Application Note

# Prevalence of Hereditary Cancer and Benefits of Multi-Gene Panel Testing

## Introduction

Most human cancers develop by chance and every person has some risk of developing cancer during his or her lifetime. These are called sporadic cancers and may arise as a natural consequence of aging or when DNA in a cell has been damaged. Somatic mutations that occur randomly in one or a few cells of the body are the reason for sporadic cancer occurrence. These mutations have effects only on the body cell concerned and are not passed down from parents to their children.

When many cases of cancer occur in a family, it could be due to chance alone or because family members have been exposed to a common risk factor. However, sometimes cancers in a family are part of a hereditary cancer syndrome and are strongly linked to inherited gene mutations. In the hereditary forms of cancer, the disease develops due to DNA mutations that originate in germline, sperm or egg cells. Germline mutations are present in nearly every cell in the body and they can be passed down in families. Persons who inherit germline mutations do not necessarily get cancer, but their risk of developing the disease at some point during their life is higher than average.

Hereditary cancers are usually characterized by an earlier age of disease onset, more than one type of cancer in a single person, cancers occurring in both of a pair of organs (such as both breasts or both ovaries) and often a family history of cancers that run through many generations. Also, there is a specific spectrum of tumors in families with hereditary cancer syndrome. For example, colon cancer and endometrial cancer tend to group together in Lynch syndrome families (also known as hereditary non-polyposis colorectal cancer, HNPCC) and breast cancer and ovarian cancer are frequent in families with hereditary breast and ovarian cancer syndrome (HBOC). Pancreatic and prostate cancers can also occur in HBOC families.

Hereditary cancers account for only about 5% of all malignancies. Nevertheless, it is very important to recognize these individuals and their family members because, unlike patients with sporadic cancers, they require long-term specific clinical care to permit early detection and/or risk reduction measures. Most of the hereditary cancer syndromes are inherited in the autosomal dominant manner so risk of inheriting a mutation among first degree relatives of a known mutation carrier is 50%.

Sometimes, hereditary cancer syndromes are caused by a single gene mutation (monogenic hereditary disease). For example, persons who inherit CDH1 mutation have an increased risk for developing hereditary diffuse gastric cancer (HDGC). However, some hereditary cancers follow the polygenic pattern of inheritance with sets of genes whose mutations carry certain level of risk. Thus, mutations in MLH1, MSH2, MSH6, PMS2 and EPCAM confer a high risk for colon and endometrial carcinoma in the context of Lynch syndrome.

The complexity of the genetic architecture of cancer predisposition is supported by the fact that there are also other, still undiscovered causative genes and numerous cancer susceptibility syndromes (1). In fact, the risk of cancer at a specific site may be elevated by mutations in one or a number of different genes, but a single mutation in a particular gene may increase the risk for more than one type of cancer. For example, breast cancer may occur as a consequence of BRCA2 mutation but the same mutation may also elevate the risk of ovarian cancer, pancreatic cancer and melanoma. Also, there are genes with high penetrability that result in hereditary predisposition indicated by the family history and there are moderate- and low-penetrance genes in which mutations might not segregate with cancer patterns in families. The list of the contributing genes grows with each new study, and for many of them the reality of increased risk has not yet been clearly established.

## Hereditary cancer syndromes prevalence

### Hereditary breast and ovarian cancer syndrome (HBOC)

Breast cancer is the most commonly diagnosed cancer among American women. It's estimated that about 30% of newly diagnosed cancers in women (around 276,480.00) will be breast cancers in 2020. According to the American Cancer Society, about 1 in 8 U.S. women (about 12%) will develop invasive breast cancer over the course of her lifetime (2). A man's lifetime risk of breast cancer is about 1 in 883 with estimated 2,620.00 new cases in 2020 in the U.S. Female breast cancer is the most commonly diagnosed cancer in Europe as well. Over 355,000.00 women in the EU-27 are estimated to be diagnosed with breast cancer in 2020 (13.3% of all cancer diagnoses) (3).

Less than 15% of persons diagnosed with breast cancer have a family member diagnosed with this disease and about 5-10% of all cases can be associated with the mutations in high-risk BRCA1/2 genes as part of the HBOC syndrome. According to the numbers, more than 70,000.00 patients in Europe and more than 50,000.00 patients in the U.S. would require genetic testing yearly in the context of hereditary disease.

