune disease and infections and they are also critical in the context of transplant surgery where better outcomes are observed if the donor and recipient are HLA-compatible.

More recently, HLA variants have been associated with susceptibility to Type B adverse drug reactions. For example, HLA-B variants have been associated with severe hypersensitivity reactions to abacavir (used to treat HIV), allopurinol (used to treat gout), and the antiepileptic drugs, carbamazepine and phenytoin.

**Gene: HLA-B*15:02**

Individuals who carry one or two copies of the high risk HLA-B*15:02 allele are known as HLA-B*15:02 positive (Table 2).
Table 2. 2014 Assignment of likely HLA-B phenotype based on genotype (CPIC)

<table>
<thead>
<tr>
<th>Likely phenotype(^a)</th>
<th>Genotype</th>
<th>Examples of diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No copies of high-risk HLA-B*15:02 allele</td>
<td>(\star X/\star X)(^b)</td>
</tr>
<tr>
<td>High-risk HLA-B*15:02 allele not detected (constitutes (\approx) 98.6% of patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Homozygous or heterozygous for high-risk HLA-B*15:02 allele</td>
<td>(15:02/\star X), (15:02/15:02)</td>
</tr>
<tr>
<td>Detection of high-risk HLA-B*15:02 allele (constitutes (\approx) 1.4% of patients)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Global frequencies presented in parentheses. Haplotype frequencies vary among populations; please see (2) for individual population frequencies.

\(^b\) Where \(\star X\) = any genotype other than \(\star 15:02\).


Note: The nomenclature used in this table reflect the standardized pharmacogenetic terms proposed by CPIC (3).

The association between the HLA-B*15:02 allele and SJS/TEN was first reported with the use of carbamazepine in the Han Chinese population. In the initial study, all patients who had carbamazepine-induced SJS/TEN were found to be a carrier of the HLA-B*15:02 allele (44/44, 100%), whereas the allele was much less common among carbamazepine-tolerant patients (3/101, 3%) (12). In subsequent studies, this association was replicated, with a HLA-B*15:02 carrier frequency of 70% among cases of carbamazepine-induced SJS/TEN (13).

The HLA-B*15:02 allele was later associated with phenytoin-induced hypersensitivity reactions, including phenytoin-induced SJS in a Thai population and phenytoin-induced SJS/TEN in Chinese Asians (14, 15).

There are fewer studies on phenytoin-induced hypersensitivity then carbamazepine, and the strength of association between phenytoin and SJS/TEN is weaker than that of carbamazepine and SJS/TEN. However, from the evidence available, the FDA recommends consideration of avoiding phenytoin as an alternative treatment to carbamazepine in individuals who are carriers of HLA-B*15:02 (2).

The prevalence of carbamazepine-induced SJS/TEN is higher in populations where HLA-B*15:02 is more common. Of note, the HLA-B*15:02 allele frequency is highest in Southeast Asia, as populations from Hong Kong, Thailand, Malaysia, Vietnam, and parts of the Philippines have an allele frequency > 15%. It is slightly lower (~ 10-13%) in Taiwan and Singapore, and around 4% in North China. South Asians, including Indians, appear to have a HLA-B*15:02 allele frequency of ~2 to 4%, with higher frequencies in some subpopulations (12-14, 16-27).
The *HLA-B*15:02 allele is rare (< 1%) in East Asia (Japan and Korea) and among individuals who are not of Asian descent. For example, the variant is very rare in Europeans, Hispanics, Africans, African Americans, and Native Americans (13, 18).

**Gene: CYP2C9**

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

The CYP2C9 gene is highly polymorphic, with more than 50 known alleles. Variation in CYP2C9 is thought to contribute to the pharmacogenetic variability in phenytoin metabolism.

CYP2C9*1 is the wild-type allele and is associated with normal enzyme activity (2). Individuals who have two normal-function alleles (e.g., CYP2C9 *1/*1) are classified as “normal metabolizers” (Table 3). For individuals who are CYP2C9 normal metabolizers, the recommended starting maintenance dose of phenytoin does not need to be adjusted based on genotype (2).

**Table 3.** 2014 Assignment of likely CYP2C9 phenotype based on genotype (CPIC)

<table>
<thead>
<tr>
<th>Likely phenotypea</th>
<th>Genotype</th>
<th>Examples of diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal metabolizer (normal activity) ( constitutes ~91% of patients)</td>
<td>An individual carrying two normal-function alleles</td>
<td>*1/*1</td>
</tr>
<tr>
<td>Intermediate metabolizer (heterozygote or intermediate activity) (constitutes ~8% of patients)b</td>
<td>An individual carrying one normal-function allele plus one decreased-function allele</td>
<td>*1/*3, *1/*2</td>
</tr>
<tr>
<td>Poor metabolizer (homozygous variant, low or deficient activity) (constitutes ~1% of patients)</td>
<td>An individual carrying two decreased function alleles</td>
<td>*2/*2, *3/*3, *2/*3</td>
</tr>
</tbody>
</table>

a Global frequencies presented in parentheses. Haplotype frequencies vary among populations; please see (2) for individual population frequencies
b The enzyme activity in this grouping varies widely. Please see (2) for activity ranges.


Note: The nomenclature used in this table reflect the standardized pharmacogenetic terms proposed by CPIC (3).

Two allelic variants associated with reduced enzyme activity are CYP2C9*2 and *3. The *2 allele is more common in Caucasian (10-20%) than Asian (1-3%) or African (0-6%) populations, whereas the *3 allele is less common (<10% in most populations) and is extremely rare in African populations (24, 25, 28-30).
Individuals with one decreased function allele (e.g., CYP2C9*1/*2 and *1/*3) have mild to moderately reduced clearance of phenytoin; these individuals are classified as CYP2C9 intermediate metabolizers. The CPIC recommendations for CYP2C9 intermediate metabolizers include “to consider at least a 25% reduction of the recommended starting maintenance dose” (2).

Individuals with two decreased function alleles (e.g., CYP2C9*2/*2, *3/*3) have reduced clearance of phenytoin and are classified as CYP2C9 poor metabolizers. CPIC recommendations for CYP2C9 poor metabolizers include “to consider at least a 50% reduction of the starting maintenance dose” (2).

In African Americans, the CYP2C9*5, *6, *8 and *11 variants are more common, and these variants are also associated with a decrease in phenytoin metabolism (31).

**Genetic Testing**

The NIH’s Genetic Testing Registry provides examples of the genetic tests that are currently available for the phenytoin drug response, the HLA-B gene, and the CYP2C9 gene.

The genotype results for an HLA allele such as HLA-B*15:02 can either be “positive” or “negative.” There are no intermediate phenotypes because the HLA genes are expressed in a codominant manner.

A positive result indicates the individual is either “heterozygous” or “homozygous” for the variant, depending upon whether they are carrying one or two copies of the *15:02 allele, respectively.

A negative result indicates that the individual does not carry the HLA-B*15:02 allele. However, a negative result does not rule out the possibility of a patient developing phenytoin-induced SJS/TEN. Therefore, clinicians should carefully monitor all patients according to standard practices.

For CYP2C9, the variants that are routinely tested for include CYP2C9*2 and *3. Results are typically reported as a diplotype, such as CYP2C9 *1/*2.

**Therapeutic Recommendations based on Genotype**

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA)

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1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
Regarding HLA-B:

Studies in patients of Chinese ancestry have found a strong association between the risk of developing SJS/TEN and the presence of HLA-B*1502, an inherited allelic variant of the HLA B gene, in patients using carbamazepine. Limited evidence suggests that HLA-B*1502 may be a risk factor for the development of SJS/TEN in patients of Asian ancestry taking other antiepileptic drugs associated with SJS/TEN, including phenytoin. Consideration should be given to avoiding phenytoin as an alternative for carbamazepine in patients positive for HLA-B*1502.

The use of HLA-B*1502 genotyping has important limitations and must never substitute for appropriate clinical vigilance and patient management. The role of other possible factors in the development of, and morbidity from, SJS/TEN, such as antiepileptic drug (AED) dose, compliance, concomitant medications, comorbidities, and the level of dermatologic monitoring have not been studied.

Regarding CYP2C9:

In most patients maintained at a steady dosage, stable phenytoin serum levels are achieved. There may be wide interpatient variability in phenytoin serum levels with equivalent dosages. Patients with unusually low levels may be noncompliant or hypermetabolizers of phenytoin. Unusually high levels result from liver disease, variant CYP2C9 and CYP2C19 alleles, or drug interactions which result in metabolic interference. The patient with large variations in phenytoin plasma levels, despite standard doses, presents a difficult clinical problem. Serum level determinations in such patients may be particularly helpful. As phenytoin is highly protein bound, free phenytoin levels may be altered in patients whose protein binding characteristics differ from normal.

Please review the complete therapeutic recommendations that are located here: (1).

2014 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

Regarding HLA-B: […] Therefore, regardless of the CYP2C9 genotype and the individual’s ancestry or age, if the HLA-B*15:02 test result is positive, the recommendation is to consider using an anticonvulsant other than carbamazepine and phenytoin, unless the benefits of treating the underlying disease clearly outweigh the risks. Some evidence exists linking SJS/TEN with the HLA-B*15:02 allele in association with the use of alternative medications such as oxcarbazepine, eslicarbazepine acetate, and lamotrigine, and thus caution should be used in choosing alternatives to phenytoin.

Regarding CYP2C9: The recommended phenytoin maintenance dose does not need adjustment based on genotype for CYP2C9 extensive ["normal"] metabolizers. Available evidence does not clearly indicate the amount of dose reduction needed to prevent phenytoin-related toxicities in CYP2C9 intermediate and poor metabolizers; thus, our recommendations should be considered conservative estimates, given the variability surrounding phenytoin dosing in an individual. On the basis of the doses reported in the pharmacokinetic and pharmacogenetic studies mentioned above and in Supplementary Table S9 online, at least a 25% reduction of the recommended starting maintenance dose may be considered for CYP2C9 intermediate metabolizers, with subsequent maintenance doses adjusted based on therapeutic drug monitoring and response. For CYP2C9 poor metabolizers, consider at least a 50% reduction of starting maintenance dose, with subsequent maintenance doses adjusted based on therapeutic drug monitoring or response.

Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature of selected HLA-B alleles

<table>
<thead>
<tr>
<th>Allele name</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-B*15:02</td>
<td>rs2844682 and rs3909184</td>
</tr>
</tbody>
</table>

For the MHC region, variations in genes such as HLA-B occur across the whole sequence of the gene, not a single locus. Therefore, the HLA-B*15:02 allele is defined by its sequence rather than single coding or protein variations. If there is strong linkage disequilibrium between one or more SNPs and a specific HLA allele, the presence of these SNPs (tag SNPs) may be used for HLA typing in some populations; however, genotyping tag SNPs should not be considered diagnostic or equivalent to actual HLA testing. For HLA-B*15:02, rs2844682 and rs3909184 are the tag SNPs (32). Guidelines on nomenclature of the HLA system are available from HLA Nomenclature: http://hla.alleles.org/

Nomenclature of selected CYP2C9 alleles

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence Coding</th>
<th>Protein</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*2</td>
<td>430C&gt;T Arg144Cys</td>
<td>NM_000771.3;c.430C&gt;T</td>
<td>NP_000762.2:p.Arg144Cys</td>
<td>rs1799853</td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>1075A&gt;C Ile359Leu</td>
<td>NM_000771.3;c.1075A&gt;C</td>
<td>NP_000762.2:p.Ile359Leu</td>
<td>rs1057910</td>
</tr>
<tr>
<td>CYP2C9*5</td>
<td>1080C&gt;G Asp360Glu</td>
<td>NM_000771.3;c.1080C&gt;G</td>
<td>NP_000762.2:p.Asp360Glu</td>
<td>rs28371686</td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines
Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

Table continues on next page...
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## References


Related Summaries by Gene

Abacavir Therapy and HLA-B*57:01 Genotype

Allopurinol Therapy and HLA-B*58:01 Genotype

Carbamazepine Therapy and HLA Genotypes

Celecoxib Therapy and CYP2C9 Genotype
Prasugrel Therapy and *CYP* Genotype

Warfarin Therapy and the Genotypes *CYP2C9* and *VKORC1*

**Related Summaries by Drug Class**

Carbamazepine Therapy and *HLA* Genotypes

**Tests in GTR by Condition**

Phenytoin response

**Tests in GTR by Gene**

*HLA-B* gene

*CYP2C9* gene
Prasugrel Therapy and CYP Genotype

Laura Dean, MD
Created: April 10, 2017.

Introduction

Prasugrel is a third-generation thienopyridine platelet inhibitor used in the management of patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI). Prasugrel is used to reduce thrombotic cardiovascular events, such as stent thrombosis, myocardial infarction, and stroke in these patients. Prasugrel, along with other antiplatelet agents such as clopidogrel and ticagrelor, inhibits platelet activation by irreversibly binding to the platelet receptor, P2RY12.

Prasugrel is metabolized to its active metabolite primarily by CYP3A5 and CYP2B6, and to a lesser extent by CYP2C9 and CYP2C19. The FDA-approved label for prasugrel states that genetic variations in CYP2B6, CYP2C9, CYP2C19, or CYP3A5 genes do not have a relevant effect on prasugrel pharmacokinetics and the generation of its active metabolite or its inhibition of platelet aggregation in healthy subjects, patients with stable atherosclerosis, or ACS (1).

Another commonly prescribed antiplatelet is the second-generation thienopyridine clopidogrel, which is bioactivated primarily by CYP2C19. Consequently, clopidogrel is less effective among patients with decreased or no function variant alleles in the CYP2C19 gene. In contrast, CYP2C19 variants are not associated with a decrease in effectiveness of prasugrel, which is a more potent antiplatelet agent than clopidogrel, but has a higher risk of bleeding (2-5).

Drug: Prasugrel

Prasugrel is a third-generation thienopyridine antiplatelet agent that binds irreversibly to the P2RY12 receptor and inhibits ADP-mediated platelet activation and aggregation. Other P2RY12 receptor blockers include clopidogrel and ticagrelor.

As an antiplatelet agent, prasugrel inhibits the formation of blood clots in the coronary, peripheral, and cerebrovascular arteries among patients with acute coronary syndrome (ACS).

ACS reflects a decreased blood flow in the coronary arteries, and includes unstable angina, which occurs suddenly, often at rest or with minimal exertion. Unstable angina may be new in onset or it may occur with less exertion than previously. Another form of ACS is a myocardial infarction (MI), which may be classified as “STEMI” or “NSTEMI”

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
based on EKG findings. EKG findings that include ST-segment elevation is termed “ST segment elevation MI” (STEMI). If no ST segment elevation is present but myocardial biomarkers such as troponin I or T are increased, the term “non-ST segment elevation MI” (NSTEMI) is applied.

Patients with ACS are usually treated with a P2Y12 receptor blocker and aspirin (called dual antiplatelet therapy, DAPT) to reduce the risk of developing a coronary artery thrombus. Platelet adhesion and aggregation are early stages in the formation of a thrombus, which may occlude the coronary artery. Patients who undergo PCI are at risk of stent occlusion via this mechanism.

A large trial, TRITON-TIMI 38, compared prasugrel with clopidogrel in 13,608 patients with ACS who were undergoing PCI. Prasugrel was found to provide more potent platelet inhibition than clopidogrel: after 15 months, the patients treated with prasugrel had a lower incidence of the combined endpoint of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke as compared with patients treated with clopidogrel (9.9% vs. 12.1%) (2, 3). However, prasugrel was associated with a higher risk of bleeding, leading to the FDA warning that prasugrel use is contraindicated in patients with active pathological bleeding, or a history of stroke or transient ischemic attack (TIA) (4, 5).

Prasugrel inhibits ADP-induced platelet aggregation by selectively binding to the platelet purinergic receptor, P2RY12. Because prasugrel is a pro-drug, it requires conversion into an active metabolite before it can act as an antiplatelet agent. Prasugrel is rapidly metabolized to thiolactone, which is then converted to an active metabolite by CYP3A5 and CYP2B6, and to a lesser extent by CYP2C9 and CYP2C19.

The active prasugrel metabolite (R-138727) contains a reactive thiol group, which forms a disulfide bridge with a free cysteine residue on the P2RY12 receptor. Once irreversibly bound to prasugrel, the receptor is unable to bind ADP, and platelet activation via this pathway is prevented for the rest of the platelet’s lifespan of about 10 days (6).

Despite the general efficacy of clopidogrel as an antiplatelet agent, interindividual variability in metabolite levels, platelet inhibition, and clinical response has been reported. It has been estimated that between 16–50% of patients treated with clopidogrel have high on-treatment platelet reactivity (HTPR), indicating that despite clopidogrel treatment, a portion of P2RY12 receptors are not blocked (7). This is due, in part, to genetic variants in the CYP2C19 gene, which encodes the principal hepatic enzyme involved in converting clopidogrel to its active metabolite. Patients that carry no function CYP2C19 alleles (e.g., CYP2C19*2) have reduced plasma active clopidogrel metabolites and an increased risk for HTPR.

In contrast, there is no relevant effect of genetic variation in CYP3A5, CYP2B6, CYP2C9, or CYP2C19 on the prasugrel pharmacokinetics and generation of active metabolites, or its inhibition of platelet aggregation (8-12). Therefore, although both clopidogrel and prasugrel form active metabolites with similar potency, prasugrel is a more potent
antiplatelet agent than clopidogrel due to the more efficient formation of the active metabolite from the prodrug (13).

Although prasugrel is more effective than standard-dose clopidogrel, DAPT with clopidogrel and aspirin remains the standard of care at some institutions for some patients with ACS undergoing PCI (14). This is mainly because clopidogrel has a lower bleeding risk and is less expensive (15). However, the availability of CYP2C19 genetic testing can facilitate personalized antiplatelet therapy, as individuals with impaired CYP2C19 activity could be identified and offered an alternative antiplatelet agent, such as prasugrel (16-19). Recent studies have found that CYP2C19-genotype guided antiplatelet therapy results in a higher likelihood of achieving a therapeutic level of on-treatment platelet reactivity (20-22), which may also be cost effective among ACS patients undergoing PCI (23).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are highly polymorphic and can result in no, decreased, normal, or increased enzyme activity.

CYP2C19, CYP2C9, CYP3A5, and CYP2B6 are involved in the metabolism of prasugrel, but genetic variations in these genes do not appear to influence the pharmokinetics of prasugrel. In contrast, genetic variation in the CYP2C19 gene may lead to decreased effectiveness of the related drug, clopidogrel. To read more about CYP variants and the clopidogrel drug response, please see “Clopidogrel Therapy and CYP2C19 Genotype”.

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the genes CYP2C19, CYP2C9, CYP3A5, and CYP2B6. Given that the formation of the active metabolite of prasugrel is not known to be affected by CYP variants, genetic testing prior to the use of prasugrel is not currently recommended.

For clopidogrel, its effectiveness is dependant on its activation to an active metabolite, principally by CYP2C19. Therefore, the FDA states that tests that identify a patient’s CYP2C19 genotype can be used as an aid to determining therapeutic strategy.

Therapeutic Recommendations based on Genotype

This section contains excerpted1 information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA): In healthy subjects, patients with stable atherosclerosis, and patients with ACS receiving prasugrel, there was
no relevant effect of genetic variation in CYP2B6, CYP2C9, CYP2C19, or CYP3A5 on the pharmacokinetics of prasugrel's active metabolite or its inhibition of platelet aggregation.

[...]

In TRITON-TIMI 38, prasugrel reduced ischemic events (mainly nonfatal MIs) and increased bleeding events relative to clopidogrel. The findings are consistent with the intended greater inhibition of platelet aggregation by prasugrel at the doses used in the study. There is, however, an alternative explanation: both prasugrel and clopidogrel are pro-drugs that must be metabolized to their active moieties. Whereas the pharmacokinetics of prasugrel's active metabolite are not known to be affected by genetic variations in CYP2B6, CYP2C9, CYP2C19, or CYP3A5, the pharmacokinetics of clopidogrel's active metabolite are affected by CYP2C19 genotype, and approximately 30% of Caucasians are reduced-metabolizers. Moreover, certain proton pump inhibitors, widely used in the ACS patient population and used in TRITON-TIMI 38, inhibit CYP2C19, thereby decreasing formation of clopidogrel's active metabolite. Thus, reduced-metabolizer status and use of proton pump inhibitors may diminish clopidogrel's activity in a fraction of the population, and may have contributed to prasugrel's greater treatment effect and greater bleeding rate in TRITON-TIMI 38. The extent to which these factors were operational, however, is unknown.

Acknowledgments

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References


\(^1\) The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Carisoprodol Therapy and CYP2C19 Genotype
Celecoxib Therapy and CYP2C9 Genotype
Clopidogrel Therapy and CYP2C19 Genotype
Diazepam Therapy and CYP2C19 Genotype
Esomeprazole Therapy and CYP2C19 Genotype
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Omeprazole Therapy and CYP2C19 Genotype
Phenytoin Therapy and HLA-B*15:02 and CYP2C9 Genotypes
Warfarin Therapy and the Genotypes CYP2C9 and VKORC1
Related Summaries by Drug Class
Clopidogrel Therapy and CYP2C19 Genotype

Tests in GTR by Condition
Prasugrel response

Tests in GTR by Gene
CYP2C19 gene
CYP2C9 gene
CYP3A5 gene
CYP3A5 gene
CYP2B6 gene
Propafenone Therapy and CYP2D6 Genotype
Laura Dean, MD
Created: April 4, 2017.

Introduction
Propafenone is an antiarrhythmic medication. It is used to prevent the reoccurrence of atrial fibrillation in patients with episodic atrial fibrillation who do not have underlying structural heart disease (propafenone may provoke proarrhythmic events in patients with structural heart disease).

Propafenone belongs to class IC of antiarrhythmic agents and acts on cardiac sodium channels to inhibit action potentials. In general, because of the lack of evidence that antiarrhythmic agents improve survival, they should only be used to treat arrhythmias that are thought to be life-threatening.

Propafenone is metabolized by CYP2D6, CYP3A4, and CYP1A2 enzymes. Approximately 6% of Caucasians in the US lack CYP2D6 activity, and are known as “CYP2D6 poor metabolizers” (Table 1) (1). Standard doses of propafenone will lead to higher plasma drug concentrations in poor metabolizers, compared to normal metabolizers. In addition, drugs that inhibit CYP2D6, CYP3A4, and CYP1A2 may also increase propafenone levels, which may lead to cardiac arrhythmia episodes.

The FDA-approved drug label for propafenone states that the recommended dosing regimen of propafenone is the same for all patients (CYP2D6 poor metabolizers and normal metabolizers). However, the label also cautions that the simultaneous use of propafenone with both a CYP2D6 inhibitor (or in patients with CYP2D6 deficiency) and a CYP3A4 inhibitor should be avoided, because of the increased risk of causing arrhythmias and other adverse events (1).

A guideline from The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Pharmacists Association (KNMP) provides dosing recommendations for propafenone, based on CYP2D6 genotype. For CYP2D6 poor metabolizers, the guideline recommends reducing the initial dose of propafenone by 70%, ECG monitoring, and monitoring plasma concentrations. For intermediate and ultrarapid metabolizers, the guideline states there is insufficient data to allow for a calculation of dose adjustment. Therefore, it is recommended to adjust the dose in response to plasma concentration and to monitor with ECG, or select an alternative drug (e.g., sotalol, disopyramide, quinidine, amiodarone) (2, 3) (Table 2).

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
**Drug class: Antiarrhythmics**

Antiarrhythmic agents suppress abnormal heart rhythms (cardiac arrhythmias), which can originate from the atria (e.g., atrial fibrillation, atrial flutter) or the ventricles (e.g., ventricular tachycardia, ventricular fibrillation).

There are five main classes of antiarrhythmic agents, based on their primary site of action:

- **Class I**: block sodium (Na+) channels e.g., quinidine (class IA), lidocaine (class IB), propafenone (class IC)
- **Class II**: block beta adrenoreceptors e.g., carvedilol, metoprolol, propranolol
- **Class III**: block potassium (K+) channels e.g., amiodarone, sotalol
- **Class IV**: block calcium (Ca2+) channels e.g., verapamil, diltiazem
- **Class V**: work by other or unknown mechanisms e.g., adenosine, digoxin

**Drug: Propafenone**

Propafenone is an antiarrhythmic used to prevent the recurrence of atrial fibrillation in patients who have episodic atrial fibrillation and no underlying structural heart disease. Propafenone is also used in the management of paroxysmal supraventricular tachycardia and atrial flutter (1).

Because there are no well-controlled studies in pregnant women, the FDA-approved drug label states that propafenone should only be used during pregnancy if the benefit justifies the potential risk to the fetus. The label also states that the safety and effectiveness of propafenone in pediatric patients have not been established.

Atrial fibrillation is the most common type of harmful cardiac arrhythmias. It is more common in men than women, and the risk of developing atrial fibrillation increases with age. Atrial fibrillation may be paroxysmal (intermittent), persistent (persists for at least 7 days), long-standing (more than 12 months), or permanent.

The symptoms of atrial fibrillation range from no symptoms, to feeling dizzy, short of breath, and experiencing palpitations. The pulse feels irregular, and an ECG will show an absence of P waves and an irregular QRS complex. Atrial fibrillation can lead to reduced cardiac output, increase the risk of thrombosis and stroke, and affected patients may be at an increased risk for mortality (4). Management typically includes antithrombotic therapy and rhythm control.

Propafenone is a class IC antiarrhythmic agent. All class I agents have a "membrane stabilizing effect"—by reducing the fast influx of sodium ions into the cardiac muscle cells, they inhibit the propagation of action potentials. Propafenone also has some Class II activity—it can act as a beta blocker. Side effects of this action include bradycardia and bronchospasm (5, 6).

The class IC agents encainide and flecainide have been associated with increasing the risk of cardiac arrest or death, compared to placebo. Consequently all class IC agents,
including propafenone, are considered to have a significant risk of provoking proarrhythmic events in patients with structural heart disease. Therefore, propafenone should not be used in patients with underlying structural heart disease. Its use is contraindicated in a number of conditions, including heart failure, conduction disorders, bradycardia, and recent myocardial infarction (within the last 3 months) (1, 7-9).

Propafenone is metabolized into two active metabolites: 5-hydroxypropafenone, which is formed by CYP2D6, and norpropafenone, which is formed by both CYP3A4 and CYP1A2. Multiple studies have found that genetic variants in the CYP2D6 gene influence the plasma drug levels of propafenone (10-13).

In patients who lack CYP2D6 activity, metabolism of propafenone is slower, so the 5-hydroxy metabolite is not formed or is formed at very slow rates. In these patients, high doses of propafenone (850mg daily) lead to plasma concentrations of propafenone that are about twice those of patients who have normal CYP2D6 activity. At lower initial doses, the difference between propafenone and 5-hydroxy metabolite concentrations is even greater (1, 14).

