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## Autism genes keep turning up chromatin

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### Abstract

Autism-spectrum disorders (ASD) are complex genetic disorders collectively characterized by impaired social interactions and language as well as repetitive and restrictive behaviors. Of the hundreds of genes implicated in ASD, those encoding proteins acting at neuronal synapses have been most characterized by candidate gene studies. However, recent unbiased genome-wide analyses have turned up a multitude of novel candidate genes encoding nuclear factors implicated in chromatin remodeling, histone demethylation, histone variants, and the recognition of DNA methylation. Furthermore, the chromatin landscape of the human genome has been shown to influence the location of de novo mutations observed in ASD as well as the landscape of DNA methylation underlying neurodevelopmental and synaptic processes. Understanding the interactions of nuclear chromatin proteins and DNA with signal transduction pathways and environmental influences in the developing brain will be critical to understanding the relevance of these ASD candidate genes and continued uncovering of the “roots” of autism etiology.

### Keywords

epigenetics; epigenomics; genetics; genomics; neurodevelopment; environment; nutrition; metabolism

## 1. Introduction

The functional and cognitive deficits of autism-spectrum disorders characterized by deficits in social interactions and communication, as well as repetitive interests and behaviors appear to be by nature disorders of the neuronal synapse<sup>1</sup>. So perhaps not surprising has been a prioritization of genes for intense investigation in ASD research to include genes encoding neurotransmitters and their receptors, neuronal adhesion molecules, synaptic signal transduction pathways, and neuronal growth factors. Yet, a number of rare Mendelian disorders, such as Rett syndrome, Cornelia de Lange syndrome, and Coffin-Siris syndrome have pointed to the importance of chromatin remodeling factors and DNA methylation in human brain development. Thus, analysis of ASD by human genetics has led to the greater appreciation of “epigenetics”, a term used to describe the additional layers and players on top of DNA that confer long-lasting and reversible gene expression modifications without changing the underlying genetic sequence<sup>2</sup>.

An excellent example of a recently uncovered connection between nuclear epigenetic and transcriptionally regulatory factors in ASD is a recent meta-analysis of four exome sequencing publications<sup>3</sup>, together representing 965 ASD probands and 121 predicted disruptive mutations in protein-coding genes<sup>4-7</sup>. This study demonstrated a significant over-

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representation of genes with functions in chromatin regulation and early developmental expression with variants found in ASD probands but not unaffected siblings<sup>3</sup>.

Here, I will attempt to demystify chromatin and to summarize the recent crop of chromatin genes implicated in ASD with the goal of future understanding their functional relevance to the human chromatin landscape underlying synaptic function.

### 1.1. Autism genes acting in nucleosome packaging

Chromatin can be defined simply and collectively as genomic DNA and associated proteins within the nucleus. Not so simple is the vast assortment of chromatin factors dedicated to the fine-tuning of DNA packaging and the enzymatic functions involved in changing chromatin states as cells undergo tissue and developmental differentiation. Nucleosomes are the primary unit of chromatin organization that serve to keep DNA molecules condensed and regulated by only releasing genes into the open conformation when their accessibility is needed. Nucleosomes are made up of histone core protein subunits H2A/B, H3, and H4 that form the spool-like nucleosome and linker subunit H1 that connects the nucleosomes. The tightness of wrapping at specific genes or genomic locations within nucleosome arrays is influenced by a number of variables affecting histone protein subunits, namely variant histone proteins and post-translational modifications.

Post-translational modifications are covalent changes to specific amino acids in the core histone subunits that can be detected by specific antibodies and examined genome-wide. Certain histone modifications, such as histone H3K4 trimethylation (H3K4me3) and H3K27 or H3K9 acetylation mark active gene promoters, while marks such as H3K27 or H3K9 trimethylation (H3K27me3, H3K9me3) are associated with transcriptionally silent genes<sup>2</sup>. However, in pluripotent stem cells, a subgroup of developmental genes regulated by polycomb group complexes contain both active (H3K4me3) and repressive (H3K27me3) marks, but remain in an inactive but poised state, waiting for an external signal. The regulation of each histone modification requires specific enzymes that add or remove the methyl or acetyl group. Interestingly, several genes found mutated in ASD encode histone demethylases, such as KDM5C, a demethylase of histone H3K4 implicated in gene repression and JMJD1C, a demethylase for histone H3K9 implicated in hormone-dependent transcriptional activation (Table 1).

Histone variant proteins are generally encoded by separate genes and can substitute for the canonical histone subunit in specific situations. For instance, the histone H2A subunit has a variant encoded by the autosomal gene H2AFY/MACROH2A1.1 that was identified as an autism candidate gene by a genome-wide association study (GWAS)<sup>8</sup>. MACROH2A1.1 is generally associated with repressed chromatin, such as the inactive X chromosome<sup>9</sup>. But because it also contains binding sites for cellular metabolites in through the macro domain, MACROH2A1.1 may have a more dynamic role in modulation of gene expression in response to environmental signals<sup>10</sup>. A related family member encoding gene, *MACROD2* was also found on a separate chromosome in an ASD GWAS<sup>11</sup>. The macro domain of MACROD2 binds a cellular metabolite that emerges from histone deacetylation reactions<sup>10</sup>, linking both histone variant and histone modification events.

