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**Supplementary Table 1:** BRCA1 (a) and BRCA2 (b) variants in cholangiocarcinoma cohort with interpretation and corresponding variant frequencies.

**a) BRCA1, n=12**

| Protein Change | Exon | Effect | Interpretation | N | Average Variant Frequency (%) | SD of variant frequency |
|---|---|---|---|---|---|---|
| Q148X | 7 | Nonsense | Mutated, Pathogenic | 1 | 60 | 0 |
| A1708E | 17 | Missense | Mutated, Pathogenic | 1 | 70 | 0 |
| Q1395X | 11 | Nonsense | Mutated, Pathogenic | 1 | 52 | 0 |
| C61G | 4 | Missense | Mutated, Pathogenic | 1 | 66 | 0 |
| E836X | 10 | Nonsense | Mutated, Pathogenic | 1 | 36 | 0 |
| H971Q | 10 | Missense | Mutated, Variant of Unknown Significance | 1 | 10 | 0 |
| I171T | 7 | Missense | Mutated, Variant of Unknown Significance | 1 | 13 | 0 |
| S140R | 6 | Missense | Mutated, Variant of Unknown Significance | 1 | 14 | 0 |
| H1860L | 23 | Missense | Mutated, Variant of Unknown Significance | 1 | 13 | 0 |
| c.4987-2A>T | 16 | Splicing | Mutated, Pathogenic | 1 | 13 | 0 |
| E23fs | 2 | Frameshift | Mutated, Pathogenic | 2 | 66 | 24.04 |

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**b) BRCA2, n=51**

| Protein Change | Exon | Effect | Interpretation | N | Average Variant Frequency (%) | SD of variant frequency |
|---|---|---|---|---|---|---|
| F529fs | 10 | Frameshift | Mutated, Pathogenic | 1 | 31 | 0 |
| I1874fs | 11 | Frameshift | Mutated, Pathogenic | 1 | 50 | 0 |
| Q2823X | 19 | Nonsense | Mutated, Pathogenic | 1 | 52 | 0 |
| c.8755-1G>A | 22 | Splicing | Mutated, Pathogenic | 1 | 75 | 0 |
| I505T | 10 | Missense | Mutated, Benign | 1 | 52 | 0 |
| N1706fs | 11 | Frameshift | Mutated, Pathogenic | 1 | 35 | 0 |
| R2842C | 20 | Missense | Mutated, Variant of Unknown Significance | 1 | 41 | 0 |
| N1626fs | 11 | Frameshift | Mutated, Pathogenic | 1 | 40 | 0 |
| Q3350R | 27 | Missense | Mutated, Presumed Benign | 1 | 56 | 0 |
| I1859fs | 11 | Frameshift | Mutated, Pathogenic | 1 | 63 | 0 |
| S2835P | 20 | Missense | Mutated, Benign | 1 | 30 | 0 |
| N2699fs | 18 | Frameshift | Mutated, Pathogenic | 1 | 23 | 0 |
| T3085fs | 24 | Frameshift | Mutated, Pathogenic | 1 | 31 | 0 |
| S2378X | 14 | Nonsense | Mutated, Pathogenic | 1 | 40 | 0 |
| T203A | 7 | Missense | Mutated, Variant of Unknown Significance | 1 | 25 | 0 |
| F2638fs | 17 | Frameshift | Mutated, Pathogenic | 1 | 69 | 0 |
| C711X | 11 | Nonsense | Mutated, Pathogenic | 1 | 45 | 0 |
| Y600H | 10 | Missense | Mutated, Benign | 1 | 34 | 0 |
| R2502C | 15 | Missense | Mutated, Presumed Benign | 1 | 30 | 0 |
| c.8488-1G>A | 20 | Splicing | Mutated, Pathogenic | 1 | 41 | 0 |
| I1633F | 11 | Missense | Mutated, Variant of Unknown Significance | 1 | 8 | 0 |
| L2753fs | 18 | Frameshift | Mutated, Pathogenic | 1 | 19 | 0 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| G267X | 10 | Nonsense | Mutated, Pathogenic | 1 | 56 | 0 |
| K2496X | 15 | Nonsense | Mutated, Pathogenic | 1 | 26 | 0 |
| L1466fs | 11 | Frameshift | Mutated, Pathogenic | 1 | 46 | 0 |
| T2967fs | 22 | Frameshift | Mutated, Pathogenic | 1 | 10 | 0 |
| T2766fs | 18 | Frameshift | Mutated, Pathogenic | 1 | 72 | 0 |
| R645fs | 11 | Frameshift | Mutated, Pathogenic | 1 | 67 | 0 |
| K1691fs | 11 | Frameshift | Mutated, Pathogenic | 1 | 10 | 0 |
| D1769fs | 11 | Frameshift | Mutated, Pathogenic | 1 | 37 | 0 |
| T1346fs | 11 | Frameshift | Mutated, Pathogenic | 1 | 84 | 0 |
| E97X | 3 | Nonsense | Mutated, Pathogenic | 1 | 14 | 0 |
| C1820R | 11 | Missense | Mutated, Variant of Unknown Significance | 1 | 9 | 0 |
| C1820X | 11 | Nonsense | Mutated, Pathogenic | 1 | 11 | 0 |
| N1784fs | 11 | Frameshift | Mutated, Pathogenic | 2 | 23 | 14.14 |
| K2162fs | 11 | Frameshift | Mutated, Pathogenic | 2 | 63 | 4.24 |
| D946fs | 11 | Frameshift | Mutated, Pathogenic | 2 | 30.5 | 13.44 |
| T3033fs | 23 | Frameshift | Mutated, Pathogenic | 2 | 14 | 5.66 |
| S1982fs | 11 | Frameshift | Mutated, Pathogenic | 2 | 78 | 16.97 |
| I605fs | 10 | Frameshift | Mutated, Pathogenic | 3 | 41.67 | 27.54 |
| W1692fs | 11 | Frameshift | Mutated, Pathogenic | 4 | 14.5 | 6.86 |

