

## Multi-Gene Panels in Hereditary Cancer: Cause for Caution?

### Introduction

For years, Sanger sequencing was used for targeted sequencing of a single small area in DNA or a small number of samples. Historically it has been the gold standard for detecting DNA mutations. However, Sanger sequencing has its limitations due to the inability to perform parallel testing of multiple targets and due to its restricted sensitivity. Recent advances in the NGS technologies made a huge impact on the research and clinical domains comparing to prior sequencing practices.

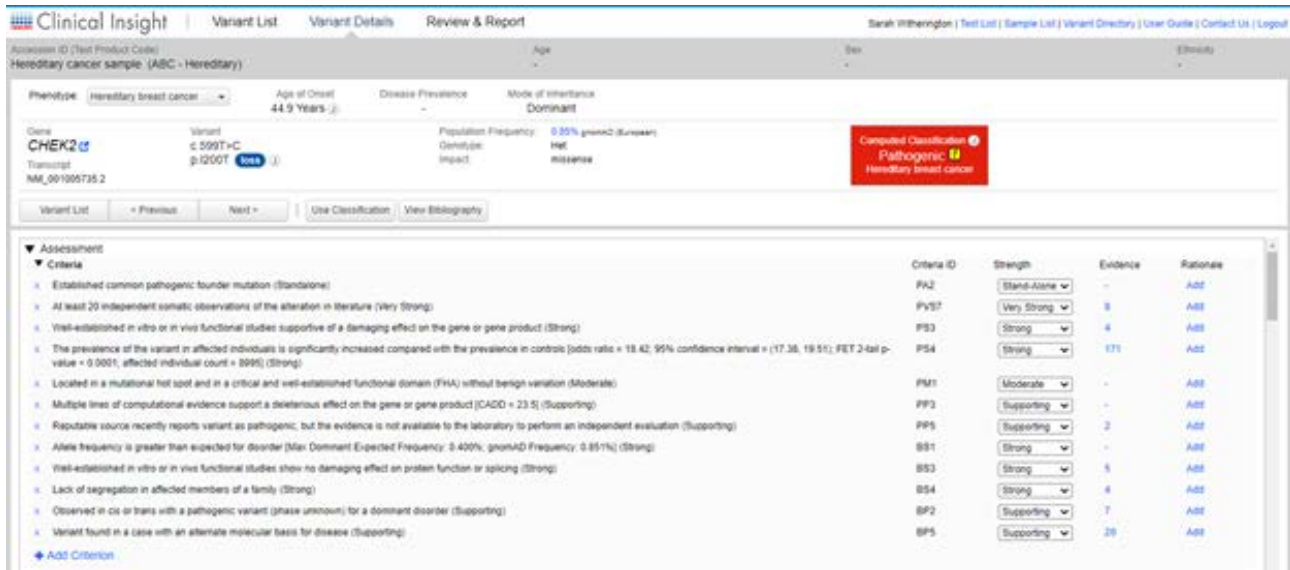
Thanks to the technology development and large sequencing projects, many new genes with moderate and low risk for hereditary cancers were revealed. With the reduction of sequencing costs and increasing sensitivity of new technologies, there was an immediate increase in the number of genes that could be evaluated simultaneously. Whole-exome sequencing (WES) and whole-genome sequencing (WGS) entered the market in a number of laboratories becoming an integral part of clinical diagnostics and a common option for cancer genetic testing.

The list of genes contributing to hereditary cancer grows with each new study, and for many of them the reality of increased risk has not yet been clearly established. Because of the rising complexity of genetic testing, difficulties in variant annotation and challenges of risk stratification, issues surrounding clinical utility of multi-gene genetic testing, clinical and analytical validity of genetic tests as well as their accessibility have become important issues that should be addressed in each individual case.

### Cause for caution in the use of multi-gene panels

#### **Clinical utility**

In the context of genetic testing clinical utility refers to the ability of a genetic test to prevent or ameliorate adverse health outcomes through the adoption of effective treatments which are based on test results (4). Clinical utility of a genetic test broadly refers to any use of genetic results to inform clinical decision-making or, even broader, to any outcomes considered important to individuals and families (e.g. reproductive decisions, lifestyle choices).



