

OncoGeneDx: Melanoma Panel

Panel Gene List: *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.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. This DNA is enriched for the complete coding regions and splice site 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). 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. For *MITF*, only c.952G>A (p.Glu318Lys) is analyzed and reported. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data by next generation sequencing (NGS). Reported clinically significant variants are confirmed by an appropriate method. Sequence and copy number variants are reported according to the Human Genome Variation Society (HGVS) or International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively. 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
<i>BAP1</i> ^{10,11}	UBIQUITIN CARBOXYL-TERMINAL HYDROLASE BAP1	AD	Uveal/cutaneous melanoma, mesothelioma, & renal cancer
<i>BRCA2</i> 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
<i>CDK4</i> ^{6,7,22}	CYCLIN-DEPENDENT KINASE 4	AD	Melanoma, non-melanoma skin & pancreatic cancer
<i>CDKN2A</i> ^{4,7,23-26}	CYCLIN-DEPENDENT KINASE INHIBITOR 2A, TUMOR SUPPRESSOR ARF	AD	Familial atypical multiple mole melanoma (FAMMM) syndrome: melanoma, pancreatic cancer & astrocytoma
<i>MITF</i> ²⁷⁻²⁹	MICROPHthalmia-ASSOCIATED TRANSCRIPTION FACTOR	AD	Renal cancer & melanoma
<i>POT1</i> ³⁰⁻³⁵	PROTECTION OF TELOMERES 1	AD	Melanoma & brain glial tumors

<i>PTEN</i> ^{36–40}	PHOSPHATIDYLINOSITOL 3,4,5-TRISPHOSPHATE 3-PHOSPHATASE AND DUAL-SPECIFICITY PROTEIN PHOSPHATASE PTEN	AD	<i>PTEN</i> hamartoma tumor syndrome (PHTS): breast, thyroid, endometrial, colon, melanoma & renal cancer, gastrointestinal polyps, Lhermitte-Duclos Disease
<i>RB1</i> ^{41–45}	RETINOBLASTOMA-ASSOCIATED PROTEIN	AD	Hereditary retinoblastoma: retinoblastoma, sarcoma, leukemia, melanoma, & pineoblastoma
<i>TP53</i> ^{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

References:

1. Probability of Developing or Dying of Cancer - Surveillance Research Program. Available at: <https://surveillance.cancer.gov/devcan/>. (Accessed: 18th September 2017)
2. Hayward, N. K. Genetics of melanoma predisposition. *Oncogene* **22**, 3053–3062 (2003).
3. Goldstein, A. M. Familial melanoma, pancreatic cancer and germline CDKN2A mutations. *Hum. Mutat.* **23**, 630 (2004).
4. Goldstein, A. M. *et al.* Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. *J. Med. Genet.* **44**, 99–106 (2007).
5. Sekulic, A. *et al.* Malignant melanoma in the 21st century: the emerging molecular landscape. *Mayo Clin. Proc.* **83**, 825–846 (2008).
6. Puntervoll, H. E. *et al.* Melanoma prone families with CDK4 germline mutation: phenotypic profile and associations with MC1R variants. *J. Med. Genet.* **50**, 264–270 (2013).
7. Leachman, S. A. *et al.* Identification, genetic testing, and management of hereditary melanoma. *Cancer Metastasis Rev.* **36**, 77–90 (2017).
8. van der Rhee, J. I. *et al.* Clinical and histologic characteristics of malignant melanoma in families with a germline mutation in CDKN2A. *J. Am. Acad. Dermatol.* **65**, 281–288 (2011).
9. Ransohoff, K. J. *et al.* Familial skin cancer syndromes: Increased melanoma risk. *J. Am. Acad. Dermatol.* **74**, 423–434; quiz 435–436 (2016).
10. Pilarski, R., Rai, K., Cebulla, C. & Abdel-Rahman, M. BAP1 Tumor Predisposition Syndrome. in *GeneReviews®* (eds. Adam, M. P. *et al.*) (University of Washington, Seattle, 1993).
11. Rai, K., Pilarski, R., Cebulla, C. M. & Abdel-Rahman, M. H. Comprehensive review of BAP1 tumor predisposition syndrome with report of two new cases. *Clin. Genet.* **89**, 285–294 (2016).
12. Ford, D. *et al.* Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. *Am. J. Hum. Genet.* **62**, 676–689 (1998).
13. Antoniou, A. *et al.* Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *Am. J. Hum. Genet.* **72**, 1117–1130 (2003).
14. Biron-Shental, T., Drucker, L., Altaras, M., Bernheim, J. & Fishman, A. High incidence of BRCA1-2 germline mutations, previous breast cancer and familial cancer history in Jewish patients with uterine serous papillary carcinoma. *Eur. J. Surg. Oncol. J. Eur. Soc. Surg. Oncol. Br. Assoc. Surg. Oncol.* **32**, 1097–1100 (2006).
15. Chen, S. & Parmigiani, G. Meta-analysis of BRCA1 and BRCA2 penetrance. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **25**, 1329–1333 (2007).
16. Graeser, M. K. *et al.* Contralateral breast cancer risk in BRCA1 and BRCA2 mutation carriers. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **27**, 5887–5892 (2009).

