OncoGeneDx: Colorectal Cancer Panel

PANEL GENE LIST

APC, ATM, AXIN2, BMPR1A, CDH1, CHEK2, CTNNA1, EPCAM, MLH1, MSH2, MSH3, MSH6, MUTYH, NTHL1, PMS2, POLD1, POLE, PTEN, SCG5/GREM1*, SMAD4, STK11, TP53*

*Testing includes sequencing and deletion/duplication analysis for all genes except *EPCAM* (del/dup only) and *SCG5/GREM1* (del/dup only).

CLINICAL FEATURES

Individuals in the general population have an approximately 4.3% lifetime risk of developing colorectal cancer.[1] Most cases of colorectal cancer develop sporadically. Approximately 5-10% of colorectal cancers are due to a hereditary predisposition. Individuals with hereditary colorectal cancer syndromes often have a high risk of developing gastrointestinal cancers and require increased screening and surveillance to reduce their cancer risk. The features suggestive of a hereditary colorectal cancer predisposition include young age at diagnosis, history of colorectal cancer or multiple polyps in one or more close relatives, multiple primary cancers in a single individual, and several relatives affected with cancer spanning multiple generations.

Of the cases that are suspected of having a hereditary predisposition to colorectal cancer, the most common causes are Lynch syndrome, due to pathogenic *MLH1, MSH2, MSH6, PMS2* variants and *EPCAM* deletions, as well as Familial Adenomatous Polyposis (FAP) and Attenuated FAP (AFAP) due to pathogenic *APC* variants. The other 16 genes on this panel account for an additional proportion of hereditary colorectal cancer cases.

The genes included on this panel have been shown to cause an increased risk for colorectal cancer and, in many cases, other cancers as well. Newer genes, such as *AXIN2, MSH3,* and *NTHL1*, have been identified in families with colorectal cancer and polyposis and have been included in the panel to make it as comprehensive as possible. The evidence available to date may be derived from a small number of patients with wide confidence intervals or is based upon an ethnic cohort with one specific variant. Accurate risk assessment may be complicated by the low penetrance of pathogenic variants in these genes and/or ascertainment bias.

INHERITANCE PATTERN

Most genes on this panel are associated with an autosomal dominant cancer risk with the exception of *MSH3, MUTYH* and *NTHL1*, which are associated with an autosomal recessive 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). For *PTEN* nucleotides c.-700 through c.-1300 in the promoter region, and for *APC*, promoters 1A and 1B 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. Concurrent *MSH2* Exons 1-7 Inversion analysis from NGS data is also performed. For *EPCAM* and *SCG5*, deletion/duplication analysis, but not sequencing, is performed. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. 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 22 genes included in the OncoGeneDx Colorectal 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
APC[2,3]	ADENOMATOUS POLYPOSIS COLI PROTEIN	AD	Familial Adenomatous Polyposis (FAP)-associated condition: colorectal, duodenal or periampullary, gastric, thyroid, pancreatic, brain (medulloblastoma) & liver (hepatoblastoma) cancers, desmoid tumors, gastrointestinal polyps
ATM[4–8]	SERINE-PROTEIN KINASE ATM	AD	Breast, pancreatic, prostate & colon cancer
		AR	Ataxia telangiectasia
AXIN2[9,10]	AXIN-2	AD	Colon cancer, colon polyps
BMPR1A[11,12]	BONE MORPHOGENETIC PROTEIN RECEPTOR TYPE-1A	AD	Juvenile Polyposis syndrome (JPS): colorectal, gastric (if gastric polyps), small bowel & pancreatic cancer, gastrointestinal polyps

Gene	Protein	Inheritance	Disease Associations
CDH1[13–16]	CADHERIN 1	AD	Hereditary Diffuse Gastric Cancer (HDGC) syndrome: gastric (diffuse), breast & colon (signet ring) cancer
CHEK2[17–21]	SERINE/THREONINE-PROTEIN KINASE CHK2	AD	Breast, colon, prostate, gastric & thyroid cancer
CTNNA1[13,22]	CATENIN ALPHA-1	AD	Diffuse gastric cancer
EPCAM[23–25]	EPITHELIAL CELL ADHESION MOLECULE	AD	Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, pancreatic, biliary tract, urinary tract, small bowel, prostate & brain cancer, sebaceous neoplasms
		AR	Constitutional mismatch repair deficiency syndrome
MLH1[26,27]	DNA MISMATCH REPAIR PROTEIN MLH1	AD	Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, pancreatic, biliary tract, urinary tract, small bowel, prostate & brain cancer, sebaceous neoplasms
		AR	Constitutional mismatch repair deficiency syndrome
MSH2[26,27]	DNA MISMATCH REPAIR PROTEIN MSH2	AD	Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, pancreatic, biliary tract, urinary tract, small bowel, prostate & brain cancer, sebaceous neoplasms
		AR	Constitutional mismatch repair deficiency syndrome
MSH3[28,29]	DNA MISMATCH REPAIR PROTEIN MSH3	AR	Colorectal cancer, colonic polyposis
MSH6[26,27]	DNA MISMATCH REPAIR PROTEIN MSH6	AD	Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, pancreatic, biliary tract, urinary tract, small bowel, prostate & brain cancer, sebaceous neoplasms
		AR	Constitutional mismatch repair deficiency syndrome
MUTYH[30,31]	ADENINE DNA GLYCOSYLASE	AR	*MUTYH*-associated polyposis (MAP): colorectal, small bowel & endometrial serous cancer, gastrointestinal polyps

Gene	Protein	Inheritance	Disease Associations
NTHL1[29,32]	ENDONUCLEASE III-LIKE 1	AR	Colon cancer, colon polyps
PMS2[26,27]	MISMATCH REPAIR ENDONUCLEASE PMS2	AD	Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, pancreatic, biliary tract, urinary tract, small bowel, prostate & brain cancer, sebaceous neoplasms
		AR	Constitutional mismatch repair deficiency syndrome
POLD1[33,34]	DNA POLYMERASE DELTA CATALYTIC SUBUNIT	AD	Colon & endometrial cancer, colon polyps
POLE[34,35]	DNA POLYMERASE EPSILON CATALYTIC SUBUNIT A	AD	Colon cancer, gastrointestinal polyps
		AR	Intrauterine growth restriction, metaphyseal dysplasia, congenital adrenal hypoplasia, and genitourinary anomalies in males (IMAGe) with variable immunodeficiency
PTEN[36]	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
SCG5/ GREM1[37–39]	NEUROENDOCRINE PROTEIN 7B2/GREMLIN-1	AD	Hereditary Mixed Polyposis syndrome (HMPS): colon cancer, colon polyps
SMAD4[40,41]	MOTHERS AGAINST DECAPENTAPLEGIC HOMOLOG 4	AD	Juvenile Polyposis syndrome (JPS): colorectal, gastric (if gastric polyps), small bowel & pancreatic cancer, gastrointestinal polyps
STK11[42–44]	SERINE/THREONINE-PROTEIN KINASE STK11	AD	Peutz-Jeghers syndrome (PJS): breast, colorectal, pancreatic, gastric, small bowel, ovarian, lung, cervical & endometrial cancer, testicular tumors (LCCSCT), gastrointestinal polyps
TP53[45]	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
LCCSCT – Large cell-calcifying Sertoli cell tumors
MLPA - Multiplex ligation-dependent probe amplification

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