OncoGeneDx: Lynch/Colorectal Cancer High Risk Panel

PANEL GENE LIST

APC, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2*
*Testing includes sequencing and deletion/duplication analysis for all genes except *EPCAM* (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 with no family history of the cancer; however, approximately 5-10% of cases are thought to be due to a hereditary cancer predisposition syndrome. Features suggestive of hereditary cancer predisposition include: young age at diagnosis (before age 50), multiple primary cancers in a single individual, multiple colon polyps, diagnosis of an uncommon cancer type (such as ovarian cancer, ampullary cancer or pancreatic cancer) and several relatives affected with related cancers spanning multiple generations.

The OncoGeneDx Lynch/Colorectal Cancer High Risk Panel includes genes associated with well-described hereditary cancer predisposition syndromes, including classic and attenuated Familial Adenomatous Polyposis (*APC*), Lynch syndrome/Hereditary Non-Polyposis Colorectal Cancer syndrome (*EPCAM, MLH1, MSH2, MSH6* and *PMS2*), and *MUTYH*-associated polyposis (*MUTYH*). Lynch syndrome is the most common hereditary colon cancer syndrome and is estimated to account for approximately 2% of all colorectal cancer diagnoses.[2] Polyposis-associated colon cancer syndromes are somewhat less common, and it is estimated that *APC*- and *MUTYH*-associated polyposis each account for less than 1% of all colorectal cancer diagnoses.[3,4]

Familial Adenomatous Polyposis (*APC*): Classic Familial Adenomatous Polyposis (FAP) predisposes pathogenic variant carriers to develop many adenomatous colon polyps, colorectal cancer, and other cancers. Individuals with classic FAP typically develop hundreds to thousands of adenomatous polyps by age 35 and, on average, are diagnosed with colon cancer by the age of 39. The age-related risk for colon cancer in untreated individuals is 7% by age 21, 87% by age 45, and 93% by age 50.[5] Other cancer risks in individuals with FAP include a 5% risk for duodenal or periampullary cancer and a ≤2% risk for stomach, thyroid, pancreatic, brain (typically medulloblastoma), and liver (hepatoblastoma) cancers. Upper gastrointestinal tract polyps and fundic gland polyps develop in most cases of FAP. Other findings associated with classic FAP include desmoid tumors, osteomas, epidermoid cysts, fibromas, dental abnormalities, and congenital hypertrophy of the retinal pigment epithelium (CHRPE).

Attenuated Familial Adenomatous Polyposis (AFAP) predisposes pathogenic variant carriers to develop many adenomatous polyps, colorectal cancer, and other cancers. AFAP is distinguished from classic FAP primarily by lower polyp burden and later age at presentation. Individuals with AFAP develop an average of about 30 polyps and are typically diagnosed with colon cancer between the ages of 50 and 55. Other cancer risks in individuals with AFAP include a 5% risk for duodenal or periampullary cancer and ≤2% risk for stomach, thyroid, and pancreatic cancers.[6] Upper gastrointestinal tract polyps and fundic gland polyps develop in most cases of AFAP. Other findings associated with AFAP include desmoid tumors, osteomas, epidermoid cysts, fibromas, dental abnormalities, and congenital hypertrophy of the retinal pigment epithelium (CHRPE); however, these findings are observed less frequently in AFAP as compared to classic FAP.

Lynch Syndrome (*EPCAM, MLH1, MSH2, MSH6*, and *PMS2*): The predominant cancers that individuals with Lynch syndrome are at risk to develop are colorectal cancer and endometrial cancer. The lifetime risk of colon cancer has been estimated to be 15%-80% for both male and female pathogenic variant carriers while the lifetime risk for endometrial cancer has been estimated to be 15%-61% for female pathogenic variant carriers. Importantly,

cancer risks vary among the Lynch syndrome-associated genes with some conferring greater cancer risks than others.[7–11] In general, the lifetime cancer risks are thought to be lower for those harboring *MSH6* and *PMS2* pathogenic variants compared to those with *MLH1, MSH2* and *EPCAM* pathogenic variants. Individuals with Lynch syndrome also have an increased risk of ovarian (≤20%), gastric (≤7%), urothelial (≤8%), small bowel (≤ 4%) and brain cancers (≤3%, Turcot variant).[7,12] Additionally, some individuals with Lynch syndrome also have an increased risk of sebaceous neoplasms and keratoacanthomas of the skin (Muir-Torre variant).

***MUTYH*-Associated Polyposis (*MUTYH*):** *MUTYH*-associated polyposis (MAP) causes an increased risk for biallelic pathogenic variant carriers to develop colon polyps and colon cancer. The risk for colon cancer in individuals with homozygous or compound heterozygous pathogenic variants in *MUTYH* is estimated to be 43% at age 60 and 80% at age 70.[13,14] Most individuals with biallelic *MUTYH* pathogenic variants, ascertained because of a personal or family history of polyps, develop between 10-100 polyps, but individuals can develop hundreds of polyps. Further, up to one third of biallelic *MUTYH* pathogenic variant carriers develop colorectal cancer in the absence of polyposis, indicative of incomplete penetrance.[3,15–18] Adenomas are the most common type of polyp in MAP, but serrated and hyperplastic polyps have also been observed.[15,19] Duodenal polyps and gastric fundic gland polyps have been observed in a minority of individuals with MAP. The lifetime risk for duodenal cancer is estimated to be 4% and there is some evidence that risk for other extra-intestinal cancers such as endometrial, ovarian, bladder, breast, and skin may also be increased.[20,21] The risk of cancer in individuals heterozygous for a pathogenic variant in *MUTYH* is still under investigation. Although an increased risk for colon cancer, endometrial cancer, and breast cancer has been reported in carriers of a single MUTYH pathogenic variant[13,22,23], other studies provide conflicting data regarding such associations.[24,25]

INHERITANCE PATTERN

Most genes on this panel are associated with an autosomal dominant cancer risk with the exception of *MUTYH*, which is 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 *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*, 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 7 genes included in the OncoGeneDx Lynch/Colorectal Cancer High Risk 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	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
EPCAM	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[26,27]
MLH1	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[26,27]
MSH2	DNA MISMATCH REPAIR	AD	Lynch syndrome (LS):

	PROTEIN MSH2		colorectal, endometrial, ovarian, gastric, pancreatic, biliary tract, urinary tract, small bowel, prostate & brain cancer, sebaceous neoplasms
		AR	Constitutional mismatch repair deficiency syndrome[26,27]
MSH6	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[26,27]
MUTYH	ADENINE DNA GLYCOSYLASE	AR	*MUTYH*-associated polyposis (MAP): colorectal, small bowel & endometrial serous cancer, gastrointestinal polyps
PMS2	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[26,27]

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.

Abbreviations:
AD – Autosomal Dominant
AR – Autosomal Recessive
CGH – Comparative genomic hybridization
MLPA – Multiplex ligation-dependent probe amplification

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