OncoGeneDx: Melanoma Cancer Panel

Panel Gene List: \textit{BAP1, BRCA2, CDK4, CDKN2A, MITF*, POT1, PTEN, RB1, TP53}

*Testing includes sequencing and deletion/duplication analysis for all genes except MITF (only c.952G>A (p.Glu318Lys) will be analyzed and reported)

Clinical Features:
In the general population, approximately 1 in 50 individuals (2\%) will develop melanoma in their lifetime.\textsuperscript{1} Several individual factors and exposures, such as fair skin, light hair, freckling, multiple moles, a history of sunburn, excessive ultraviolet light exposure, and sun sensitivity, are important risk factors. While most cases of malignant melanoma (MM) develop sporadically with no family history, approximately 10\% of individuals diagnosed with MM have at least one first- or second-degree relative with a history of the disease.\textsuperscript{2} Of those individuals with familial MM, 10 to 40\% are thought to be due to pathogenic germline variants in the \textit{CDKN2A} gene depending on inclusion criteria.\textsuperscript{2-7} The prevalence of pathogenic germline variants in the other genes included on this panel among familial MM cases is not well described.

The features suggestive of a hereditary MM predisposition may include, but are not limited to: early onset of disease, multiple primary melanomas and/or other cancers in a single individual, history of MM in one or more close relatives, and several relatives affected with MM spanning multiple generations.\textsuperscript{6,8}

Hereditary MM can be divided into two categories: melanoma-predominant or melanoma-dominant syndromes and melanoma-including or melanoma-subordinate syndromes.\textsuperscript{7,9} Melanoma-predominant syndromes are caused by pathogenic variants in \textit{BAP1, CDK4, CDKN2A, MITF,} and \textit{POT1} and may confer up to a 90\% lifetime risk to develop MM.\textsuperscript{9} Families with pathogenic variants in these genes generally display clustering of MM which will often be the most predominant cancer in these families. Individuals with germline pathogenic variants in \textit{CDKN2A} and \textit{CDK4} are often referred to as having Familial Atypical Multiple Mole Melanoma syndrome (FAMMM).

Melanoma-including syndromes are caused by pathogenic variants in \textit{BRCA2, PTEN, RB1,} and \textit{TP53}. These syndromes are not defined by their MM risk; however, MM can be part of the associated cancer spectrum. Individuals with a personal and/or family history of MM and other related cancers should be considered at risk for these conditions.

Begg et al. (2005) found that individuals who test negative for a previously identified familial \textit{CDKN2A} pathogenic variant may still have an increased risk for MM likely due to multifactorial effects, although their risk is lower compared to individuals with the familial germline pathogenic variant.
Inheritance Pattern:
All of the genes on this panel are associated with an autosomal dominant cancer risk. Some of the genes on this panel are also associated with extremely rare conditions when inherited in an autosomal recessive fashion. The specifics of this inheritance are outlined in the table below.

Test Methods:
Genomic DNA from the submitted specimen is enriched for the complete coding region and splice site junctions of the genes on the panel using a proprietary targeted capture system developed by GeneDx. (For *PTEN*, approximately nucleotides c.-700 through c.-1300 in the promoter region are also captured.) The products are sequenced on an Illumina HiSeq instrument with 2x100 paired-end reads. The sequence is aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Capillary sequencing is used to confirm all variants with clinical or uncertain significance and to analyze regions with inadequate coverage by Next Generation sequencing (NGS). If present, apparently homozygous variants are confirmed using alternate primer pairs to significantly reduce the possibility of allele drop-out. All sequence alterations are described according to the Human Genome Variation Society (HGVS) nomenclature guidelines. Concurrent deletion/duplication analysis from NGS data is performed for all relevant genes on the panel to detect multi-exonic and most single-exon deletions and duplications. For specimens with insufficient copy number data and for confirmation of identified copy number changes, exon-level array CGH, MLPA or other appropriate methods are used. For *MITF*, only c.952G>A (p.Glu318Lys) is analyzed and reported. Copy-number alterations are reported according to the International System for Human Cytogenetic Nomenclature (ISCN) guidelines. Benign and likely benign variants, if present, are not reported but are available upon request.

Test Sensitivity:
The clinical sensitivity of sequencing and deletion/duplication analysis of the 9 genes included in the OncoGeneDx Melanoma Cancer Panel depends in part on the patient’s clinical phenotype and family history. In general, the sensitivity is highest for individuals with features suggestive of hereditary predisposition to cancer as outlined above. DNA sequencing will detect nucleotide substitutions and small insertions and deletions, while NGS-CNV analysis, array CGH, or MLPA will detect exon-level deletions and duplications. These methods are expected to be greater than 99% sensitive in detecting pathogenic variants identifiable by sequencing or CNV technology. The likelihood of a false positive result is expected to be <1%.

