OncoGeneDx: Endometrial Cancer Panel

Panel Gene List: *BRCA1, BRCA2, CHEK2, EPCAM*, MLH1, MSH2, MSH6, MUTYH, PMS2, POLD1, PTEN, TP53*

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

Clinical Features:
Women in the general population have approximately a 5% lifetime risk to develop endometrial cancer, also known as uterine cancer (SEER). Most cases of endometrial cancer develop sporadically. However, approximately 5-10% of endometrial cancer cases are due to a hereditary predisposition. The features of a personal and/or family history of cancer that are suggestive of a hereditary cancer predisposition include: young ages at diagnosis, multiple primary cancers in a single individual, and several relatives affected with related cancers spanning multiple generations.

Of the cases that are suspected of having a hereditary predisposition to endometrial cancer, approximately 2-3% are associated with Lynch syndrome due to pathogenic variants in the *MLH1, MSH2, MSH6*, or *PMS2* genes (Hampel 2007). A subset of individuals with Lynch syndrome carry exon-level deletions in the 3' end of the *EPCAM* gene (Tutlewska 2013). Approximately 5% of individuals with uterine serous carcinoma have a pathogenic variant in a non-Lynch syndrome associated gene (Pennington 2013). The additional 7 genes on this panel besides the Lynch syndrome genes account for additional causes of hereditary endometrial cancer cases. All of these genes are associated with increased risk of endometrial cancer and, in many cases, other cancers as well. Newer genes that have been identified in families with endometrial cancer have been included in the panel to make it as comprehensive as possible. These genes include *CHEK2* and *POLD1*. 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 *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 *PTEN*, nucleotides c.-700 through c.-1300 in the promoter region 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. 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 but are available upon request. Data analysis is performed using gene-specific filtering; the genes evaluated by this test are listed on the first page of the report.

Test Sensitivity:
The clinical sensitivity of sequencing and deletion/duplication analysis of the 12 genes included in the OncoGeneDx Endometrial 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 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* and *CHEK2*, 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.

Gene	Protein	Inheritance	Disease Associations
BRCA1	BREAST CANCER TYPE 1 SUSCEPTIBILITY PROTEIN	AD	Hereditary Breast and Ovarian Cancer (HBOC) syndrome: breast, ovarian, pancreatic, prostate & endometrial serous cancer
BRCA2	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
CHEK2	SERINE/THREONINE-PROTEIN KINASE CHK2	AD	Breast, colon, prostate, thyroid, endometrial & ovarian cancer
EPCAM	EPITHELIAL CELL ADHESION MOLECULE	AD	Lynch syndrome: colorectal, endometrial, ovarian, gastric pancreatic, biliary tract, urinary tract, small bowel & brain cancer, sebaceous neoplasms
		AR	Constitutional mismatch repair deficiency syndrome
MLH1	DNA MISMATCH REPAIR PROTEIN MLH1	AD	Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, pancreatic, Biliary tract, Urinary tract, small bowel & brain cancer, sebaceous neoplasms
		AR	Constitutional mismatch repair deficiency syndrome

MSH2	DNA MISMATCH REPAIR PROTEIN MSH2	AD	Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, urinary tract, pancreatic, biliary tract, small bowel & brain cancer, sebaceous neoplasms
		AR	Constitutional mismatch repair deficiency syndrome
MSH6	DNA MISMATCH REPAIR PROTEIN MSH6	AD	Lynch syndrome (LS): colorectal, endometrial, ovarian, gastric, pancreatic, biliary tract, small bowel & brain cancer, sebaceous neoplasms
		AR	Constitutional mismatch repair deficiency syndrome
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 & brain cancer, sebaceous neoplasms
		AR	Constitutional mismatch repair deficiency syndrome
POLD1	DNA POLYPMERASE DELTA CATALYTIC SUBUNIT	AD	Colon & endometrial cancer, colon polyps
PTEN	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
TP53	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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