### 1.2. Chromatin remodeling and the importance of energy

Changing chromatin states during neuronal lineage commitment is an active process requiring the appropriate external signals, as well as energy in the form of ATP. The engines that carry out the active process of changing chromatin are called “chromatin remodeling complexes”. Each chromatin remodeling complex contains an ATPase with a variable group of associated protein factors. A neuron-specific protein ATPase subunit BAF53b defines a

neuronal chromatin remodeling complex that is required for long term memory and synaptic plasticity in mice<sup>12</sup>.

In humans, mutations in chromatin remodeling complex factor subunit genes appear to be a recurrent theme in neurodevelopmental disorders and autism (Table I). Several components of the SWI/SNF specific chromatin remodeling complexes, including SMARCC1, SMARCC2, ARID1A, ARID1B, and ATRX are encoded by genes in which rare autism mutations have been observed<sup>5, 6, 13–15</sup>. In addition, several exome sequencing studies in autism have identified rare mutations in genes encoding the ATP-dependent chromatin helicases CHD8, with additional variants found in family members CHD1, CHD3, and CHD7<sup>5, 6, 16</sup>. CHD8 serves as an important regulator of beta-catenin and Wnt signaling pathways in neuronal development<sup>17</sup>.

### 1.3. The dynamics of histone methylation and DNA methylation in development

Mammalian neurons require extensive methyl modifications throughout development and postnatal life for many molecules, particularly nucleotides and proteins. As mentioned above, several important post-translational modifications of histone core subunits within nucleosomes involve methylation, including the activating H3K4me3 and polycomb repressive H3K27me3 marks. Histone methylation is more stable than acetylation or phosphorylation, suggesting a long-lived component to these epigenetic marks<sup>18</sup>. The other major epigenetic layer of information is DNA methylation. In the mammalian genome, CpG sites are targets for methylation carried out by a family of DNA methyltransferases (DNMTs). Over evolutionary history, eukaryotic organisms have gradually acquired more DNA methylation, with humans having a nearly saturated genome of ~80% of possible CpG sites methylated in human embryonic stem cells<sup>19</sup>.

Both histone and DNA methylation patterns are highly dynamic processes in early development that correlate with dynamic changes in cell lineage and differentiation events. Interestingly, mutations in autism have been found in several genes encoding proteins involved in demethylase reactions, which are the removal of methyl groups from histones or DNA (Table 1). For example, mutations in the X-linked gene *KDM5C* have been found in individuals with intellectual disability (ID) and autism spectrum disorder, and this gene encodes a histone demethylase enzyme that removes the active H3K4me3 mark, thus repressing gene expression. Genome-wide, prefrontal cortex neurons from human infants in their first year of life exhibit a large excess of H3K4me3 actively marked genes compared to later ages, suggesting that histone demethylation reactions are rampant in early postnatal life<sup>20</sup>. Interestingly, generalized disruption of the H3K4me3 landscape was observed in autism frontal cortex samples compared to controls, including several genes with known neurodevelopmental functions<sup>21</sup>. Together these results suggest that histone demethylation reactions may be critical early life events that may become dysregulated in autism.

## 2. Chromatin influences on de novo mutations in autism

In addition to chromatin genes being mutated in autism, chromatin itself has been recently shown to influence the genomic locations of *de novo* mutations observed in monozygotic twins concordant for autism<sup>22</sup>. In this whole genome sequencing study, *de novo* variants were not randomly distributed throughout the genome, but instead clustered into “hotspots” enriched for simple repeats and DNase I hypersensitivity in embryonic stem cells, a mark of open active chromatin not bound by nucleosomes. DNA methylation also impacts mutation rates, as spontaneous deamination of methylated CpG sites is a frequent mutation type found in mammals and accounted for 15% of the *de novo* variants found in autism.

### 3. Chromatin influences on DNA methylation and synaptic genes

Chromatin can also influence DNA methylation levels in human tissues and cell lines. Genome-wide, ES cells and mature human tissues have high saturation of CpG methylation, except at conserved clusters of CpGs called CpG islands that have been protected from DNA methylation and are found at many gene promoters. However, genome-wide DNA methylation detection has revealed the presence of methylome landscape features called “partially methylated domains” (PMDs) which are genomic landscape features of the human methylome characterized by lower levels of methylation in the range of 40–70% compared to the >70% methylation observed over the rest of the genome<sup>19</sup>. PMDs are also characterized by reduced gene expression compared to highly methylated domains (HMDs) and the more repressive histone marks such as H3K27me<sub>3</sub> and H3K9me<sub>3</sub>. What is particularly interesting about PMDs is that they are both tissue-specific and developmentally regulated and they are highly enriched for tissue-specific and developmental genes, particularly those involved in neuronal development, immune responses, and synaptic transmission<sup>23, 24</sup>. While the presence of PMDs was previously thought to be limited to primary and tumor cell lines, we recently identified placenta as a normal human tissue that contains PMDs covering 37% of the genome<sup>23</sup>.