However, the FDA recommends that the dosing regimen of propafenone should be the same for all patients, regardless of their CYP2D6 activity levels. This is because even at high doses, the effects of high propafenone levels are mitigated by the lack of the active 5-hydroxy metabolite in the slow metabolizers, and also because steady-state conditions are achieved after 4 to 5 days of titrating the dose in all patients. But the FDA also recommends that because of the large variation in plasma drug levels between individuals, the dose of propafenone should be individually titrated on the basis of response and tolerance, with close attention paid to clinical and ECG evidence of toxicity (1).

The FDA-approved drug label for propafenone cautions against the simultaneous use of propafenone with both a CYP2D6 inhibitor and a CYP3A4 inhibitor. This is because the combination of CYP3A4 inhibition and either CYP2D6 inhibition or deficiency may increase propafenone exposure, which may trigger new cardiac arrhythmias and exaggerate beta adrenoreceptor blockage (1).

**The Cytochrome P450 Superfamily**

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

**Gene: CYP2D6**

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described (15). CYP2D6*1 is the reference (or wild-type) allele encoding enzyme with normal activity. The CYP2D6*2, *33, and *35 alleles are also considered to confer normal activity (Table 1).
Table 1. Activity status of selected CYP2D6 alleles

<table>
<thead>
<tr>
<th>Allele type</th>
<th>CYP2D6 Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal function</td>
<td>*1, *2, *33, *35</td>
</tr>
</tbody>
</table>

For a detailed list of CYP2D6 alleles, please see (15).

Individuals who have more than two normal function copies of the CYP2D6 (CYP2D6*xN) gene are “ultrarapid metabolizers,” whereas individuals who carry two normal or one normal and one decreased function allele are classified as “normal metabolizers.”

Individuals with one normal and one no function allele or two decreased function alleles are categorized as “normal metabolizers” by recent nomenclature guidelines (16), but have also been categorized as “intermediate metabolizers” in the literature. Subjects with one decreased and one no function allele are predicted to be intermediate metabolizers and those with two no function alleles are classified as poor metabolizers.

The most common no function alleles include CYP2D6*3, *4, *5, and *6 (17-20), and the most common decreased function alleles include CYP2D6*9, *10, *17, *29 and *41 (5, 6, 18, 20, 21) (Table 1).

There are large inter-ethnic differences in the frequency of these alleles. For example, CYP2D6*4 is the most common no function allele in Caucasians, but is less abundant in subjects with African ancestry, and is rare in Asians. In contrast, the decreased function allele CYP2D6*10 is the most common allele in Asians, and CYP2D6*17 is almost exclusively found in individuals with African ancestry (22).

Consequently, the phenotype frequencies vary substantially among the major ethnicities and may vary among populations. Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function CYP2D6*4 and *5 alleles (17, 23).

Genetic Testing

The NIH’s Genetic Testing Registry (GTR) lists genetic tests currently available for propafenone response and the CYP2D6 gene.

Results are typically reported as a diplotype, such as CYP2D6 *1/*1 (wild type). A result for copy number, if available, is also important when interpreting CYP2D6 results (19).

Therapeutic Recommendations based on Genotype

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.
Propafenone Therapy and CYP2D6 Genotype

2016 Statement from the US Food and Drug Administration (FDA): Propafenone is metabolized by CYP2D6, CYP3A4, and CYP1A2 isoenzymes. Approximately 6% of Caucasians in the US population are naturally deficient in CYP2D6 activity and other demographic groups are deficient to a somewhat lesser extent. Drugs that inhibit these CYP pathways (such as desipramine, paroxetine, ritonavir, sertraline for CYP2D6; ketoconazole, erythromycin, saquinavir, and grapefruit juice for CYP3A4; and amiodarone and tobacco smoke for CYP1A2) can be expected to cause increased plasma levels of propafenone.

Increased exposure to propafenone may lead to cardiac arrhythmias and exaggerated beta-adrenergic blocking activity. Because of its metabolism, the combination of CYP3A4 inhibition and either CYP2D6 deficiency or CYP2D6 inhibition in users of propafenone is potentially hazardous. Therefore, avoid simultaneous use of propafenone with both a CYP2D6 inhibitor and a CYP3A4 inhibitor.

Please review the complete therapeutic recommendations that are located here: (1).

2016 Summary of recommendations from The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Pharmacists Association (KNMP): For CYP2D6 poor metabolizers (PMs), defined as patients carrying two defective alleles, dose reductions are recommended for clomipramine, flecainide, haloperidol, zuclopenthixol (all 50%); doxepin, nortriptyline (both 60%); imipramine, propafenone (both 70%); and metoprolol (75%).

[...].

For CYP2D6 intermediate metabolizers (IMs), defined as patients carrying two decreased-activity alleles or one active/decreased-activity allele and one inactive allele, dose reductions ranging from 20 to 50% are advised for doxepin, amitriptyline, zuclopenthixol, imipramine, nortriptyline, and metoprolol. There were insufficient data to calculate dose adjustments for clomipramine, oxycodone, propafenone, risperidone, and venlafaxine (Table 2).

Please review the complete therapeutic recommendations that are located here: (2, 3).

† The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
Table 2. CYP2D6 phenotypes and the therapeutic recommendations for propafenone therapy, from The Dutch Pharmacogenetics Working Group (2016)

<table>
<thead>
<tr>
<th>CYP2D6 Phenotype</th>
<th>Recommendations for propafenone therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>Insufficient data to allow calculation of dose adjustment. Adjust dose in response to plasma concentration and record ECG or select alternative drug (e.g., sotalol, disopyramide, quinidine, amiodarone).</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>Insufficient data to allow calculation of dose adjustment. Adjust dose in response to plasma concentration and record ECG or select alternative drug (e.g., sotalol, disopyramide, quinidine, amiodarone).</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>Reduce dose by 70%, record ECG, monitor plasma concentration</td>
</tr>
</tbody>
</table>


Nomenclature of selected CYP2D6 alleles

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*4</td>
<td>1846G&gt;A</td>
<td>NM_000106.5.c.506-1G&gt;A</td>
<td>rs3892097</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td>Variant results in a whole gene deletion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>1707 del T Trp152Gly CYP2D6T</td>
<td>NM_000106.5.c.454delT</td>
<td>rs5030655</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>100C&gt;T (Pro34Ser)</td>
<td>NM_000106.5.c.100C&gt;T</td>
<td>rs1065852</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>1023C&gt;T[1] (Thr107Ile)</td>
<td>NM_000106.5.c.320C&gt;T</td>
<td>rs28371706</td>
</tr>
<tr>
<td></td>
<td>2850C&gt;T[2] (Cys296Arg)</td>
<td>NM_000106.5.c.886T&gt;C</td>
<td>rs16947</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>2850C&gt;T[2] (Cys296Arg)</td>
<td>NM_000106.5.c.886T&gt;C</td>
<td>rs16947</td>
</tr>
<tr>
<td></td>
<td>2988G&gt;A</td>
<td>NM_000106.5.c.985+39G&gt;A</td>
<td>rs28371725</td>
</tr>
</tbody>
</table>

[1] In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
[2] In the literature, 2850C>T is also referred to as 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)
Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

Acknowledgments

The author would like to thank the following individuals for reviewing this summary: JT Callaghan, M.D., Ph.D., Associate Dean of Veterans Affairs Research, Associate Professor of Medicine, and Pharmacology and Toxicology, Department of Veterans Affairs, and Indiana University School of Medicine; Ben Kong, PharmD, BCPS, Clinical Pharmacist, Oregon Health & Science University, Oregon; Gouri Mukerjee, Scientific Officer at Geneyouin Inc., Toronto, Canada; Mohamed Nagy, Clinical Pharmacist, Head of the Personalised Medication Management Unit, Department of Pharmaceutical Services, Children’s Cancer Hospital, Egypt; Mandy van Rhenen, Secretary of the Dutch Pharmacogenetics Working Group (DPWG), Centre for Information on Medicines, Royal Dutch Pharmacists Association (KNMP); and DeeAnn Visk, PhD, a medical writer, editor, and member of the Clinical Pharmacogenetics Implementation Consortium (CPIC).

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15. The Human Cytochrome P450 (CYP) Allele Nomenclature Database [Internet]. CYP2D6 allele nomenclature [Cited Dember 14, December 2015]. Available from: http://www.cypalleles.ki.se/cyp2d6.htm


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Aripiprazole Therapy and CYP2D6 Genotype
Atomoxetine Therapy and CYP2D6 Genotype
Clozapine Therapy and CYP2D6, CYP1A2, and CYP3A4 Genotypes
Codeine Therapy and CYP2D6 Genotype
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Metoprolol Therapy and CYP2D6 Genotype
Risperidone Therapy and CYP2D6 Genotype
Tamoxifen Therapy and CYP2D6 Genotype
Thioridazine Therapy and CYP2D6 Genotypes
Tramadol Therapy and CYP2D6 Genotype
Venlafaxine Therapy and CYP2D6 Genotype

Tests in GTR by Condition

Propafenone response

Tests in GTR by Gene

CYP2D6 gene
Risperidone Therapy and CYP2D6 Genotype

Laura Dean, MD

Created: April 10, 2017.

Introduction

Risperidone is the most commonly prescribed antipsychotic medication in the US. It is an atypical (second generation) antipsychotic used in the treatment of schizophrenia, bipolar disorder, severe dementia, and irritability associated with autism.

Risperidone is metabolized to the active metabolite 9-hydroxyrisperidone by the enzyme CYP2D6 and to a lesser extent by CYP3A4. Individuals who carry two inactive copies of the CYP2D6 gene are termed “poor metabolizers” and may have a decreased capacity to metabolize risperidone. These individuals may be at a higher risk of adverse effects because of increased exposure to plasma risperidone, compared to normal metabolizers, who carry two active copies of CYP2D6. Individuals who are CYP2D6 ultrarapid metabolizers (who carry more than two functional copies of CYP2D6) may have a decreased response to therapy, resulting from lower steady-state risperidone concentrations.

The FDA-approved drug label states that analysis of clinical studies involving a modest number of poor metabolizers (n=70) does not suggest that poor and extensive (normal) metabolizers have different rates of adverse effects (1). In addition, the Dutch Pharmacogenetics Working Group (DPWG) recently changed its dosing recommendations to “no action is needed” for CYP2D6 poor metabolizers taking risperidone (2).

Drug: Risperidone

Risperidone is an atypical antipsychotic primarily used in the treatment of schizophrenia and manic or mixed episodes in bipolar disorder. Risperidone may also be used as part of the management of aggression and/or psychosis in severe dementia and irritability associated with autistic disorder in children and adolescents (1).

The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine. Known as “first-generation” or “typical” antipsychotics, these drugs are used to treat psychosis (regardless of the underlying cause), chronic psychotic disorders (e.g., schizophrenia), and other psychiatric conditions.

All antipsychotics, with the exception of aripiprazole, are dopamine receptor antagonists. Blockade of the D2 dopamine receptor in the brain’s limbic system is thought to improve...
the “positive” symptoms of schizophrenia, such as delusions and hallucinations, which are signs of psychosis.

However, typical antipsychotics also block dopamine receptors in the nigrostriatal pathway. This can cause movement disorders known as extrapyramidal side effects. These disorders include akathisia (motor restlessness), dystonia (abnormal muscle tone), and tardive dyskinesia (involuntary and repetitive movements).

Newer antipsychotics, known as “second generation” or “atypical” antipsychotics, have a lower risk of extrapyramidal side effects. Risperidone is an atypical antipsychotic. The most common side effects of risperidone therapy are sedation and dry mouth, but the rates of both appear to be low, at around 5% (3). Other atypical antipsychotics approved by the FDA include aripiprazole, asenapine, brexpiprazole, cariprazine, clozapine, lurasidone, olanzapine, quetiapine, and ziprasidone.

Atypical antipsychotics, such as risperidone, are thought to transiently occupy D2 receptors and then rapidly dissociate, to allow for normal dopamine neurotransmission (4). Because risperidone has high affinity for the D2 receptor but binds it “loosely”, it does not block dopamine receptors in the nigrostriatal pathway and extrapyramidal side effects are less likely (5).

Risperidone also blocks serotonin receptors, alpha 1 adrenergic receptors, and, to a lesser extent, histamine H1 and alpha 2 adrenergic receptors.

The main route of risperidone metabolism is in the liver by the enzyme CYP2D6. The major active metabolite, 9-hydroxyrisperidone, contributes to the pharmacological effects of this drug (5). While risperidone and 9-hydroxyrisperidone are often regarded as equipotent, they display different affinities towards the two target receptors (D2 and 5HT2A), where risperidone appears to be approximately 2-fold more potent than 9-hydroxyrisperidone. There is also a difference in brain distribution; risperidone is distributed more to the CNS (6).

Genetic variations in the CYP2D6 gene may contribute to an increased risk of adverse events associated with risperidone therapy (7). Individuals who are “CYP2D6 poor metabolizers” carry two no function copies of the CYP2D6 gene. In these individuals, standard doses of risperidone may lead to increased plasma levels of risperidone and decreased levels of 9-hydroxyrisperidone.

However, it is unclear to the extent to which CYP2D6 genotype influences the efficacy and safety of risperidone therapy. One small study of 76 patients with schizophrenia reported that CYP2D6 poor metabolism was associated with greater clinical improvement in the total Positive and Negative Syndrome Scale (PANSS) (8). Other studies have reported a higher rate of adverse reactions and drug discontinuations in CYP2D6 poor metabolizers compared to normal metabolizers (5, 9, 10).

The ratio of risperidone to 9-hydroxyrisperidone, which largely reflects CYP2D6 phenotype, may be a risk factor for different side effects (11). Because prolactin levels
mainly correlate with 9-hydroxyrisperidone levels, CYP2D6 ultrarapid metabolizers may experience different side effects than normal metabolizers (12). In addition, because elderly patients accumulate 9-hydroxyrisperidone due to reduced renal function, older patients who are CYP2D6 poor metabolizers (and others with reduced renal function) are at particular risk of side effects during risperidone treatment (5).

Individuals who are “CYP2D6 ultrarapid metabolizers” may have decreased plasma levels of risperidone, due to increased CYP2D6 activity—these individuals carry more than two functional copies of the CYP2D6 gene. A small study of 85 patients taking long-lasting risperidone showed that the plasma concentrations of risperidone and its active metabolite were subtherapeutic in three individuals who were CYP2D6 ultrarapid metabolizers. The study, however, did not report whether these changes affected the effectiveness or tolerability of the drug in these patients (13).

Overall, it remains unclear whether the accurate determination of an individual’s CYP2D6 genotype, together with therapeutic drug monitoring, has the potential to optimize the response of CYP2D6 poor metabolizers and ultrarapid metabolizers to antipsychotic therapy (9, 14).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing or detoxifying lipids, hormones, toxins, and drugs. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

**Gene: CYP2D6**

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described (15). CYP2D6*1 is the reference (or wild-type) allele encoding an enzyme with normal activity. The CYP2D6*2, *33, and *35 alleles are also considered to confer normal enzyme activity (Table 1).

<table>
<thead>
<tr>
<th>Allele type</th>
<th>CYP2D6 Alleles</th>
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</thead>
<tbody>
<tr>
<td>Normal function</td>
<td>*1, *2, *33, *35</td>
</tr>
</tbody>
</table>

For a detailed list of CYP2D6 alleles, please see (15).

Individuals who have more than two normal function copies of the CYP2D6 gene are classified as “ultrarapid metabolizers,” whereas individuals who carry two normal or one normal and one decreased function allele are classified as “normal metabolizers” (also referred to as “extensive metabolizers”).
Individuals with one normal and one no function allele or two decreased function alleles are also categorized as “normal metabolizers” by recent nomenclature guidelines (16), but have also been categorized as “intermediate metabolizers” elsewhere in the literature. Subjects with one decreased and one no function allele are predicted to be “intermediate metabolizers” and those with two no function alleles are considered to be “poor metabolizers” (Table 2).

### Table 2: 2016 Assignment of CYP2D6 phenotypes by CPIC

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Activity Score</th>
<th>Genotypes</th>
<th>Examples of diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6 ultrarapid metabolizer</td>
<td>Greater than 2.0</td>
<td>An individual carrying duplications of functional alleles</td>
<td>(*1/*1)xN, (*1/*2)xN, (*2/*2)xN&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(approximately 1–20% of patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6 normal metabolizer</td>
<td>1.0 – 2.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>An individual carrying two normal function alleles or two decreased function alleles or one normal and no function allele or one normal function and decreased function allele or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0</td>
<td>*1/*1, *1/*2, *2/*2, *1/*9, *1/*41, *41/*41, *1/*5, *1/*4</td>
</tr>
<tr>
<td>(approximately 72–88% of patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6 intermediate metabolizer</td>
<td>0.5</td>
<td>An individual carrying one decreased function and one no function allele</td>
<td>*4/*41, *5/*9, *4/*10</td>
</tr>
<tr>
<td>(approximately 1–13% of patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6 poor metabolizer</td>
<td>0</td>
<td>An individual carrying two no function alleles</td>
<td>*4/*4, *4/*4xN, *3/*4, *5/*5, *5/*6</td>
</tr>
<tr>
<td>(approximately 1–10% of patients)</td>
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</table>

<sup>a</sup> For population-specific allele and phenotype frequencies, please see (17).

<sup>b</sup> Where xN represents the number of CYP2D6 gene copies (N is 2 or more).

<sup>c</sup> Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.

For more information about activity scores, please see the Genetic Testing section.


The most common no function alleles include CYP2D6*3, *4, *5, and *6 (18–21), and the most common decreased function alleles include CYP2D6*9, *10, *17, *29 and *41 (19, 21–24). There are large inter-ethnic differences in the frequency of these alleles. For
example, CYP2D6*4 is the most common no function allele in Caucasians, but is less abundant in subjects with African ancestry and is rare in Asians. In contrast, the decreased function allele CYP2D6*10 is the most common allele in Asians, and CYP2D6*17 is almost exclusively found in individuals with African ancestry (25).

Consequently, the phenotype frequencies also vary substantially among the major ethnicities and may vary among populations. Approximately 6–10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function CYP2D6*4 and *5 alleles (26, 27).

**Genetic Testing**

The NIH’s Genetic Testing Registry provides examples of the genetic tests that are currently available for risperidone response and for the CYP2D6 gene.

Results are typically reported as a diplotype, such as CYP2D6 *1/*1. A result for copy number, if available, is also important when interpreting CYP2D6 genotyping results (28). However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (29).

If the test results include an interpretation of the patient’s predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1 for each copy of a normal function allele). The phenotype is defined by the sum of the two scores:

- A normal (previously referred to as “extensive”) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2 (17, 30)

**Therapeutic Recommendations based on Genotype**

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**2016 Statement from the US Food and Drug Administration (FDA):** Risperidone is extensively metabolized in the liver. The main metabolic pathway is through hydroxylation of risperidone to 9-hydroxyrisperidone by the enzyme, CYP 2D6. A minor metabolic pathway is through N-dealkylation. The main metabolite, 9-

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1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
hydroxyrisperidone, has similar pharmacological activity as risperidone. Consequently, the clinical effect of the drug results from the combined concentrations of risperidone plus 9-hydroxyrisperidone.

CYP 2D6, also called debrisoquin hydroxylase, is the enzyme responsible for metabolism of many neuroleptics, antidepressants, antiarrhythmics, and other drugs. CYP 2D6 is subject to genetic polymorphism (about 6%–8% of Caucasians, and a very low percentage of Asians, have little or no activity and are "poor metabolizers") and to inhibition by a variety of substrates and some non-substrates, notably quinidine. Extensive\(^2\) CYP 2D6 metabolizers convert risperidone rapidly into 9-hydroxyrisperidone, whereas poor CYP 2D6 metabolizers convert it much more slowly. Although extensive metabolizers have lower risperidone and higher 9-hydroxyrisperidone concentrations than poor metabolizers, the pharmacokinetics of risperidone and 9-hydroxyrisperidone combined, after single and multiple doses, are similar in extensive and poor metabolizers.

Please review the complete therapeutic recommendations that are located here: (1).

2017 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):

**CYP2D6 Poor metabolizers:**

No action is needed for this gene-drug interaction.

The genetic variation can result in both an increase in side effects and a stronger decrease in schizophrenia symptoms. In addition to this, the genetic variation may lead to a decrease in the required maintenance dose. However, as the effect on the dose is smaller than that of the normal biological variation, action is not useful.

**CYP2D6 intermediate metabolizers:**

No action is needed for this gene-drug interaction.

There is little evidence to support an increase in side effects caused by the genetic variation. The genetic variation may lead to a decrease in the required maintenance dose. However, as the effect on the dose is smaller than that of the normal biological variation, action is not useful.

---

CYP2D6 ultrarapid metabolizers:

No action is needed for this gene-drug interaction.

Genetic variation may lead to an increase in the required maintenance dose. However, as the effect is smaller than that of the normal biological variation, action is not useful.

Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature

Nomenclature for selected CYP2D6 alleles

<table>
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<tr>
<th>Common allele name</th>
<th>Alternative names / Major SNP</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
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<tbody>
<tr>
<td>CYP2D6*4</td>
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<td>NM_000106.5:c.506-1G&gt;A</td>
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<tr>
<td>CYP2D6*5</td>
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<td></td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>1707 del T Trp152Gly</td>
<td>NM_000106.5:c.454delT</td>
<td>NP_000097.3:p.Trp152Glyfs rs5030655</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>100C&gt;T Pro34Ser</td>
<td>NM_000106.5:c.100C&gt;T</td>
<td>NP_000097.3:p.Pro34Ser rs1065852</td>
</tr>
<tr>
<td>CYP2D6*17</td>
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<td>NM_000106.5:c.320C&gt;T</td>
<td>NP_000097.3:p.Thr107Ile rs28371706</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NM_000106.5:c.886T&gt;C</td>
<td>NP_000097.3:p.Cys296Arg rs16947</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>2988G&gt;A</td>
<td>NM_000106.5:c.985+39G&gt;A</td>
<td>Not applicable - variant occurs in a non-coding region rs28371725</td>
</tr>
</tbody>
</table>

SNP= Single Nucleotide Polymorphism
*In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

Acknowledgments

The author would like to thank the following individuals for reviewing this summary:

John T. Callaghan, M.D., Ph.D., Associate Dean of Veterans Affairs Research, Associate Professor of Medicine, and Adjunct Associate Professor of Pharmacology and Toxicology,
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8. Almoguera B., Riveiro-Alvarez R., Lopez-Castroman J., Dorado P., et al. CYP2D6 poor metabolizer status might be associated with better response to risperidone


15. The Human Cytochrome P450 (CYP) Allele Nomenclature Database [Internet]. CYP2D6 allele nomenclature [Cited Dember 14, December 2015]. Available from: http://www.cypalleles.ki.se/cyp2d6.htm


Related Summaries by Gene
Amitriptyline Therapy and **CYP2D6 and CYP2C19 Genotype**
Aripiprazole Therapy and **CYP2D6 Genotype**
Atomoxetine Therapy and **CYP2D6 Genotype**
Clozapine Therapy and **CYP2D6, CYP1A2, and CYP3A4 Genotypes**
Codeine Therapy and **CYP2D6 Genotype**
Imipramine Therapy and **CYP2D6 and CYP2C19 Genotype**
Metoprolol Therapy and CYP2D6 Genotype
Propafenone Therapy and CYP2D6 Genotype
Tamoxifen Therapy and CYP2D6 Genotype
Thioridazine Therapy and CYP2D6 Genotypes
Tramadol Therapy and CYP2D6 Genotype
Venlafaxine Therapy and CYP2D6 Genotype

Related Summaries by Drug Class
Aripiprazole Therapy and CYP2D6 Genotype
Clozapine Therapy and CYP2D6, CYP1A2, and CYP3A4 Genotypes

Tests in GTR by Condition
Risperidone response

Tests in GTR by Gene
CYP2D6 gene
Simeprevir Therapy and IFNL3 Genotype

Laura Dean, MD

Created: September 15, 2016.

Introduction

Simeprevir is a hepatitis C virus (HCV) protease inhibitor used in combination with other drugs to treat chronic hepatitis genotype 1 or 4 infection (1).

Previously, the standard care of patients with HCV infection was peginterferon alfa and ribavirin, but ~40-50% of patients with HCV genotype 1 infection had a suboptimal sustained virological response (SVR) (2).

A SVR is defined as undetectable HCV RNA by the end of treatment and at a specific number of weeks after the end of treatment. The addition of simeprevir increased the SVR in patients with HCV genotype 1 infection who were previously untreated. However, there were reports of treatment failure, most commonly in adults, who failed to respond to previous peginterferon and ribavirin treatment (3).

The FDA-approved drug label for simeprevir contains information regarding a genetic variant near the IFNL3 gene (a C to T change; rs12979860), which is a strong predictor of response to peginterferon alfa and ribavirin treatment. The label states that in phase 3 clinical trials, SVR rates were lower in patients with CT and TT genotypes, compared to patients with the CC genotype. However, patients of all IFNL3 genotypes had highest SVR rates when being treated with regimens that included simeprevir.

In addition, the label strongly recommends patients with HCV genotype 1a infection should be screened for the presence of virus with the S3 Q80K polymorphism. If Q80K is detected, the label strongly recommends that alternative therapy be considered (4).

Drug class: HCV Protease Inhibitors

The treatment of hepatitis C virus (HCV) has evolved over the years. Initially, interferon (IFN) was used as monotherapy. This was followed by the addition of the antiviral agent ribavirin (a nucleoside analogue) to peginterferon (PEG-IFN), and more recently, the addition of antiviral protease inhibitors such as simeprevir.

Protease inhibitors are the first direct-acting antivirals to be approved for the treatment of HCV, and simeprevir is the first second-generation agent to become available. Simeprevir has largely replaced the use of the first-generation protease inhibitors, boceprevir and telaprevir, which have less favorable side effect profiles.
Successful treatment of hepatitis C is confirmed when no trace of HCV can be found after treatment has finished. This is referred to as the SVR, which is defined as undetectable HCV RNA by a quantification assay at the end of treatment, and typically 12 (SVR12) or 24 weeks (SVR24) after the end of treatment.

The addition of simeprevir to a PEG-IFN and ribavirin treatment regimen increases the SVR in patients with chronic hepatitis caused by genotype type 1 or 4 hepatitis C virus, and the response to treatment is influenced by the patient’s IFNL3 genotype.

The FDA-approved drug label for simeprevir states that simeprevir should only be used in combination with other antiviral drugs, such as in combination with PEG-IFN and ribavirin; or in combination with sofosbuvir (HCV nucleotide-analogue NS5B polymerase inhibitor) (1). However, because IFN-free regimes are fast becoming the current standard of care for hepatitis C, simeprevir tends to be prescribed with sofosbuvir rather than IFNs.

**Drug: Simeprevir**

Acute infection with HCV is usually asymptomatic, and about 15-45% of people who are infected clear the virus within 6 months of infection without any treatment. The remaining 55-85% of people will develop chronic HCV infection, which may also be asymptomatic for many years. It is thought that over 180 million people are infected with HCV worldwide (5).