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**Supplementary Table 2:** gene mutation frequencies in BRCA-MT and WT cohorts. Highlighted are p-values ≤0.05

| Test | Positive | Negative | Total | % BRCA-MT BTC | Positive | Negative | Total | % BRCA-WT BTC | P value |
|---|---|---|---|---|---|---|---|---|---|
| NGS-TP53 | 25 | 20 | 45 | 55.6% | 485 | 724 | 1209 | 40.1% | 0.038423 |
| NGS-ARID1A | 12 | 11 | 23 | 52.2% | 170 | 286 | 456 | 37.3% | 0.151064 |
| NGS-KRAS | 12 | 34 | 46 | 26.1% | 224 | 1019 | 1243 | 18.0% | 0.164805 |
| NGS-KMT2D | 8 | 32 | 40 | 20.0% | 22 | 975 | 997 | 2.2% | 4.6E-11 |
| NGS-KMT2C | 4 | 26 | 30 | 13.3% | 31 | 722 | 753 | 4.1% | 0.016592 |
| NGS-CDKN2A | 5 | 33 | 38 | 13.2% | 80 | 917 | 997 | 8.0% | 0.257927 |
| NGS-ASXL1 | 4 | 28 | 32 | 12.5% | 7 | 888 | 895 | 0.8% | 1.8E-09 |
| NGS-MLH1 | 5 | 41 | 46 | 10.9% | 6 | 1236 | 1242 | 0.5% | 5.58E-14 |
| NGS-PBRM1 | 4 | 36 | 40 | 10.0% | 44 | 999 | 1043 | 4.2% | 0.081242 |
| NGS-PTCH1 | 3 | 28 | 31 | 9.7% | 2 | 786 | 788 | 0.3% | 3.92E-11 |
| NGS-PTEN | 4 | 41 | 45 | 8.9% | 16 | 1184 | 1200 | 1.3% | 7.56E-05 |
| NGS-RB1 | 4 | 41 | 45 | 8.9% | 20 | 1182 | 1202 | 1.7% | 0.000533 |
| NGS-NF1 | 3 | 34 | 37 | 8.1% | 27 | 935 | 962 | 2.8% | 0.063718 |
| NGS-RNF43 | 3 | 37 | 40 | 7.5% | 24 | 1026 | 1050 | 2.3% | 0.037297 |
| NGS-APC | 3 | 43 | 46 | 6.5% | 31 | 1212 | 1243 | 2.5% | 0.094139 |
| NGS-PIK3CA | 3 | 43 | 46 | 6.5% | 77 | 1159 | 1236 | 6.2% | 0.935932 |
| NGS-KDM6A | 2 | 30 | 32 | 6.3% | 8 | 819 | 827 | 1.0% | 0.006266 |
| NGS-ARID2 | 2 | 37 | 39 | 5.1% | 45 | 984 | 1029 | 4.4% | 0.821477 |
| CNA-FGFR1 | 2 | 37 | 39 | 5.1% | 3 | 1005 | 1008 | 0.3% | 1.76E-05 |
| NGS-BCL9 | 2 | 38 | 40 | 5.0% | 4 | 1046 | 1050 | 0.4% | 0.000107 |
| NGS-FLCN | 2 | 38 | 40 | 5.0% | 3 | 1047 | 1050 | 0.3% | 1.49E-05 |
| NGS-KMT2A | 2 | 38 | 40 | 5.0% | 6 | 1026 | 1032 | 0.6% | 0.001443 |
| NGS-MEN1 | 2 | 38 | 40 | 5.0% | 5 | 1045 | 1050 | 0.5% | 0.000439 |
| NGS-SF3B1 | 2 | 38 | 40 | 5.0% | 17 | 1026 | 1043 | 1.6% | 0.111108 |
| NGS-ATM | 2 | 44 | 46 | 4.3% | 41 | 1201 | 1242 | 3.3% | 0.697971 |
| NGS-BRAF | 2 | 44 | 46 | 4.3% | 39 | 1202 | 1241 | 3.1% | 0.647636 |
| NGS-HNF1A | 2 | 44 | 46 | 4.3% | 5 | 1226 | 1231 | 0.4% | 0.000378 |
| NGS-NOTCH1 | 2 | 44 | 46 | 4.3% | 3 | 1234 | 1237 | 0.2% | 1.14E-05 |
| NGS-SMARCB1 | 2 | 44 | 46 | 4.3% | 2 | 1241 | 1243 | 0.2% | 5.34E-07 |
| NGS-RAD50 | 1 | 29 | 30 | 3.3% | 1 | 753 | 754 | 0.1% | 0.000654 |
| NGS-PRKDC | 1 | 34 | 35 | 2.9% | 6 | 969 | 975 | 0.6% | 0.116258 |
| NGS-BCOR | 1 | 36 | 37 | 2.7% | 6 | 944 | 950 | 0.6% | 0.140785 |
| NGS-FUBP1 | 1 | 36 | 37 | 2.7% | 1 | 966 | 967 | 0.1% | 0.000501 |
| NGS-AMER1 | 1 | 38 | 39 | 2.6% | 8 | 1030 | 1038 | 0.8% | 0.227112 |
| NGS-FANCE | 1 | 38 | 39 | 2.6% | 0 | 989 | 989 | 0.0% | 4.7E-07 |
| NGS-BRIP1 | 1 | 39 | 40 | 2.5% | 6 | 1028 | 1034 | 0.6% | 0.138746 |
| NGS-CIC | 1 | 39 | 40 | 2.5% | 4 | 968 | 972 | 0.4% | 0.06486 |
| NGS-CREBBP | 1 | 39 | 40 | 2.5% | 6 | 1035 | 1041 | 0.6% | 0.136628 |
| NGS-ERBB3 | 1 | 39 | 40 | 2.5% | 14 | 1037 | 1051 | 1.3% | 0.533546 |
| NGS-FH | 1 | 39 | 40 | 2.5% | 1 | 1026 | 1027 | 0.1% | 0.000568 |
| NGS-JAK1 | 1 | 39 | 40 | 2.5% | 1 | 1034 | 1035 | 0.1% | 0.000538 |
| NGS-MAP2K1 | 1 | 39 | 40 | 2.5% | 5 | 1046 | 1051 | 0.5% | 0.089299 |
| NGS-MSH6 | 1 | 39 | 40 | 2.5% | 7 | 1020 | 1027 | 0.7% | 0.190887 |
| NGS-MUTYH | 1 | 39 | 40 | 2.5% | 14 | 1033 | 1047 | 1.3% | 0.536098 |
| NGS-NBN | 1 | 39 | 40 | 2.5% | 6 | 1027 | 1033 | 0.6% | 0.139052 |
| NGS-NSD1 | 1 | 39 | 40 | 2.5% | 1 | 1039 | 1040 | 0.1% | 0.00052 |
| NGS-PALB2 | 1 | 39 | 40 | 2.5% | 11 | 1038 | 1049 | 1.0% | 0.388133 |
| NGS-GNAS | 1 | 45 | 46 | 2.2% | 9 | 1234 | 1243 | 0.7% | 0.271069 |
| NGS-IDH1 | 1 | 45 | 46 | 2.2% | 110 | 1132 | 1242 | 8.9% | 0.112738 |
| NGS-JAK2 | 1 | 45 | 46 | 2.2% | 3 | 1217 | 1220 | 0.2% | 0.022178 |
| NGS-NRAS | 1 | 45 | 46 | 2.2% | 36 | 1208 | 1244 | 2.9% | 0.773887 |
| NGS-SMAD4 | 1 | 45 | 46 | 2.2% | 90 | 1151 | 1241 | 7.3% | 0.187025 |