Autism candidate genes with mutations found from genetic studies are highly enriched in PMDs compared to highly methylated parts of the genome<sup>24</sup>. Specifically, genes that are highly methylated in neuronal cells, but within PMDs in placenta or fibroblasts include many genes acting at the synapse and implicated in autism, including *CNTNAP2*, *CACANA1C*, *GABRB3*, *CHRNA7*, *SYNGAP1*, *NRXN1*, *SCNA1*, and *SHANK3* (Figure 1)<sup>23</sup>. Furthermore a recent study of monozygotic twins characterized for ASD-associated traits and DNA methylation differences identified several differentially methylated genes that also localize to PMDs (*NRXN1*, *GABRB3*, *SNRPN*, *SNURF*) or chromatin genes (*JMJD1C*, *MBD4*) already implicated in ASD<sup>25</sup>. While understanding the relationship between genomic methylation patterns and gene regulation is still in its infancy, the first glimpses of the DNA methylation and chromatin landscape is beginning to uncover a pathway of synaptic genes that may be coordinately epigenetically regulated and dysregulated by both genes and environmental factors.

## 4. The multiple factors influencing chromatin and ASDs

### 4.1. Genetics

The examples in Table 1 are of rare mutations found in genes that encode proteins involved in chromatin regulation, but a larger genetic effect is likely to come from mutations and genetic variants that lie outside the protein coding exons but influence the binding and actions of chromatin factors and DNA methylation patterns. While it is tempting to think about epigenetic layers as completely independent of the DNA sequence, the genetic code ultimately determines the chromatin state that occurs during developmental programming. As an example, CpG islands are defined by CpG density at the sequence level and are protected from DNA methylation by a property called G-C skew, also at the sequence level<sup>26</sup>. But in Fragile X syndrome, an expanded CCG triplet repeat at the CpG island promoter of *FMR1* alters this protection, resulting in methylation and transcriptional repression<sup>27</sup>. But in addition to the obvious gene regulatory regions, repetitive regions of the genome classically considered to be “off the map” for consideration as disease-causing mutation may also impact neighboring chromatin and gene expression. As a clear example, facioscapulohumeral muscular dystrophy results from a loss of a critical number of repeats in a microsatellite repeat array on chromosome 4 that results in chromatin and gene expression changes to the *DUX4* retrogene<sup>28</sup>. As genome-wide sequencing technologies have now opened up the entire genome for examination, many more examples of genetic

variation, particularly repeats causing methylation and chromatin changes relevant to phenotypes are expected.

#### 4.2. Sex differences

Differences between males and females in phenotypes and disease susceptibilities is foremost a genetic difference, due to the chromosomal differences of XX versus XY. However, the differential developmental program enacted in males versus females results in large differences in sex hormones, which can also have effects on epigenetics as well as phenotype. Since autism has a strong male bias for susceptibility, it is important to consider both chromosomal and hormonal influences that may be influencing gene expression and phenotypes.

The epigenetic process of X chromosome inactivation that occurs in females largely serves as a mechanism of dosage compensation by inactivating one of the two X chromosomes in each cell. The inactive X chromosome creates a large heterochromatic domain within the nucleus, called the Barr body. Interestingly, simply having the Barr body present appears to create global sex differences in DNA methylation levels, as females have detectably lower global levels of DNA methylation, and increasing the number of X chromosomes further reduces the methylation on autosomes<sup>29</sup>. Furthermore, human brain transcriptome data analyzed for sex differences revealed that male-biased transcripts were enriched for chromatin functions, as well as roles in extracellular matrix formation/glycoproteins, immune response, and cell cytoskeleton<sup>30</sup>.

In addition, not all genes on the inactive X chromosome are inactivated, and the genes that “escape” X inactivation in females are revealing some interesting insights in to sex differences in chromatin<sup>31</sup>. *KDM5C/JARID1C*, listed in Table 1 for its involvement in ASD and ID, is a gene that escapes X chromosome inactivation<sup>32</sup>. In addition, the gene encoding O-linked-*N*-acetylglucosamine (O-GlcNAc) transferase (OGT) that regulates chromatin remodeling factors, is expressed lower in males than females and further reduced by prenatal stress<sup>33</sup>. These inherent epigenetics and brain transcriptional sex differences should be further examined in understanding the female protective effect in autism.

#### 4.3. Environmental toxins

Modern humans are surrounded by a stunning array of environmental toxins, primarily man-made chemicals that are in our air, water, food, and furniture. While a single chemical compound is unlikely to arise a “smoking gun” for autism risk, some exposures have been demonstrated to modestly increase risk for ASD<sup>34</sup>. In the nascent field of environmental epigenetics of relevance to ASDs, there appear to be two emerging themes from multiple studies. First, many different exposures individually appear to result in reduced levels of DNA methylation<sup>35</sup>. Second, is that there are sex differences in susceptibility to environmental factors. In our recent mouse model of perinatal exposure to the common flame retardant polybrominated biphenyl ether in a genetically and epigenetically susceptible *MeCP2* mouse mutant, we observed deficits in sociability, early postnatal growth, and brain DNA methylation levels only in females, and the interaction effects in spatial learning were only observed in females<sup>36</sup>. The males of this model were more genetically susceptible because *Mecp2* is X-linked, but this and other studies raise the question of whether females, which have a lower baseline level for DNA methylation saturation, may be more susceptible to the multiple chemical insults on the dynamic methylome described in the previous section.