The HCV is classified by genotype, based on the RNA viral strands. There are 6 classes of genotype, numbered 1-6, with multiple subtypes e.g., 1a, 1b, 2a, 2b. In the US, approximately 70% of people with HCV infection have genotype 1, with genotype 1a more common than 1b (6). Genotype 1 is the most difficult to treat, as it is less likely than genotypes 2 and 3 to respond to therapy.

Simeprevir has been FDA-approved for use in combination with other drugs, for the treatment of adults with chronic hepatitis C, caused by an infection with genotype 1 or 4 HCV.

During the natural course of HCV infection, patients develop liver fibrosis, which, without treatment, can progress to liver cancer (hepatocellular carcinoma). Approximately 45% of patients with chronic hepatitis C will develop liver cancer within 20 years from the initial infection.

Until recently, the standard of care for hepatitis C infection was based on therapy with peginterferon and ribavirin. Approximately half of the patients cleared the HCV infection, as shown by a SVR, but adverse effects were common and sometimes life-threatening (2). Treatment was expensive and inconvenient, lasting up to 48 weeks.

Protease inhibitors such as simeprevir were specifically developed to improve the effectiveness of peginterferon and ribavirin therapy. Teleprevir was the first drug to be developed, but severe dermatological adverse effects and liver toxicity limited its use.
Simeprevir belongs to the second generation of drugs, and has an improved therapeutic index.

Simeprevir prevents maturation of the HCV by blocking viral protein synthesis. Specifically, simeprevir inhibits the viral protease NS3/4A which is responsible for cleaving and processing the HCV polyprotein precursor (7). Several mutations in this viral NS3/4A protease are associated with a reduced susceptibility to simeprevir. One of the most common and clinically significant mutations is the Q80K polymorphism. The FDA-approved drug label states that patients with HCV genotype 1a infection should be screened for the presence of virus with the Q80K polymorphism. If Q80K is detected, the label strongly recommends that alternative therapy be considered (1).

The combination of protease inhibitors such as simeprevir with peginterferon and ribavirin therapy has led to a much more effective treatment of hepatitis C in patients who were “treatment naïve” (no history of HCV treatment) and among “relapers” (patients who had relapsed after previous HCV therapy). This was evidenced by improvement in the SVR and reduction of treatment from 48 to 24 weeks, without any increase in peginterferon and ribavirin adverse effects (3, 8).

The treatment options for hepatitis C continue to evolve. Currently, IFN-free treatment regimes for hepatitis C are considered to be the standard of care. The IFN-free combination of simeprevir plus sofosbuvir has been found to be a highly effective treatment, with studies reporting high SVR12 rates for the majority of patients with chronic HCV infection (from about 84% to 94%) (9-11).

Genetic variants in the IFNL3 gene have been shown to strongly influence treatment response to PEG interferon-alpha-based regimens (including regimens with simeprevir) in previously untreated patients with HCV genotype 1 infection (4). However, data are currently lacking on how IFNL3 variants influence an individual’s response to simeprevir when used with sofosbuvir in an IFN-free regimen.

**Gene: IFNL3**

The IFNL3 gene, previously known as IL28B, encodes interferon lambda-3 (IFN-λ3) and is involved in the immune response to hepatitis C.

When a person is infected by a virus, their immune response includes the production of interferons. These signaling proteins induce changes in infected and uninfected cells to block viral replication and stop the spread of virus. Interferons are given as part of treatment for HCV to strengthen this innate response.

There are three classes of IFNs: type I (IFN-α/β), type II (IFN-γ) and type III (IFN-λ). The IFNL3 is a type III interferon, and as such, induces a strong antiviral state in responsive cells with a higher risk of viral infection, such as mucosal cells (12).

IFNL3 is only highly expressed in hepatocytes and epithelial cells, in contrast to other similar interferons, such as IFN-α, which are expressed in most cell types. IFNL3 exerts its
actions by interacting with a cytokine receptor complex, which is composed of the IL10RB and IL28RA receptor chains (4).

The first two IFNL3 variants to be commonly tested for are rs12979860 and rs8099917. These variants are in close proximity to each other near the IFNL3 gene, and are in strong linkage disequilibrium. HCV genotype 1 patients with the “favorable” genotypes (CC for rs12979860 and TT for rs8099917) respond better to treatment as they are associated with an approximate 2-fold increase in SVR. However, the exact mechanism how these variants influence treatment outcome is not yet known (4).

In a US cohort of mixed ethnicity, variants in rs12979860 predicted treatment response in HCV genotype 1 infection patients: CC genotype individuals were more likely to spontaneously clear acute HCV infection and TT genotype individuals had the poorest response to treatment. Accordingly, CT genotype individuals had an intermediate response that was between those of the CC and TT genotype patients (4).

The response to HCV treatment varies across different populations, which can be largely explained by differences in allele frequencies. The rs12979860 ‘C’ allele is commonly found in East Asians (allele frequency nearly 0.9), followed by Caucasians (0.63) and Hispanics (0.55), and is the least common among individuals of African origin (0.39) (4).

Among Asians and individuals of European descent, the rs8099917 variant best predicts treatment response (13-15). Moreover, recently a variant in the IFNL4 gene (rs368234815), was found to be superior to rs12979860 in predicting treatment outcome in individuals of African ancestry. Together with another IFNL4 variant (rs117648444), the combination of these two variants was found to have greater treatment response prediction compared to testing for single variants (12).

**Genetic Testing**

Genetic testing for IFNL3 is available, and is used to predict response to peg-IFN and RBV in HCV genotype 1 patients. The results can help clinicians and patients make informed decisions on how to best manage their HCV infection.

The rs12979860 variant is most commonly tested, and the results are typically reported in the following format:

rs12979860 CC, favorable genotype

rs12979860 CT, unfavorable genotype

rs12979860 TT, unfavorable genotype (4).

Before starting a treatment regimen with simeprevir in patients with HCV genotype 1a infection, the FDA strongly recommends screening patients for the presence of virus with the “NS3 Q80K” polymorphism. The FDA states that an alternative therapy to simeprevir should be considered if Q80K is detected (1).
Therapeutic Recommendations based on Genotype

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): A genetic variant near the gene encoding interferon-lambda-3 (IL28B rs12979860, a C [cytosine] to T [thymine] substitution) is a strong predictor of response to Peg-IFN-alfa and RBV (PR). In the Phase 3 trials, IL28B genotype was a stratification factor.

Overall, SVR rates were lower in subjects with the CT and TT genotypes compared to those with the CC genotype. Among both treatment-naïve subjects and those who experienced previous treatment failures, subjects of all IL28B genotypes had the highest SVR rates with simeprevir-containing regimens.

Please review the complete therapeutic recommendations that are located here: (1)

Nomenclature

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<thead>
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<th>Alternative names</th>
<th>HGVS reference sequence</th>
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Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Acknowledgments

The author would like to thank Jitesh Kawedia, Pharmaceutical/Pharmacy Research Specialist at the University of Texas MD Anderson Cancer Center; Stuart Scott, Assistant Professor of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai; and Professor Alex Thompson, Director of the Department of Gastroenterology at St Vincent's Hospital, Melbourne, Senior Research Fellow at the Victorian Infectious Diseases Reference Laboratory, and Adjunct Assistant Professor of the Department of Gastroenterology, Duke University Medical Centre, USA; for reviewing this summary.

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1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
References


**Tests in GTR by Gene**

IFNL3 gene
Sofosbuvir Therapy and IFNL4 Genotype

Laura Dean, MD
Created: January 25, 2017.

Introduction

Sofosbuvir is an antiviral agent used in the treatment of chronic hepatitis C virus (HCV) infection. Sofosbuvir is FDA-approved to treat patients infected with HCV genotypes 1, 2, 3, and 4, as part of a combination antiviral treatment regimen (1). HCV genotype 1 is the most prevalent worldwide and HCV genotype 3 is the next most prevalent (2). Sofosbuvir may also be used as part of the treatment regimen of HCV genotypes 5 or 6 (3).

About 180 million people worldwide are infected with chronic hepatitis C, which is a major cause of chronic liver disease, cirrhosis, and liver cancer. Viral eradication is suboptimal with peginterferon plus ribavirin-based therapy, with only about half of patients with HCV genotype 1 infection achieving a sustained virological response (SVR) after 24 weeks (4). A SVR is defined as undetectable HCV RNA by the end of treatment or at a specific number of weeks after the initiation of treatment, e.g., undetectable HCV RNA at 12 weeks is annotated (SVR12).

Direct-acting antivirals (DAAs), such as sofosbuvir, were developed to improve viral eradication rates. They target HCV-encoded proteins involved in viral replication and infection. Sofosbuvir, the first and thus far only DAA, targets NS5B polymerase, the viral enzyme required for HCV RNA replication.

Sofosbuvir may be used in combination with peginterferon. The genetic variant rs12979860, located in the INFL4 gene, is a strong predictor of response to peginterferon-based therapies. The variant is a C to T change—individuals with the favorable “C/C” genotype have about a 2-fold higher likelihood of achieving SVR compared to individuals with CT or TT genotypes (5). (Note, because the association of rs12979860 with treatment response was reported several years before the discovery of IFNL4, the variant is commonly, but mistakenly, referred to as IL28B, which is the previous name for the IFNL3 gene.)

For specific treatment regimens that include sofosbuvir, although the IFNL4 variant still influences treatment outcomes, the SVR remains relatively high for all IFNL4 genotypes. For example in the NEUTRINO study, which is referred to in the FDA-approved drug label for sofosbuvir, the SVR12 rate was 99% in individuals with baseline C/C alleles and 87% in individuals with baseline non-C/C alleles. The individuals in this study had HCV genotype 1 or 4 infection, and were receiving sofosbuvir plus peginterferon plus ribavirin therapy (1, 6).

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
The drug label for sofosbuvir also discusses viral resistance. In cell culture, the amino acid substitution S282T in the viral NS5B polymerase is associated with reduced susceptibility to sofosbuvir (7). During the ELECTRON trial, this substitution was transiently detected in one individual who relapsed during sofosbuvir monotherapy. However, the clinical significance of such substitutions remains unknown (1).

**Drug Class: Direct Acting Antivirals for HCV**

The treatment of hepatitis C virus (HCV) has evolved over the years. Initially, interferon (IFN) was used as monotherapy. This was followed by the addition of the antiviral agent ribavirin (a nucleoside analogue) to peginterferon. However, only about half of the HCV genotype 1-infected patients cleared their infection, and adverse effects were common and sometimes life-threatening (4). Treatment was also expensive and inconvenient, lasting up to 48 weeks.

Direct-acting antivirals (DDAs) improved the effectiveness of peginterferon and ribavirin therapy. These agents target specific viral proteins required for viral replication and infection.

HCV is a single-stranded RNA virus that encodes structural proteins (to encode the viral capsid and envelope) and non-structural proteins (required for viral replication). The DDAs target several of the non-structural proteins, the viral protease (NS3/NS4A), the viral RNA polymerase (NS5B), and a viral protein thought to regulate replication and viral assembly (NS5A).

Currently, there are four classes of drugs in clinical use or in development, which are classified by their therapeutic target:

- Protease inhibitors e.g., simeprevir, grazoprevir, paritaprevir
- Nucleoside polymerase inhibitors e.g., sofosbuvir
- Non-nucleoside polymerase inhibitors
- NS5A inhibitors e.g., ledipasvir

Successful treatment of hepatitis C is confirmed when no trace of HCV can be found after treatment has finished. This is referred to as the SVR, which is defined as undetectable HCV RNA by a quantification assay at the end of treatment, and typically 12 (SVR12) or 24 weeks (SVR24) after the end of treatment.

**Drug: Sofosbuvir**

Sofosbuvir is a nucleotide analogue used in the treatment of chronic HCV infection as part of a combination antiviral treatment regimen.

The early stages of infection with HCV are usually asymptomatic—about 15-45% of people spontaneously clear the virus within 6 months of infection without any treatment. The remaining 55-85% of people will develop chronic HCV infection, which may also be asymptomatic for many years (8).
However, during the natural course of HCV infection, patients develop liver fibrosis, which, without treatment, can progress to liver cirrhosis and liver cancer (hepatocellular carcinoma). The risk of developing liver cancer for a patient with HCV-related cirrhosis is approximately 2-6% per year (9).

HCV is classified by genotype, based on the nucleotide sequence of the viral RNA. There are six major classes of genotype, numbered 1-6, with multiple subtypes e.g., 1a, 1b, 2a, 2b. In the US, approximately 70% of people with HCV infection have genotype 1, with genotype 1a more common than 1b (8). Genotype 1 was formerly the most difficult to cure with interferon-based therapies, as it was less likely than genotypes 2 and 3 to respond to therapy. With the introduction of DAA-based, interferon-free treatments, this is no longer the case.

Sofosbuvir is indicated for the treatment of genotype 1, 2, 3 or 4 chronic HCV infection and is generally considered to have moderate to high efficacy for all six genotypes (10). For the treatment of genotype 1 or 4 infections, the drug label recommends a combination therapy of sofosbuvir plus peginterferon alfa plus ribavirin. For the treatment of genotype 2 or 3 infections, the combination therapy of sofosbuvir plus ribavirin is recommended (1).

Sofosbuvir is a NS5B nucleotide analogue and a prodrug. Once inside a liver cell, sofosbuvir is activated by phosphorylation to a nucleoside triphosphate that competes with nucleotides during viral replication. Binding of the analogue to the viral NS5B polymerase results in RNA chain termination, thus inhibiting the virus from replicating its genome (11).

The safety and efficacy of sofosbuvir has been established in several clinical trials. The usual dose of sofosbuvir is a 400mg tablet, taken once a day for 12 weeks, in combination with other antiviral agents. Sofosbuvir is generally well tolerated, with no side effects beyond those associated with placebo therapy (10, 12).

Sofosbuvir forms the backbone of several treatment regimens including DAA such as sofosbuvir/velpatasvir and sofosbuvir/velpatasvir/voxilaprevir. The regimen sofosbuvir/ledipasvir has been found to result in high SVR rates in shorter periods of time, but costs may be prohibitive (7).

Genetic variants in the IFNL4 gene have been shown to strongly influence treatment response to peginterferon-based regimens in previously untreated patients with HCV genotype 1 infection (5, 13). Such variants also appear to influence the outcomes of treatment regimens that include sofosbuvir. For example, the rs12979860 genotype predicts the response to 8 weeks of treatment with sofosbuvir/ledipasvir (14).

In addition, several substitutions that occur with the viral NS5B polymerase have been reported. Most notably, a S282T polymorphism has been associated with sofosbuvir resistance (15). In cell cultures, the S282T substitution is associated with a reduced susceptibility to sofosbuvir. However, the clinical significance of such substitutions is not yet known, as they appear to be detrimental to viral fitness. So far, the S282T substitution...
has only been detected in one patient who experienced a relapse while being treated with sofosbuvir monotherapy in a trial, and the substitution was no longer detectable at week 12 post-treatment (1).

**Gene: IFNL4**

The IFNL4 gene encodes interferon lambda-4 (IFN-λ4) and is involved in the immune response to hepatitis C.

When a person is infected by viruses, including HCV, their immune response includes the production of interferons. These signaling proteins induce changes in infected and uninfected cells to block the viral replication cycle and stop the spread of virus. Interferons are given as part of treatment for HCV to strengthen this innate response.

Three classes of IFNs exist: type I (IFN-α/β), type II (IFN-γ), and type III (IFN-λ). The most recent interferon to be discovered, IFNL4, belongs to the type III class. It is located upstream of IFNL3 and is a functional gene in the majority (>95%) of the African population. But in about 50% of the European population and in most of the east Asian population, IFNL4 is a pseudogene, created by a frameshift-causing deletion polymorphism (rs368234815) (16-18).

As a type III interferon, IFNL4, induces an antiviral state in responsive cells with a higher risk of viral infection, such as mucosal cells (17). IFNL4 exerts its actions by interacting with a cytokine receptor complex, which is composed of the IL10RB and IFNLR1 receptor chains (5). Expression of IFNLR1 is largely restricted to cells of epithelial origin, which includes hepatocytes. In contrast, receptors for type I interferons, such as IFN-α, are expressed in most cell types.

The first two variants to be commonly tested for are rs12979860 (located in IFNL4) and rs8099917, which lies proximate to IFNL4. These variants are in close proximity to each other and are in strong linkage disequilibrium (5). Linkage disequilibrium means that the variants are linked to treatment response more than would be expected in the general population.

HCV genotype 1 patients with the “favorable” genotypes (CC for rs12979860 and TT for rs8099917) respond better to interferon-based treatment—favorable genotypes are associated with an approximate 2-fold increase in SVR (5). However, for specific treatment regimens which include sofosbuvir, although an individual’s IFNL4 genotype still influences treatment outcomes, the SVR for non-favorable genotypes remains relatively high (1).

In the NETURINO study, patients with HCV genotype 1 or 4 who had not received previous treatments for HCV infection were treated with a regimen of sofosbuvir plus peginterferon plus ribavirin for 12 weeks. The SVR12 rate was 99% (89/90) in subjects with baseline rs12979860 C/C alleles and 87% (200/230) in subjects with baseline rs12979860 non-C/C alleles (6).
Similarly, in the PHOTON trial, patients with HCV genotype 1 infection and co-infection with HIV were treated with a combination of sofosbuvir and ribavirin. The SVR12 rates were 80% (24/30) in subjects with baseline rs12979860 C/C allele and 75% (62/83) in subjects with baseline rs12979860 non-C/C alleles (1).

The frequency of the rs12979860 ‘C’ allele varies globally across different populations—it is commonly found in East Asians (allele frequency nearly 0.9), followed by Caucasians (0.63) and Hispanics (0.55), and is the least common among individuals of African origin (0.39) (5).

In individuals of African ancestry, the rs368234815 variant is superior to rs12979860, and together with another IFNL4 variant (rs117648444), the combination of testing these two variants gives a greater treatment response prediction compared to testing for single variants (16, 17).

**Genetic Testing**

Genetic testing for IFNL4 is used to predict response to peginterferon and ribavirin in HCV genotype 1 patients. The results can help clinicians and patients make informed decisions on how to manage HCV infection.

The rs12979860 variant is most commonly tested, and the results are typically reported in the following format:

- rs12979860 CC, favorable genotype
- rs12979860 CT, unfavorable genotype
- rs12979860 TT, unfavorable genotype (5).

**Therapeutic Recommendations based on Genotype**

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**Statement from the US Food and Drug Administration (FDA):** NEUTRINO was an open-label, single-arm trial that evaluated 12 weeks of treatment with sofosbuvir in combination with peginterferon alfa 2a and ribavirin in treatment-naïve subjects with genotype 1, 4, 5 or 6 HCV infection compared to pre-specified historical control. [...] SVR12 rates were 99% (89/90) in subjects with genotype 1 or 4 HCV and baseline IL28B C/C allele and 87% (200/230) in subjects with genotype 1 or 4 HCV and baseline IL28B non-C/C alleles

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1. The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
It is estimated that the SVR12 in patients who previously failed pegylated interferon and ribavirin therapy will approximate the observed SVR12 in NEUTRINO subjects with multiple baseline factors traditionally associated with a lower response to interferon-based treatment. The SVR12 rate in the NEUTRINO trial in genotype 1 subjects with IL28B non-C/C alleles, HCV RNA greater than 800,000 IU/mL and Metavir F3/F4 fibrosis was 71% (37/52).

[...]

In a pooled analysis of 982 subjects who received sofosbuvir in Phase 3 trials, 224 subjects had post- baseline NS5B genotypic data from next generation nucleotide sequencing (assay cutoff of 1%).

Treatment-emergent substitutions L159F (n=6) and V321A (n=5) were detected in post-baseline samples from GT3a-infected subjects across the Phase 3 trials. No detectable shift in the phenotypic susceptibility to sofosbuvir of subject isolates with L159F or V321A substitutions was seen. The sofosbuvir-associated resistance substitution S282T was not detected at baseline or in the failure isolates from Phase 3 trials. However, an S282T substitution was detected in one genotype 2b subject who relapsed at Week 4 post-treatment after 12 weeks of sofosbuvir monotherapy in the Phase 2 trial P7977-0523 [ELECTRON]. The isolate from this subject displayed a mean 13.5-fold reduced susceptibility to sofosbuvir. For this subject, the S282T substitution was no longer detectable at Week 12 post-treatment by next generation sequencing with an assay cutoff of 1%.

Please review the complete therapeutic recommendations that are located here: (1).

### Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12979860</td>
<td>/</td>
<td>NM_001276254.2: c.151-152G&gt;A</td>
<td>rs12979860</td>
</tr>
<tr>
<td>rs8099917</td>
<td>/</td>
<td>N/A</td>
<td>rs8099917</td>
</tr>
</tbody>
</table>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

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2 Note: Recent studies report that the rs12979860 variant is in the IFNL4 gene, and not the IFNL3 gene (previously called IL28B). Therefore, a more accurate term for describing an individual's genotype would be “rs12979860 C/C”, instead of “IL28B C/C”. 
Acknowledgments

The author would like to thank Teresa Beam, Ph.D., Chair, Department of Pharmaceutical Sciences, Manchester University, Indiana; David Kisor, B.S., Pharm.D., Professor and Director of Pharmacogenomics Education, Pharmacogenomics Program, Manchester University, Indiana; Martin Lagging, MD, PhD, Professor, Department of Infectious Medicine / Virology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Sweden; and Thomas R. O’Brien, M.D., M.P.H., Senior Investigator, National Cancer Institute, Division of Cancer Epidemiology & Genetics, Infections and Immunoepidemiology Branch; for reviewing this summary.

References


Tests in GTR by Gene

IFNL4 gene
Tamoxifen Therapy and CYP2D6 Genotype

Laura Dean, MD


Introduction

Tamoxifen is a selective estrogen receptor modulator (SERM) which is used in the treatment and prevention of breast cancer (1).

The CYP2D6 enzyme metabolizes a quarter of all prescribed drugs, and is one of the main enzymes responsible for converting tamoxifen into its major active metabolite, endoxifen. Variants in the CYP2D6 allele may lead to reduced (“intermediate metabolizer”) or absent (“poor metabolizer”) enzyme activity. Individuals who carry these variant alleles may have reduced plasma concentrations of endoxifen and benefit less from tamoxifen therapy.

At this time, the FDA-approved drug label for tamoxifen does not discuss genetic testing for CYP2D6. The National Comprehensive Cancer Network (NCCN) does not recommend CYP2D6 testing as a tool to determine the optimal adjuvant endocrine strategy (2), and this recommendation is consistent with the 2010 guidelines from the American Society of Clinical Oncology (ASCO) (2, 3).

In contrast, the Dutch Pharmacogenetics Working Group has made recommendations for tamoxifen therapy based on CYP2D6 genotypes. For both poor and intermediate metabolizers, their recommendation is to consider using aromatase inhibitors for postmenopausal women due to an increased risk for relapse of breast cancer with tamoxifen. They also recommend that intermediate metabolizers avoid the concomitant use of CYP2D6 inhibitors (Table 1) (4).

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Therapeutic recommendation for tamoxifen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>More than two copies of functional alleles</td>
<td>None</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>One active allele and one inactive allele, or two decreased</td>
<td>Increased risk for relapse of breast cancer. Avoid concomitant use of</td>
</tr>
</tbody>
</table>


Table 1. continues on next page...
Table 1. continued from previous page.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Therapeutic recommendation for tamoxifen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>activity alleles, or one decreased activity allele and one inactive allele</td>
<td>CYP2D6 inhibitors. Consider aromatase inhibitor for postmenopausal women</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>Two inactive alleles</td>
<td>Increased risk for relapse of breast cancer. Consider aromatase inhibitor for postmenopausal women</td>
</tr>
</tbody>
</table>


Table 2. Activity status of CYP2D6 alleles

<table>
<thead>
<tr>
<th>Allele type</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>*1, *2, *33, *35</td>
</tr>
</tbody>
</table>

Note: The most clinically significant variants are highlighted in bold.

Drug: Tamoxifen

Tamoxifen is a selective estrogen receptor modulator (SERM) that is used in the treatment and prevention of breast cancer. In both men and women, tamoxifen is used to treat metastatic breast cancer—patients with tumors that are estrogen receptor positive (ER+) are more likely to benefit. In post-menopausal women with breast cancer, tamoxifen is used as an adjuvant treatment following surgery and radiation—patients with four or more positive axillary nodes may benefit the most. And tamoxifen is also used to prevent breast cancer in women who have an increased risk, and to reduce the risk of invasive breast cancer in women with ductal carcinoma in situ (DCIS) (1).

Tamoxifen acts on the estrogen receptor (ER) and has both estrogenic and anti-estrogenic actions, depending on the target tissue. In the breast tissue, it acts as an anti-estrogen (inhibitory effect) and competitively inhibits cancerous ER+ cells from receiving the estrogen they need to grow (5, 6).

In other tissues, such as the endometrium, tamoxifen acts as an estrogen agonist (stimulatory effect) leading to some of the adverse effects associated with tamoxifen therapy. These include endometrial hyperplasia, endometrial polyps, and about a 2.5 times higher risk of developing endometrial cancer. Hot flashes are the most common side effect.

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
associated with tamoxifen use, which affect up to 80% of women, and there is also an increased risk of depression (5, 7).

Tamoxifen increases the risk of thromboembolic events, such as deep vein thrombosis (DVT) and pulmonary embolism. The risk of tamoxifen-associated thromboembolic events (TTE) is further increased when tamoxifen is coadministered with chemotherapy. The drug label for tamoxifen states that the risks and benefits of tamoxifen therapy should be carefully considered in women with a history of thromboembolic events.

Some studies suggest that clinicians should consider screening breast cancer patients before prescribing adjuvant tamoxifen to identify women who are at risk of thrombotic embolic disease because of Factor V Leiden (R506Q in the F5 gene); or have an increased risk of TTE because of a variant in the estrogen receptor gene (ESR1) (8-11). However, a small substudy (N=81) of the NSABP P-1 trial found no benefit in screening women for Factor V Leiden or prothrombin thrombophilia (G20210A in the F2 gene) as a means to identify women who may not be appropriate candidates for tamoxifen therapy due to the propensity of thromboembolic side effects (1, 12).

Tamoxifen is a pro-drug that is metabolized to active metabolites in the liver. The metabolites 4-hydroxytamoxifen and 4-hydroxy-N-desmethyltamoxifen (endoxifen) are thought to be mainly responsible for the clinical effects of tamoxifen. Both of these metabolites have about a 100-fold higher affinity for the ER compared to tamoxifen, but endoxifen is thought to be the major metabolite because plasma levels of endoxifen tend to be several-fold higher than that of 4-hydroxytamoxifen (5, 13). Endoxifen formation mainly occurs via the conversion of the inactive primary metabolite N-desmethyltamoxifen, mediated by CYP2D6.