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**Supplementary Table 3:** Determination of MSI-H/MMR status by different test platform (2A) and inter-platform concordance (2B).

2A

| Biomarker Tested | Platform | Testing Time Range | N of tumors Tested | Alterations detected | Overall MSI-H/dMMR (combination of FA, IHC and NGS) |
|---|---|---|---|---|---|
| **Microsatellite Instability** | **FA** | **Aug 2015-Sep 2018** | 102 | 2.0% (2/102) | 2.4% (24/1089) |
| **Mismatch Repair Deficiency** | **IHC** | **Aug 2015-Jan 2019** | 397 | 4.7% (19/397) | |
| **Microsatellite Instability** | **NGS** | **Mar 2017-Jan 2019** | 1046 | 2.4% (25/1046) | |

2B

| | | **FA** | | | | **FA** | | | | **IHC** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **MSI-H** | **MSS** | | | **MSI-H** | **MSS** | | | **dMMR** | **pMMR** |
| | **dMMR** | 1 | 0 | | **MSI-H** | 2 | 2 | | **MSI-H** | 17 | 0 |
| **IHC** | **pMMR** | 1 | 30 | **NGS** | **MSS** | 0 | 87 | **NGS** | **MSS** | 1 | 349 |
| **Concordance** | | 0.96875 | | **Concordance** | | 0.978022 | | **Concordance** | | 0.997275 | |