A causative mutation in BRCA1/2 genes can only be detected in about 50% of families in which there is suspicion of HBOC. Other genes such as ATM, BRIP1, CDH1, CHEK2, NBN, NF1, PALB2, RAD51C, and RAD51D with high, intermediate, and low impact on cancer risk have also been identified in HBOC families. Besides risk for breast and ovarian cancers, mutations in these cancer susceptibility genes elevate the risk for other cancers such as prostate and pancreatic cancers.

For example, variants in multiorgan cancer susceptibility gene CHEK2, can be associated with thyroid, breast, and prostate cancers (4). A specific missense variant I157T in CHEK2 is also associated with the increased risk of colon cancer and kidney cancer in addition to risks for breast, prostate and thyroid cancers (4).

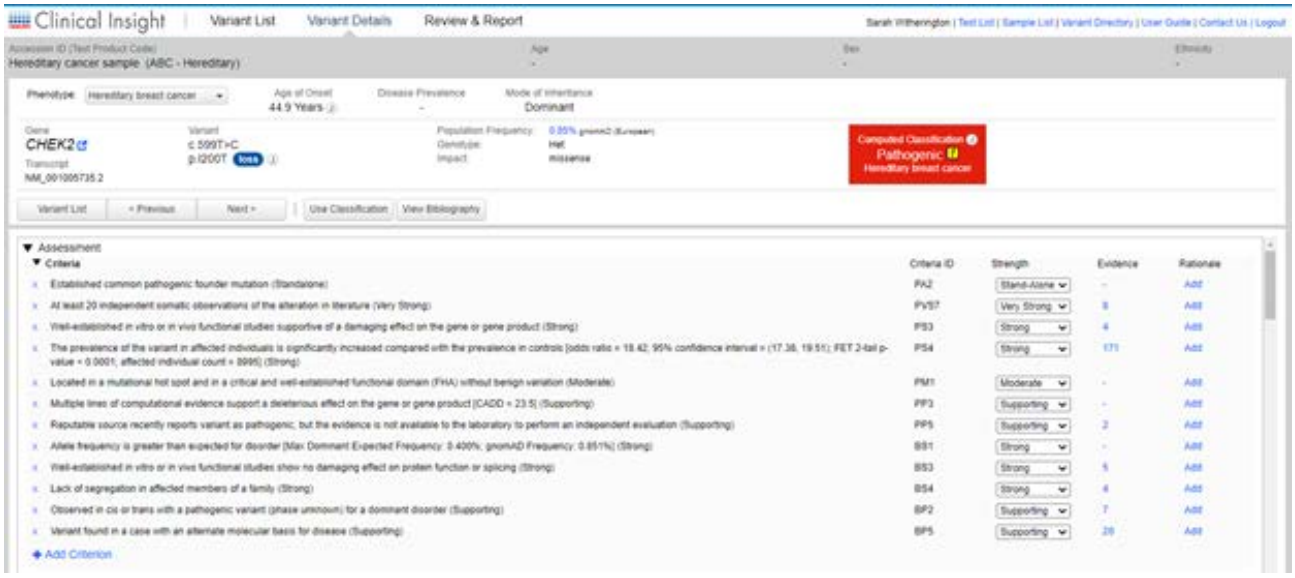


**Figure 1.** Example of a CHEK2 mutation in QCI Interpret with the different lines of evidence from the ACMG criteria that were triggered for the variant based on evidence from the QIAGEN Knowledge Base, an extensive proprietary database.

## Lynch syndrome- hereditary nonpolyposis colorectal cancer (HNPCC)

After breast cancer, colon cancer is the second most diagnosed malignancy in Europe (341,000.00, 12.7%) and the third most commonly diagnosed malignancy in the U.S. (104,610.00 new cases in 2020). About 3% of these cases involve one of the hereditary colon cancer predisposition syndromes. Lynch syndrome (LS) is the most common inherited cause of colorectal cancer. The estimated population frequency is 1 in 370 to 1 in 2,000.00 in Western populations. In the U.S., it is estimated that 1 in 279 individuals have a gene mutation associated with LS (5).

LS is a highly penetrant autosomal dominant hereditary cancer syndrome caused by defects in the DNA mismatch repair genes, including MLH1, MSH2, MSH6, PMS2, and EPCAM. Besides colorectal cancer, LS accounts for most cases of hereditary uterine cancer and is the second most common cause of inherited ovarian cancer (after HBOC).