17. King, M.-C., Marks, J. H., Mandell, J. B. & New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science* **302**, 643–646 (2003).
18. Liede, A., Karlan, B. Y. & Narod, S. A. Cancer risks for male carriers of germline mutations in BRCA1 or BRCA2: a review of the literature. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **22**, 735–742 (2004).
19. Levine, D. A. *et al.* Fallopian tube and primary peritoneal carcinomas associated with BRCA mutations. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **21**, 4222–4227 (2003).
20. Risch, H. A. *et al.* Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. *J. Natl. Cancer Inst.* **98**, 1694–1706 (2006).
21. Claus, E. B., Schildkraut, J. M., Thompson, W. D. & Risch, N. J. The genetic attributable risk of breast and ovarian cancer. *Cancer* **77**, 2318–2324 (1996).
22. Goldstein, A. M. *et al.* High-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. *Cancer Res.* **66**, 9818–9828 (2006).
23. Kefford, R. F., Newton Bishop, J. A., Bergman, W. & Tucker, M. A. Counseling and DNA testing for individuals perceived to be genetically predisposed to melanoma: A consensus statement of the Melanoma Genetics Consortium. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **17**, 3245–3251 (1999).
24. Czajkowski, R., Placek, W., Drewa, G., Czajkowska, A. & Uchańska, G. FAMMM syndrome: pathogenesis and management. *Dermatol. Surg. Off. Publ. Am. Soc. Dermatol. Surg.* **30**, 291–296 (2004).
25. Canto, M. I. *et al.* International Cancer of the Pancreas Screening (CAPS) Consortium summit on the management of patients with increased risk for familial pancreatic cancer. *Gut* **62**, 339–347 (2013).
26. Soura, E., Eliades, P. J., Shannon, K., Stratigos, A. J. & Tsao, H. Hereditary melanoma: Update on syndromes and management: Emerging melanoma cancer complexes and genetic counseling. *J. Am. Acad. Dermatol.* **74**, 411–420; quiz 421–422 (2016).
27. Bertolotto, C. *et al.* A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. *Nature* **480**, 94–98 (2011).
28. Ghorzo, P. *et al.* Prevalence of the E318K MITF germline mutation in Italian melanoma patients: associations with histological subtypes and family cancer history. *Pigment Cell Melanoma Res.* **26**, 259–262 (2013).
29. Potrony, M. *et al.* Prevalence of MITF p.E318K in Patients With Melanoma Independent of the Presence of CDKN2A Causative Mutations. *JAMA Dermatol.* **152**, 405–412 (2016).
30. Shi, J. *et al.* Rare missense variants in POT1 predispose to familial cutaneous malignant melanoma. *Nat. Genet.* **46**, 482–486 (2014).
31. Robles-Espinoza, C. D. *et al.* POT1 loss-of-function variants predispose to familial melanoma. *Nat. Genet.* **46**, 478–481 (2014).
32. Wilson, T. L.-S. *et al.* A new POT1 germline mutation-expanding the spectrum of POT1-associated cancers. *Fam. Cancer* (2017). doi:10.1007/s10689-017-9984-y
33. Calvete, O. *et al.* A mutation in the POT1 gene is responsible for cardiac angiosarcoma in TP53-negative Li-Fraumeni-like families. *Nat. Commun.* **6**, 8383 (2015).