#### 4.4. Nutrition and metabolism

Fortunately, nutritional factors, particularly folate, B vitamins and choline, can help to counteract the assault by chemical pollutants on DNA methylation levels. A likely pathway of this action is the one carbon metabolism cycle, which supplies the methyl donors from the diet for methylation reactions mediated by SAM to both DNA and proteins. Multiple chemical exposures utilize the SAM inhibitor glutathione for detoxification and therefore prevent the high saturation of DNA methylation in brain<sup>34, 37</sup>.

There are several additional examples of cellular and organismal metabolic cycles serving to regulate gene expression through modifications to chromatin reviewed elsewhere<sup>18</sup>. Oxygen and glycolysis are required for the action of the JMJC histone demethylases (family includes JMJD1C from Table 1). Acetyl-CoA produced from the citrate cycle provides the donor for histone acetylation reactions and the sexually dimorphic regulator OGT mentioned above uses a byproduct of the hexosamine biosynthetic pathway derived from glycolysis. The histone deacetylase SIRT1 is modulated by NADH and diurnal metabolic cycles. And as mentioned above, multiple chromatin proteins contain macro domains that translate metabolic changes into chromatin and gene expression changes<sup>10</sup>.

A central signal transduction pathway regulating the nutrient sensing and metabolic changes to chromatin is mediated by the mammalian target of rapamycin (mTOR). mTOR mediates the signals from the PI3K/AKT signal transduction cascade, promoting protein synthesis and anabolism, and is also becoming a central pathway disrupted in several syndromic forms of ASD, including Fragile X syndrome and tuberous sclerosis<sup>38</sup>.

#### 4.5. Immune responses

There is accumulating evidence for immune dysregulation playing a role in the pathogenesis of ASD. For example, maternal fever or influenza infection during pregnancy increases ASD risk and several animal models that mimic an acute maternal immune response result in autistic-like features in the offspring. Mothers of children with ASD exhibit autoantibodies and altered cytokine profiles indicative of systemic immune activation<sup>39</sup>.

While there is much to be still learned in this area, neuronal and immune dysfunction could be occurring in parallel during the pathogenesis of ASD through chromatin pathways. Both T and B cell lineages of adaptive immune responses undergo coordinated changes in DNA methylation and chromatin marks that could become dysregulated by a variety of genetic and environmental risk factors. For example, *FOXP3* is a marker of regulatory T cells (Tregs), a subset of CD4<sup>+</sup> T cells primed in early life to recognize common environmental antigens and inhibit later inappropriate immune responses. Interestingly, Treg fate determination is an epigenetic event of *FOXP3* promoter demethylation induced by repeated Ca<sup>+2</sup>-mediated signal transduction and prevented by the mTOR pathway<sup>40, 41</sup>.

### 5. Concluding remarks

Considering the many roles that chromatin plays at the interface of genetic and environmental factors in regulating gene expression and epigenetic states, it is perhaps not surprising that genomic approaches keep uncovering chromatin encoding genes. An ongoing understanding of the complex dynamic changes that chromatin undergoes in the developing brain is likely to help to make sense of the regulatory pathways connecting the diversity of genes implicated in ASD. Furthermore, since chromatin events are integrated with environmental, nutrient, and metabolic cellular sensors, they may help explain how these complex genetic disorders are further modified by environmental risk and protective factors.