Technical Limitations: Neither sequencing, exon-level array CGH nor MLPA can reliably detect mosaicism, and cannot detect chromosomal aberrations. Deletions involving more than 20bp and insertions involving more than 10bp are not reliably detected by the sequencing
methodology, and deletions or duplications of less than 250bp are not reliably detected by NGS-CNV analysis or array CGH. Regions of certain genes have inherent sequence properties that yield suboptimal data, potentially impairing accuracy of the results. In the absence of mRNA/cDNA studies, we cannot completely exclude the possibility of undetectable clinically significant variants in certain regions of these genes. False negatives may also occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. In individuals with active leukemia or lymphoma or with known chronic myeloid or lymphoid neoplasms (such as low grade MDS, CML, ET, P. vera, PMF, CLL), there is a possibility that testing of specimens containing leukocytes may detect an acquired somatic variant, resulting in a false positive result. In this situation, please contact one of our genetic counselors to discuss the utility of submitting an alternate specimen. The ability to detect genetic variants and naming conventions can differ among laboratories. Rare false negatives, therefore, may occur when testing for a specific variant identified at a laboratory other than GeneDx, if a positive control is not provided. Based on the specific array design and technology used, the reported coordinates of duplications and deletions at the exon or gene level can slightly differ among family members tested but, in general, relatives are expected to have the same copy number variant.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Inheritance</th>
<th>Disease Associations</th>
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<tbody>
<tr>
<td>BAP1</td>
<td>UBIQUITIN CARBOXYL-TERMINAL HYDROLASE BAP1</td>
<td>AD</td>
<td>Uveal/cutaneous melanoma, mesothelioma, &amp; renal cancer</td>
</tr>
<tr>
<td>BRCA2</td>
<td>BREAST CANCER TYPE 2 SUSCEPTIBILITY PROTEIN</td>
<td>AD</td>
<td>Hereditary Breast and Ovarian Cancer (HBOC) syndrome: breast, ovarian, pancreatic, prostate, melanoma, &amp; endometrial serous cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AR</td>
<td>Fanconi Anemia</td>
</tr>
<tr>
<td>CDK4</td>
<td>CYCLIN-DEPENDENT KINASE 4</td>
<td>AD</td>
<td>Melanoma, non-Melanoma skin &amp; pancreatic cancer</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>CYCLIN-DEPENDENT KINASE INHIBITOR 2A, TUMOR SUPPRESSOR ARF</td>
<td>AD</td>
<td>Familial Atypical Multiple Mole Melanoma (FAMMM) Syndrome: Melanoma &amp; pancreatic cancer</td>
</tr>
<tr>
<td>MITF</td>
<td>MICROPHTHALMIA-ASSOCIATED TRANSCRIPTION FACTOR</td>
<td>AD</td>
<td>Renal cancer &amp; melanoma</td>
</tr>
<tr>
<td>POT1</td>
<td>PROTECTION OF TELOMERES 1</td>
<td>AD</td>
<td>Melanoma &amp; glial tumors</td>
</tr>
<tr>
<td>PTEN</td>
<td>PHOSPHATIDYLINOSITOL 3,4,5-TRISPHOSPHATE 3-</td>
<td>AD</td>
<td>PTEN hamartoma tumor syndrome (PHTS): breast,</td>
</tr>
</tbody>
</table>
PHOSPHATASE AND DUAL-SPECIFICITY PROTEIN PHOSPHATASE PTEN  |  thyroid, endometrial, colon, melanoma & renal cancer, gastrointestinal polyps, Lhermitte-Duclos Disease

RB1  |  RETINOBLASTOMA-ASSOCIATED PROTEIN  |  Hereditary retinoblastoma: retinoblastoma, sarcoma, leukemia, melanoma, & pineoblastoma

TP53  |  CELLULAR TUMOR ANTIGEN P53  |  Li-Fraumeni syndrome (LFS): breast cancer, sarcoma, brain cancer, hematologic malignancies, adrenocortical carcinoma, among others

Because of evolving and expanding phenotypes, this list of cancer/tumor types is not exhaustive. Gene-specific risk for some of the cancers and other features listed are not well-defined.

** High overall risk of cancer: 75% lifetime risk for males to develop cancer, nearly 100% risk for females.

**Abbreviations:**
AD – Autosomal dominant
AR – Autosomal recessive
CGH – Comparative genomic hybridization
MLPA – Multiplex ligation-dependent probe amplification

**References:**