34. Bainbridge, M. N. *et al.* Germline mutations in shelterin complex genes are associated with familial glioma. *J. Natl. Cancer Inst.* **107**, 384 (2015).
35. Speedy, H. E. *et al.* Germ line mutations in shelterin complex genes are associated with familial chronic lymphocytic leukemia. *Blood* **128**, 2319–2326 (2016).
36. Jasperson, K. W. Genetic testing by cancer site: colon (polyposis syndromes). *Cancer J. Sudbury Mass* **18**, 328–333 (2012).
37. Bubián, V. *et al.* High cumulative risks of cancer in patients with PTEN hamartoma tumour syndrome. *J. Med. Genet.* **50**, 255–263 (2013).
38. Hobert, J. A. & Eng, C. PTEN hamartoma tumor syndrome: an overview. *Genet. Med. Off. J. Am. Coll. Med. Genet.* **11**, 687–694 (2009).
39. Nieuwenhuis, M. H. *et al.* Cancer risk and genotype-phenotype correlations in PTEN hamartoma tumor syndrome. *Fam. Cancer* **13**, 57–63 (2014).
40. Tan, M.-H. *et al.* Lifetime cancer risks in individuals with germline PTEN mutations. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* **18**, 400–407 (2012).
41. Lohmann, D. R. & Gallie, B. L. Retinoblastoma. in *GeneReviews*(®) (eds. Pagon, R. A. *et al.*) (University of Washington, Seattle, 1993).
42. de Jong, M. C. *et al.* Trilateral retinoblastoma: a systematic review and meta-analysis. *Lancet Oncol.* **15**, 1157–1167 (2014).
43. Dimaras, H. *et al.* Loss of RB1 induces non-proliferative retinoma: increasing genomic instability correlates with progression to retinoblastoma. *Hum. Mol. Genet.* **17**, 1363–1372 (2008).
44. Marees, T. *et al.* Risk of second malignancies in survivors of retinoblastoma: more than 40 years of follow-up. *J. Natl. Cancer Inst.* **100**, 1771–1779 (2008).
45. Kleinerman, R. A. *et al.* Risk of soft tissue sarcomas by individual subtype in survivors of hereditary retinoblastoma. *J. Natl. Cancer Inst.* **99**, 24–31 (2007).
46. Pennington, K. P. *et al.* BRCA1, TP53, and CHEK2 germline mutations in uterine serous carcinoma. *Cancer* **119**, 332–338 (2013).
47. Chompret, A. *et al.* P53 germline mutations in childhood cancers and cancer risk for carrier individuals. *Br. J. Cancer* **82**, 1932–1937 (2000).
48. Gonzalez, K. D. *et al.* Beyond Li Fraumeni Syndrome: clinical characteristics of families with p53 germline mutations. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **27**, 1250–1256 (2009).
49. Olivier, M. *et al.* Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and TP53 genotype. *Cancer Res.* **63**, 6643–6650 (2003).
50. Ruijs, M. W. G. *et al.* TP53 germline mutation testing in 180 families suspected of Li-Fraumeni syndrome: mutation detection rate and relative frequency of cancers in different familial phenotypes. *J. Med. Genet.* **47**, 421–428 (2010).
51. Hisada, M., Garber, J. E., Fung, C. Y., Fraumeni, J. F. & Li, F. P. Multiple primary cancers in families with Li-Fraumeni syndrome. *J. Natl. Cancer Inst.* **90**, 606–611 (1998).