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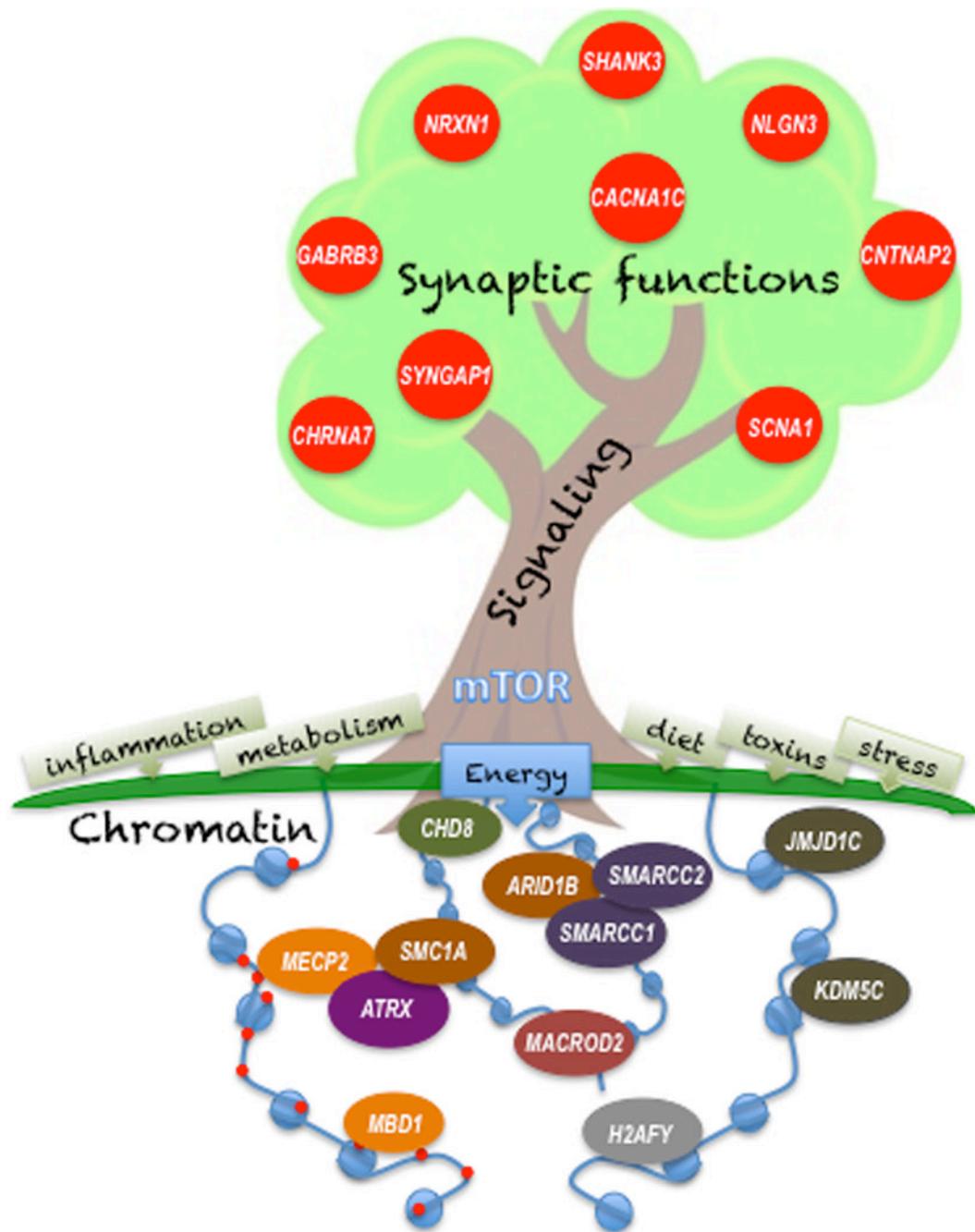
## References

1. Zoghbi HY. Postnatal neurodevelopmental disorders: meeting at the synapse? *Science*. 2003 Oct 31; 302(5646):826–830. [PubMed: 14593168]
2. LaSalle J, Powell WT, Yasui DH. Epigenetic layers and players underlying neurodevelopment. *Trends Neurosci*. 2013 in press.
3. Ben-David E, Shifman S. Combined analysis of exome sequencing points toward a major role for transcription regulation during brain development in autism. *Molecular psychiatry*. 2012 Nov 13.
4. Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, et al. De novo gene disruptions in children on the autistic spectrum. *Neuron*. 2012 Apr 26; 74(2):285–299. [PubMed: 22542183]
5. Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, Sabo A, et al. Patterns and rates of exonic de novo mutations in autism spectrum disorders. *Nature*. 2012 May 10; 485(7397):242–245. [PubMed: 22495311]
6. O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature*. 2012 May 10; 485(7397):246–250. [PubMed: 22495309]
7. Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature*. 2012 May 10; 485(7397):237–241. [PubMed: 22495306]
8. Philippi A, Tores F, Carayol J, Rousseau F, Letexier M, Roschmann E, et al. Association of autism with polymorphisms in the paired-like homeodomain transcription factor 1 (PITX1) on chromosome 5q31: a candidate gene analysis. *BMC medical genetics*. 2007; 8:74. [PubMed: 18053270]
9. Li H, Yamagata T, Mori M, Yasuhara A, Momoi MY. Mutation analysis of methyl-CpG binding protein family genes in autistic patients. *Brain & development*. 2005 Aug; 27(5):321–325. [PubMed: 15967618]
10. Posavec M, Timinszky G, Buschbeck M. Macro domains as metabolite sensors on chromatin. *Cellular and molecular life sciences : CMLS*. 2013 May; 70(9):1509–1524. [PubMed: 23455074]
11. Anney R, Klei L, Pinto D, Regan R, Conroy J, Magalhaes TR, et al. A genome-wide scan for common alleles affecting risk for autism. *Human Molecular Genetics*. 2010 Oct 15; 19(20):4072–4082. [PubMed: 20663923]
12. Vogel-Ciernia A, Matheos DP, Barrett RM, Kramar EA, Azzawi S, Chen Y, et al. The neuron-specific chromatin regulatory subunit BAF53b is necessary for synaptic plasticity and memory. *Nature neuroscience*. 2013 Mar 24.
13. Kernohan KD, Jiang Y, Tremblay DC, Bonvissuto AC, Eubanks JH, Mann MR, et al. ATRX partners with cohesin and MeCP2 and contributes to developmental silencing of imprinted genes in the brain. *Developmental cell*. 2010 Feb 16; 18(2):191–202. [PubMed: 20159591]
14. Nord AS, Roeb W, Dickel DE, Walsh T, Kusenda M, O'Connor KL, et al. Reduced transcript expression of genes affected by inherited and de novo CNVs in autism. *European journal of human genetics : EJHG*. 2011 Jun; 19(6):727–731. [PubMed: 21448237]
15. Halgren C, Kjaergaard S, Bak M, Hansen C, El-Schich Z, Anderson CM, et al. Corpus callosum abnormalities, intellectual disability, speech impairment, and autism in patients with haploinsufficiency of ARID1B. *Clinical genetics*. 2012 Sep; 82(3):248–255. [PubMed: 21801163]
16. O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, et al. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nature Genetics*. 2011 Jun; 43(6):585–589. [PubMed: 21572417]