The mechanism of action of tamoxifen is complex and involves tamoxifen metabolites binding to the ER and inducing a conformational change that blocks or changes the expression of estrogen-dependent genes. It is also likely that tamoxifen interacts with other protein cofactors (both activators and repressors), and binds with different estrogen receptors (ER-alpha or ER-beta), to produce estrogenic and anti-estrogenic effects in different tissues. Certain tamoxifen metabolites such as norendoxifen have also been found to act as aromatase inhibitors in vitro (albeit at high concentrations)—decreasing the amount of estrogen available by inhibiting the conversion of steroids to estradiol (14).

The response to tamoxifen (i.e., clinical efficacy and side effects) varies widely between individuals; this may be partly caused by differences in metabolism because of variations in genes such as CYP2D6 (15).

**Gene: CYP2D6**

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are often polymorphic and can result in reduced, absent, or increased enzyme activity.
CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. CYP2D6 is the main enzyme that catalyzes the rate-limited step in the metabolism of tamoxifen to its potent metabolite, endoxifen. Other CYP enzymes involved in tamoxifen metabolism include CYP2C19, CYP2B6, CYP3A4, and CYP3A5.

CYP2D6 is a particularly complex gene that is difficult to genotype because of the large number of variants (more than 100 alleles have been described (16)), and the presence of gene deletions, duplications, multiplications and pseudogenes. The complexity of genetic variation complicates making a correct determination of CYP2D6 genotype.

There is substantial variation in CYP2D6 genotypes among different populations. CYP2D6*1 is the wild-type allele and is associated with normal enzyme activity and the normal “extensive metabolizer” phenotype. The CYP2D6 alleles *2, *33, and *35 are also considered to have near-normal activity. Other alleles include variants that produce a non-functioning enzyme (e.g., *3, *4, *5, and *6) (17-20) or an enzyme with reduced activity (e.g., *10, *17, and *41) (21-23) (see Table 2). There are large inter-ethnic differences in the frequency of these alleles, with *3, *4, *5, *6, and *41 being more common in the Caucasian population, *17 more common in Africans, and *10 more common in Asians (24).

Individuals who are intermediate or poor metabolizers carry copies of reduced-activity or non-functioning CYP2D6 alleles (Table 1). Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. In these populations, only half of CYPD6 alleles are fully functional, with the reduced function *10 variant being very common (~40%, compared to ~2% in Caucasians) (25). As a result, Asians are more likely to be intermediate metabolizers than Caucasians (24). Similarly, in Africans and African Americans, only half of CYPD6 alleles are functional. However, a wider range of variants account for the remaining alleles (24, 26, 27).

Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to the more prevalent nonfunctional *4 and *5 alleles (24). Notably, less than 40% are homozygous extensive metabolizers (carrying two copies of *1 allele), and more than 50% belong to a mixed group of intermediate metabolizers and heterozygote carriers of one functional allele in combination with either a deficient or non-functional allele (20, 28-30).

Because CYP2D6 is the main enzyme involved in converting tamoxifen into its most potent anti-estrogenic metabolites, endoxifen and 4-hydroxytamoxifen, genetic polymorphisms of CYP2D6 may influence tamoxifen metabolism (31). High plasma levels of endoxifen require the presence of fully functional CYP2D6 alleles, in poor metabolizers, endoxifen levels are decreased (13, 32).

The role of CYP2D6 in tamoxifen response has yet to be fully determined (33). Some studies suggest that genetic polymorphisms of CYP2D6 may be important predictors of the clinical outcomes of tamoxifen treatment for patients with metastatic breast cancer.
(34) and for patients with early breast cancer who receive tamoxifen as an adjuvant treatment following surgery (32, 35-37).

However, the high degree of inter-individual variability of tamoxifen metabolism and treatment outcomes is not fully accounted for by CYP2D6 variation (38). Additional contributors may include genetic variation in other metabolic pathways and the sequestration of lipophilic tamoxifen metabolites into fat tissues (13, 32).

**Genetic Testing**

Genetic testing is available for many (~30) of the variant CYP2D6 alleles. Usually a patient’s result is reported as a diplotype, such as CYP2D6 *1/*1. A result for copy number is also important when interpreting results for this gene.

If the test results include an interpretation of the patient’s predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores, e.g., poor metabolizers have an activity score of 0; intermediate metabolizers have an activity score between 0.5 and 1.5 and metabolizers have an activity score of 2 and up (39).

**Therapeutic Recommendations based on Genotype**

This section contains excerpted1 information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**2013 Statement from the US Food and Drug Administration (FDA):** “Tamoxifen is extensively metabolized after oral administration. N-desmethyl tamoxifen is the major metabolite found in patients’ plasma. The biological activity of N-desmethyl tamoxifen appears to be similar to that of tamoxifen. 4-Hydroxytamoxifen and a side chain primary alcohol derivative of tamoxifen have been identified as minor metabolites in plasma. Tamoxifen is a substrate of cytochrome P-450 3A, 2C9 and 2D6, and an inhibitor of P-glycoprotein.”

Please review the complete therapeutic recommendations that are located here: (1).

**2014 Statement from the National Comprehensive Cancer Network (NCCN):** “The cytochrome P-450 (CYP450) enzyme, CYP2D6, is involved in the conversion of tamoxifen to endoxifen. Over 100 allelic variants of CYP2D6 have been reported in the literature. Individuals with wild-type CYP2D6 alleles are classified as extensive metabolizers of tamoxifen. Those with one or two variant alleles with either reduced or no

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1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
activity are designated as intermediate metabolizers and poor metabolizers, respectively. A large retrospective study of 1325 patients found that time to disease recurrence was significantly shortened in poor metabolizers of tamoxifen. However, the BIG 1-98 trial reported on the outcome based on CYP2D6 genotype in a subset of postmenopausal patients with endocrine-responsive, early invasive breast cancer. The study found no correlation between CYP2D6 allelic status and disease outcome or between CYP2D6 allelic status and tamoxifen-related adverse effects. A genetic analysis of the ATAC trial found no association between CYP2D6 genotype and clinical outcomes. Given the limited and conflicting evidence at this time, the NCCN Breast Cancer Panel does not recommend CYP2D6 testing as a tool to determine the optimal adjuvant endocrine strategy. This recommendation is consistent with the ASCO Guidelines.”

Please review the complete therapeutic recommendations that are located here: (2)

2010 Excerpt from the American Society of Clinical Oncology (ASCO) guideline:\n
“Are There Specific Patient Populations That Derive Differing Degrees of Benefit From an AI Compared With Tamoxifen?

Recommendation: Direct evidence from randomized trials does not identify a specific marker or clinical subset that predicted which adjuvant treatment strategy—tamoxifen, AI monotherapy, or sequential therapy—would maximally improve outcomes for a given patient. Among men with breast cancer, tamoxifen remains the standard adjuvant endocrine treatment. The Update Committee recommends against using CYP2D6 genotype to select adjuvant endocrine therapy. The Committee encouraged caution with concurrent use of CYP2D6 inhibitors (such as bupropion, paroxetine, fluoxetine; see Table 11 in the full guideline for a complete list of inhibitors) and tamoxifen because of the known drug-drug interactions.

Comment: The adjuvant endocrine therapy recommendations in this update are for all women, irrespective of any specific clinical subset or prognostic marker. AI therapy has not been evaluated in men, thus the continued recommendation that men with breast cancer receive adjuvant tamoxifen.

Data suggest that variability in tamoxifen metabolism affects the likelihood of cancer recurrence in patients treated with tamoxifen. Factors that contribute to this variability include concurrent use of other drugs that inhibit the CYP2D6 isoenzyme and pharmacogenetic variation (polymorphisms) in CYP2D6 alleles. It is not yet known whether these variations account for differences in outcomes among patients treated with tamoxifen.

Available data on CYP2D6 pharmacogenetics are insufficient to recommend testing as a tool to determine an adjuvant endocrine strategy. Patients who clearly benefit from

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2 The 2014 ASCO practice guideline focused update does not address pharmacogenetic testing (JCO July 20, 2014 vol. 32 no. 212255-2269).
known CYP2D6 inhibitors might consider avoiding tamoxifen because of potential pharmacologic interactions. Conversely, patients who receive tamoxifen may prefer to avoid concurrent use of known CYP2D6 inhibitors if suitable alternatives are available.

Please review the complete therapeutic recommendations that are located here: (3)

2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): “For CYP2D6 poor metabolizers (PMs), defined as patients carrying two defective alleles […] With respect to tamoxifen, an increased risk for breast cancer relapse is present, and it is advised that an aromatase inhibitor be considered for treating postmenopausal women with breast cancer. Other recommendations included the selection of an alternative drug, therapeutic drug monitoring, increased alertness to adverse drug events and to reduced efficacy, and the recording of an electrocardiogram.

For CYP2D6 intermediate metabolizers (IMs), defined as patients carrying two decreased-activity alleles or one active/decreased-activity allele and one inactive allele […] For tamoxifen, the use of an aromatase inhibitor for treating postmenopausal women with breast cancer and the avoidance of concomitant use of a CYP2D6 inhibitor are advised. Other recommendations are comparable to the recommendations for PMs.”

Please review the complete therapeutic recommendations that are located here: (4).

Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
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<td>CYP2D6*4</td>
<td></td>
<td>NM_000106.5:c.506-1G&gt;A</td>
<td>Not applicable—variant occurs in a non-coding region</td>
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<tr>
<td>CYP2D6*5</td>
<td></td>
<td></td>
<td>Not applicable—variant results in a whole gene deletion</td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>1707 del T Trp152Gly</td>
<td>NM_000106.5:c.454delT</td>
<td>NP_000097.3:p.Trp152Glyfs</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>100C&gt;T Pro34Ser</td>
<td>NM_000106.5:c.100C&gt;T</td>
<td>NP_000097.3:p.Pro34Ser</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>Includes at least two functional variants*: 1023C&gt;T (Thr107Ile) 2850C&gt;T (Cys296Arg)</td>
<td>NM_000106.5:c.886T&gt;C</td>
<td>NP_000097.3:p.Thr107Ile</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NM_000106.5:c.320C&gt;T</td>
<td>NP_000097.3:p.Cys296Arg</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>2988G&gt;A</td>
<td>NM_000106.5:c.985+39G&gt;</td>
<td>Not applicable—variant occurs in a non-coding region</td>
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</tbody>
</table>

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

Acknowledgments

First edition:

The author would like to thank Harold Burstein, Associate Professor of Medicine, Harvard Medical School; and Hiltrud Brauch, Deputy Head of the Fischer-Bosch-Institute of Clinical Pharmacology (IKP) and Head of the Breast Cancer Susceptibility and Pharmacogenomics IKP Department, for reviewing this summary.

Version History

To view an earlier version of this summary (Created: October 7, 2014), please click here.

References


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Aripiprazole Therapy and CYP2D6 Genotype
Atomoxetine Therapy and CYP2D6 Genotype
Clozapine Therapy and CYP2D6, CYP1A2, and CYP3A4 Genotypes
Codeine Therapy and CYP2D6 Genotype
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Metoprolol Therapy and CYP2D6 Genotype
Propafenone Therapy and CYP2D6 Genotype
Risperidone Therapy and CYP2D6 Genotype
Thioridazine Therapy and CYP2D6 Genotypes
Tramadol Therapy and CYP2D6 Genotype
Venlafaxine Therapy and CYP2D6 Genotype

Tests in GTR by Condition

Tamoxifen response

Tests in GTR by Gene

CYP2D6 gene
Thioguanine Therapy and *TPMT* Genotype

Laura Dean, MD


**Introduction**

Thioguanine is an antineoplastic agent that belongs to the drug class of thiopurines. It is used in the treatment of acute myeloid leukemia (1).

Thioguanine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), the major active metabolites. Thiopurine S-methyltransferase (TPMT) inactivates thioguanine, leaving less parent drug available to form TGNs.

An adverse effect of thioguanine therapy is bone marrow suppression, which can occur in any patient, is dose-dependent, and may be reversed by reducing the dose of thioguanine. However, patients who carry two nonfunctional *TPMT* alleles universally experience life-threatening myelosuppression when treated with thioguanine, due to high levels of TGNs. Patients who carry one nonfunctional *TPMT* allele may also be unable to tolerate conventional doses of thioguanine (2, 3).

The FDA-approved drug label for thioguanine states that there are individuals with an inherited deficiency of the thiopurine methyltransferase (TPMT) enzyme who may be unusually sensitive to the myelosuppressive effects of thioguanine and prone to developing rapid bone marrow suppression following treatment initiation. Substantial dosage reductions may be required to avoid the development of life-threatening bone marrow suppression in these patients. Prescribers should be aware that some laboratories offer testing for TPMT deficiency.

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published recommendations for *TPMT* genotype-based thioguanine dosing. These recommendations include:

Start with reduced doses of thioguanine for patients with one nonfunctional *TPMT* allele, or drastically reduced doses for patients with malignancy and two nonfunctional alleles; adjust dose based on degree of myelosuppression and disease-specific guidelines. Consider alternative nonthiopurine immunosuppressant therapy for patients with nonmalignant conditions and two nonfunctional alleles (see Table 1) (2-4).

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1 NCBI; Email: dean@ncbi.nlm.nih.gov.
Table 1. *TPMT* phenotypes and the therapeutic recommendations for thioguanine therapy, adapted from CPIC

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Phenotype details</th>
<th><em>TPMT</em> Genotype</th>
<th>Examples of diplotypes</th>
<th>Therapeutic recommendations for thioguanine (TG)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Homozygous wild-type (“normal”)</strong></td>
<td>High enzyme activity. Found in approximately 86–97% of patients.</td>
<td>Two or more functional <em>TPMT</em> alleles</td>
<td>*1/*1</td>
<td>Start with normal starting dose. Adjust doses of TG and of other myelosuppressive therapy without any special emphasis on TG. Allow 2 weeks to reach steady state after each dose adjustment.</td>
</tr>
<tr>
<td><strong>Heterozygous</strong></td>
<td>Intermediate enzyme activity. Found in approximately 3–14% of patients.</td>
<td>One functional <em>TPMT</em> allele plus one nonfunctional <em>TPMT</em> allele</td>
<td>*1/*2</td>
<td>*1/*3A</td>
</tr>
<tr>
<td><strong>Homozygous variant</strong></td>
<td>Low or deficient enzyme activity. Found in approximately 1 in 178 to 1–3736 patients.</td>
<td>Two nonfunctional <em>TPMT</em> alleles</td>
<td>*3A/*3A</td>
<td>*2/*3A</td>
</tr>
</tbody>
</table>

The strength of therapeutic recommendations is “moderate” for heterozygous individuals, and “strong” for the other phenotypes.

Table is adapted from Relling M.V. et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clinical pharmacology and therapeutics. 2011;89(3):387–91 (2, 3).
**Drug Class: Thiopurines**

Thiopurines are used as anticancer agents and as immunosuppressants in inflammatory bowel disease, rheumatoid arthritis, and other autoimmune conditions. Three thiopurines are used clinically: thioguanine, mercaptopurine, and azathioprine (a prodrug for mercaptopurine). All three agents have similar effects but are typically used for different indications. Thioguanine is most commonly used in the treatment of myeloid leukemias, mercaptopurine is used for lymphoid malignancies, and mercaptopurine and azathioprine are used for immune conditions.

Thiopurines are either activated to form TGNs (the major active metabolite) or deactivated by TPMT. Individuals who carry two non-functional TPMT alleles (“TPMT homozygotes”) universally experience life-threatening bone marrow suppression because of high levels of TGNs when treated with conventional doses. Individuals who carry one non-functional TPMT allele (“TPMT heterozygotes”) may also be unable to tolerate conventional doses of thiopurines due to increased levels of TGNs.

**Drug: Thioguanine**

Thioguanine is a neoplastic agent used in the treatment of acute myeloid leukemia (AML). AML is the most common acute leukemia in adults, accounting for approximately 80% of cases, and the incidence increases with age. It is a less common cause of acute leukemia in children accounting for less than 10% of cases.

AML is characterized by a proliferation of the myeloid lineage of blood cells, causing an accumulation of abnormal and immature cells in the blood, bone marrow, and sometimes other tissues. This causes a disruption in the production of normal red blood cells, platelets, and mature granulocytes, leading to anemia, bleeding, and an increased risk of infection.

Combination chemotherapy for AML, which includes thioguanine, more frequently induces remission and a longer duration of remission than using thioguanine alone, but because of the high risk of liver toxicity, thioguanine is not recommended for long-term use. Younger patients with AML tend to have a better response to thioguanine than older patients (1).

Like all thiopurines, thioguanine is a purine analogue, and acts as an antimetabolite. Thioguanine is metabolized by two main pathways—it is either activated by HPRT1 (hypoxanthine phosphoribosyltransferase) and metabolized to form TGNs, or deactivated by TPMT. The cytotoxicity of thioguanine is due, in part, to the incorporation of TGNs into DNA. In addition to inhibiting de novo purine synthesis, thioguanine may also inhibit purine nucleotide interconversions (1).

The most frequent adverse reaction to thioguanine is myelosuppression, which can occur in any patient, and can usually be reversed by decreasing the dose of thioguanine. However, all patients who carry two nonfunctional TPMT alleles (approximately 0.3%)
experience life-threatening myelosuppression after starting treatment with conventional doses of thioguanine.

Individuals who are heterozygous for nonfunctional \textit{TPMT} alleles (approximately 3–14\%) are at an increased risk of moderate to severe bone marrow suppression, whereas individuals who are homozygous for wild-type \textit{TPMT} alleles have a lower risk of bone marrow suppression. However, all individuals receiving thioguanine require close monitoring (2, 3, 5, 6).

The FDA-approved drug label for thioguanine states that substantial dosage reductions may be required to avoid the development of life-threatening bone marrow suppression in patients with an inherited deficiency of TPMT. A concern among oncologists may be that a reduced dose of thioguanine will have less anti-tumor efficacy. However, CPIC recommendations (table 1) state that “dose adjustments based on \textit{TPMT} genotype have reduced thiopurine-induced adverse effects without compromising desired antitumor and immunosuppressive therapeutic effects in several clinical settings”.

Another adverse effect of thioguanine treatment is hyperuricemia, which frequently occurs because of the rapid lysis of tumor cells. In addition, liver toxicity associated with vascular endothelial damage has been reported when thioguanine is used for maintenance, or for similar long-term continuous therapy. Liver toxicity usually presents as the clinical syndrome of hepatic veno-occlusive disease (hyperbilirubinemia, tender hepatomegaly, weight gain due to fluid retention, and ascites) or with signs of portal hypertension (splenomegaly, thrombocytopenia, and oesophageal varices). For this reason, the long-term use of thioguanine is not recommended (1).

**Gene: \textit{TPMT}\text**

The \textit{TPMT} gene encodes one of the important enzymes of phase II metabolism, thiopurine S-methyltransferase. \textit{TPMT} is one of the main enzymes involved in the metabolism of thiopurines, such as thioguanine. \textit{TPMT} activity is inherited as a co-dominant trait, as the \textit{TPMT} gene is highly polymorphic with over 40 reported variant alleles (7-10).

The wild-type \textit{TPMT}*1 allele is associated with normal enzyme activity. Individuals who are homozygous for \textit{TPMT}*1 (TPMT normal metabolizers) are more likely to have a typical response to thioguanine and a lower risk of myelosuppression. This accounts for the majority of patients (~86–97\%) (2, 3).

Individuals who are TPMT poor (approximately 0.3\%) or intermediate (approximately 3–14\%) metabolizers carry variant \textit{TPMT} alleles that encode reduced or absent enzyme activity. Three variant \textit{TPMT} alleles account for over 90\% of the reduced or absent activity \textit{TPMT} alleles (11, 12):

- \textit{TPMT}*2 (c.238G>C)
- \textit{TPMT}*3A (c.460G>A and c.719A>G)
- \textit{TPMT}*3B (c.460G>A)
• **TPMT**<sup>∗</sup>3C (c.719A>G)

The frequency of **TPMT** alleles varies among different populations. In the United States, the most common low-activity allele in the Caucasian population is **TPMT**<sup>∗</sup>3A (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently (7, 13).

In East Asian, African-American, and some African populations, the most common variant is **TPMT**<sup>∗</sup>3C (~2%), although **TPMT**<sup>∗</sup>8 may be more common in African populations than previously thought (~2%). In general, **TPMT**<sup>∗</sup>2 occurs much less commonly, and **TPMT**<sup>∗</sup>3B occurs rarely (7, 14).

**Genetic Testing**

Genetic testing is available for several **TPMT** variant alleles, which most commonly includes **TPMT**<sup>∗</sup>2, <sup>∗</sup>3A, and <sup>∗</sup>3C as they account for >90% of inactivating alleles. Of note, rare and/or previously undiscovered variants will not be detected by variant-specific genotyping methods (2, 3, 15-18).

TPMT phenotype enzyme activity testing is also available by measuring TPMT activity in red blood cells directly (5). In adult patients taking thioguanine as an immunosuppressive agent, there is strong evidence of a near 100% concordance between phenotype and genotype testing. Inflammatory disease processes do not interfere with the accuracy of TPMT activity measurements if the blood sample is taken under standard conditions (e.g., not within two months of a blood transfusion).

However in patients with leukemia, the concordance between TPMT phenotype and genotype is poor (19). By the time of diagnosis, red cell TPMT activity is typically greatly reduced because of atypical hematopoiesis. Therefore, phenotype testing may wrongly identify an individual as having a TPMT deficiency, e.g., a patient who has two functional copies of the **TPMT** gene (homozygous wild-type) may be determined as having only one functional copy and one nonfunctional variant (**TPMT** heterozygous); and a patient who is **TPMT** heterozygous may be wrongly determined to be **TPMT** homozygous (two copies of nonfunctional **TPMT** variants). In addition, during the course of chemotherapy, **TPMT** phenotype testing may reveal excessively high TPMT activity. This is thought to be due to an excess of young red blood cells with their associated higher level of TPMT enzyme activity. Therefore, to avoid an incorrect TPMT status, genotype testing is recommended for patients with leukemia (19).

Finally, one study reported that **TPMT** genotyping was more reliable than phenotyping in identifying patients at risk of adverse reactions from thiopurine treatment (20), and several studies reported that the **TPMT** genotype is a better indicator than TPMT activity for predicting TGN accumulation or treatment outcome (6, 21-23).
Therapeutic Recommendations based on Genotype

This section contains excerpted\(^1\) information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2013 Statement from the US Food and Drug Administration (FDA): There are individuals with an inherited deficiency of the enzyme thiopurine methyltransferase (TPMT) who may be unusually sensitive to the myelosuppressive effects of thioguanine and prone to developing rapid bone marrow suppression following the initiation of treatment. Substantial dosage reductions may be required to avoid the development of life-threatening bone marrow suppression in these patients. Prescribers should be aware that some laboratories offer testing for TPMT deficiency. Since bone marrow suppression may be associated with factors other than TPMT deficiency, TPMT testing may not identify all patients at risk for severe toxicity. Therefore, close monitoring of clinical and hematologic parameters is important. Bone marrow suppression could be exacerbated by coadministration with drugs that inhibit TPMT, such as olsalazine, mesalazine, or sulphasalazine.

Please review the complete therapeutic recommendations that are located here: (1).

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Testing for TPMT status is recommended prior to starting thioguanine therapy so that the starting dosages can be adjusted accordingly—see Table 1 for dosing recommendations. In homozygous variant individuals, consider an alternative agent for nonmalignant conditions and drastically reduce doses in malignant conditions. In heterozygous individuals, the starting doses should be reduced. In both patient groups, a longer period of time should be left after each dose adjustment to allow for a steady state to be reached.

Please review the complete therapeutic recommendations that are located here: (2, 3).

Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence Coding</th>
<th>Protein</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT*2</td>
<td>238G&gt;C</td>
<td>NM_000367.2:c.238G&gt;C</td>
<td>NP_000358.1:p.Ala80Pro</td>
<td>rs1800462</td>
</tr>
</tbody>
</table>

\(^1\) The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
Table continued from previous page.

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence Coding</th>
<th>Protein</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT*3A</td>
<td>This allele contains two variants in cis: c.460G&gt;A and c.719A&gt;G</td>
<td>NM_000367.2:c.460G&gt;A</td>
<td>NP_000358.1:p.Ala154Thr</td>
<td>rs1800460</td>
</tr>
<tr>
<td>TPMT*3B</td>
<td>460G&gt;A Ala154Thr</td>
<td>NM_000367.2:c.719A&gt;G</td>
<td>NP_000358.1:p.Tyr240Cys</td>
<td>rs1142345</td>
</tr>
<tr>
<td>TPMT*3C</td>
<td>719A&gt;G Tyr240Cys</td>
<td>NM_000367.2:c.719A&gt;G</td>
<td>NP_000358.1:p.Tyr240Cys</td>
<td>rs1142345</td>
</tr>
</tbody>
</table>

The TPMT Nomenclature Committee defines the nomenclature and numbering of novel TPMT variants: [http://www.imh.liu.se/tpmtalleles](http://www.imh.liu.se/tpmtalleles)

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

### Acknowledgments

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**First edition:**

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The Pharmacogenomics Knowledgebase: [http://www.pharmgkb.org](http://www.pharmgkb.org)


### Version History

To view an earlier version of this summary (Update: March 18, 2013), please click here.

### References


10. TPMT Nomenclature Committee [Internet]. Sweden: Linköping University. Table of TPMT alleles. [Cited 2016 February 02]. Available from: http://www.imh.liu.se/tpmtalleles/tabell-over-tpmt-alleler?l=en


Related Summaries by Gene
Azathioprine Therapy and TPMT Genotype
Mercaptopurine Therapy and TPMT Genotype

Related Summaries by Drug Class
Azathioprine Therapy and TPMT Genotype
Mercaptopurine Therapy and TPMT Genotype

Tests in GTR by Condition
Thioguanine response

Tests in GTR by Gene
TPMT gene
Thioridazine Therapy and CYP2D6 Genotypes

Laura Dean, MD
Created: February 9, 2017.

Introduction

Thioridazine is an antipsychotic used in the treatment of schizophrenia and psychosis. Its use is reserved for patients who have failed to respond to or cannot tolerate other antipsychotics.

Thioridazine has been shown to prolong the QT interval (the time taken for the heart ventricles to depolarize and repolarize) in a dose related manner. Drugs with this potential have been associated with the life-threatening ventricular tachycardia, “torsades de pointes”.

The CYP2D6 enzyme is involved in metabolizing thioridazine. About 7% of the population has reduced enzyme activity because of variants in the CYP2D6 gene. In individuals with low CYP2D6 activity, standard doses of thioridazine may lead to higher drug levels in the plasma, and increase the risk of cardiac arrhythmias.

The FDA-approved drug label for thioridazine states that thioridazine is contraindicated in individuals who are known to have reduced levels of CYP2D6 activity. The label also states it is contraindicated to coadminister thioridazine with drugs that inhibit CYP2D6 (e.g., fluoxetine, paroxetine) or inhibit the metabolism of thioridazine (e.g., fluvoxamine, propranolol, and pindolol) (1).

