PREDICT™

Comprehensive Hereditary Cancer Risk Assessment

LABSOLUTIONS

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LABSOLUTIONS *is pleased to offer its clients a comprehensive suite of genetic tests, allowing the physicians we serve to provide* ***truly personalized healthcare.*** Coupling the latest in laboratory technologies with highly trained genomic scientists and counselors keeps our clients cutting edge. Next Generation Sequencing (NGS) based screening for mutations known to effect an individuals risk of developing hereditary cancer is performed at our state-of-the-art, CLIA-certified facility. **PREDICT**™ test results, along with a treating physicians' expertise, are used to make better informed treatment and/or monitoring decisions.

INTRODUCTION

Hereditary or "predisposition" genetic testing looks for specific inherited changes (mutations) in a person's genetic make-up. Genetic mutations can have harmful, beneficial, neutral, or uncertain effects on health. Mutations that are harmful may increase a person's chance, or risk, of developing a disease such as cancer. Overall, inherited mutations are thought to play a role in 5-10% of all cancers. These particular disease states are known as hereditary cancers, and proper genetic testing can be used to determine an individual's risk.

Cancer is a disorder in which normal control of cell growth is lost—causing abnormal proliferation of the effected cells. Inherited genetic mutations can increase a person's risk of developing cancer through a variety of mechanisms, depending on the function of the mutated gene. Mutations in genes that control the repair of damaged DNA and cell growth are particularly likely to be associated with an increased risk of cancer.

Some people inherit mutation(s) in the germline, potentially allowing for the cancers associated with the mutation(s) to be passed on. These mutation(s) occur in two classes of cellular genes: *oncogenes* and *tumor suppressor genes.* Often, multiple genetic mutations in a single individual are responsible for the development of hereditary cancers.

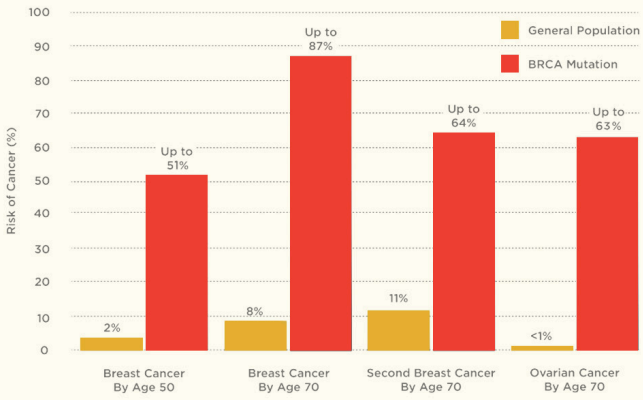

FIGURE: *Risk increase of Hereditary Breast and Ovarian Cancer, associated with BRCA mutation*

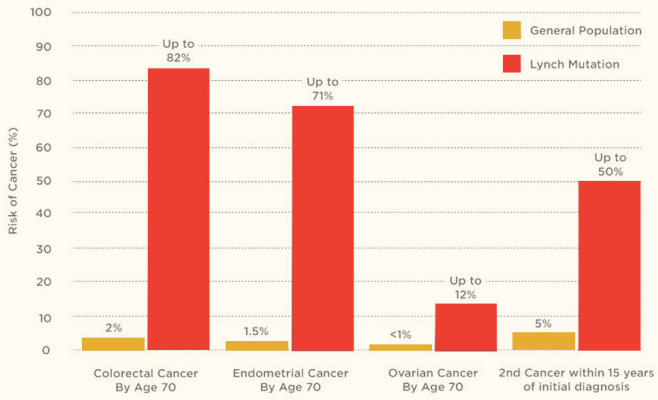

FIGURE: *Risk increase of Hereditary Colon and Uterine Cancer, associated with Lynch Mutation*

Cancer can sometimes appear to "run in families" even if it is not caused by an inherited mutation. For example, a shared environment or lifestyle, such as tobacco use, can cause similar cancers to develop among family members. However, certain patterns—such as the types of cancer that develop, other non-cancer conditions/symptoms that are present, and the ages at which cancers develop—may suggest the cancer is in-fact hereditary in nature and due to inherited genetic mutation(s).