17. Nishiyama M, Skoultchi AI, Nakayama KI. Histone H1 recruitment by CHD8 is essential for suppression of the Wnt-beta-catenin signaling pathway. *Molecular and cellular biology*. 2012 Jan; 32(2):501–512. [PubMed: 22083958]
18. Badeaux AI, Shi Y. Emerging roles for chromatin as a signal integration and storage platform. *Nature reviews Molecular cell biology*. 2013 Apr; 14(4):211–224.
19. Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature*. 2009 Oct 14.
20. Cheung I, Shulha HP, Jiang Y, Matevossian A, Wang J, Weng Z, et al. Developmental regulation and individual differences of neuronal H3K4me3 epigenomes in the prefrontal cortex. *Proc Natl Acad Sci U S A*. May 11; 107(19):8824–8829. [PubMed: 20421462]
21. Shulha HP, Cheung I, Whittle C, Wang J, Virgil D, Lin CL, et al. Epigenetic signatures of autism: trimethylated H3K4 landscapes in prefrontal neurons. *Archives of general psychiatry*. 2012 Mar; 69(3):314–324. [PubMed: 22065254]
22. Michaelson JJ, Shi Y, Gujral M, Zheng H, Malhotra D, Jin X, et al. Whole-genome sequencing in autism identifies hot spots for de novo germline mutation. *Cell*. 2012 Dec 21; 151(7):1431–1442. [PubMed: 23260136]
23. Schroeder DI, Blair JD, Lott P, Yu HO, Hong D, Crary F, et al. The human placenta methylome. *Proceedings of the National Academy of Sciences of the United States of America*. 2013 Mar 25.
24. Schroeder DI, Lott P, Korf I, LaSalle JM. Large-scale methylation domains mark a functional subset of neuronally expressed genes. *Genome Research*. 2011 Oct; 21(10):1583–1591. [PubMed: 21784875]
25. Wong CC, Meaburn EL, Ronald A, Price TS, Jeffries AR, Schalkwyk LC, et al. Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. *Molecular psychiatry*. 2013 Apr 23.
26. Ginno PA, Lott PL, Christensen HC, Korf I, Chedin F. R-Loop Formation Is a Distinctive Characteristic of Unmethylated Human CpG Island Promoters. *Molecular cell*. 2012 Feb 29.
27. McLennan Y, Polussa J, Tassone F, Hagerman R. Fragile × syndrome. *Curr Genomics*. 2011 May; 12(3):216–224. [PubMed: 22043169]
28. Statland JM, Tawil R. Facioscapulohumeral muscular dystrophy: molecular pathological advances and future directions. *Current opinion in neurology*. 2011 Oct; 24(5):423–428. [PubMed: 21734574]
29. Singer H, Walier M, Nusgen N, Meesters C, Schreiner F, Woelfle J, et al. Methylation of L1Hs promoters is lower on the inactive X, has a tendency of being higher on autosomes in smaller genomes and shows inter-individual variability at some loci. *Human Molecular Genetics*. 2012 Jan 1; 21(1):219–235. [PubMed: 21972244]
30. Ziats MN, Rennert OM. Sex-biased gene expression in the developing brain: implications for autism spectrum disorders. *Mol Autism*. 2013; 4(1):10. [PubMed: 23651621]
31. Carrel L, Willard HF. X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature*. 2005 Mar 17; 434(7031):400–404. [PubMed: 15772666]
32. Xu J, Deng X, Disteché CM. Sex-specific expression of the X-linked histone demethylase gene *Jarid1c* in brain. *PLoS One*. 2008; 3(7):e2553. [PubMed: 18596936]
33. Howerton CL, Morgan CP, Fischer DB, Bale TL. O-GlcNAc transferase (OGT) as a placental biomarker of maternal stress and reprogramming of CNS gene transcription in development. *Proceedings of the National Academy of Sciences of the United States of America*. 2013 Mar 26; 110(13):5169–5174. [PubMed: 23487789]
34. LaSalle JM. A genomic point-of-view on environmental factors influencing the human brain methylome. *Epigenetics : official journal of the DNA Methylation Society*. 2011 Jul; 6(7):862–869. [PubMed: 21617367]
35. Baccarelli A, Bollati V. Epigenetics and environmental chemicals. *Curr Opin Pediatr*. 2009 Apr; 21(2):243–251. [PubMed: 19663042]
36. Woods R, Vallero RO, Golub MS, Suarez JK, Ta TA, Yasui DH, et al. Long-lived epigenetic interactions between perinatal PBDE exposure and *Mecp2308* mutation. *Human Molecular Genetics*. 2012 Jun 1; 21(11):2399–2411. [PubMed: 22343140]