Drug Class: Antipsychotics

The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine, followed by other agents, including fluphenazine, loxapine, phephenazine, pimozide, thioridazine, thiiothixene, and trifluoperazine.

Known as “first generation” or “typical” antipsychotics, these drugs were used to treat psychosis (regardless of the cause), chronic psychotic disorders (e.g., schizophrenia), and other psychiatric conditions. However, prominent adverse effects included extrapyramidal side effects such as tardive dyskinesia, muscle rigidity, and tremors, i.e., Parkinsonian-like symptoms.

Newer antipsychotics, known as “second generation” or “atypical” antipsychotics, have a lower risk of extrapyramidal side effects. However, many have serious metabolic effects.
These antipsychotics include aripiprazole, clozapine, iloperidone, olanzapine, and risperidone.

**Drug: Thioridazine**

Thioridazine is a first generation “typical” antipsychotic used in the treatment of schizophrenia. Schizophrenia is a severe neurodevelopmental disorder with a worldwide prevalence of around 0.3–0.7% (2). The etiology of schizophrenia is unknown, but it is thought to result from a combination of complex genetic and environmental factors. Before the discovery of the first antipsychotics in the 1950s, the management of schizophrenia relied heavily upon sedation, electroconvulsive therapy, and institutionalization.

The symptoms of schizophrenia fall into three main categories: positive, negative, and cognitive. Positive symptoms are generally not found in healthy individuals, but may come and go or persist in individuals with schizophrenia. Positive symptoms include reality distortion (e.g., delusions, hallucinations), and thought disorders. These symptoms often respond well to treatment.

Negative symptoms are deficits in normal emotions and behavior, and may be mistaken for depression. Symptoms divide into reduced expression of emotion (e.g., speaking without moving or with a monotonous voice) and avolition (a lack of motivation to start or continue with a task). No treatment has established efficacy for these pathologies.

Cognitive symptoms may also be difficult to recognize. They include poor executive functioning (understanding information and using it to make decisions) and trouble focusing or paying attention. And again, no treatment has established efficacy.

The use of thioridazine is reserved for patients who have failed to respond to or cannot tolerate the side effects of other antipsychotics. The FDA-approved drug label for thioridazine strongly recommends that prior to starting thioridazine, a patient should be given at least two trials, each with a different antipsychotic drug product, at an adequate dose, for an adequate duration of time. The label also states that for patients who do require chronic treatment with thioridazine, the smallest dose and the shortest duration of treatment should be sought and the need for continued treatment should be reassessed periodically; and cautions that the efficacy of thioridazine in treating patients with refractory schizophrenia is unknown (1).

The main action of both first-generation and second-generation antipsychotics appears to be the post-synaptic blockade of D2 dopamine receptors in the brain. (An exception is aripiprazole, which is a D2 partial agonist.) Blockade of the D2 receptor in the brain's limbic system is thought to improve the “positive” symptoms of schizophrenia (3).

However, because the first-generation antipsychotics also block dopamine receptors in the nigrostriatal pathway, they cause movement disorders known as extrapyramidal side effects. These disorders include akathisia (motor restlessness), dystonia (abnormal muscle tone), and tardive dyskinesia (involuntary and repetitive movements).
Compared to other first generation antipsychotics, thioridazine shares a similar efficacy, but has a lower risk of extrapyramidal side effects (4-6). However, a higher level of EKG changes is associated with thioridazine therapy (6).

Antipsychotics, and thioridazine in particular, can inhibit cardiac ion channels. Most first generation antipsychotics block the cardiac potassium channel KCNH2, previously known as the human ether-a-go-go-related gene (hERG) (7, 8). Blockade of this channel reduces inward potassium current, resulting in longer cardiac repolarization times. On the EKG, this manifests as a prolonged QT interval. In extreme cases, this can lead to a life-threatening ventricular tachycardia known as torsades de pointes (“twisting of the points”) (9-11).

At one point, thioridazine was one of the most commonly used medications for major mental health disorders. However, numerous case reports of sudden, unexpected death led to label changes in 2000, which recommended that thioridazine be used as a last resort (12). In 2005, the manufacturer Novartis discontinued the branded form of thioridazine because of its association with QT prolongation, but generic forms are still available in the US (13, 14).

Thioridazine is metabolized by CYP2D6 to the active metabolite mesoridazine, which is further metabolized to sulforidazine, both of which are more potent than thioridazine. In addition, both thioridazine and mesoridazine have similar effects on the QT interval (15, 16).

Recent research has found that thioridazine is active against multidrug resistant tuberculosis, when used in combination with other antituberculosis drugs. Thioridazine increases the permeability of the cell-envelope, enabling the enhanced uptake of antibiotics (17).

The FDA drug label states that no teratogenic effect has been shown with thioridazine to date. However, all drugs should be kept to a minimum during pregnancy, so thioridazine should be given only when the benefits exceed the possible risks to mother and fetus. Of note, neonates exposed to antipsychotic drugs during the third trimester are at risk for extrapyramidal and/or withdrawal symptoms following delivery which vary in severity; while in some cases symptoms have been self-limited, in other cases neonates have required intensive care unit support and prolonged hospitalization.

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.
Gene: CYP2D6

CYP2D6 is highly polymorphic; over 100 star (*) alleles are described and currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (18). CYP2D6*1 is the reference (or wild-type) allele encoding enzyme with normal activity. The CYP2D6*2, *33, and *35 alleles are also considered to confer normal activity (Table 1).

**Table 1.** Activity status of selected CYP2D6 alleles

<table>
<thead>
<tr>
<th>Allele type</th>
<th>CYP2D6 Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal function</td>
<td>*1, *2, *33, *35</td>
</tr>
</tbody>
</table>

For a detailed list of CYP2D6 alleles, please see (18).

An activity score can be assigned to each CYP2D6 allele, e.g., 1 for each functional allele, 0.5 for a decreased function allele, and 0 for a no function allele. Individuals who carry more than two normal function copies (e.g., multiple copies) of the CYP2D6 gene are “ultrarapid metabolizers”, whereas individuals who are “normal metabolizers” either carry two normal function copies of CYP2D6, or a combination of normal/decreased/no function alleles that result in an activity score between 1.0 and 2.0. Individuals who are intermediate or poor metabolizers carry copies of decreased or no function CYP2D6 alleles, respectively (Table 2).

**Table 2.** 2016 Assignment of CYP2D6 phenotypes by CPIC

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Activity Score</th>
<th>Genotypes</th>
<th>Examples of diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6 Ultrarapid metabolizer</td>
<td>Greater than 2.0</td>
<td>An individual carrying duplications of functional alleles</td>
<td>(*1/*1)xN, (*1/*2)xN, (*2/*2)xN</td>
</tr>
<tr>
<td>(approximately 1-20% of patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6 Normal metabolizer</td>
<td>1.0 – 2.0$^c$</td>
<td>An individual carrying two normal function alleles or two decreased function alleles</td>
<td>*1/*1, *1/*2, *2/*2, *1/*9</td>
</tr>
<tr>
<td>(approximately 72-88% of patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ For population-specific allele and phenotype frequencies, please see
$^b$ Where xN represents the number of CYP2D6 gene copies (N is 2 or more).
$^c$ Patients with an activity score of 1.0 may be classified as intermediate metabolizers by some reference laboratories.

For more information about activity scores, please see the Genetic Testing section.

### Table 2. continued from previous page.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Activity Score</th>
<th>Genotypes</th>
<th>Examples of diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>or one normal and no function allele</td>
<td>*1/*41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or one normal function and decreased function allele</td>
<td>*41/*41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0</td>
<td>*1/*5, *1/*4</td>
</tr>
<tr>
<td>CYP2D6 Intermediate metabolizer</td>
<td>0.5</td>
<td>An individual carrying one decreased function and one no function allele</td>
<td>*4/*41, *5/*9, *4/*10</td>
</tr>
<tr>
<td>(approximately 1-13% of patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6 Poor metabolizer</td>
<td>0</td>
<td>An individual carrying two no function alleles</td>
<td>*4/*4, *4/*xN, *3/*4, *5/*5, *5/*6</td>
</tr>
<tr>
<td>(approximately 1-10% of patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* For population-specific allele and phenotype frequencies, please see

*b* Where xN represents the number of CYP2D6 gene copies (N is 2 or more).

*c* Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.

For more information about activity scores, please see the Genetic Testing section.


The most common no function alleles include CYP2D6*3, *4, *5, and *6 (20-23), and the most common decreased function alleles include CYP2D6*9, *10, *17, *29 and *41 (24-28). There are large inter-ethnic differences in the frequency of these alleles. For example, CYP2D6*4 is the most common no function allele in Caucasians, but is less abundant in subjects with African ancestry, and is rare in Asians. In contrast, the decreased function allele CYP2D6*10 is the most common allele in Asians, and CYP2D6*17 is almost exclusively found in individuals with African ancestry (29).

Consequently, the phenotype frequencies also vary substantially among the major ethnicities and may vary among populations. Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function CYP2D6*4 and *5 alleles (30, 31).

In individuals who are CYP2D6 poor metabolizers, standard doses of thioridazine may lead to the drug accumulating in the plasma. Since a dose-related side effect of thioridazine is prolongation of the QTc interval, which is a potentially life threatening event, the FDA has stated that the use of thioridazine is contraindicated in individuals who are known to have reduced CYP2D6 activity (1, 32). In addition, the label also states...
it is contraindicated to coadminister thioridazine with other drugs that inhibit CYP2D6 activity (e.g., the antidepressants fluoxetine and paroxetine) or inhibit the metabolism of thioridazine (e.g., the beta-blockers propranolol and pindolol, and the antidepressant fluvoxamine) (1).

Genetic Testing

The NIH’s Genetic Testing Registry, GTR, provides examples of the genetic tests that are currently available for the thioridazine response and the CYP2D6 gene.

Results are typically reported as a diplotype, such as CYP2D6 *1/*1. A result for copy number, if available, is also important when interpreting CYP2D6 results (33). However, it needs to be noted that the number of variants tested varies substantially among laboratories and there is no standardized way to report results (34).

If the test results include an interpretation of the patient’s predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1 for each copy of a normal function allele). The phenotype is defined by the sum of the two scores:

- An extensive (normal) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score of greater than 2 (19, 35)

Therapeutic Recommendations based on Genotype

This section contains excerpted1 information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Reduced cytochrome P450 2D6 isozyme activity drugs that inhibit this isozyme (e.g., fluoxetine and paroxetine) and certain other drugs (e.g., fluvoxamine, propranolol, and pindolol) appear to appreciably inhibit the metabolism of thioridazine. The resulting elevated levels of thioridazine would be expected to augment the prolongation of the QTc interval associated with thioridazine and may increase the risk of serious, potentially fatal, cardiac arrhythmias, such as Torsades de pointes type arrhythmias. Such an increased risk may result also from the additive effect of coadministering thioridazine with other agents that prolong the QTc interval. Therefore, thioridazine is contraindicated with these drugs as

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1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
well as in patients, comprising about 7% of the normal population, who are known to have a genetic defect leading to reduced levels of activity of P450 2D6.

Please review the complete therapeutic recommendations that are located here: (1).

**Nomenclature**

**Nomenclature of selected CYP2D6 alleles**

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP2D6*4</strong></td>
<td>1846G&gt;A</td>
<td>NM_000106.5:c.506-1G&gt;A</td>
<td>rs3892097</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variant occurs in a non-coding region (splice variant causes a frameshift)</td>
<td></td>
</tr>
<tr>
<td><strong>CYP2D6*5</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CYP2D6*6</strong></td>
<td>1707 del T</td>
<td>NM_000106.5:c.454delT</td>
<td>rs5030655</td>
</tr>
<tr>
<td></td>
<td>Trp152Gly</td>
<td>NP_000097.3:p.Trp152Glyfs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• CYP2D6T</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CYP2D6*10</strong></td>
<td>100C&gt;T</td>
<td>NM_000106.5:c.100C&gt;T</td>
<td>rs1065852</td>
</tr>
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[1] In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T. 
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Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

**Acknowledgments**

The author would like to thank David Kisor, B.S., Pharm.D., Professor and Director of Pharmacogenomics Education, Pharmacogenomics Program, Manchester University, Indiana; Mohamed Nagy, Clinical Pharmacist, Head of the Personalised Medication Management Unit, Department of Pharmaceutical Services, Children’s Cancer Hospital, Egypt; and Yolande Saab, Pharm.D., Ph.D., Associate Professor of Pharmacogenomic,
References


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
Aripiprazole Therapy and CYP2D6 Genotype
Atomoxetine Therapy and CYP2D6 Genotype
Clozapine Therapy and CYP2D6, CYP1A2, and CYP3A4 Genotypes
Codeine Therapy and CYP2D6 Genotype
Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
Metoprolol Therapy and CYP2D6 Genotype
Propafenone Therapy and CYP2D6 Genotype
Risperidone Therapy and CYP2D6 Genotype
Tamoxifen Therapy and CYP2D6 Genotype
Tramadol Therapy and CYP2D6 Genotype
Venlafaxine Therapy and CYP2D6 Genotype
Tests in GTR by Condition
Thioridazine response

Tests in GTR by Gene
CYP2D6 gene
Tramadol Therapy and CYP2D6 Genotype
Laura Dean, MD
Created: September 10, 2015.

Introduction
Tramadol is an analgesic used to treat moderate to moderately severe pain. It is a synthetic opioid, related to codeine, and is used to treat both acute and chronic pain. Tramadol is often prescribed for post-operative pain, and pain caused by cancer, osteoarthritis, and other musculoskeletal diseases (1).

The CYP2D6 enzyme metabolizes a quarter of all prescribed drugs, including tramadol. Individuals who carry two inactive copies of CYP2D6 are known as poor metabolizers and have higher plasma concentrations of tramadol compared with individuals who have two copies of normal activity alleles (1). Individuals who carry one or more reduced or inactive copies of CYP2D6 are known as intermediate metabolizers, and individuals who carry more than two active copies of CYP2D6 are known as ultrarapid metabolizers.

The FDA states that the levels of tramadol are approximately 20% higher in poor metabolizers compared to extensive (“normal”) metabolizers, while concentrations of the tramadol metabolite, M1, are 40% lower. Inhibitors of CYP2D6, such as fluoxetine and amitriptyline, also inhibit the metabolism of tramadol, and the full pharmacological impact of these alterations of tramadol dose in terms of either efficacy or safety is unknown (1).

A guideline from the Dutch Pharmacogenetics Working Group includes dose recommendations for poor metabolizers (either select an alternative drug—not oxycodone or codeine—or be alert to the symptoms of insufficient pain relief). It also contains dose recommendations for intermediate metabolizers (be alert to decreased efficacy of tramadol, consider increasing the dose and if the response is still inadequate, either select an alternative drug—not oxycodone or codeine, or be alert to the symptoms of insufficient pain relief) and ultrarapid metabolizers (either reduce the dose of tramadol by 30% and be alert to adverse drug events, or select an alternative drug e.g., acetaminophen, NSAID, morphine—not oxycodone or codeine) (see Table 1) (2).
Table 1. CYP2D6 phenotypes and the therapeutic recommendations for tramadol therapy

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Therapeutic recommendation for tramadol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>More than two copies of functional alleles</td>
<td>Reduce dose by 30% and be alert to ADEs (e.g., nausea, vomiting, constipation, respiratory depression, confusion, urinary retention) or select alternative drug (e.g., acetaminophen, NSAID, morphine—not oxycodone or codeine)</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>One active allele and one inactive allele, or two decreased activity alleles, or one decreased activity allele and one inactive allele</td>
<td>Be alert to decreased efficacy. Consider dose increase. If response is still inadequate, select alternative drug—not oxycodone or codeine—or be alert to symptoms of insufficient pain relief</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>Two inactive alleles</td>
<td>Select alternative drug—not oxycodone or codeine—or be alert to symptoms of insufficient pain relief</td>
</tr>
</tbody>
</table>

ADE: Adverse Drug Event

Table 2. Activity status of CYP2D6 alleles

<table>
<thead>
<tr>
<th>Allele type</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>*1, *2, *33, *35</td>
</tr>
</tbody>
</table>

Note: The most clinically significant variants are highlighted in bold.

Drug: Tramadol

Tramadol is an analgesic that is used to treat moderate to moderately severe pain. Tramadol is commonly prescribed for postoperative, cancer, and musculoskeletal pain. In the US, tramadol is classified as a Schedule IV controlled substance (1, 3).

Tramadol is a centrally acting analgesic that is structurally related to codeine and morphine, and belongs to the same drug class of opiate drugs. Tramadol, however, is a synthetic opioid, and it is administered as a racemic mixture of two enantiomers, (+) and (-) tramadol (4).

Although opiates have been used for pain control for several thousands of years, the receptors upon which they act were discovered relatively recently, in the 1960s. The exact mechanism of action of tramadol is not known, but it is thought that both enantiomers contribute to its analgesic effect in different ways. Tramadol has some activity at mu-
opioid receptor (less than codeine) and it also inhibits the synaptic reuptake of serotonin and norepinephrine which inhibits pain transmission at the spinal cord (4, 5).

Tramadol is extensively metabolized within the liver and has one main major metabolite, O-desmethyltramadol, known as M1. Both the parent drug and M1 contribute to the analgesic effect, but M1 has a significantly higher affinity for opioid receptors than tramadol (6). The enzyme CYP2D6 catalyzes the production of M1, and other CYP enzymes (CYP2B6 and CYP3A4) catalyze the production of M2, an inactive metabolite (7).

The adverse effects of tramadol therapy are similar to that of other weak opioids. Common side effects include dizziness, nausea, constipation, and headache. But an additional risk of tramadol therapy is the risk of seizures, especially in patients who are already taking antidepressants or other drugs that decrease the seizure threshold. There is also an increased risk of suicide, and therefore tramadol should not be prescribed for patients who are suicidal or prone to addictions—the use of non-narcotic analgesics should be considered instead (1).

Because tramadol has mu-opioid agonist activity, there is a risk of abuse and addiction, even under appropriate medical use. Therefore, as for all patients treated with opioids, there should be careful monitoring of patients taking tramadol. In addition, the longer a patient is on continuous tramadol therapy, the greater the risk of tolerance (the need to increase the dose of drug to maintain a defined level of analgesia in the absence of disease progression). Physical dependence upon tramadol is manifested by withdrawal symptoms after the use of tramadol is stopped abruptly. Symptoms include restlessness, rhinorrhea, lacrimation, and chills (1).

Serotonin syndrome is a potentially life-threatening syndrome that may occur with the use of tramadol, especially if other medications such as antidepressants or other drugs that impair the metabolism of tramadol (CYP2D6 and CYP3A4 inhibitors) are used concurrently. Symptoms include changes in mental status (e.g., agitation, hallucinations, coma), autonomic instability (e.g., tachycardia, labile blood pressure, hyperthermia), neuromuscular aberrations (e.g., hyperreflexia, incoordination) and/or gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea) (1, 8).

**Gene: CYP2D6**

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. The CYP2D6 gene is highly polymorphic, with more than 100 star (*) alleles described (9).
CYP2D6*1 is the wild-type allele and is associated with normal enzyme activity and the “extensive metabolizer” phenotype. The CYP2D6 alleles *2, *33, and *35 are also considered to have near-normal activity.

Other alleles include variants that produce a non-functioning enzyme (e.g., *3, *4, *5, and *6) (10-13) or an enzyme with reduced activity (e.g., *10, *17, and *41) (2, 14, 15) (see Table 2). There are large inter-ethnic differences in the frequency of these alleles, with *3, *4, *5, *6, and *41 being more common in Caucasians, *17 more common in Africans, and *10 more common in Asians (16).

Individuals who are intermediate or poor metabolizers carry copies of decreased-functioning and inactive CYP2D6 alleles (see Table 1 and 2). In these individuals, the metabolic capacity of CYP2D6 is decreased which may result in higher levels of tramadol.

The FDA-approved drug label for tramadol includes a study where concentrations of tramadol were approximately 20% higher in "poor metabolizers" versus "extensive metabolizers", while M1 concentrations were 40% lower. The label also states that other factors, such as the concurrent use of CYP2D6 inhibitors (e.g., fluoxetine and its metabolite norfluoxetine, amitriptyline and quinidine) could also result in increases in tramadol concentrations and decreased concentrations of M1, and that the “full pharmacological impact of these alterations in terms of either efficacy or safety is unknown” (1).

The Dutch Pharmacogenetics Working Group recommendations state that for poor metabolizers, “either select an alternative drug (not oxycodone or codeine) or be alert to the symptoms of insufficient pain relief” (2).

Poor metabolizers are commonly found in European Caucasians (6-10%). The most common allele in this population is the functional CYP2D6*1 (70%), and the most common nonfunctional alleles include CYP2D6*4 and *5, which largely account for the poor metabolizer phenotype in these populations (16). About 2% of African Americans are poor metabolizers, due to a wide range of variants that include the nonfunctional *4 and *5 alleles (17-19).

For intermediate metabolizers, the Dutch Pharmacogenetics Working Group recommendations state to be alert to decreased efficacy of tramadol. Consider increasing the dose of tramadol and if the response is still inadequate, either select an alternative drug (not oxycodone or codeine), or be alert to the symptoms of insufficient pain relief (2).

Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. In these populations, only half of CYPD6 alleles are fully functional, with the reduced function *10 variant being very common (~40%, compared to ~2% in Caucasians) (20). As a result, Asians are more likely to be intermediate metabolizers than Caucasians (16).
Individuals who have multiple functional copies of the \textit{CYP2D6} gene are “ultrarapid metabolizers” (UM). Each allele contributes to the metabolism of venlafaxine to the active metabolite, M1. The Dutch Pharmacogenetics Working Group recommendations state that for ultrarapid metabolizers, either reduce the dose of tramadol by 30% and be alert to adverse drug events (e.g., nausea, vomiting, constipation, respiratory depression, confusion, urinary retention), or select an alternative drug (e.g., acetaminophen, NSAID, morphine—not oxycodone or codeine).

The ultrarapid metabolizer phenotype is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; up to 10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (16).

**Genetic Testing**

Genetic testing is available for many of the more common variant \textit{CYP2D6} alleles. Results are typically reported as a diplotype, such as \textit{CYP2D6 *1/*1} (21). A result for copy number, if available, is also important when interpreting \textit{CYP2D6} results.

If the test results include an interpretation of the patient’s predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- An extensive (normal) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2

**Therapeutic Recommendations based on Genotype**

This section contains excerpted\(^1\) information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**Statement from the US Food and Drug Administration (FDA):** Approximately 7% of the population has reduced activity of the \textit{CYP2D6} isoenzyme of cytochrome P-450. These individuals are "poor metabolizers" of debrisoquine, dextromethorphan, tricyclic antidepressants, among other drugs. Based on a population PK [pharmacokinetic] analysis of Phase I studies in healthy subjects, concentrations of tramadol were approximately 20% higher in "poor metabolizers" versus "extensive metabolizers", while M1 [tramadol metabolite] concentrations were 40% lower. Concomitant therapy with

\(^1\) The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt and may have inserted text in brackets, to explain some of the terms used. The FDA may not have labeled all formulations containing the generic drug.
inhibitors of CYP2D6 such as fluoxetine, paroxetine and quinidine could result in significant drug interactions. In vitro drug interaction studies in human liver microsomes indicate that inhibitors of CYP2D6 such as fluoxetine and its metabolite norfluoxetine, amitriptyline and quinidine inhibit the metabolism of tramadol to various degrees, suggesting that concomitant administration of these compounds could result in increases in tramadol concentrations and decreased concentrations of M1. The full pharmacological impact of these alterations in terms of either efficacy or safety is unknown. Concomitant use of SEROTONIN re-uptake INHIBITORS and MAO INHIBITORS may enhance the risk of adverse events, including seizure and serotonin syndrome.

Please review the complete therapeutic recommendations that are located here: (1)

Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): For ultrarapid metabolizers, either reduce the dose of tramadol by 30% and be alert to adverse drug events (e.g., nausea, vomiting, constipation, respiratory depression, confusion, urinary retention), or select an alternative drug (e.g., acetaminophen, NSAID, morphine—not oxycodone or codeine).

For intermediate metabolizers, be alert to decreased efficacy of tramadol. Consider increasing the dose of tramadol and if the response is still inadequate, either select an alternative drug (not oxycodone or codeine), or be alert the symptoms of insufficient pain relief.

For poor metabolizers, either select an alternative drug (not oxycodone or codeine) or be alert to the symptoms of insufficient pain relief.

Please review the complete therapeutic recommendations that are located here: (2)

Nomenclature

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<td>NP_000097.2:p.Thr107Ile NP_000097.2:p.Cys296Arg</td>
<td>rs28371706 rs16947</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>2988G&gt;A</td>
<td>NM_000106.4:c.985+39G&gt;A</td>
<td>Not applicable—variant occurs in a non-coding region</td>
<td>rs28371725</td>
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### Acknowledgments

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### References


## Related Summaries by Gene

- Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
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- Tamoxifen Therapy and CYP2D6 Genotype
- Thioridazine Therapy and CYP2D6 Genotypes
- Venlafaxine Therapy and CYP2D6 Genotype

## Tests in GTR by Condition

- Tramadol response

## Tests in GTR by Gene

- CYP2D6 gene
Trastuzumab (Herceptin) Therapy and ERBB2 (HER2) Genotype

Laura Dean, MD

Created: August 5, 2015.

Introduction

Trastuzumab (brand name, Herceptin) is a monoclonal antibody used in the treatment of breast and gastric/gastroesophageal cancer. It targets an epidermal growth factor receptor encoded by the ERBB2 gene, which is commonly referred to as the HER2 gene.

The HER2 gene is overexpressed in 15-20% of breast cancers and is also overexpressed in some cases of gastric cancer. Overall, “HER2 positive” tumors are associated with a faster rate of growth and a poorer prognosis. The use of trastuzumab in treatment regimes improves outcomes, but adverse effects of therapy include cardiac toxicity.

The FDA-approved drug label for trastuzumab states that trastuzumab should only be used to treat patients with tumors that have either HER2 protein overexpression or HER2 gene amplification, as determined by an accurate and validated FDA-approved assay, specific for the type of tumor tested (breast or gastric). This is because these are the only patients studied for whom benefit has been shown (1).

A guideline from ASCO/CAP states that oncologists must request HER2 testing on every primary invasive breast cancer (and on a metastatic site, if stage IV and if specimen available) from a patient with breast cancer to guide decision to pursue HER2-targeted therapy. This should be especially considered for a patient who previously tested HER2 negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of HER2-positive or triple-negative disease (2).

Drug: Trastuzumab (Herceptin)

Trastuzumab (brand name, Herceptin) is a monoclonal antibody that targets ERBB2 (a tyrosine kinase receptor, also known as HER2 or HER-2/neu). Trastuzumab is only used to treat specific tumors that overexpress ERBB2; these tumors are known as “HER2-positive” tumors.