Advancements in gene sequencing technologies have allowed for genetic mutations that cause many hereditary cancers to be well described, and sophisticated mutation screening can confirm whether a cancer is, indeed, the result of an inherited mutation. Genetic testing is also performed to determine whether assymptomatic individuals with family members effected by cancer have inherited the causal genetic mutation.

Approximately 12% of women in the general population will develop breast cancer sometime during their lives. By contrast, according to the most recent estimates, 55 to 65% of women who inherit a harmful BRCA1 gene mutation and 45% of women who inherit a harmful BRCA2 mutation, will develop breast cancer by the age 70. Similarly, 1.3% of women in the general population will develop ovarian cancer sometime during their lives—as opposed to 39% and 11-17% of women with a BRCA1 or BRCA2 mutation present, respectively.

EXAMPLES OF COMMON HEREDITARY CANCERS & CANCER SYNDROMES

- Hereditary Breast and Ovarian Cancer (BRCA1, BRCA2)
- Colon Cancer (APC, BMPR1A, EPCAM)
- Uterine Cancer (MLH1, MSH2, EPCAM, MSH6, PMS2)
- Endometrial Cancer (EPCAM, MLH1, MSH2, MSH6)
- Lynch Syndrome (MLH1, MSH2, MSH6, PMS2, EPCAM)
- Cowden Syndrome (PTEN)
- Li-Fraumeni Syndrome (TP53)

WHO SHOULD GET TESTED

If you suspect that you or someone you know may have an increased risk for cancer–-based on factors like abnormal familial cancer history or membership to an at-risk ethnic population (such as Ashkenazi Jewish ancestry)—you may want to discuss advanced genomic testing options like **PREDICT™** with your healthcare provider.

If one of your family members, however distant, has been diagnosed with cancer, there is a chance that you inherited a gene mutation that not only increases your personal risk of developing cancer, but may also be passed on to your offspring—potentially increasing their risk of developing cancer.

A PREDICT™ Cancer History Questionnaire form is included at the end of this summary to aid healthcare providers in determining proper utilization and eligibility.

Mutation of the genes known to be associated with an increased risk of developing cancer, like those involved in DNA repair, often result in cancers that appear unique. An individual's familial cancer history may include a number of seemingly distinct cancer cases. This mistakingly leads to the belief that the cancers are un-related, and not caused by a hereditary mutation.

BENEFITS OF TESTING

Those who are carriers of hereditary cancer gene mutations, may be at risk of developing cancer earlier in life, as compared to members of the general population. ***The sooner genetic testing is performed, the more likely it is that this increased risk can be managed appropriately.***

Numerous professional practice guidelines describe increasingly stringent monitoring protocol—published specifically for management of patients in which deleterious mutation has been identified. These protocols may suggest the increased use of routine screening tools like mammograms and colonoscopies. Depending on the severity of the identified mutation(s), they may also suggest discussion of more aggressive options like prophylactic surgical intervention. Remember, ***your healthcare professional is your most valuable source of information.***

GENES EVALUATED BY PREDICT™

APC	CDK4	MET	NTRK1	RAD51C
ATM	P16(CDKN2A)	MLH1	PALB2	RAD51D
BARD1	CHEK2	MRE11a	PALLD	RET
BMPR1A	ELAC2	MSH2	PMS2	SMAD4
BRCA1	EPCAM	MSH6	PTCH1	STK11
BRCA2	FANCC	MUTYH	PTEN	TP53
BRIP1	HRAS1	NBN	RAD50	VHL
CDH1	MEN1	NF1	RAD51	

COLLECTION DEVICES & SPECIMEN REQUIREMENTS

- **Whole Blood:** 2 EDTA (lavender top) tubes containing 4ml each whole sterile blood
- **Oral Fluids:** Saliva collected using the LabSolutions Oral Fluid collection kit
- **Extracted DNA:** >20ug (please inquire as to proper protocol for acceptance)