37. Lee DH, Jacobs DR Jr, Porta M. Hypothesis: a unifying mechanism for nutrition and chemicals as lifelong modulators of DNA hypomethylation. *Environ Health Perspect*. 2009 Dec; 117(12):1799–1802. [PubMed: 20049195]
38. Sawicka K, Zukin RS. Dysregulation of mTOR signaling in neuropsychiatric disorders: therapeutic implications. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2012 Jan; 37(1):305–306. [PubMed: 22157871]
39. Goines PE, Ashwood P. Cytokine dysregulation in autism spectrum disorders (ASD): possible role of the environment. *Neurotoxicology and teratology*. 2013 Mar-Apr;36:67–81. [PubMed: 22918031]
40. Sauer S, Bruno L, Hertweck A, Finlay D, Leleu M, Spivakov M, et al. T cell receptor signaling controls Foxp3 expression via PI3K, Akt, mTOR. *Proceedings of the National Academy of Sciences of the United States of America*. 2008 Jun 3; 105(22):7797–7802. [PubMed: 18509048]
41. Miyao T, Floess S, Setoguchi R, Luche H, Fehling HJ, Waldmann H, et al. Plasticity of Foxp3(+) T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. *Immunity*. 2012 Feb 24; 36(2):262–275. [PubMed: 22326580]
42. Weitzel JM, Buhrmester H, Stratling WH. Chicken MAR-binding protein ARBP is homologous to rat methyl-CpG-binding protein MeCP2. *Molecular and cellular biology*. 1997 Sep; 17(9):5656–5666. [PubMed: 9271441]
43. Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, et al. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat Genet*. 1998; 19(2):187–191. [PubMed: 9620779]
44. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nature Genetics*. 1999 Oct; 23(2):185–188. [PubMed: 10508514]
45. Young JI, Hong EP, Castle JC, Crespo-Barreto J, Bowman AB, Rose MF, et al. Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. *Proc Natl Acad Sci U S A*. 2005 Dec 6; 102(49):17551–17558. [PubMed: 16251272]
46. Nagarajan RP, Hogart AR, Gwyne Y, Martin MR, Lasalle JM. Reduced MeCP2 expression is frequent in autism frontal cortex and correlates with aberrant MECP2 promoter methylation. *Epigenetics*. 2006 Oct; 1(4):172–182.
47. Gonzales ML, Adams S, Dunaway KW, LaSalle JM. Phosphorylation of distinct sites in MeCP2 modifies cofactor associations and the dynamics of transcriptional regulation. *Molecular and cellular biology*. 2012 Jul; 32(14):2894–2903. [PubMed: 22615490]
48. Nusinow DA, Hernandez-Munoz I, Fazzio TG, Shah GM, Kraus WL, Panning B. Poly(ADP-ribose) polymerase 1 is inhibited by a histone H2A variant, MacroH2A, and contributes to silencing of the inactive X chromosome. *The Journal of biological chemistry*. 2007 Apr 27; 282(17):12851–12859. [PubMed: 17322296]
49. Tahiliani M, Mei P, Fang R, Leonor T, Rutenberg M, Shimizu F, et al. The histone H3K4 demethylase SMCX links REST target genes to X-linked mental retardation. *Nature*. 2007 May 31; 447(7144):601–605. [PubMed: 17468742]
50. Iwase S, Lan F, Bayliss P, de la Torre-Ubieta L, Huarte M, Qi HH, et al. The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases. *Cell*. 2007 Mar 23; 128(6):1077–1088. [PubMed: 17320160]
51. Jensen LR, Amende M, Gurok U, Moser B, Gimmel V, Tzschach A, et al. Mutations in the JARID1C gene, which is involved in transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. *American Journal of Human Genetics*. 2005 Feb; 76(2):227–236. [PubMed: 15586325]
52. Adegbola A, Gao H, Sommer S, Browning M. A novel mutation in JARID1C/SMCX in a patient with autism spectrum disorder (ASD). *American journal of medical genetics Part A*. 2008 Feb 15; 146A(4):505–511. [PubMed: 18203167]
53. Cukier HN, Rabionet R, Konidari I, Rayner-Evans MY, Baltos ML, Wright HH, et al. Novel variants identified in methyl-CpG-binding domain genes in autistic individuals. *Neurogenetics*. 2010 Jul; 11(3):291–303. [PubMed: 19921286]