Other neoplasms associated with LS include gastric cancer, small-bowel cancer, hepatobiliary cancer, renal, pelvis and ureteral cancer, and potentially some types of breast cancer, certain brain tumors, and sebaceous skin tumors (6).

For example, defects in genes that result in microsatellite instability are the most common mutations in women with Lynch syndrome and a gynecologic malignancy as their sentinel cancer. The highest cumulative risk for endometrial carcinoma was observed in the MSH6 mutation carriers (61%), while the cumulative risks for MLH1 and MSH2 mutation carriers were 25% and 49% respectively (7).

## Li Fraumeni syndrome

Li–Fraumeni syndrome (LFS) is autosomal dominant disorder that increases the risk of developing several types of cancer, especially in children and young adults. LFS is estimated to occur in 1 in 5,000.00 to 1 in 20,000.00 people worldwide.

Approximately 70% of individuals clinically diagnosed with LFS have a germline mutation in TP53 (8). The cancers most often associated with LFS include breast cancer, osteosarcoma, soft tissue sarcomas, brain tumors, leukemia, and adrenocortical carcinoma. LFS is highly penetrant with the risk of TP53 mutation carriers for developing multiple cancers in different organs during their lifetime is reported to be as high as 90% (9).

**Other hereditary cancer syndromes**

Cowden syndrome is caused by pathogenic variants in the PTEN gene and is relatively rare with a population prevalence of 1 in 200,000.00 (10). It is a disorder characterized by multiple noncancerous, tumor-like growths and an increased risk of developing certain cancers (breast, endometrial, thyroid). Other cancers such as colorectal and kidneyare also found in people with this syndrome.

Estimated lifetime risks for PTEN mutation carriers are 85.2% for breast cancer, 35.2% for thyroid cancer, 28.2% for endometrial cancer, 9.0% for colon cancer, 33.6% for kidney cancer, and 6% for melanoma (11). Mutations in the promoter region are usually associated with breast cancer while nonsense mutations are usually associated with colorectal cancer.

Peutz-Jeghers Syndrome (PJS) is caused by pathogenic variants in the serine/threonine kinase 11 (*STK11*) gene. PJS is a rare disorder with the prevalence estimated to be between 1 in 50,000.00 and 1 in 200,000.00. It is characterized by the development of noncancerous growths called hamartomatous polyps in the gastrointestinal tract and a greatly increased risk of developing cancers of the gastrointestinal tract, pancreas, cervix, ovary, and breast.

Hereditary diffuse gastric cancer (HDGC) is a rare cancer representing approximately 2% of all gastric cancers (12). HDGC is characterized by an increased risk of diffuse gastric cancer, lobular breast cancer, and colorectal cancer, and is attributable to mutations in the CDH1 gene. CDH1 mutation carriers have a lifetime risk for diffuse gastric cancer estimated to be up to 83% by age 80. Lobular breast cancer is diagnosed in women with a mutation in the CDH1 gene with the risk of 39% to 52% by age 80.

## Benefits of hereditary cancer NGS testing

Sanger sequencing was developed in the 1970s and it was the first sequencing method to be commercialized. It is still used for targeted sequencing of a single small area in DNA or a small number of samples. However, Sanger sequencing has its limitations.