TESTING METHODS

Genomic DNA from the submitted specimen was enriched for the complete coding regions and splice site junctions of genes assessed by **PREDICT™** using a proprietary targeted capture system developed by LabSolutions. The products were sequenced on either an Illumina NextSeq or HiSeq instrument with 2x150 or 2x100 paired-end reads, respectively. The sequence was aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Capillary sequencing was used to confirm all variants with clinical or uncertain significance and to analyze regions with inadequate coverage by next generation sequencing based approaches. If present, apparently homozygous variants were confirmed using alternate primer pairs to significantly reduce the possibility of allele drop-out. Concurrent deletion/duplication testing was performed for all of the genes on the panel using either exon-level array CGH or MLPA. Confirmation of copy number changes was performed by MLPA, qPCR, or repeat aCGH analysis. For PTEN, nucleotides c.-700 through c.-1300 in the promoter region are also sequenced. For EPCAM, SCG5 and the first exon of GREM1, deletion/duplication analysis, but not sequencing, was performed. All sequence alterations are described according to nomenclature guidelines set forth by the Human Genome Variation Society (HGVS). Benign and likely benign variants, if present, are not reported but are available upon request.

ADDITIONAL HEREDITARY CANCER INFORMATION

BREAST CANCER is the most common cancer in women in developed countries, effecting about 1 in 8 (12.5%) women in their lifetime.[1] The National Cancer Institute (NCI) estimates that approximately **231,840 new cases of female breast cancer and 2,350 new cases of male breast cancer will be diagnosed in the U.S. in 2015.**[2]

The majority of breast cancers are sporadic, but 5-10% are due to inherited causes. Hereditary breast cancer tends to occur earlier in life than non-inherited sporadic cases, and is more likely to occur in both breasts. The highly penetrant genes, BRCA1 and BRCA2, appear to be responsible for around half of hereditary breast cancer.[3-5] However, additional genes have been discovered that are associated with increased breast cancer risk as well.[3-7] Mutations in the genes included in **PREDICT™** can confer an estimated 20–87% lifetime risk for breast cancer. Some of these genes have also been associated with increased risks for other cancers, such as pancreatic cancer with PALB2, ovarian cancer with BRCA1, BRCA2, RAD51C (and others), and sarcoma with TP53.[8-12]

OVARIAN CANCER is the fifth most common cancer among women in developed countries, effecting approximately 1 in 71 (1.4%) women in their lifetime.[1] The NCI estimates that approximately **21,290 new cases of ovarian cancer will be diagnosed and 14,180 ovarian cancer deaths will occur in the U.S. in 2015.**[2] It is the leading cause of death from gynecologic malignancy, usually characterized by advanced presentation with regional dissemination in the peritoneal cavity. Epithelial ovarian cancer is the most common form, and up to 25% of epithelial cases may be due to inherited gene mutations.[13,14] BRCA1 and BRCA2 are the most common causes of hereditary ovarian cancer, but several other genes are associated with increased ovarian cancer risk as well.[11,13,15,16]

COLORECTAL CANCER (CRC) effects about 1 in 20 (5%) men and women in their lifetime.[1] The NCI estimates that approximately 132,700 new cases will be diagnosed and 49,700 CRC deaths will occur in the U.S. in 2015.[2] The majority of CRC is sporadic, but approximately 30% are familial, a subset of which have a strong genetic cause. Lynch syndrome is the most common form of hereditary CRC, but several other genes are associated with increased CRC risk as well.[17]

UTERINE CANCER affects about 1 in 38 (2.6%) women in their lifetime.[1] The NCI estimates that approximately **54,870 new cases of uterine cancer will be diagnosed and 10,170 uterine cancer deaths will occur in the U.S. in 2015.**[2] Increased risk for uterine cancer has been identified in a number of hereditary cancer syndromes, including Lynch syndrome and Cowden syndrome.

PANCREATIC CANCER affects about 1 in 65 (1.5%) of men and women in their lifetime.[1] The NCI estimates that approximately **48,960 new cases of pancreatic cancer will be diagnosed in the U.S. in 2015.**[2] Approximately 95% of pancreatic cancers are pancreatic adenocarcinomas of the exocrine gland (which produces enzymes for food digestion). Neuroendocrine/islet cell tumors of the endocrine gland (a gland that produces insulin and regulates blood sugar) make up the other 5% of pancreatic cancer subtypes. While the majority of pancreatic cancers are sporadic, approximately 5-10% of pancreatic cancer cases are familial, often occurring in families with multiple affected individuals.[18] Multiple genes are associated with increased pancreatic cancer susceptibility.