Trastuzumab is typically used as an adjuvant treatment of early-stage HER2-positive breast cancer. Adjuvant therapies are used after primary treatment (such as surgery) to increase the chance of long-term disease-free survival. An example chemotherapy treatment regime is "AC→TH", which stands for Adriamycin, Cytoxan, then Taxol and...
Herceptin. Trastuzumab is also used in the treatment of HER2-positive metastatic breast cancer and HER2-positive metastatic gastric cancer (1).

Recently, HER2 targeted therapy has been approved by the FDA for use in the neoadjuvant setting. Neoadjuvant therapy is given before primary therapy, for example, to shrink a tumor to an operable size or to allow for breast-conserving surgery, and to increase the chance of long-term, disease-free survival. In the neoadjuvant setting, pertuzumab, along with trastuzumab and docetaxel (a chemotherapy agent) can be given pre-operatively (3-5).

Before treatment with trastuzumab begins, overexpression of the ERBB2 protein or amplification of the ERBB2 gene must first be determined. The FDA recommends that testing be performed using an FDA-approved test for the specific tumor type (breast or gastric tumor), in a laboratory with demonstrated proficiency with the technology being used. This is because the benefits of trastuzumab have only been proven in patients with tumors that overexpress ERBB2. In addition, although trastuzumab is generally well tolerated, the risks of treatment include infusion reactions, pulmonary toxicity, and cardiomyopathy that can result in cardiac failure (1).

Trastuzumab targets the ERBB2 receptor by binding to the juxtamembrane portion of the extracellular domain. This binding limits the receptor’s ability to activate its intrinsic tyrosine kinase, which in turn, limits the activation of numerous signaling pathways that can promote the growth of cancerous cells.

A number of proposed mechanisms may underlie the anti-tumor effects of trastuzumab. One such mechanism is that trastuzumab blocks the HER3 receptor from binding to ERBB2. The ERBB2-HER3 dimerized receptor is thought to be highly active, triggering many signaling cascades in the absence of a “true” ligand (6).

Another proposed mechanism is antibody-dependent cellular cytotoxicity (ADCC). Once trastuzumab has bound to a cancer cell, immune cells (typically activated natural killer cells) bind to trastuzumab and initiate lysis of the cancer cell (7). Trastuzumab may also mediate the enhanced internalization and degradation of the ERBB2 receptor, inhibit angiogenesis, and inhibit ERBB2 shedding by preventing the cleavage of ERBB2 and the subsequent release of its extracellular domain (8, 9).

Unfortunately, breast cancer may start to progress again during trastuzumab therapy. Possible mechanisms that may facilitate disease progression during treatment include increased signaling from the HER family of receptors, an upregulation of downstream signaling pathways, and an increased level of insulin growth factor -1 receptor (10, 11).

At the time of writing, four drugs have been approved to target ERBB2 (trastuzumab, lapatinib, pertuzumab, and T-DM1), with more drugs in clinical trials.
Gene: **ERBB2 (HER2)**

The human epidermal growth factor receptor (HER) family consists of four members: the epidermal growth factor receptor (EGFR), HER2, HER3, and HER4 (see Nomenclature). All four members are transmembrane tyrosine kinase receptors, and they regulate a number of important cellular processes, such as cell growth, survival, and differentiation (12).

HER2, along with EGFR, are proto-oncogenes. Proto-oncogenes are a group of genes that, when mutated or expressed at abnormally high levels, can contribute to normal cells becoming cancerous cells. The mutated version of the proto-oncogene is called an oncogene. Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. All these are important biological processes. However, the increased production of these proteins, caused by oncogenes, can lead to the proliferation of poorly differentiated cancer cells (13).

The official gene symbol for HER2 is ERBB2, which is derived from a viral oncogene with which the receptor shares homology; “v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2.” However, clinicians commonly refer to the ERBB2 gene as “HER2” (Human Epidermal growth factor Receptor 2) or “HER2/neu” (neu was the name given to the gene that caused cancer derived from a rodent neuro/glioblastoma). HER2 is an alternate gene symbol for ERBB2 and is more commonly used by the community.

One unique feature of ERBB2 compared to the other receptors in the HER family is the absence of a known ligand. It is therefore thought that this receptor may permanently be in an activated state, or it may become activated during heterodimerization with one of the other members of the HER family (9). And, one unique feature of HER3 is that it has very little enzymatic activity compared to the other tyrosine kinase receptors in the HER family. It is therefore thought that an important role of HER3 is to act as a heterodimerization partner for ERBB2 (14, 15).

When a partner such as HER3 binds to ERBB2, the heterodimer undergoes activation, which stimulates the intrinsic tyrosine kinase activity of the receptor. Autophosphorylation of several key residues of the receptor triggers the downstream activation of many commonly used growth factor signaling pathways, such as the PI3K/AKT/mTOR pathway and the RAS/RAF/MEK/ERK pathway (16, 17). Impaired ERBB2 signaling is associated with the development of neurodegenerative diseases, such as multiple sclerosis and Alzheimer disease, whereas excessive ERBB2 signaling is associated with the development of cancers.

ERBB2 is overexpressed in approximately 15-20% of breast tumors, as a result of amplification of the ERBB2 gene, and tumors with increased ERBB2 usually have a higher growth rate and more aggressive clinical behavior (2, 18-20). Although gene amplification is frequently seen in cancer and other degenerative disorders, the underlying basis for amplification remains largely unknown (21). And in the case of ERBB2, although sequence variants have been identified, it is nearly always the wildtype ERBB2 gene that is
overexpressed in tumors (22). In about 1% of breast cancers, activating mutations in ERBB2 can be identified that are likely to drive tumorigenesis, without ERBB2 amplification (23).

**Tumor Testing for ERBB2 (HER2)**

There are two main methods used for HER2 testing: testing for overexpression of the HER2 protein using immunohistochemistry (IHC), or testing for gene amplification using in-situ hybridization (ISH). Each assay type has diagnostic pitfalls that must be avoided, and so the pathologist who reviews the histologic findings should determine the optimal assay (IHC or ISH) for the determination of HER2 status (2, 20).

In an IHC assay, a slice of tumor tissue is stained, along with a control sample that contains high levels of HER2. The tumor sample is then examined by light microscopy to assess the intensity of membrane staining—the amount of staining correlates with the quantity of HER2 protein and is typically graded from 0 to 3+:

- **IHC 0** means no visible staining and is an “HER2 negative” result
- **IHC 1+** is also an “HER2 negative” result—there is a staining pattern with weak and incomplete staining, or weak and complete staining of very few tumor cells
- **IHC 2+** is an “HER2 equivocal result”—there is a staining pattern with moderately intense staining, or intense staining of very few tumor cells
- **IHC 3+** is an “HER2 positive result”—there is a staining pattern with intense membrane staining on more than 10% of tumor cells, indicating a higher than normal level of HER2

For an equivocal (IHC 2+) result, either a reflex test must be ordered (same specimen using ISH), or a new test must be ordered (using a new specimen, if available, using IHC or ISH) to confirm the results.

The ISH assay, or FISH assay (fluorescence in situ hybridization), measures HER2 gene amplification by measuring HER2 DNA—the actual number of copies of the HER2 genes are counted. Under the microscope, the genes appear as red signals or dots, in a blue-stained cancer cell nucleus. The result is usually either FISH negative (normal level of HER2 gene) or FISH positive (at least twice as much as normal level of HER2 gene), but in a small number of cases the FISH result will be equivocal due to a low level of HER2 amplification. The use of a control helps distinguish between a negative result and a non-informative result caused by an error. Approximately 25% of patients who have an IHC 2+ result will have a FISH positive result (24).

**For the complete algorithms for evaluation of HER2 protein expression using IHC or ISH, please see the American Society of Clinical Oncology (ASCO) / College of American Pathologists (CAP) clinical practice guideline update, located here (2)**
Therapeutic Recommendations based on HER2 Testing

This section contains excerpted\(^1\) information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA):

Detection of HER2 protein overexpression is necessary for selection of patients appropriate for trastuzumab therapy because these are the only patients studied and for whom benefit has been shown. Due to differences in tumor histopathology, use FDA-approved tests for the specific tumor type (breast or gastric/gastroesophageal adenocarcinoma) to assess HER2 protein overexpression and HER2 gene amplification. Tests should be performed by laboratories with demonstrated proficiency in the specific technology being utilized. Improper assay performance, including use of suboptimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results.

Several FDA-approved commercial assays are available to aid in the selection of breast cancer and metastatic gastric cancer patients for trastuzumab therapy. Users should refer to the package inserts of specific assay kits for information on the Intended Use, and the validation and performance of each assay.

Limitations in assay precision make it inadvisable to rely on a single method to rule out potential Herceptin benefit.

Please review the complete therapeutic recommendations that are located here: (1)

FDA-approved medical devices for HER2 are listed here.

Excerpted recommendations from the American Society of Clinical Oncology / College of American Pathologists 2013 clinical practice guideline update:

Key Recommendations for Oncologists

- Must request HER2 testing on every primary invasive breast cancer (and on metastatic site, if stage IV and if specimen available) from a patient with breast cancer to guide decision to pursue HER2-targeted therapy. This should be especially considered for a patient who previously tested HER2 negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of HER2-positive or triple-negative disease.

\(^1\) The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.
• Should recommend HER2-targeted therapy if HER2 test result is positive, if there is no apparent histopathologic discordance with HER2 testing and if clinically appropriate.
• Must delay decision to recommend HER2-targeted therapy if initial HER2 test result is equivocal. Reflex testing should be performed on the same specimen using the alternative test if initial HER2 test result is equivocal or on an alternative specimen.
• Must not recommend HER2-targeted therapy if HER2 test result is negative and if there is no apparent histopathologic discordance with HER2 testing.
• Should delay decision to recommend HER2-targeted therapy if HER2 status cannot be confirmed as positive or negative after separate HER2 tests (HER2 test result or results equivocal). The oncologist should confer with the pathologist regarding the need for additional HER2 testing on the same or another tumor specimen.
• If the HER2 test result is ultimately deemed to be equivocal, even after reflex testing with an alternative assay (i.e., if neither test is unequivocally positive), the oncologist may consider HER2-targeted therapy. The oncologist should also consider the feasibility of testing another tumor specimen to attempt to definitely establish the tumor HER2 status and guide therapeutic decisions. A clinical decision to ultimately consider HER2-targeted therapy in such cases should be individualized on the basis of patient status (comorbidities, prognosis, and so on) and patient preferences after discussing available clinical evidence.

Please review the complete therapeutic recommendations, including Key Recommendations for Pathologists that are located here (2).

Nomenclature

<table>
<thead>
<tr>
<th>Common gene symbols</th>
<th>Alternative gene symbols</th>
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<tr>
<td>EGFR</td>
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</tr>
<tr>
<td></td>
<td>ERBBB1</td>
</tr>
<tr>
<td></td>
<td>ERBBB</td>
</tr>
<tr>
<td></td>
<td>HER1</td>
</tr>
<tr>
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<td>HER2</td>
</tr>
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<td>HER4</td>
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Acknowledgments

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Trastuzumab (Herceptin) Therapy and ERBB2 (HER2) Genotype

David G. Hicks, Director of Surgical Pathology and Professor of Pathology and Laboratory Medicine at the University of Rochester Medical Center

Stanley Lipkowitz, Chief of the Women’s Malignancies Branch, National Cancer Institute

Tracy G. Lively, Deputy Associate Director of the Cancer Diagnosis Program, National Cancer Institute

References


22. V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOCENE HOMOLOG 2; ERBB2, in OMIM.


Related Summaries by Gene

Pertuzumab Therapy and ERBB2 (HER2) Genotype

Related Summaries by Drug Class

Pertuzumab Therapy and ERBB2 (HER2) Genotype
Tests in GTR by Gene

ERBB2 gene
Vemurafenib Therapy and *BRAF* and *NRAS* Genotype

Laura Dean, MD

Created: August 15, 2017.

**Introduction**

Vemurafenib is a kinase inhibitor used in the treatment of patients with unresectable or metastatic melanoma with the *BRAF V600E* variant.

*BRAF* is an intracellular kinase in the mitogen-activated protein kinases (MAPK) pathway. *BRAF* is involved in regulating important cell functions such as cell growth, division, differentiation, and apoptosis. *BRAF* is also a proto-oncogene—when mutated it has the ability to transform normal cells into cancerous cells.

Variation in the kinase domain of *BRAF* have been associated with various cancers. The most common *BRAF* variant, V600E, constitutively activates the kinase, and causes cell proliferation in the absence of growth factors that would normally be required. The V600E variant is detected in approximately 50% of melanomas (1, 2).

The FDA-approved drug label for vemurafenib states that the presence of *BRAF V600E* mutation in tumor specimens should be confirmed, using an FDA-approved test, before starting treatment with vemurafenib. The label also states that vemurafenib is not indicated for treatment of patients with wild-type *BRAF* melanoma (3).

Variations in *NRAS*, also an oncogene, are found in up to 30% of all malignancies and in approximately 15-20% of melanomas. *NRAS* variants activate MAPK and have been implicated in in acquired resistance to *BRAF* inhibitors. Vemurafenib’s label warns that one adverse effect associated with therapy may be the progression of pre-existing chronic myelomonocytic leukemia with *NRAS* mutation (3). Other adverse effects include arthralgia, rash, alopecia, photosensitivity reaction, pruritus, and skin papilloma.

**Drug: Vemurafenib**

Vemurafenib is a *BRAF* kinase inhibitor indicated for the treatment of patients with unresectable or metastatic melanoma with the *BRAF V600E* variant, as detected by an FDA-approved test. It was one of the first molecularly targeted agents to receive FDA approval for advanced melanoma (3). Off-label uses of vemurafenib include the treatment of other *BRAF V600E* positive tumors that are not responding to traditional treatments, e.g., refractory hairy cell leukemia (4).

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1 NCBI; Email: dean@ncbi.nlm.nih.gov.
Skin cancer is the most common of all cancers. Although melanoma is the least common type of skin cancer, accounting for approximately 1% of cases, it is responsible for the majority of deaths from skin cancer. In the US, the lifetime risk of melanoma is approximately 2.5% for whites, 0.5% for Hispanics, and 0.1% for blacks (5).

Most cases of malignant melanoma are diagnosed at an early stage, when the tumor is localized and surgical excision can be curative. However, the 5-year survival rate drops from 98% for localized disease, to only 16% for patients with metastatic disease.

For patients with advanced metastatic or unresectable malignant melanoma, treatment options typically include immunotherapy and targeted therapy. Although chemotherapy was once widely used, it does not increase survival and therefore its use is now limited to patients who are not candidates for further treatment with either immunotherapy or targeted therapy, and for whom there is no appropriate clinical trial.

High-dose interleukin2 (IL2) therapy may be successful in a minority of cases, but can only be used in select patients with good organ function because of the risk of severe toxicity. Immunotherapy drugs include antibodies that target programmed cell death protein 1 (PD1), e.g., nivolumab and pembrolizumab (6); and ipilimumab, a monoclonal antibody that targets cytotoxic T-lymphocyte-associated protein 4 (CTLA4). Oncolytic virus therapy with T-VEC (talimogene laherparepvec) is one of the newer immunotherapy drugs approved for melanoma.

Targeted therapies are designed to inhibit components of the MAPK signaling pathway, primarily when it is constitutively activated in melanomas with the activating BRAF mutation, V600E. Drugs in this category include vemurafenib and dabrafenib, which inhibit BRAF, and trametinib and cobimetinib, which target downstream kinases MEK1 and MEK2, respectively.

Vemurafenib is a potent inhibitor of the kinase domain of the variant BRAF V600E. It acts by decreasing signaling through the MAPK pathway, leading to the reduced transcription of genes involved in various cellular responses. Combining vemurafenib with MEK inhibitors may potentiate these effects and has been shown to extend survival (7, 8).

Both targeted therapy with vemurafenib and immunotherapy regimens (e.g., nivolumab plus ipilimumab) have been shown to improve overall survival in patients with metastatic melanoma compared with chemotherapy (9, 10). However, at this time there are no randomized trials that compare targeted therapy with immunotherapy, and there are little data regarding the appropriate combinations and sequencing of these therapies for patients with a BRAF V600E variant.

In the BRIM3 trial, vemurafenib improved overall survival (13.6 versus 9.7 months) and progression-free survival (6.9 versus 1.6 months) when compared to cytotoxic chemotherapy (dacarbazine)(11). However, virtually every patient treated with a BRAF inhibitor eventually demonstrated disease progression (12). Most patients developed mechanisms of acquired resistance, which is sometimes associated with NRAS variants, and approximately 15% of patients did not achieve tumor regression at all (11, 13-17).
The most common adverse events associated with vemurafenib are skin lesions (benign and malignant), fever, arthralgia, and fatigue. Skin lesions, such as cutaneous squamous cell carcinoma, tend to occur during the first 8 weeks of treatment. Regular evaluation of the skin is recommended, with excision of suspicious lesions (18). Liver enzymes (transaminases, alkaline phosphatase, and bilirubin) should also be monitored because of the risk of liver injury. Combining BRAF with MEK inhibitors helps reduce the odds of these side effects.

Approximately 50% of cases of metastatic melanoma are found to have the \textit{BRAF} V600E activating variant (1, 2). Because vemurafenib targets the kinase with this variant, patients without \textit{BRAF} variants or with a different type of \textit{BRAF} variant (e.g., V600K) should not be treated with vemurafenib; they will not benefit from vemurafenib therapy and will be needlessly exposed to adverse events. In addition, the FDA drug label warns that BRAF inhibitors have been shown to increase cell proliferation in \textit{BRAF} wild-type cells \textit{in vitro}.

\textbf{Gene: \textit{BRAF}}

\textit{RAF} is a family of intracellular kinases within the MAPK signaling pathway. The \textit{RAF} family has three members, ARAF, BRAF, and CRAF (19). \textit{RAF}, along with RAS (see below), are proto-oncogenes.

Proto-oncogenes are genes that, when mutated or expressed at abnormally high levels, can transform normal cells into cancerous cells. Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. The increased production of oncogenic proteins can lead to the proliferation of poorly differentiated cancer cells (20).

Germline mutations in \textit{BRAF}, as well as other components of the MAPK signaling pathway, are associated with birth defects, such as cardiofaciocutaneous syndrome, characterized by heart defects, mental retardation, and a distinctive facial dysmorphology. Somatic \textit{BRAF} mutations are also associated with several malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, colorectal carcinoma, and malignant melanoma.

Variations in \textit{BRAF} are detectable in approximately 50% of malignant melanomas, and drive progression of the disease (1, 2). The \textit{BRAF} variant V600E accounts for approximately 90% of variants. This variant is a substitution of adenine for thymine at position 1799 and results in the substitution of valine for glutamate at codon 600. The variant \textit{BRAF} protein kinase is constitutively active and a highly potent oncogene, with an increase in kinase activity by as much as 500-fold compared to the wild-type (21). The second most common \textit{BRAF} variant is V600K. Substitutions at other sites are rarer (22, 23).

Several drugs are being developed to target \textit{BRAF} mutations, and so far, two drugs have been FDA-approved: vemurafenib and dabrafenib. Unfortunately, less progress has been made in developing targeted therapies for melanoma with wild-type \textit{BRAF}. There are
fewer treatment options available, but these include immunotherapy and MEK inhibitors (6, 24).

**Gene: NRAS**

The RAS family contains three genes, HRAS, NRAS, and KRAS, which are essential components of a number of signaling pathways. They act as signal transducers, coupling cell surface receptors to intracellular signaling pathways.

RAS proteins have intrinsic GTPase activity, they are activated by a guanine nucleotide-exchange factor, and inactivated by a GTPase activating protein. RAS proteins regulate cell signal transduction by acting as a switch; they cycle between "on" (GTP-bound) or "off" (GDP-bound) conformations. In the "on" position, RAS proteins transmit extracellular growth signals to the nucleus, primarily via the MAPK pathway. Cells are subsequently stimulated to grow, divide, mature, and differentiate.

Variations in RAS genes lead to RAS proteins that are resistant to GTPase, so that GTP-remains permanently bound and the receptor remains "on" providing a continual growth stimulus to cells. Such activating RAS variants are common, having been detected in colorectal cancer, lung cancer, pancreatic cancer, and melanoma.

Variations in NRAS are detectable in 15–30% of melanomas, clustering at codons 12, 13, and 61 (25, 26). These NRAS variants are the second most common oncogenic “driver” mutation in malignant melanomas, behind alternations in *BRAF* (26).

NRAS variants are associated with more aggressive melanomas, and generally a poorer prognosis (26). Currently, no therapies that specifically target NRAS have been approved. However, in the near future newer targeted therapies will likely provide effective treatment options for NRAS-variant melanoma (26, 27). Off-label, MEK inhibitors, especially in combination with other agents, have exhibited some efficacy in NRAS-variant melanoma.

NRAS variants are also associated with a number of other conditions, including Noonan syndrome (type 6), somatic rectal cancer, follicular thyroid cancer, autoimmune lymphoproliferative syndrome, and juvenile myelomonocytic leukemia.

**Genetic Testing**

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the genes *BRAF* and *NRAS*.

The FDA-approved label for vemurafenib states that the presence of the *BRAF* V600E mutation should be confirmed in tumor specimens using an FDA-approved test before starting treatment with vemurafenib. The label also states that vemurafenib is not indicated for treatment of patients with wild-type *BRAF* melanoma.
Therapeutic Recommendations based on Genotype

This section contains excerpted\(^1\) information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

**2016 Statement from the US Food and Drug Administration (FDA):**

Vemurafenib is indicated for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E mutation as detected by an FDA-approved test.

Limitation of Use: Vemurafenib is not indicated for treatment of patients with wild-type BRAF melanoma.

Patient Selection: Confirm the presence of BRAF V600E mutation in tumor specimens prior to initiation of treatment with Vemurafenib. Information on FDA-approved tests for the detection of BRAF V600 mutations in melanoma is available at [http://www.fda.gov/CompanionDiagnostics](http://www.fda.gov/CompanionDiagnostics).

Please review the complete therapeutic recommendations that are located here: (3)

**Nomenclature**

**Selected BRAF variants**

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<th>dbSNP reference identifier for allele location</th>
</tr>
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Table continues on next page...
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

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**References**


Related Summaries by Gene
Dabrafenib Therapy and BRAF and G6PD Genotype

Related Summaries by Drug Class
Dabrafenib Therapy and BRAF and G6PD Genotype

Tests in GTR by Gene
NRAS gene
BRAF gene
Venlafaxine Therapy and CYP2D6 Genotype

Laura Dean, MD

Created: July 27, 2015.

Introduction

Venlafaxine is an antidepressant used in the treatment of major depressive order, anxiety, and panic disorders. Venlafaxine belongs to the drug class of serotonin and norepinephrine reuptake inhibitors (SNRIs) (1).

The CYP2D6 enzyme metabolizes a quarter of all prescribed drugs, including venlafaxine. This enzyme converts venlafaxine to the active metabolite, O-desmethylvenlafaxine (ODV). Individuals who carry two inactive copies of CYP2D6 (“poor metabolizers”) may have decreased capacity to metabolize venlafaxine, resulting in less active metabolites in their system. In contrast, individuals who carry more than two copies of functional CYP2D6 alleles (“ultrarapid metabolizers”) may have an enhanced capacity to metabolize venlafaxine, resulting in more increased active metabolites in their system.

The FDA states that because the total exposure of venlafaxine and ODV is similar in poor and extensive (normal) metabolizers, there is no need for different venlafaxine dosing regimens for these individuals (1). However, the Dutch Pharmacogenetics Working Group recommends that both poor and intermediate metabolizer genotypes should be treated with an alternative drug, or lower doses of venlafaxine based on clinical response and drug levels. For ultrarapid metabolizer genotypes, they recommend that either the dose of venlafaxine be increased up to 150% of the normal dose, or an alternative drug used (see Table 1 and 2) (2).

Table 1. CYP2D6 phenotypes and therapeutic recommendations for venlafaxine therapy

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Recommendations for venlafaxine therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>More than two copies of functional alleles</td>
<td>Be alert to decreased venlafaxine and increased O-desmethylvenlafaxine plasma concentration. Titrate dose to a maximum of 150% of the normal dose or select alternative drug (e.g., citalopram, sertraline).</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>One active allele and one inactive allele, or two decreased activity alleles, or one decreased activity</td>
<td>Insufficient data to allow calculation of dose adjustment. Select alternative drug (e.g., citalopram, sertraline) or adjust dose to clinical response and monitor O-desmethylvenlafaxine plasma concentration</td>
</tr>
</tbody>
</table>


Table 1. continues on next page...

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
Table 1. continued from previous page.

<table>
<thead>
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<th>Phenotype</th>
<th>Genotype</th>
<th>Recommendations for venlafaxine therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor metabolizer</td>
<td>allele and one inactive allele</td>
<td>Insufficient data to allow calculation of dose adjustment. Select alternative drug (e.g., citalopram, sertraline) or adjust dose to clinical response and monitor O-desmethylvenlafaxine plasma concentration.</td>
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Table 2. Activity status of CYP2D6 alleles

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<tr>
<td>Active</td>
<td>*1, *2, *33, *35</td>
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Drug: Venlafaxine

Venlafaxine is an antidepressant that is used for the treatment of a range of psychiatric disorders that include major depressive disorder, generalized anxiety disorder (GAD), social anxiety disorder, and panic disorder (1).

Venlafaxine is thought to exert its antidepressant effect by blocking the transporter reuptake proteins for key neurotransmitters affecting mood, thereby leaving more active neurotransmitters in the synapse. This is known as the “potentiation of neurotransmission.”

Venlafaxine belongs to the drug class of serotonin-norepinephrine reuptake inhibitors (SNRIs). However, because venlafaxine also weakly inhibits dopamine reuptake, it is also referred to as a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI).

Venlafaxine is metabolized in the liver to its major active metabolite, O-desmethylvenlafaxine (ODV). Venlafaxine and ODV are both potent inhibitors of neuronal serotonin and norepinephrine reuptake and weak inhibitors of dopamine reuptake. The formation of ODV is catalyzed by the enzyme CYP2D6. A high ratio of venlafaxine to ODV is a marker of low CYP2D6 activity. Other hepatic enzymes (CYP3A4, CYP2C19, and CYP2C9) metabolize venlafaxine and ODV to minor, less active metabolites (1).

As for all antidepressants, the FDA-approved drug label for venlafaxine includes a black box warning about the risk of suicide: “Antidepressants increased the risk compared to placebo of suicidal thinking and behavior (suicidality) in children, adolescents, and young adults in short-term studies of Major Depressive Disorder (MDD) and other psychiatric disorders.”
disorders. Anyone considering the use of venlafaxine or any other antidepressant in a child, adolescent, or young adult must balance this risk with the clinical need.” (1)

The toxicity of venlafaxine appears to be higher than for other drugs of the same class. Adverse events include an increase in anxiety, insomnia, and nervousness; the precipitation of mania or hypomania in patients with bipolar disorder; weight loss, reduced appetite, hyponatremia, seizures, cardiac conduction abnormalities, and an increased risk of bleeding events. There is also a risk of discontinuation syndrome, which may occur if therapy is stopped abruptly (a gradual reduction in the dose of venlafaxine is recommended whenever possible) (1, 3).

