OncoGeneDx: Melanoma Panel

Panel Gene List: *BAP1, BRCA2, CDK4, CDKN2A, MITF, POT1, PTEN, RB1, TP53*

Clinical Features:
In the general population, approximately 1 in 50 individuals (2%) will develop melanoma in their lifetime.[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.[2] Of those individuals with familial MM, 10 to 40% are thought to be due to pathogenic germline variants in the *CDKN2A* gene, depending on inclusion criteria.[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.[6,8]

Hereditary MM can be divided into two categories: melanoma-predominant or melanoma-dominant syndromes and melanoma-including or melanoma-subordinate syndromes.[7,9] Melanoma-predominant syndromes are caused by pathogenic variants in *BAP1*, *CDK4*, *CDKN2A*, *MITF*, and *POT1* and may confer up to a 90% lifetime risk to develop MM.[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 *CDKN2A* and *CDK4* are often referred to as having Familial Atypical Multiple Mole Melanoma syndrome (FAMMM).

Melanoma-including syndromes are caused by pathogenic variants in *BRCA2*, *PTEN*, *RB1*, and *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 *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 is extracted from the submitted specimen. For skin punch biopsies, fibroblasts are cultured and used for DNA extraction. The DNA is enriched for the complete coding regions and splice junctions of the genes on this panel using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). For *PTEN*, nucleotides c.-700 through c.-1300 in the promoter region are also captured. The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data by NGS. Reported clinically significant variants are confirmed by an appropriate method. Sequence variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Copy number variants are reported based on the probe coordinates, the coordinates of the exons involved, or precise breakpoints when known. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely 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 a 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.

Genetic testing using the methods applied at GeneDx is expected to be highly accurate. Normal findings do not rule out the diagnosis of a genetic disorder since some genetic

abnormalities may be undetectable by this test. The methods used cannot reliably detect deletions of 20bp to 250bp in size, or insertions of 10bp to 250 bp in size. Sequencing cannot detect low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect mosaicism and cannot identify balanced chromosome aberrations. Rarely, incidental findings of large chromosomal rearrangements outside the gene of interest may be identified. Regions of certain genes have inherent sequence properties (for example: repeat, homology, or pseudogene regions, high GC content, rare polymorphisms) that yield suboptimal data, potentially impairing accuracy of the results. False negatives may also occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. In individuals with active or chronic hematologic neoplasms or conditions, there is a possibility that testing may detect an acquired somatic variant, resulting in a false positive result. As the ability to detect genetic variants and naming conventions can differ among laboratories, rare false negative results may occur when no positive control is provided for testing of a specific variant identified at another laboratory. The chance of a false positive or false negative result due to laboratory errors incurred during any phase of testing cannot be completely excluded. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate. Consultation with a genetics professional is recommended for interpretation of results.

Gene	Protein	Inheritance	Disease Associations
BAP1[10,11]	UBIQUITIN CARBOXYL-TERMINAL HYDROLASE BAP1	AD	Uveal/cutaneous melanoma, mesothelioma, & renal cancer
BRCA2[12–21]	BREAST CANCER TYPE 2 SUSCEPTIBILITY PROTEIN	AD	Hereditary breast and ovarian cancer (HBOC) syndrome: breast, ovarian, pancreatic, prostate, melanoma, & endometrial serous cancer
		AR	Fanconi anemia
CDK4[6,7,22]	CYCLIN-DEPENDENT KINASE 4	AD	Melanoma, non-melanoma skin & pancreatic cancer
CDKN2A[4,7,23–26]	CYCLIN-DEPENDENT KINASE INHIBITOR 2A, TUMOR SUPPRESSOR ARF	AD	Familial atypical multiple mole melanoma (FAMMM) syndrome: melanoma, pancreatic cancer & astrocytoma
MITF[27–29]	MICROPHTHALMIA-ASSOCIATED TRANSCRIPTION FACTOR	AD	Renal cancer & melanoma
POT1[30–35]	PROTECTION OF TELOMERES 1	AD	Melanoma & brain glial tumors

PTEN[36–40]	PHOSPHATIDYLINOSITOL 3,4,5-TRISPHOSPHATE 3-PHOSPHATASE AND DUAL-SPECIFICITY PROTEIN PHOSPHATASE PTEN	AD	*PTEN* hamartoma tumor syndrome (PHTS): breast, thyroid, endometrial, colon, melanoma & renal cancer, gastrointestinal polyps, Lhermitte-Duclos Disease
RB1[41–45]	RETINOBLASTOMA-ASSOCIATED PROTEIN	AD	Hereditary retinoblastoma: retinoblastoma, sarcoma, leukemia, melanoma, & pineoblastoma
TP53[46–51]	CELLULAR TUMOR ANTIGEN P53	AD	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

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