KIDNEY CANCER affects about 1 in 60 (1.6%) of men and women in the U.S. in their lifetime and it is the seventh and eighth most common cancer in men and women, respectively.[1] Renal cell carcinoma (RCC) is a complex disease with a diverse spectrum of tumor subtypes, including clear cell or conventional (70-80%), papillary type 1 and type 2 (10-15%), chromophobe (3-5%), and collecting duct (1%).[19] Approximately 3-5% of RCC cases are hereditary[20-22] and occur as a result of an inherited mutation in one or more genes. Unlike sporadic RCC cases, hereditary RCC is often characterized by earlier disease onset and/or multifocal or bilateral tumors.[19]

PARAGANGLIOMAS (PGLs) are often benign, neuroendocrine tumors of the autonomic nervous system originating from the external ganglia. Pheochromocytomas (PCCs) are PGLs that are confined to the adrenal medulla. PGLs are further subdivided into sympathetic and parasympathetic tumors, depending upon their site of origin. Sympathetic PGLs commonly hypersecrete catecholamines and are typically located in the chest, abdomen and pelvis. Parasympathetic PGLs are primarily non-secretory and occur along the nerves in the head, the neck, and the upper mediastinum (termed head and neck PGLs or HNPGLs).[23,24] The prevalence of PGLs in the U.S. is 1 in 2,500 to 1 in 6,500, although this is likely an underestimate. The average age of diagnosis is between 40-50 years.[24,25] Approximately 75% of PGL/PCCs are benign; however, morbidity and mortality are associated with high levels of circulating catecholamines, which can lead to hypertension and stroke.[23,25] Published population studies have found that at least 10-30% individuals with PGL/PCCs have an inherited germline mutation in one of the known susceptibility genes.

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Cancer History Questionnaire

Patient Name	*Date of Birth*	*Gender*	*Ethnicity*

Phone	*Email*	*Date Completed*

Please complete the below questionnaire to assist your healthcare provider in determining if your personal or family history may be placing you or other family members at increased risk to develop cancer, and if you may be eligible for genetic testing.

	YOU	IMMEDIATE BLOOD RELATIVES		EXTENDED BLOOD RELATIVES (AUNTS, UNCLES, GRANDPARENTS, ETC.)			
Breast & Ovarian Cancer	Age at Diagnosis	Parents, Siblings or Children	Age at Diagnosis	Mother's Side	Age at Diagnosis	Father's Side	Age at Diagnosis
Example: Woman with Breast Cancer at age ≤50	45	Mother Sister	49 36	Maternal Aunt	46	Paternal First Cousin	50
Woman with Breast Cancer at age ≤50							
Woman with Breast Cancer >50							
"Triple Negative" Breast Cancer (Estrogen Receptor (ER) negative, Progesterone Receptor (PR) negative, HER2neu negative)							
Ovarian, fallopian tube, or primary peritoneal cancer							
A woman who has been diagnosed with both breast and ovarian cancer in her lifetime (two separate cancers)							
Male breast cancer							
Bilateral breast cancer (cancer in both breasts) or two breast primaries *Please specify*							
Ashkenazi (Eastern/Central European) Jewish ancestry with breast or ovarian cancer							
Pancreatic or Prostate Cancer *Please specify*							
Colorectal & Endometrial (Uterine) Cancer	**Age at Diagnosis**	**Parents, Siblings or Children**	**Age at Diagnosis**	**Mother's Side**	**Age at Diagnosis**	**Father's Side**	**Age at Diagnosis**
Colorectal cancer or several pre-cancerous polyps (adenomas) at an age ≤50							
An individual who has been diagnosed with two or more colon cancers (not reoccurrences, but two separate primary cancers)							
A woman who has been diagnosed with endometrial (uterine) cancer at age ≤50 OR both colorectal and endometrial (uterine) cancer *Please specify*							
10 or more total pre-cancerous polyps (adenomas) in a person's lifetime							
Relatives with any of the below related cancers* *Please specify*							

** Related cancers include colon, endometrial (uterine), ovarian, stomach, pancreas, ureter, kidney, biliary tract, brain, small intestine, and sebaceous gland tumors/cancers.*