**Gene: CYP2D6**

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. The CYP2D6 gene is highly polymorphic, with more than 100 star (*) alleles described (4).

*CYP2D6*1 is the wild-type allele and is associated with normal enzyme activity and the “extensive metabolizer” phenotype. The *CYP2D6*2, *33, and *35 alleles are also considered to have near-normal activity. Other alleles include variants that produce a non-functioning enzyme (e.g., *3, *4, *5, and *6) (5-8) or an enzyme with reduced activity (e.g., *10, *17, and *41) (9-11) (see Table 2). There are large inter-ethnic differences in the frequency of these alleles, with *3, *4, *5, *6, and *41 being more common in Caucasians, *17 more common in Africans, and *10 more common in Asians (12).

Individuals who are intermediate or poor metabolizers carry copies of decreased-function and inactive *CYP2D6* alleles (see Table 1 and 2). In these individuals, the metabolic capacity of CYP2D6 is decreased, which may result in higher levels of venlafaxine and lower levels of ODV.

The FDA-approved drug label for venlafaxine states that although poor metabolizers have increased levels of venlafaxine and decreased levels of ODV compared to individuals with normal CYP2D6 activity, the differences between poor and extensive (normal) metabolizers are not thought to be clinically important because “the sum of venlafaxine and ODV is similar in the two groups and venlafaxine and ODV are pharmacologically approximately equiactive and equipotent.” (1) However, the results of some reported studies suggest that side effects are more common in poor metabolizers, and that *CYP2D6* genotyping prior to the initiation of venlafaxine may prevent potential side effects (13, 14). Some of the adverse effects of venlafaxine therapy that have been reported to occur more frequently in poor metabolizers include gastrointestinal side effects, such as
vomiting and diarrhea; and cardiovascular side effects, such as hypertension, tachycardia, and prolonged QTc interval (14, 15).

The Dutch Pharmacogenetics Working Group recommendations state that for poor and intermediate metabolizers, there is insufficient data to calculate the dose adjustment for venlafaxine, and an alternative drug should be used (e.g., citalopram, sertraline). Or, the dose of venlafaxine should be adjusted according to the clinical response, and ODV plasma levels should be monitored (2).

Poor metabolizers are commonly found in European Caucasians. The functional CYP2D6*1 allele is the most common (~70%), and the most common nonfunctional alleles include CYP2D6*4 and *5, which largely account for the poor metabolizer phenotype in these populations (16, 17).

In individuals of Asian descent, only about 50% of CYPD6 alleles are functional, with the reduced function CYP2D6*10 variant being very common (~40%). As a result, Asians are more likely to be intermediate metabolizers than Caucasians (12). Similarly, in Africans and African Americans, only 50% of CYPD6 alleles are functional; however, a wider range of variants account for the remaining alleles (18, 19).

Individuals who have multiple functional copies of the CYP2D6 gene are “ultrarapid metabolizers” (UM). Each allele contributes to the metabolism of venlafaxine to the active metabolite, ODV. Data suggest that the ultrarapid metabolizer phenotype does not have a significant effect on treatment with venlafaxine (efficacy or side effects) but as a precaution, drug levels should be monitored and an increased dose of venlafaxine may be required (13, 14, 20). The Dutch Pharmacogenetics Working Group recommendations state that for ultrarapid metabolizers, there is a need to be alert to decreased venlafaxine and increased ODV concentrations. The dose of venlafaxine should be titrated to a maximum of 150% of the normal dose, or an alternative drug (e.g., citalopram, sertraline) should be considered (2), in patients with normal renal clearance (21).

The ultrarapid metabolizer phenotype is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; ~10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (22).

**Genetic Testing**

Genetic testing is available for many of the more common variant CYP2D6 alleles. Results are typically reported as a diplotype, such as CYP2D6 *1/*1 (23). A result for copy number, if available, is also important when interpreting CYP2D6 results.

If the test results include an interpretation of the patient’s predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- An extensive (normal) metabolizer phenotype has an activity score of 1 to 2
Therapeutic Recommendations based on Genotype

This section contains excerpted information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA): Plasma concentrations of venlafaxine were higher in CYP2D6 poor metabolizers than extensive metabolizers. Because the total exposure (AUC) of venlafaxine and ODV was similar in poor and extensive metabolizer groups, however, there is no need for different venlafaxine dosing regimens for these two groups.

Please review the complete therapeutic recommendations that are located here: (1).

Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): For individuals who are poor or intermediate metabolizers, there are insufficient data to calculate a dose adjustment for venlafaxine. Select an alternative drug (e.g., citalopram, sertraline), or adjust the dose of venlafaxine based on the clinical response, and monitor (O-desmethyl)venlafaxine plasma concentration. For individuals who are ultrarapid metabolizers, physicians should be alert to decreased venlafaxine and increased (O-desmethyl)venlafaxine plasma concentration. The dose of venlafaxine should be titrated up to a maximum of 150% of the normal dose or an alternative drug used (e.g., citalopram, sertraline).

Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature

<table>
<thead>
<tr>
<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
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</tr>
</tbody>
</table>

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Table continues on next page...

† The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.
<table>
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<th>Common allele name</th>
<th>Alternative names</th>
<th>HGVS reference sequence</th>
<th>dbSNP reference identifier for allele location</th>
</tr>
</thead>
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</table>

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to as 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): [http://www.hgvs.org/content/guidelines](http://www.hgvs.org/content/guidelines)

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: [http://www.cypalleles.ki.se/](http://www.cypalleles.ki.se/)

### Acknowledgments

The author would like to thank Stuart Scott, Assistant Professor of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai; and Bruce G. Pollock, Vice President of Research and Director of the Campbell Family Mental Health Research Institute, CAMH and Professor of Psychiatry & Pharmacology at the University of Toronto; for reviewing this summary.

### References


Related Summaries by Gene

Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype

Aripiprazole Therapy and CYP2D6 Genotype

Atomoxetine Therapy and CYP2D6 Genotype

Clozapine Therapy and CYP2D6, CYP1A2, and CYP3A4 Genotypes

Codeine Therapy and CYP2D6 Genotype

Imipramine Therapy and CYP2D6 and CYP2C19 Genotype

Metoprolol Therapy and CYP2D6 Genotype

Propafenone Therapy and CYP2D6 Genotype

Risperidone Therapy and CYP2D6 Genotype

Tamoxifen Therapy and CYP2D6 Genotype

Thioridazine Therapy and CYP2D6 Genotypes

Tramadol Therapy and CYP2D6 Genotype

Tests in GTR by Condition

Venlafaxine response
Tests in GTR by Gene

CYP2D6 gene
Warfarin Therapy and the Genotypes CYP2C9 and VKORC1

Laura Dean, MD

Created: March 8, 2012; Updated: June 8, 2016.

Introduction

Warfarin is an anticoagulant that acts by reducing the activity of vitamin K-dependent clotting factors. It is used in the prevention and treatment of thrombotic disorders. The dose of warfarin must be tailored for each patient according to the patient's INR response and the condition being treated.

A patient's CYP2C9 and VKORC1 genotype can be used to help determine the optimal starting dose of warfarin. The CYP2C9 gene encodes one of the main enzymes involved in the metabolism of warfarin. Several variant CYP2C9 alleles are associated with reduced enzyme activity and lower clearance rates of warfarin. Patients who carry at least one copy of such a variant allele (such as CYP2C9*2 and CYP2C9*3) have reduced metabolism leading to higher warfarin concentrations. On average, they require a lower daily warfarin dose than patients who are homozygous for the wild-type CYP2C9*1 allele.

The VKORC1 gene encodes the vitamin K epoxide reductase enzyme, the target of warfarin. Patients who carry the -1639G>A polymorphism in the promoter region of the VKORC1 gene are more sensitive to warfarin and require lower doses.

The FDA-approved warfarin drug label provides a dosing table based on CYP2C9 and VKORC1 genotypes (Table 1). The label states if the patient's CYP2C9 and/or VKORC1 genotype are known, to consider these ranges in choosing the initial doses, but whether this strategy reduces warfarin-related adverse events is controversial. The label also states that patients with CYP2C9 *1/*3, *2/*2, *2/*3, and *3/*3 may require more time (longer than 2 to 4 weeks) to achieve maximum INR effect for a given dosage regimen than patients without these CYP variants (1).

However, the Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends that this dosing table should only be used when electronic access is not possible. Instead, CPIC recommends that whenever possible, the pharmacogenetic algorithms available on http://www.warfarindosing.org should be used to predict the optimal warfarin dose (2). Although one randomized trial found that genotype-guided dosing might improve INR control after warfarin initiation (3), the largest completed trial found no benefit. (4). The largest trial of pharmacogenetic dosing of warfarin
(ClinicalTrials.gov Identifier: NCT01006733) is expected to have results in December 2016.

Table 1. Three Ranges of Expected Maintenance Warfarin Doses based on CYP2C9 and VKORC1 Genotypes, adapted from the FDA drug label.

<table>
<thead>
<tr>
<th>VKORC1</th>
<th>CYP2C9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*1/*1</td>
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<tr>
<td>GG</td>
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</tr>
<tr>
<td>AG</td>
<td>5-7 mg</td>
</tr>
<tr>
<td>AA</td>
<td>3-4 mg</td>
</tr>
</tbody>
</table>

Ranges are derived from multiple published clinical studies. VKORC1 –1639G>A (rs9923231) variant is used in this table. Other co-inherited VKORC1 variants may also be important determinants of warfarin dose. This table is adapted from the FDA-approved drug label for Coumadin (warfarin) (1).

Drug: Warfarin

Warfarin is an anticoagulant used in the prevention and treatment of venous thrombosis, pulmonary embolism, and the complications associated with atrial fibrillation and/or cardiac valve replacement. Warfarin is sometimes prescribed to reduce the risk of stroke after a myocardial infarction (MI).

Warfarin has no direct effect on an established thrombus. However, once a thrombus has occurred (e.g., deep venous thrombosis), the goal of warfarin therapy is to prevent further extension of the formed clot and to prevent secondary thromboembolic complications that may be fatal (e.g., pulmonary embolism).

Warfarin exerts its anticoagulant effect by inhibiting the enzyme encoded by VKORC1, which catalyzes the conversion of vitamin K epoxide to the active reduced form of vitamin K, vitamin K hydroquinone. Vitamin K hydroquinone is an essential cofactor in the synthesis of several clotting factors—it promotes the synthesis of γ-carboxyglutamic acid residues in the proteins essential for biological activity. The decreased availability of vitamin K hydroquinone leads to decreased activity of the clotting factors II, VII, IX, and X, and the anticoagulant proteins C and S (5).

Warfarin is administered as a racemic mixture of the R and S stereoisomers. (S)-warfarin is two to five times more potent than (R)-warfarin, and is mainly metabolized by CYP2C9. (R)-warfarin is mainly metabolized via CYP3A4, with involvement of several other cytochrome P450 enzymes (6).

The initial and maintenance dosing of warfarin must be individualized for each patient. The goal of warfarin therapy is to achieve an international normalized ratio (INR) in a target range for the condition being treated (most commonly 2-3). This involves selecting an initial starting dose, followed by regular testing of the INR so that the dose of warfarin can be adjusted until the appropriate daily maintenance dose is determined. In general,
the duration of anticoagulant therapy varies by clinical indication and should be continued until the danger of thrombosis and embolism has passed.

Selecting the initial dose of warfarin should be based on the expected maintenance dose, having taken into account the factors known to influence warfarin dose. Using an optimal starting dose for an individual may reduce the time taken to reach a stable INR, and reduce the risk of having either a high INR (with a risk of bleeding) or a low INR (with a risk of thrombosis) (2). Appropriate dosing of warfarin varies widely between individuals, and not all factors responsible for the variability in warfarin dose are known or easily quantified.

Known factors that influence an individual's response to the first dose of warfarin include clinical factors (e.g., age, race, body weight, sex, concomitant medications—including those that compete for binding to albumin, comorbidities, diet, nutritional status) and genetic factors (e.g., CYP2C9 and VKORC1 genotypes). Therefore, the initial dose should be modified to take into account these and any additional patient-specific factors that may influence warfarin response.

The FDA-approved drug label for warfarin suggests considering a lower initial and maintenance dose of warfarin for elderly and/or debilitated patients, and in Asian patients. The drug label recommends against the routine use of loading doses because this practice may increase hemorrhagic and other complications and does not offer more rapid protection against clot formation.

Warfarin can cause major or fatal bleeding. Bleeding is more likely to occur within the first month, and the risk factors include a high intensity of anticoagulation (INR greater than 4), age greater than or equal to 65, and a history of highly variable INRs. Other serious adverse events associated with warfarin therapy include necrosis of the skin and other tissues, particularly when used prematurely to manage thrombosis associated with heparin-induced thrombocytopenia (HIT).

**Gene: CYP2C9**

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

CYP450 isoenzymes involved in the metabolism of warfarin include CYP2C9 and CYP3A4. The more potent warfarin S-enantiomer is metabolized by CYP2C9 while the R-enantiomer is metabolized by CYP1A2 and CYP3A4. The FDA-drug label for warfarin states that drugs that inhibit or induce CYP2C9, CYP1A2, and/or CYP3A4 have the potential to alter the effect (INR) of warfarin by altering the exposure of warfarin.

**CYP2C9*1** is the wild-type allele and is associated with normal enzyme activity and the normal metabolizer phenotype.
Two common allelic variants associated with reduced enzyme activity are CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu). Compared to normal metabolizers, patients who inherit one or two copies of *2 or *3 are more sensitive to warfarin—they require lower doses and are at a greater risk of bleeding during warfarin initiation (7-10).

The frequencies of the CYP2C9 alleles vary between different ethnic groups (11-13). The *2 allele is more common in Caucasian (10-20%) than Asian (1-3%) or African (0-6%) populations (14). The *3 allele is less common (<10% in most populations) and extremely rare in African populations (15). In African Americans, it is likely that other CYP2C9 variants such as CYP2C9*5, *6, *8, and *11 contribute to the variability in patient response to warfarin (2).

**Gene: VKORC1**

The VKORC1 gene encodes the vitamin K epoxide reductase enzyme. It catalyzes the rate-limiting step in vitamin K recycling, and it is the target of the drug warfarin.

A common non-coding variant, -1639G>A, is associated with an increased sensitivity to warfarin (16). The polymorphism occurs in the promoter region of VKORC1 and is thought to alter a transcription factor binding site, leading to lower protein expression. As a result, patients starting warfarin therapy who are −1639A carriers require lower initial and maintenance doses of the drug than −1639G carriers.

The −1639G>A allele frequency varies among different ethnic groups. It is the major allele (around 90%) in Asian populations, and may be a contributing factor for lower warfarin dosing requirements often observed in patients of Asian descent. It is also common in Caucasians (around 40%) and African Americans (around 14%) (17-19).

Less commonly, missense mutations in VKORC1 can lead to warfarin resistance (20, 21).

**Genetic Testing**

VKORC1 and CYP2C9 genotypes are the most important genetic determinants of warfarin dosing. The contribution of VKORC1 to the variation in dose requirement is larger (approximately 30%) than the contribution of CYP2C9 (usually less than 10%) (22).

Individuals who are most likely to benefit from genetic testing are those who have yet to start warfarin therapy. However, genotype-guided warfarin dosing is not the standard of care in most healthcare systems, and most (but not all) recent studies have reported that, in general, the use of genotype-guided dosing algorithms did not improve anticoagulation control in the first few weeks of warfarin therapy (4, 23-27).

Genetic testing is available for CYP2C9 and VKORC1. The variants that are routinely tested for are CYP2C9*2, CYP2C9*3, and −1639G>A. These variants are used in the FDA table to guide therapy, and also in the International Warfarin Pharmacogenomics Consortium (IWPC) algorithm.
Other variants that are not routinely tested for include the CYP2C9*6 and *8, alleles, the genes CYP4F2, EPHX1, and GGX (which all have a role in the vitamin-K cycle), and the gene CALU (a cofactor in the VKOR complex) (2, 28). Including these additional genotypes in an expanded dosing algorithm improves warfarin dose prediction in African-Americans, while maintaining high performance in European-Americans (29).

Therapeutic Recommendations based on Genotype

This section contains excerpted1 information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA):

Dosing Recommendations without Consideration of Genotype

If the patient's CYP2C9 and VKORC1 genotypes are not known, the initial dose of warfarin is usually 2 to 5 mg once daily. Determine each patient’s dosing needs by close monitoring of the INR response and consideration of the indication being treated. Typical maintenance doses are 2 to 10 mg once daily.

Dosing Recommendations with Consideration of Genotype

Table 1 displays three ranges of expected maintenance COUMADIN doses observed in subgroups of patients having different combinations of CYP2C9 and VKORC1 gene variants [...]. If the patient's CYP2C9 and/or VKORC1 genotype are known, consider these ranges in choosing the initial dose. Patients with CYP2C9 *1/*3, *2/*2, *2/*3, and *3/*3 may require more prolonged time (>2 to 4 weeks) to achieve maximum INR effect for a given dosage regimen than patients without these CYP variants.

Please review the complete therapeutic recommendations that are located here: (1)

2014 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): The pharmacogenetic algorithms available on http://www.warfarindosing.org should be used whenever possible to determine the dose of warfarin required. Such algorithms have been derived from large studies across different ethnic populations, and they take into account both the genetic and non-genetic factors that influence the variability in warfarin response. The existence of rare genetic variants may be responsible for individuals whose warfarin dosing is not well predicted. However, overall the dosing equations are well validated and fairly precise. Only if electronic access to a pharmacogenetic algorithm is not possible should the table-based dosing approach be used, which is preferable to a fixed-dose approach.

1 The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.
Please review the complete therapeutic recommendations that are located here: (2, 30).

Table 2. Recommended daily warfarin doses (mg/day) to achieve a therapeutic INR based on CYP2C9 and VKORC1 genotype using the warfarin product insert approved by the US Food and Drug Administration

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<tr>
<td>GG</td>
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Nomenclature

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<td>NP_000762.2:p.Arg144Cys</td>
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Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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The author would like to thank Brian F. Gage, MD, MSC, Professor of Medicine, Washington University, St. Louis; and Sol Schulman, MD, Clinical Fellow in Medicine, Division of Hemostasis and Thrombosis, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston; for reviewing this summary.

First edition:

The Pharmacogenomics Knowledgebase: http://www.pharmgkb.org
The Clinical Pharmacogenetics Implementation Consortium: http://www.pharmgkb.org/page/cpic

References


Related Summaries by Gene

Celecoxib Therapy and CYP2C9 Genotype

Phenytoin Therapy and HLA-B*15:02 and CYP2C9 Genotypes

Prasugrel Therapy and CYP Genotype

Tests in GTR by Condition

Warfarin response

Tests in GTR by Gene

CYP2C9 gene

VKORC1 gene
Genetic variants and disease
ABO Blood Group

Laura Dean, MD

Created: October 1, 2012; Updated: July 27, 2015.

Characteristics

There are four common blood groups in the ABO system: O, A, B, and AB. The blood groups are defined by the presence of specific carbohydrate sugars on the surface of red blood cells, N-acetylgalactosamine for the A antigen, and D-galactose for the B antigen. Both of these sugars are built upon the H antigen—if the H antigen is left unmodified, the resulting blood group is O because neither the A nor the B antigen can attach to the red blood cells.

Individuals will naturally develop antibodies against the ABO antigens they do not have. For example, individuals with blood group A will have anti-B antibodies, and individuals with blood group O will have both anti-A and anti-B. Before a blood transfusion takes place, routine serological testing checks the compatibility of the ABO (and Rh) blood groups. An ABO incompatible blood transfusion can be fatal, due to the highly immunogenic nature of the A and B antigens, and the corresponding strongly hemolytic antibodies (1).

Compared to other blood groups, individuals with blood group O may have a lower risk of pancreatic cancer and thromboembolic disease (2, 3). In addition, in certain African populations, individuals with the blood group O may be protected from life-threatening malaria (4). However, this blood group is not more common in some regions where malaria is endemic. This might be because individuals with blood group O are at higher risk of cholera and severe diarrhea due to Vibrio cholerae 01, with individuals with the AB blood group being the most protected (5, 6).

Over 80 ABO alleles have been reported. The common alleles include A1, A2, B1, O1, O1v, and O2 (7). Whereas the A and B alleles each encode a specific glycosyl-transferring enzyme, the O allele appears to have no function. A single-base deletion in the O allele means that individuals with blood group O do not produce either the A or B antigens. Blood type frequencies vary in different racial/ethnic groups. In the US, in Caucasians, the ratio of blood group O, A, B, and AB is 45%, 40%, 11%, and 4% respectively. In Hispanics, the distribution is 57%, 31%, 10%, and 3%; and in Blacks, 50%, 26%, 20%, and 4% (8).
Diagnosis/testing

Serological testing is sufficient to determine an individual's blood type (e.g., blood group A) for the purposes of blood donation and transfusion. Molecular genetic testing can be used to determine an individual's ABO genotype (e.g., genotype AO or AA). This may be useful in the research setting, for example, to investigate the link between ABO blood groups and particular diseases, and also in the forensic setting (9).

Management

Determining an individual's blood group is important prior to blood transfusion and prior to the donation or receiving of a kidney transplant.

Occasionally, a person's blood type may appear to change. For example, the ABO antigens can act as tumor markers. Their presence may be decreased in particular diseases, such as acute myeloid leukemia, AML (10). In contrast, occasionally the B antigen may be acquired in certain infectious diseases. A bacterial infection with specific strains of E. coli or Clostridium tertium can generate a B-like antigen from an individual who has the A1 allele (11).

Genetic counseling

The ABO blood type is inherited in an autosomal codominant fashion. The A and B alleles are codominant, and the O allele is recessive.

Acknowledgments

The author would like to thank Michael Murphy, Professor of Blood Transfusion Medicine, University of Oxford, and Consultant Haematologist, NHS Blood & Transplant and Oxford University Hospitals, Oxford, UK, for reviewing this summary.

References


ACHOO Syndrome
Laura Dean, MD

Characteristics
Autosomal Dominant Compelling Helioophthalmic Outburst (ACHOO) Syndrome is characterized by uncontrollable sneezing in response to the sudden exposure to bright light, typically intense sunlight (1). This type of sneezing is also known as photic sneezing. About one in four individuals who already have a prickling sensation in their nose will sneeze in response to sunlight, but “pure” photic sneezing is far less common (2).

Sneezing is usually triggered by contact with infectious agents or after inhaling irritants, but the cause of photic sneezing is not fully understood. It may involve an over-excitability of the visual cortex in response to light, leading to a stronger activation of the secondary somatosensory areas (3).

Diagnosis/testing
The diagnosis of ACHOO syndrome is usually made by clinical history. Affected individuals report a “prickling sensation” or sneezing in response to a bright light. This response may be reproduced in the clinical setting by asking the individual to look at a bright light, although findings are unreliable.

The genetic basis of this syndrome is not yet known.

Management
Recommendations for management of ACHOO syndrome include using a hat or sunglasses to shield the eyes from direct sunlight whenever possible. Potential hazards include the possibility of drivers having an accident caused by sneezing brought on by, for example, exiting a road tunnel on a bright day. Similarly, airline pilots may be at risk (4).

Genetic counseling
ACHOO syndrome is inherited in an autosomal dominant manner (1). As such, if one parent is affected, their child has a 50% chance of inheriting the syndrome.

1 NCBI; Email: dean@ncbi.nlm.nih.gov.
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References

McCune-Albright Syndrome

Laura Dean, MD
Created: March 8, 2012; Updated: March 6, 2017.

Characteristics

McCune-Albright Syndrome (MAS) is a rare genetic disorder originally characterized as the triad of polyostotic fibrous dysplasia of bone, precocious puberty, and café-au-lait skin pigmentation (1-3). With time other associated endocrinopathies have been recognized, including hyperthyroidism, growth hormone excess, FGF23-mediated phosphate wasting, and hypercortisolism (4, 5).

MAS is caused by an activating mutation in the GNAS gene, which encodes the alpha subunit of the stimulatory G protein involved in G-protein signaling (6, 7). A missense mutation, typically Arg201Cys or Arg201His (NM_001077488.3:c.604C>T, rs11554273), impairs the intrinsic GTPase activity of the Gsα protein, resulting in the constitutive activation of the Gsα-cAMP signaling pathway in the cells that contain the mutation.

The mutation arises early in embryogenesis and is distributed in a mosaic pattern. The clinical phenotype is therefore highly variable, depending upon the location and timing of the mutation during embryologic development. Skin manifestations are common and are usually present at or shortly after birth. The café-au-lait spots typically have irregular margins giving them a “coast of Maine” appearance, and usually show an association with the midline of the body.

In MAS, fibrous dysplasia of bone typically occurs at several sites (polyostotic), and commonly presents with fracture, deformity and/or bone pain (8). Radiographs show characteristic expansile lesions with a “ground glass” appearance. Craniofacial fibrous dysplasia can be severe in individuals who have pituitary disorders leading to hypersecretion of growth hormone. Treatment can be challenging and should begin as soon as possible.

In girls, precocious puberty is a common initial manifestation, with recurrent ovarian cysts leading to episodes of vaginal bleeding and breast development. Precocious puberty is less common in boys, presenting with penile enlargement, pubic and axillary hair, acne, body odor, and sexual behavior. However, in both girls and boys, there is a high frequency of gonadal pathology (ovarian abnormalities in girls, and testicular abnormalities in boys) (9).

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Diagnosis

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the **GNAS** gene and the **McCune-Albright Syndrome**.

Currently, the diagnosis of McCune-Albright syndrome is made clinically in most cases. This is due to the mosaic nature of the disease whereby a negative genetic test result (e.g., in blood) does not exclude the presence of the mutation in other tissues. However, newer techniques such as digital PCR may improve the sensitivity of genetic testing in individuals who have clinical signs of McCune-Albright syndrome (10, 11).

Management

Treatment is individualized based on each patient’s clinical presentation. Letrozole (12) and/or tamoxifen (13) may be effective for treatment of precocious puberty in girls. Medications and/or surgery may be used for treatment of hyperthyroidism (14, 15), growth hormone excess (16, 17), and hypercortisolism (18). Management of fibrous dysplasia of bone is palliative, with surgery as needed for fracture and deformity (19, 20). Bisphosphonates are effective for treatment of fibrous dysplasia-related pain, but have not been shown to have any long-term effect on the course of the disease (21, 22).

Genetic Counseling

McCune-Albright syndrome is caused by a new (de novo) mutation that occurs after conception, at an early stage of development. Individuals with McCune-Albright syndrome have not been observed to pass the syndrome on to their children.

Acknowledgments

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Version History

To view an earlier version (8 March 2012), please click here.

References


Tests in GTR by Condition

McCune-Albright Syndrome

Tests in GTR by Gene

GNAS gene
Methylenetetrahydrofolate Reductase Deficiency

Laura Dean, MD

Created: March 8, 2012; Updated: October 27, 2016.