**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.

Clinical utility of multi-gene panel testing in hereditary cancers is sometimes difficult to assess since clinical surveillance and management guidelines for many of the newly discovered, moderate- or low- penetrance genes still don't exist. For example, while management guidelines for high-penetrance gene carriers in HBOC (such as BRCA1, BRCA2, PALB2) are well established, guidelines on moderate-penetrance genes (such as ATM, CHEK2) are not homogenous. Thus, finding a mutation in the ATM gene which is associated with a two-fold increase risk for breast cancer, surveillance, and management recommendation will often not change even if there is a personal or family history of breast cancer. For the same gene, some recommend more extensive imaging and screening while others take family history into account more so than the test results.

Another issue with moderate-penetrance genes is related to the family implications. When a mutation in the ATM gene is detected in a family with an extensive family history of breast

cancer, a negative test in a family member shouldn't be reassuring since it is considered that ATM doesn't recap family history. Another example that shows the difficulty in establishing clinical utility is related to CDH1 mutation carriers. CDH1 is the gene most commonly associated with Hereditary diffuse gastric cancer (HDGC). When using multigene panel approach, a CDH1 mutation may be detected in the person with no relative affected with gastric carcinoma. It is questionable to perform prophylactic surgery, unless otherwise indicated when family members are affected, in the setting where no family history exists. A similar question might be raised regarding mutations in other genes because modifier factors have long been known to increase or decrease the risk of certain cancers. The additional issues with clinical utility refer to an increasing frequency of variants of unknown significance (VUS). It has been reported that as many as 44% of the patients will receive one or more VUS depending on the primary cancer site and the test ordered (5).

According to the ACMG guidelines 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 downgraded to benign or likely benign.

### **Actionability**

Actionability is often highly context-specific and different users of the term (i.e., laboratory scientists, healthcare providers, patients, and insurers) use it to convey a wide range of concepts. In the context of genetic testing, actionability refers to actions that individuals take to make meaningful changes to their lives as a consequence of test results (6).

In the era of multi-gene panel testing questions have arisen concerning what constitutes actionable results specifically in the categorization of findings that are unrelated to the indication for ordering testing, called incidental genomic findings (IFs). For example, genetic tests can identify mutations in predisposition genes that have no direct clinical utility for the patient himself but can have an immediate clinical impact on the family members. Incidental BRCA1 mutation in a young boy is an example of how IFs might have potential clinical relevance to family members despite having no immediate clinical utility/actionability for the child itself.

Difficulties might also arise when analyzing the results of panel testing in a specific syndrome. For example, HBOC panels include a list of genes such as ATM, BRIP1, CDH1, CHEK2, PALB2, RAD51C, RAD51D which are associated with hereditary breast and ovarian cancer cases. However, some of these genes (CDH1, CHEK2) are considered actionable for their increased breast cancer risk but without evidence for ovarian cancer risk. Similarly, BRIP1, RAD51C, RAD51D from the same panel might show an increased ovarian cancer risk without increasing risk for breast cancer.

### **Clinical validity**

In the context of genetic testing, clinical validity refers to whether the test accurately and reproducibly predict the clinically defined condition. Because of the limited data and the lack of information on many genes included in the multi-gene panels, clinical validity is not easy to demonstrate. For example, until recently RAD51C and RAD51D were not associated with the increased risk for breast cancer although they are an integral part of HBOC panels. A new data from the recent study, however, showed a high risk for triple-negative breast cancer (TNBC) for RAD51D mutation carriers and moderate TNBC risk for RAD51C mutation carriers (7).

Due to the low frequency of RAD51C and RAD51D mutations and the fact that TNBC represents only 15% of all breast cancers, the clinical validity of these genes in this specific subtype was difficult to demonstrate showing that classification of clinical validity is a dynamic process subject to new information. Co-segregation data, case-control studies, population data, functional data, cell, and animal models should be used to classify the strength of association between a gene and a disease risk.

### Analytical validity

Analytical validity of the test includes sensitivity and specificity of variant detection. NGS technologies, being able to detect variant at low levels (up to 1%) show higher sensitivity than Sanger sequencing (15-20%). In hereditary cancer testing high sensitivity is particularly important for detecting mutations in genes that have high de-novo mutation rate such as TP53 especially when one-fifth of these de-novo variants are mosaics.

