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 \( \leq 2\% \) 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):** Colon and endometrial cancer are the predominant Lynch syndrome-related cancers that pathogenic variant carriers are at risk to develop. 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 (\( \leq 20\% \)), gastric (\( \leq 7\% \)), urothelial (\( \leq 8\% \)), small bowel (\( \leq 4\% \)) and brain cancers (\( \leq 3\% \), 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, other studies provide conflicting data regarding such associations.\(^24,25\)

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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 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 APC, promoters 1A and 1B 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. Concurrent MSH2 Exons 1-7 Inversion analysis from NGS data is also performed. For EPCAM, deletion/duplication analysis, but not sequencing, is performed. 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.

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 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. For instance, sequence and deletion/duplication analysis of PMS2, among others, is complicated by the presence of pseudogenes or homologous sequences that involve multiple exons of these genes. 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>APC</td>
<td>ADENOMATOUS POLYPOSIS COLI PROTEIN</td>
<td>AD</td>
<td>Familial Adenomatous Polyposis (FAP)-associated condition: colorectal, duodenal or periampullary, gastric, thyroid, pancreatic, brain &amp; liver (Hepatoblastoma) cancers, desmoid tumors, gastrointestinal polyps</td>
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<tr>
<td>EPCAM</td>
<td>EPITHELIAL CELL ADHESION MOLECULE</td>
<td>AD</td>
<td>Lynch syndrome: colorectal, endometrial, ovarian, gastric, pancreatic, biliary tract, urinary tract, small bowel &amp; brain cancer, sebaceous neoplasms</td>
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<td></td>
<td>AR</td>
<td>Constitutional mismatch repair deficiency syndrome</td>
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<tr>
<td>MLH1</td>
<td>DNA MISMATCH REPAIR PROTEIN MLH1</td>
<td>AD</td>
<td>Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, pancreatic, biliary tract, urinary tract, small bowel &amp; brain cancer, sebaceous neoplasms</td>
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<tr>
<td>Gene</td>
<td>Function</td>
<td>AD</td>
<td>AR</td>
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<td><strong>MSH2</strong></td>
<td>DNA MISMATCH REPAIR PROTEIN MSH2</td>
<td>Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, urinary tract, pancreatic, biliary tract, small bowel &amp; brain cancer, sebaceous neoplasms</td>
<td>Constitutional mismatch repair deficiency syndrome</td>
</tr>
<tr>
<td><strong>MSH6</strong></td>
<td>DNA MISMATCH REPAIR PROTEIN MSH6</td>
<td>Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, pancreatic, biliary tract, small bowel &amp; brain cancer, sebaceous neoplasms</td>
<td>Constitutional mismatch repair deficiency syndrome</td>
</tr>
<tr>
<td><strong>MUTYH</strong></td>
<td>ADENINE DNA GLYCOSYLASE</td>
<td>MUTYH-associated polyposis (MAP): colorectal, small bowel &amp; endometrial serous cancer, gastrointestinal polyps</td>
<td></td>
</tr>
<tr>
<td><strong>PMS2</strong></td>
<td>MISMATCH REPAIR ENDOGUCLEASE PMS2</td>
<td>Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, pancreatic, biliary tract, urinary tract, small bowel &amp; brain cancer, sebaceous neoplasms</td>
<td>Constitutional mismatch repair deficiency syndrome</td>
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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

**References:**