Characteristics

Methylenetetrahydrofolate Reductase (MTHFR) Deficiency is the most common genetic cause of elevated levels of homocysteine in the plasma (hyperhomocysteinemia).

The MTHFR enzyme plays an important role in processing amino acids, specifically, the conversion of homocysteine to methionine. Genetic variations in the MTHFR gene can lead to impaired function or inactivation of this enzyme, which results in mildly elevated levels of homocysteine, especially in individuals who are also deficient in folate (1). In these individuals, a daily supplement of low dose folic acid may reduce and often normalize their homocysteine levels, but this has not been demonstrated to improve health outcomes (2, 3).

A common genetic variant in the MTHFR gene is a 677C>T polymorphism (NM_005957.4:c.665C>T, rs1801133). This variant encodes a thermolabile enzyme that is less active at higher temperatures. Individuals who carry two copies of this variant (“TT homozygous”) tend to have higher homocysteine levels and lower serum folate levels compared to controls.

More than 25% of Hispanics and around 10-15% of North America Caucasians are estimated to be homozygous for the “thermolabile” variant (TT genotype) (4). The TT genotype is least common in individuals of African descent (6%) (5, 6).

Another common MTHFR variant, 1298A>C (NM_005957.4:c.1286A>C, rs1801131), does not cause increased homocysteine levels in heterozygous or homozygous individuals, but combined heterozygosity of 1298A>C and 677C>T results in an outcome similar to TT homozygous individuals (7).

Until recently, it was thought that MTHFR deficiency, by causing elevated homocysteine levels, led to an increased risk of venous thrombosis, coronary heart disease, and recurrent pregnancy loss (8-11). However, more recent analysis has not found an association between elevated homocysteine levels and the risk of venous thrombosis or the risk of coronary heart disease (12).

MTHFR polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia, recurrent pregnancy loss, or for at-risk family members (4).
Rarely, more severe variants in the MTHFR gene can be a cause of an autosomal recessive inborn error or metabolism where extremely high levels of homocysteine accumulate in the urine and plasma. This can cause developmental delay, eye disorders, thrombosis, and osteoporosis. But more commonly, homocystinuria is caused by variants in a different gene (cystathionine beta-synthase, CBS). To read more about homocystinuria caused by CBS deficiency, please see GeneReviews.

**Diagnosis**

A blood test that measures total homocysteine levels can diagnose hyperhomocysteinemia.

Genetic testing of the MTHFR gene may be used to confirm the diagnosis of an inherited hyperhomocysteinemia caused by MTHFR deficiency. However, a 2013 Practice Guideline from the American College of Medical Genetics and Genomics (ACMG) states that there is growing evidence that “MTHFR polymorphism testing has minimal clinical utility and, therefore should not be ordered as a part of a routine evaluation for thrombophilia” (4).

In an infant or child in whom autosomal recessive severe MTHFR deficiency is suspected, tests for plasma homocysteine and serum amino acids levels would be expected to show a pattern of extremely elevated homocysteine and low methionine. MTHFR full gene sequencing (as opposed to targeted polymorphism testing) can confirm the suspected clinical diagnosis.

**Management**

2013 Statement from the American College of Medical Genetics and Genomics (ACMG) includes the following recommendations:

- MTHFR polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia or recurrent pregnancy loss
- MTHFR polymorphism genotyping should not be ordered for at-risk family members
- A clinical geneticist who serves as a consultant for a patient in whom an MTHFR polymorphism(s) is found should ensure that the patient has received a thorough and appropriate evaluation for his or her symptoms
- If the patient is homozygous for the “thermolabile” variant c.665C→T, the geneticist may order a fasting total plasma homocysteine, if not previously ordered, to provide more accurate counseling
- MTHFR status does not change the recommendation that women of childbearing age should take the standard dose of folic acid supplementation to reduce the risk of neural tube defects as per the general population guidelines

The management of severe autosomal recessive MTHFR deficiency is outside the scope of this review.

**Genetic Testing**

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the *MTHFR* gene and for homocysteinemia due to MTHFR deficiency.

Biochemical genetic tests may also be used, which assess the level of activity of the MTHFR enzyme or the level of analyte in the blood. GTR provides a list of biochemical tests that assess the level of homocysteine analytes and the activity of the MTHFR enzyme.

**Genetic Counseling**

The MTHFR polymorphism has been associated with many different medical complications. Individuals who are “MTHFR positive” carry one or two copies of variants in the *MTHFR* gene. However, in general, the following genotypes are unlikely to be of clinical significance:

- 677C>T heterozygote
- c.1286A→C homozygote
- (677C>T);(c.1286A→C) compound heterozygote

Individuals who are TT homozygous with normal homocysteine levels do not have an increased risk of venous thrombosis or recurrent pregnancy loss, according to recent evidence. However, women do have a modestly increased risk of having a child with a neural tube defect and this risk increases if the fetus is also homozygous.

If homocysteine levels are elevated, TT homozygotes may have a mildly increased risk of venous thrombosis or recurrent pregnancy loss, but not other previously associated conditions, such as cardiovascular disease.

Less is known about the c.1286A→C variant, but current evidence suggests that it is milder than the “thermolabile” c.665C→T variant (4).

For all individuals, it is important to determine whether medical disorders have been incorrectly attributed to their positive MTHFR status. Referral to a hematologist or maternal–fetal medicine specialist may be needed. And patients should provide their MTHFR genotype status to their physician before starting chemotherapy agents that require folate (e.g., methotrexate).

Finally, MTHFR positive individuals may decide to take vitamin B and folic acid supplements. Although safe (toxicity is rare), evidence is lacking on whether such supplements reduce the risks associated with hyperhomocysteinemia or MTHFR genotype status (4).
Acknowledgments

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References


**Tests in GTR by Condition**

MTHFR deficiency, thermolabile type

Homocysteinemia due to MTHFR deficiency

Homocystinuria due to MTHFR deficiency

**Tests in GTR by Gene**

MTHFR gene
Pitt-Hopkins Syndrome
Laura Dean, MD
Created: March 8, 2012.

Characteristics

Pitt-Hopkins syndrome is characterized by mental retardation, a wide mouth, and intermittent hyperventilation. In infancy, hypotonia is typical leading to feeding problems and a delay in reaching developmental motor milestones. In older children, an abnormal breathing pattern often develops, such as a period of hyperpnea followed by apnea. Epilepsy is less common but in children who do have seizures, their ECG is abnormal. Many children have a happy disposition, flapping their hands when excited and laughing without a clear reason. Their gait looks stiff, due to a combination of hypotonia and ataxia. Most adults with Pitt-Hopkins syndrome have moderate to severe cognitive impairment and will not be able to speak.

Pitt-Hopkins syndrome is a genetically heterogeneous condition caused by an autosomal dominant mutation in TCF4. Many different mutations have been found in the TCF4 gene of affected children and adults, including heterozygous stop, splice, and missense mutations (1, 2).

Diagnosis

The diagnosis of Pitt-Hopkins syndrome is based on clinical features and the exclusion of other conditions with similar symptoms, such as Angelman syndrome, Rett syndrome, and Mowat-Wilson syndrome.

Molecular genetic testing of TCF4 can be used to confirm the diagnosis.

Management

Treatment: Manifestations of Pitt-Hopkins syndrome are treated by a multidisciplinary team specializing in the care of children with cognitive and motor impairment, including physical therapists, speech therapists, and specialists who treat epilepsy.

Genetic Counseling

Almost all mutations in the TCF4 gene occur de novo (3). Prenatal testing may be offered to unaffected parents who have had a child with Pitt-Hopkins syndrome.

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References


Schizophrenia
Laura Dean, MD
Created: March 8, 2012; Updated: February 6, 2017.

Characteristics

Schizophrenia is a severe neurodevelopmental disorder with a worldwide prevalence of around 0.3-0.7% (1). The etiology of schizophrenia is unknown, but it is thought to result from a combination of complex genetic and environmental factors. This includes physical factors e.g., complications during pregnancy and birth, infection, and autoimmune disease; as well as psychological factors that may trigger psychosis, such as stress and drug abuse (2). Several neurotransmitter systems are thought to be involved in the pathogenesis, including dopamine, glutamate, GABA, and acetylcholine.

Schizophrenia is associated with substantial morbidity and mortality. Antipsychotics are the mainstay of treatment, however, their efficacy is poor for many patients. Antipsychotics are thought to exert their therapeutic effects by the post-synaptic blockade of D2 dopamine receptors in the brain.

The symptoms of schizophrenia fall in to three main categories: positive, negative, and cognitive. Positive symptoms are generally not found in healthy individuals, but may come and go or persist in individuals with schizophrenia. Positive symptoms include reality distortion (e.g., delusions, hallucinations), and thought disorders. These symptoms often respond well to treatment.

Negative symptoms are deficits in normal emotions and behavior, and may be mistaken for depression. Symptoms divide into reduced expression of emotion (e.g., speaking without moving or with a monotonous voice) and avolition (a lack of motivation to start or continue with a task). No treatment has established efficacy for these pathologies.

Cognitive symptoms may also be difficult to recognize. They include poor executive functioning (understanding information and using it to make decisions) and trouble focusing or paying attention. And again, no treatment has established efficacy.

Genetics

Schizophrenia is highly heritable, as shown by family, twin, and adoption studies. For example, for identical twins, if one twin develops schizophrenia, the other twin has about a 50% chance of also developing the disease. The risk of the general population developing the schizophrenia is about 0.3-0.7% worldwide (3).
The search for “schizophrenia genes” has been elusive. Initial linkage studies looked at parts of the genome associated with schizophrenia, and many candidate genes were identified, including APOE, COMT, DAO, DRD1, DRD2, DRD4, DTNBP1, GABRB2, GRIN2B, HP, IL1B, MTHFR, PLXNA2, SLC6A4, TP53, and TPH1 (4). However, some of these have later been questioned (5).

Microdeletions and microduplications have been found to be three times more common in individuals with schizophrenia, compared to controls. Because these deletions and duplications are in genes that are overexpressed in pathways related to brain development, it is possible that the inheritance of multiple rare variants may contribute to the development of schizophrenia (6).

Several genetic disorders feature schizophrenia as a clinical feature. The 22q11.2 Deletion Syndrome comprises many different syndromes, of which one of the most serious is DiGeorge syndrome. Children born with DiGeorge syndrome typically have heart defects, cleft palate, learning difficulties, and immune deficiency. Schizophrenia is a late manifestation, affecting around 30% of individuals (7). Microdeletions and duplications in chromosome 1, 2, 3, 7, 15 and 16 have also been associated with schizophrenia (8).

In 2014, a genome-wide association study looked at the genomes of over 35,000 patients and 110,000 controls. The study identified 108 SNPs that were associated with schizophrenia, 83 of which had not been previously reported. As expected, many of these loci occurred in genes that are expressed in the brain. For example, the SNPs included a gene that encodes the dopamine D2 receptor, DRD2 (the target of antipsychotic drugs), and many genes involved in glutamine neurotransmitter pathways and synaptic plasticity (e.g., GRM3, GRIN2A, SRR, GRIA1). More surprisingly, however, associations were also enriched among genes expressed in tissues with important immune functions (9).

In 2016, a study based on nearly 65,000 people investigated the association between schizophrenia and variation in the Major Histocompatibility Complex (MHC) locus—a region on chromosome 6 that is important for immune function. The study focused on the C4 gene (complement component 4) that exists as two distinct genes: C4A and C4B, which encode particularly structurally diverse alleles.

The study found that the alleles which promoted greater expression of C4A in the brain were associated with a greater risk of schizophrenia. By using mice models, the study showed that C4 is involved in the elimination of synapses during brain maturation. In humans, “synaptic pruning” is most active during late adolescence, which coincides with the typical onset of symptoms of schizophrenia. It is therefore possible that the inheritance of specific C4A alleles could lead to “run away” synaptic pruning, increasing the risk of schizophrenia. Further research may even determine C4 as a potential therapeutic target (10).
Diagnosis

Currently, the diagnosis of schizophrenia is made via a psychiatric assessment using the criteria presented in the American Psychiatric Association Manual of Psychiatric Diseases, which is now in its 5th edition, and is known as DSM-V. To make a diagnosis, specific characteristic symptoms of schizophrenia must be present for at least 6 months, together with a disruption in social or occupational function, in the absence of another diagnosis that could account for the symptoms.

The use of chromosome microarray analysis has been suggested as a diagnostic test for schizophrenia. Microarray analysis can detect copy number variants (CNVs), which are large regions of the genome that have been deleted or duplicated. The prevalence of clinically significant CNVs in schizophrenia is around 5%. For autism and intellectual disability, the prevalence is around 10-20%, and CNV testing with microarray analysis is now a routine first-line diagnostic test for these conditions.

For an individual with schizophrenia, a positive test result for CNV may have implications for medical management, because of the association of CNVs with physical diseases and genetic counseling, and because offspring have a 50% risk of inheriting the CNV (3, 11).

Management

Treatment of manifestations: Antipsychotic medications are the mainstay of treatment and help reduce symptoms and improve behaviors in patients with schizophrenia. The type, dose, and route of administration of antipsychotic medications depends upon the clinical scenario. Adverse effects are common, and may require the dose or type of drug to be altered.

Antipsychotics may be given with counseling and other types of psychosocial interventions. For refractory (treatment-resistant) symptoms, an alternative antipsychotic or an additional antipsychotic may be required.

During pregnancy, antipsychotic drugs should be given only when the benefits derived from treatment exceed the possible risks to mother and fetus. Neonates exposed during the third trimester are at risk for extrapyramidal and/or withdrawal symptoms following delivery. There have been reports of agitation, hypertonia, hypotonia, tremor, somnolence, respiratory distress, and feeding disorder. While in some cases symptoms have been self-limited, in others neonates have required intensive care unit support and prolonged hospitalization.

Surveillance: Routine monitoring for the symptoms and signs of extrapyramidal adverse effects is needed in individuals taking antipsychotics. These adverse effects include akathisia (feeling of restlessness that may be accompanied with motor restlessness), dystonias (involuntary contraction of large muscle groups), and parkinsonian syndrome. Patients should also be monitored for signs of tardive dyskinesia (involuntary facial movements) and drug-specific adverse effects. For clozapine, because of the risk of
neutropenia, the patient's white blood cell count and absolute neutrophil count must be regularly monitored. For thioridazine, the risk of prolonged QT interval may lead to Torsades de pointes.

**Prevention of secondary complications:** Patients should be regularly monitored for weight gain and metabolic problems such as hyperglycemia and hyperlipidemia, which are common side effects of antipsychotic medications.

**Genetic Testing**

Genetic testing is available for several of the susceptibility loci for schizophrenia, including clinical and research tests registered in the NIH Genetic Testing Registry (GTR). Additional tests may be found in the ‘Related section’ of the main GTR record for schizophrenia.

GTR also has registered tests for genetic conditions with schizophrenia as a clinical feature.

**Genetic Counseling**

Genetic counseling is recommended for people who have a family member with schizophrenia. Recurrence risk counseling is based on empiric familial risk for families with individuals with schizophrenia (12).

The lifetime risk of schizophrenia for the general population is estimated to be 0.2 to 0.7% (13).

The recurrence risk of schizophrenia in the siblings of a patient is 10%, and in the children of patients, the risk is approximately 10%. The risk for second-degree relatives is approximately 3-4% (14, 15).

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**Version History**

To view an earlier version (8 March 2012), please click here.

**References**


**Tests in GTR by Condition**

**Schizophrenia**

**22q11 Deletion Syndrome**

**DiGeorge sequence**
Authoring and Peer Review

Created: May 11, 2017.

Medical Genetics Summaries (MGS) for pharmacogenetics is a freely available collection of articles describing how genetics plays a role in an individual's response to drugs or predisposition to disease. The structured format of each summary makes accessing information such as genetic testing or therapeutic recommendations quick and easy to use. Medical Genetics Summaries use authoritative sources, are guideline-driven and actionable, and are subject to an extensive review process as described below.

Editorial Oversight

The editors of Medical Genetics Summaries advise on subject matter, guide the project through developments in the field, provide final approval prior to the publication of each summary, and assist in recruiting reviewers and in resolution of key issues which may be raised during the review process.

Selection of Topics

The selection of topics for new MGS chapters is guided by two factors. First, the author consults the FDA’s “Table of Pharmacogenomic Biomarkers in Drug Labeling” to select new drugs that have not yet been covered in MGS. Second, to prioritize the order of new MGS chapters, the author checks the Genetic Testing Registry (GTR) for drug response records which contain information about genetic testing, but lack summary information about the drug response. After a new MGS chapter is released to the production site, an excerpt from the chapter is displayed in the relevant GTR drug response record. Additional reciprocal links between MGS, GTR, and MedGen are also added.

Structured Format

Each MGS drug response chapter follows a structured format. Each summary has one drug section, but may have one or more gene sections, depending on how many genetic factors have been identified.

1. Introductory paragraphs detail the drug and its uses, how the genetic variants influence an individual's response to the drug, and displays dosing recommendations from the FDA and practice guidelines from authoritative professional societies.
2. The drug section begins with a description of the drug, the drug class, its mechanism of action, the indications for its use, and common side effects. This is followed by a discussion on the factors which influence the drug response.
3. The gene section reviews important facts about the gene — what role it plays in the drug metabolism or action, and the nature of the gene variants and how they impact the drug response. The common or clinically significant variants are then discussed, including their prevalence across different ethnic populations.
4. “Genetic Testing” section is a key part of the summary. Here, the summary clearly describes the genetic testing options that are available, linking to genetic test providers listed in GTR.

5. “Therapeutic Recommendations based on Genotype” excerpts clinically actionable information, e.g. dosing recommendations from the FDA drug label; and therapeutic recommendations from pharmacogenetic societies such as CPIC, CPNDS and DPWG and medical societies, such as ASCO, ACMG, NCCN.

6. Nomenclature table provides information about the different terms used for genetic variants. Terms that are commonly used in the literature and historic terms are linked to the official HGVS terms and rs identifiers when available.

7. Expert reviewers are acknowledged, and information about previous versions of the summary is given.

**Writing Process**

Each summary is written by our in-house senior medical writer, who is an MD. All phases from authoring to production are tracked in an internal ticket management system. To create the first draft of a summary:

1. The author consults the most recent FDA drug label for the drug. To gain a better understanding for the context of the drug use and impact of genetic factors, the author will use NIH resources and other clinical sites, such as UpToDate.

2. The author then identifies key guidelines and primary papers, using PubMed Clinical Queries, PubMed, CPIC and PharmGKB.

3. Finally, the author searches PubMed for the most recent publications — to both find content that has not yet been cited by guidelines, and to identify external reviewers who are actively involved in research.

**Internal Review**

Each summary undergoes internal review involving one or two NCBI staff members. Once the author has finalized the first draft of a summary, it is submitted for internal review, along with key supporting guidelines (e.g., FDA drug label, key guidelines). The internal reviewers perform the first round of expert review, using track changes to ask questions and make suggestions and corrections. Because this process occurs in a ticket management system, all versions of the document and comments from the author and reviewers are documented.

**External Review**

Following internal review, each summary goes through a scientific peer-review process involving between 2 to 9 experts from outside NCBI. Typically, the external review includes at least one individual who is a member of CPIC, and a clinical specialist, experienced in prescribing the drug and has published papers about its use. Expert reviewers comments are tracked so that the evolution of the summary can be seen, and
after the summary is released to production, all versions of the summary are stored in the document management system.

**Finalizing the Summary**

Once all the review comments are reconciled, the summary is copyedited in-house and released to production.

**Updates**

Summaries are scheduled to be updated every 2 years. An earlier update is triggered by an update to guidelines from which excerpts have been taken for the summary. The internal reviewers decide whether the nature of the updates is minor or major. All minor updates undergo internal review and copy editing, and when published — a link to the previous version of the summary is made available. For major updates, the summary is sent out for external review.
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Murphy, Michael, MD; University of Oxford, UK
- ABO Blood Group

Nagy, Mohamed, BPharm; Children’s Cancer Hospital, Egypt
- Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
- Capecitabine Therapy and DPYD Genotype
- Carisoprodol Therapy and CYP2C19 Genotype
- Fluorouracil Therapy and DPYD Genotype
- Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
- Phenytoin Therapy and HLA-B*15:02 and CYP2C9 Genotypes
- Thioridazine Therapy and CYP2D6 Genotypes

O’Brien, Thomas, MD, MPH; National Cancer Institute, Bethesda, MD, USA
- Sofosbuvir Therapy and IFNL4 Genotype

Obeng, Aniwaa Owusu, PharmD; Icahn School of Medicine at Mount Sinai, New York, NY, USA
- Maraviroc Therapy and CCR5 Genotype
  Patrinos, George, PhD; University of Patras, Greece
  - Fluorouracil Therapy and DPYD Genotype
  - Risperidone Therapy and CYP2D6 Genotype

- Pauli, Emily, PharmD; Clearview Cancer Institute, Huntsville, AL, USA
  - Capecitabine Therapy and DPYD Genotype
  - Phenytoin Therapy and HLA-B*15:02 and CYP2C9 Genotypes

- Pawloski, Pamala, PharmD; HealthPartners Institute, Bloomington, MN, USA
  - Dabrafenib Therapy and BRAF and G6PD Genotype
  - Vemurafenib Therapy and BRAF and NRAS Genotype

- Phillips, Elizabeth, MD, FIDSA; Vanderbilt University Medical Center, Nashville, TN, USA
  - Abacavir Therapy and HLA-B*57:01 Genotype

- Pirmohamed, Munir, MD, PhD; University of Liverpool, UK
  - Abacavir Therapy and HLA-B*57:01 Genotype

- Pollock, Bruce, MD, PhD; University of Toronto, Canada
  - Venlafaxine Therapy and CYP2D6 Genotype

- Pratt, Victoria, PhD, FACMG; Indiana University School of Medicine, Indianapolis, IN, USA
  - Fluorouracil Therapy and DPYD Genotype
  - Irinotecan Therapy and UGT1A1 Genotype
  - Maraviroc Therapy and CCR5 Genotype

- Rahman, Shamima, PhD, FRCP; University College London, UK
  - Gentamicin Therapy and MT-RNR1 Genotype

- Rashed, Rashed, BCNSP, MSc; Children's Cancer Hospital, Egypt
  - Irinotecan Therapy and UGT1A1 Genotype

- Rogawski, Michael, MD, PhD; University of California, Davis, CA, USA
  - Carbamazepine Therapy and HLA Genotypes
  - Phenytoin Therapy and HLA-B*15:02 and CYP2C9 Genotypes

- Saab, Yolande, PharmD, PhD; Lebanese American University, Lebanon
  - Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
- Imipramine Therapy and \textit{CYP2D6} and \textit{CYP2C19} Genotype
- Thioridazine Therapy and \textit{CYP2D6} Genotypes

Schellens, Jan, MD, PhD; The Netherlands Cancer Institute, Amsterdam, Netherlands
- Capecitabine Therapy and \textit{DPYD} Genotype

Schneeweiss, Andreas, MD; Heidelberg University Hospital, Germany
- Pertuzumab Therapy and \textit{ERBB2 (HER2)} Genotype

Schulman, Sol, MD; Harvard Medical School, Boston, MA, USA
- Warfarin Therapy and the Genotypes \textit{CYP2C9} and \textit{VKORC1}

Scott, Stuart, PhD, ABMGG; Icahn School of Medicine at Mount Sinai, New York, NY, USA
- Allopurinol Therapy and \textit{HLA-B*58:01} Genotype
- Amitriptyline Therapy and \textit{CYP2D6} and \textit{CYP2C19} Genotype
- Azathioprine Therapy and \textit{TPMT} Genotype
- Celecoxib Therapy and \textit{CYP2C9} Genotype
- Clopidogrel Therapy and \textit{CYP2C19} Genotype
- Diazepam Therapy and \textit{CYP2C19} Genotype
- Esomeprazole Therapy and \textit{CYP2C19} Genotype
- Gentamicin Therapy and \textit{MT-RNR1} Genotype
- Imipramine Therapy and \textit{CYP2D6} and \textit{CYP2C19} Genotype
- Mercaptopurine Therapy and \textit{TPMT} Genotype
- Omeprazole Therapy and \textit{CYP2C19} Genotype
- Phenytoin Therapy and \textit{HLA-B*15:02} and \textit{CYP2C9} Genotypes
- Simeprevir Therapy and \textit{IFNL3} Genotype
- Thioguanine Therapy and \textit{TPMT} Genotype
- Venlafaxine Therapy and \textit{CYP2D6} Genotype

Skaar, Todd, PhD; Indiana University, Bloomington, IN, USA
- Codeine Therapy and \textit{CYP2D6} Genotype

Sukasem, Chonlaphat, PhD; Mahidol University, Bangkok, Thailand
- Risperidone Therapy and \textit{CYP2D6} Genotype

Thirumaran, Ranjit, MPharm, PhD; Genelex Labs, Seattle, WA, USA
- Imipramine Therapy and \textit{CYP2D6} and \textit{CYP2C19} Genotype

Thompson, Alex, MD, PhD; Duke University Medical Centre, Durham, NC, USA
- Simeprevir Therapy and \textit{IFNL3} Genotype

Trenk, Dietmar, PhD; Albert Ludwig University of Freiburg, Germany
- Clopidogrel Therapy and CYP2C19 Genotype

Uppugunduri, Chakradhara, PhD; University of Geneva, Switzerland

- Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
- Celecoxib Therapy and CYP2C9 Genotype
- Diazepam Therapy and CYP2C19 Genotype
- Imipramine Therapy and CYP2D6 and CYP2C19 Genotype

van Rhenen, Mandy, MSc; Royal Dutch Pharmacists Association (KNMP), Netherlands

- Amitriptyline Therapy and CYP2D6 and CYP2C19 Genotype
- Imipramine Therapy and CYP2D6 and CYP2C19 Genotype
- Metoprolol Therapy and CYP2D6 Genotype
- Propafenone Therapy and CYP2D6 Genotype
- Risperidone Therapy and CYP2D6 Genotype

Visk, DeeAnn, PhD; San Diego, CA, USA

- Propafenone Therapy and CYP2D6 Genotype

Wadelius, Mia, PhD; Uppsala University, Sweden

- Allopurinol Therapy and HLA-B*58:01 Genotype
- Atomoxetine Therapy and CYP2D6 Genotype
- Esomeprazole Therapy and CYP2C19 Genotype
- Irinotecan Therapy and UGT1A1 Genotype
- Omeprazole Therapy and CYP2C19 Genotype

Wainberg, Mark, PhD, OC, OQ, FRSC; McGill University, Quebec, Canada

- Maraviroc Therapy and CCR5 Genotype

Wikistrand, John, MD, PhD; University of Gothenburg, Sweden

- Metoprolol Therapy and CYP2D6 Genotype

Zujewski, Jo Anne, MD; National Cancer Institute, Bethesda, MD, USA

- Pertuzumab Therapy and ERBB2 (HER2) Genotype