54. Sarraf SA, Stancheva I. Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. *Molecular cell*. 2004 Aug 27; 15(4):595–605. [PubMed: 15327775]
55. Castermans D, Vermeesch JR, Fryns JP, Steyaert JG, Van de Ven WJ, Creemers JW, et al. Identification and characterization of the TRIP8 and REEP3 genes on chromosome 10q21.3 as novel candidate genes for autism. *European journal of human genetics : EJHG*. 2007 Apr; 15(4): 422–431. [PubMed: 17290275]
56. Wolf SS, Patchev VK, Obendorf M. A novel variant of the putative demethylase gene, s-JMJD1C, is a coactivator of the AR. *Arch Biochem Biophys*. 2007 Apr 1; 460(1):56–66. [PubMed: 17353003]



**Figure 1. Chromatin factors at the roots of autism etiology**

An analogy of a tree with deep roots is used to illustrate several points about chromatin factors implicated in autism. Candidate gene approaches for ASD have justifiably prioritized the investigation of low hanging fruit (large red circles) for genes that encode proteins with known functions at neuronal synapses. But these synaptic proteins are connected to signal transduction pathways that make changes to gene expression patterns through chromatin dynamics within the neuronal nucleus. The mTOR pathway, which integrates metabolic and nutrient sensing signals into energy is central to the signaling pathways and to supplying energy for chromatin remodeling events. But just as the roots and trunk of a tree are bidirectional pathways for the tree, information within the nucleus stored in the form of

chromatin provides information back to the synapse to regulate levels of synaptic proteins during synaptic pruning and scaling. Levels of DNA methylation (small red dots) and readers of DNA methylation (MECP2, MBD1, etc) may act as chromatin sensors of many environmental factors including diet and chemical toxins during the maturation of synapses. Furthermore, chromatin factors such as MACROD2 and JMJD1C have metabolic sensing properties, so that factors such as stress and inflammation may alter chromatin dynamics of neurons with long lasting effects. Thus, in the future, it will be important to continue to dig beneath the surface to unearth the chromatin factors and epigenetic pathways in the etiology of ASD in order to fully understand these complex genetic disorders.

Table 1

Chromatin genes implicated in autism spectrum disorders

Gene name	aliases	Human chrom location	Human disease	Protein function	Interacting proteins	refs
<i>MECP2</i>	Methyl CpG binding protein 2, ARBP	Xq28	Rett syndrome (RTT), autism (rare mutation or aberrant methylation)	Binds mCpG, repression, chromatin dynamics	Sim3a, HDAC, ATRX, YB1, SMC1A	9, 42-46
<i>ATRX</i>	RAD54, XH2	Xq21.1	thalassemia, intellectual disabilities	SWI/SNF chromatin remodeling, ATPase/helicase domain	MeCP2, SMC1A	13, 47
<i>H2AFY</i>	MACROH2A1.1	5q31.1	Autism (association)	Histone H2 variant, X-chromosome inactivation	HDAC1, PARP1	8, 48
<i>SMC1A</i>	Cohesin, CDLS2	Xp11.22	Comelia de Lange syndrome (CDLS)	Chromosome cohesion	ATRX, SMC3, MeCP2, CTCF	13
<i>MACROD2</i>		20p12.1	Autism (association)	O-acetyl-ADP-ribose deacetylase, binds this metabolite from histone deacetylation		11
<i>KDM5C</i>	JARID1C, SMCX	Xp11.22	ASD, ID (rare mutations)	Histone demethylase of H3K4, gene repression	HDAC, REST	49-52
<i>MBD1</i>	CXXC3	18q21	Autism (rare mutations), also rare variants in related genes MBD4, MBD5	Binds mCpG, links mCpG to H3K9me3	SETDB1, AFT7IP	9, 53, 54
<i>ARID1B</i>	BAF250B	6p25.3	Coffin-Siris syndrome (CSS), mental retardation autosomal dominant type 12 (MRD12), autism (rare)	Component of SWI/SNF chromatin remodeling complex, AT-rich binding domain	SWI/SNF complex proteins in neuronal BAF complex (nBAF)	14, 15
<i>SMARCC1</i>	BAF155	3p31.21	Autism (rare mutation)	Component of SWI/SNF chromatin remodeling complex, and nBAF	ARID1A, ARID1B, SMARCC2,	5
<i>SMARCC2</i>	BAF170	12q13.2	Autism (rare mutation)	Component of SWI/SNF chromatin remodeling complex, and nBAF	ARID1A, SMARCC1, HDAC1/2	5
<i>JMJD1C</i>	TRIP8	10q21.3	Autism (rare mutation, translocation, abnormal methylation)	Histone demethylase for H3K9, hormone-dependent transcriptional activation	Thyroid hormone receptor, androgen receptor	5, 25, 55, 56
<i>CHD8</i>	AUTS18	14q11.2	autism (rare mutation), also rare autism variants in family members CHD1, CHD3, CHD7	ATP-dependent chromatin helicase, negative regulator of Wnt signaling pathway by regulating beta-catenin (CTNNB1)	p53, histone H1, CTNNB1, CTCF, MLL complex proteins WDR5, RBBP5, CHD7 (mutated in CHARGE)	5, 6, 16