Signature _____ Date _____

NCCN GENETIC TESTING CRITERIA FOR HEREDITARY BREAST AND OVARIAN CANCER SYNDROME

Family history of a known BRCA1 or BRCA2 mutation

Personal history of breast cancer diagnosed at age 45 or younger

Personal history of breast cancer diagnosed at age 50 or younger with one of the following:

- ≥1 close blood relative(s) with breast cancer at any age
- An unknown or limited family history
- Two breast primaries, the first of which was diagnosed at age 50 or younger

Personal history of a triple negative breast cancer diagnosed at age 60 or younger

Personal history of epithelial ovarian, fallopian tube, or primary peritoneal cancer at any age

Personal history of male breast cancer at any age

Personal history of breast cancer at any age with one or more of the following:

- ≥1 close blood relative(s) with breast cancer diagnosed at age 50 or younger
- ≥2 close blood relatives with breast cancer at any age
- ≥1 close blood relative(s) with epithelial ovarian/fallopian tube/primary peritoneal cancer
- Close male blood relative with breast cancer
- ≥2 close blood relatives with pancreatic cancer and/or prostate cancer (Gleason score ≥7) at any age
- For an individual of ethnicity associated with higher mutation frequency (e.g., Ashkenazi Jewish) no additional family history may be required*
- Personal history of pancreatic cancer or prostate cancer (Gleason score ≥7) at any age with ≥2 close blood relatives with breast and/or ovarian and/or pancreatic and/or prostate cancer (Gleason score ≥7) at any age
- For pancreatic cancer, if Ashkenazi Jewish ancestry, only one additional affected relative is needed
- Unaffected patient with a first or second-degree relative who meets any of the above criteria
- Testing unaffected individuals should only be considered when an appropriate affected family member is unavailable for testing

**Testing for Ashkenazi Jewish founder-specific mutation(s) should be performed first. Full sequencing may be considered if ancestry also includes non-Ashkenazi Jewish relatives or other criteria are met.*

NCCN TESTING CRITERIA FOR LYNCH SYNDROME (ALSO KNOWN AS HNPCC) AND POLYPOSIS SYNDROMES

Criteria for Lynch Syndrome genetic testing

Family history of a known Lynch syndrome mutation (MLH1, MSH2, MSH6, PMS2, EPCAM)

Patient has a cancer on the Lynch syndrome tumor spectrum that demonstrates microsatellite instability (MSI-H) or absence of a mismatch repair protein via immunohistochemistry (IHC)

Patient diagnosed with endometrial cancer at age 50 or younger

Meets Revised Bethesda Guidelines:

- Patient has a personal history of colorectal cancer AND meets one of the following:
  - Patient diagnosed at age 50 or younger
  - Presence of synchronous or metachronous Lynch syndrome-associated cancers, regardless of age
  - Patient diagnosed at age 60 or younger with a colorectal cancer that demonstrates MSI-high histology (tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern)
  - One or more first-degree relatives with a Lynch syndrome-associated cancer, with one of the cancers being diagnosed at age 50 or younger
  - Two or more first- or second-degree relatives with Lynch syndrome-associated cancers, regardless of age

Meets Amsterdam Criteria:

- Patient and at least two close relatives who all have or have had a cancer associated with Lynch syndrome AND all of the following criteria must be met:
  - One must be a first-degree relative of the other two;
  - At least two successive generations must be affected;
  - At least one of the cancers should be diagnosed at age 50 or younger;
  - Familial adenomatous polyposis (FAP) should be excluded

Unaffected patient with a close relative who meets any of the above criteria

- Testing unaffected individuals when no affected family member is available should be considered; significant limitations of interpreting test results should be discussed

Criteria for Adenomatous Polyposis (APC and MUTYH) genetic testing

Family history of a known APC mutation or two (biallelic) MUTYH mutations

Personal history of a total of >10 adenomas

Personal history of a desmoid tumor

Other Polyposis Syndrome Genetic Testing Criteria

Personal or family history of multiple GI hamartomatous polyps or serrated polyps

Guidelines are current as of October, 2014. Please visit www.nccn.org for the most current