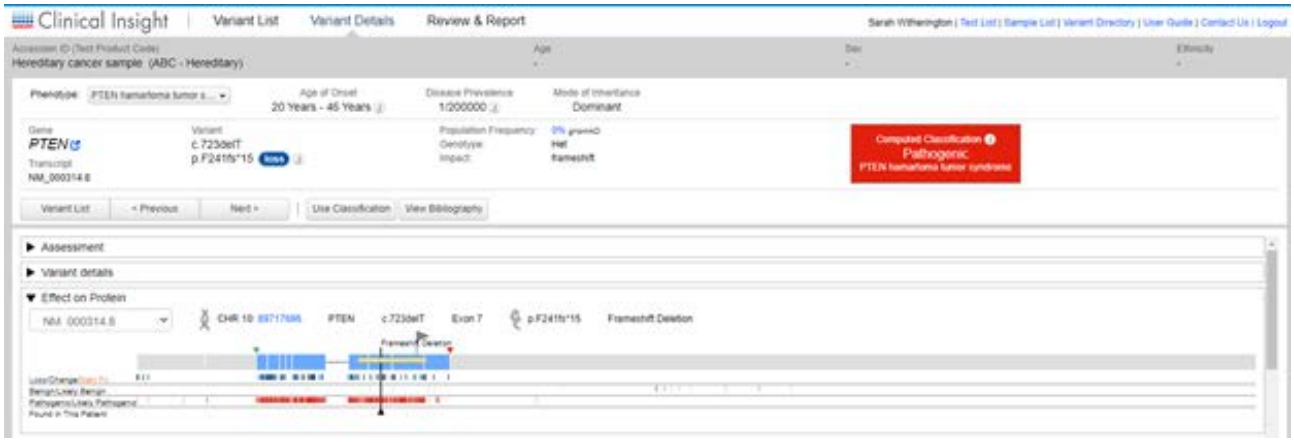


**Figure 2.** Detection of pathogenic variants in the *PTEN* gene in QCI Interpret software.

For the large genes such as BRCA1/2 with no mutational hot spots and with more than 3,500.00 mutations scattered throughout the whole coding region, first-generation sequencing is very slow and expensive. Also, Sanger sequencing is unable to perform parallel testing of multiple targets and has restricted sensitivity.

The BRCA1 gene contains multiple characterized mutations from benign to pathogenic. The QIAGEN Clinical Insights (QCI®) Interpret platform provides a way to see and investigate the classification of these mutations in the appropriate variant-phenotype context using over 1 million unpublished variant-phenotype relationships from the QIAGEN Knowledge Base. This illustration displays a frameshift deletion (black line) in BRCA1 (NM_007294.4) coding region from an HBOC sample.

The first line below the coding region indicate which variations are predicted to be loss/ gain of function. The second line represent mutation that are classified as likely benign/ benign in an HBOC context base on the computed classification. The third line indicate the likely pathogenic/ pathogenic mutations. QCI Interpret computed classification is based on manually curated evidence and the implementation of the professional guidelines from ACMG/AMP.

The beginning of the 21st century has brought several practice changing events that have led to shifting paradigms for hereditary cancer predisposition testing. DNA sequencing advances through next-generation sequencing (NGS), and NGS-based tests encompassing multi-gene panels, whole-exome sequencing (WES), and whole-genome sequencing (WGS) have been developed.


**Figure 3. Characterized mutations in BRCA1.** A large number of characterized variants can be found in BRCA1, QCI gives a visual to nearby mutations in the same gene as well as information on pathogenicity, splice site variants, and nonsense mediated decay expectations.

Costs have become significantly lower and multi-gene panels are often similar in price to single-gene testing (13). With the reduction of sequencing costs and increasing sensitivity of new technologies, there was an immediate increase in the number of genes that could be evaluated simultaneously. WES and WGS entered the market and became an integral part of the clinical diagnostics and common testing option in oncology.

NGS offers a higher sensitivity to detect low-frequency variants, the faster turnaround time for high sample volumes, higher throughput, cost efficiency, comprehensive genomic coverage, and the ability to sequence hundreds to thousands of genes simultaneously. In the context of hereditary cancers, high-throughput NGS technologies enabled a multiplex approach and allowed quick evaluation of high-, moderate-, and low- penetrance genes in a single run. It is particularly important in the situations where genetic heterogeneity exists, where several genes carry actionable mutations and when there is difficulty in predicting which gene may be affected on the basis of phenotype and family history.

Variant annotation is an important step of assigning clinical significance to the DNA variations detected by NGS. Since the number of available genetic tests is rapidly increasing, as is the number of genes included in any given test, the clinicians are handling much larger volume of genetic variants that need clinical classification every day. The process of variant annotation is based on accessing up-to-date information on variants such as their prevalence in healthy people and those with diseases, functional impact on the protein, and results from clinical trials.

Data sources that provide information on variants are numerous, heterogeneous, quickly evolving, and sometimes conflicting, which often makes variant annotation rather a challenging process that relies on probabilistic assessment that the variant is disease-causing. Because of this, a significant discrepancy in classification was shown between different laboratories which might have a tremendous impact on the clinical decision making (14). To work efficiently, clinicians need reliable variant annotation systems that will help to collect and aggregate available data from various data sources acknowledging existing uncertainty.

American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) published variant classification guidelines in 2015 (15) that are applicable to all areas of genetics. They propose a scoring system that gives different weights to different types of evidence, and an algorithm to classify variants into one of the five following classes: pathogenic (class 5), likely pathogenic (class 4), variant of unknown clinical significance (VUS) (class 3), likely benign (class2) and benign (class 1). Pathogenic and likely pathogenic variants are those that have an actual clinical impact on making diagnosis, predicting the course of treatment, and assessing the risk of disease in healthy family members.

Findings from genetic testing for which the clinical significance is currently unresolved are even more difficult to deal with. Variants are usually classified as VUS in the case when evidence for their classification conflict with each other or in the case when there is a lack of evidence for their classification. Expectedly, multi-gene panel testing has greatly increased the number of VUS encountered in clinical practice. The more genes we look at the more likely we are to find uncertain results. Unlike some other uncertain medical results whose status won't change over time, VUS in genetics can be reclassified as more data are gathered and more evidence for classification appears. Thus, they may be upgraded to pathogenic or likely pathogenic, or more likely downgraded to benign or likely benign. When reclassification occurs, amended reports should be issued and disclosed to the patients.

## Importance of education and real-time content

Single-gene testing is still the method of choice in cases when the patient`s clinical features and family history are strongly associated with a single gene indicative of a particular hereditary syndrome. Such testing is highly specific and it minimizes the likelihood of detecting incidental findings, VUS, or pathogenic mutations in genes with questionable clinical utility. The genes tested in this setting typically have well-described cancer risks and often have established guidelines for appropriate management of mutation carriers.

On the other hand, multi-gene testing approach is appropriate when the family history is not suggestive of a single specific gene or one specific hereditary syndrome. Also, multigene panel testing might be considered in cases when initial single testing is negative (for example BRCA1 negative patient from HBOC family). This approach has a huge potential to improve the detection rate of hereditary cancer syndromes to reveal associations between mutations

in different genes and clinical phenotypes and to contribute to a better understanding of family history for many different conditions.

With the increased demand for multi-gene panel testing, the availability of new technologies, and the complexity of detected variants and clinical implications they carry, there is an increased need for genetic education of all medical providers to provide adequate care in genomic medicine. Lack of education in genomics among physicians and other health care providers might not only delay appropriate clinical care but also could lead to erroneous uses with serious consequences (16). In some of the reported cases, the choice of a particular genetic test was wrong which led to inaccurate medical management recommendations as well as unnecessary testing and money expenditure.

Some of the cases also showed frequent and unnecessary duplication of tests (17). Results misinterpretation is also an important issue to consider especially when VUS are involved. These variants are particularly difficult to interpret and misinterpretation might lead to unnecessary prophylactic surgeries when being falsely interpreted as a known disease-causing mutation. Factors such as case complexity, time pressures, lack of experience, insufficient training, poor communication, and inadequate counseling all contribute to the increased likelihood that errors can occur. Clinicians without extensive training and knowledge in genetics may also not be aware of current policies, guidelines, and recommendations for testing.

WES and WGS will continue to generate a vast number of genetic variants per individual and the interpretation of how these variants impact health is likely to be far more complex involving interaction between multiple genes, polygenic risk scores, multiple SNPs, and gene-environment interactions.

Unfortunately, our ability to generate massive amounts of genetic data has far outpaced our ability to interpret their clinical significance. Thus, the proper use of genetic information poses significant challenges even for providers with extensive knowledge and experience in clinical genomics. To address these challenges and to keep the pace with the new technology advancements opportunities should be made to educate physicians and other health care providers in genomics.

To overcome the major bottleneck of accurately interpreting an individual's genetic variants from larger panels and even whole exome and genomes requires sophisticated curation methods and processes to find, prioritize, transform, and constantly update biologically and clinically relevant publications at scale.

QIAGEN Digital Insights has unparalleled experience in content curation. As the leading provider of genomic content knowledge, QIAGEN's variant interpretation software and service take advantage of different curation methods to accurately transform the literature into biological and clinical insights. Ultimately, the aggregated knowledge ensures users receive timely, accurate, reproducible, and consistent content to confidently interpret variants at scale and support evidence-based medicine.

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Learn more about QCI for hereditary cancer at

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QCI Interpret is evidence-based decision support software intended as an aid in the interpretation of variants observed in genomic next-generation sequencing data. The software evaluates genomic variants in the context of published biomedical literature, professional association guidelines, publicly available databases, annotations, drug labels, and clinical trials. Based on this evaluation, the software proposes a classification and bibliographic references to aid in the interpretation of observed variants. The software is NOT intended as a primary diagnostic tool by physicians or to be used as a substitute for professional healthcare advice. Each laboratory is responsible for ensuring compliance with applicable international, national, and local clinical laboratory regulations and other specific accreditations requirements.