When evaluating the appropriate use of new genetic tests, clinicians and health care policymakers must consider all: clinical utility/actionability, clinical validity and analytical validity which is proven to be challenging in the NGS multi-gene era and is rarely easily quantified. These are often a matter of judgment depending on the stakeholder's perspective of the supporting evidence. For example, even when a genetic test has clinical utility in a population it might not carry personal utility for a given patient and, in fact, may be the wrong choice in some cases.

### Accessibility

Clinical genomic sequencing can be a game-changer for many patients and should be made available to all who will benefit from it. Sequencing is indeed becoming more accessible thanks to the technology improvements that reduce costs and simplify analysis. However, even with these ongoing advances, many patients lack access to genomic sequencing. One of the gaps that can limit patients' access to genetic testing is related to the health insurance reimbursements. Lack of insurance coverage may raise concerns about placing an additional financial burden on the patients. In addition, providers, patients, and policymakers may not fully understand the availability and benefits of the appropriate genomic test. Additional confusion might introduce the fact that sometimes different medical guidelines exist in different societies for the same clinical application. Also, there is a general lack of genetics experts and many patients may actually lack geographic access to genetic counselors and other specialists. Issues related to racial, ethnic, and economic disparities also limit accessibility to genomic testing.

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**Figure 2.** QCI Interpret will return a list of journal articles, professional guidelines, functional studies, external database reports, and many other pieces of evidence that are variant specific.



## Family implications

Unlike other health-related information, genetic information has a unique shared nature. This means that genetic information is not related only to the person tested but extends to the family members as well. Once an individual is identified as a germline mutation carrier this information is important to the unaffected family members so they too can be proactive in terms of preventive measures if they are identified to also have the familial mutation.

Considerable debate has been generated over the issue of informing the family members about the test results and who should be the one to disclose the information. Should a patient diagnosed with a genetic disorder inform the family members himself or should the physician be the one to recommend genetic testing to family members? Respect for patients' autonomy, privacy, and doctor-patient confidentiality are directly opposed to the duty of beneficence directed to the family members that could carry mutation related to a serious health condition. The physicians could find themselves in a difficult position being protective of the people with potential risks yet not be abandoned in the duty to respect personal privacy. A compromise should be made and patients are usually counseled to inform their family members of the potential risks and to share their positive genetic test results with their relatives especially in the case when steps to prevent or ameliorate symptomatic disease exist.

Psychological and social implications of family members' genetic testing are also important to consider. For example, family members who tested negative for a family mutation may feel guilt that their loved ones are affected when they are not. In some cases, family members may not wish to know their genetic information.

If that is the case, simple disclosure on the carrier status of their relatives potentially violates their autonomy and privacy. Taking into account all the issues that may arise, family-level perspective should be carefully evaluated and the ways to improve family risk communication and genetic testing uptake should be considered.

## Clinical classification of variants

### **Appropriate clinical interpretation of detected variants**

Variant annotation is an important step of assigning clinical significance to the DNA variations detected by NGS. 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.

The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) published variant classification guidelines in 2015 and proposed 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 (class 2) and benign (class 1). The evidence for variant classification can be found in various data sources including population data, computational and predictive data, functional data, segregation data, de-novo data, and allelic data. After gathering all the evidence for a particular variant, they are further combined according to the scoring rules to choose a classification from the five-tier system. Lack of data on the variant may implicate its status of a variant of unknown clinical significance (VUS).

Thanks to a large number of sequencing projects, it was discovered that genetic susceptibility to cancer can be driven by low-, moderate-, or high-penetrance genes in accordance

A. Phenotype: Hereditary breast and/or o... Age of Onset Variable 1/4000 Disease Prevalence 1/4000 Mode of Inheritance Dominant

Gene: **RAD51C** Variant: c.97C>T p.Q33\* **loss** Population Frequency: 0.006% gnomAD (African) Genotype: Hom Impact: stop.gain

Computed Classification: Pathogenic Hereditary breast and/or ovarian c...

Variant List < Previous Next > Use Classification View Bibliography

▼ Assessment

Criteria

Criteria	Criteria ID	Strength	Evidence	Rationale
Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, copy number loss, single or multi exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease (Very Strong)	PVS1	Very Strong	-	Add
The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls (odds ratio = 6.57; 95% confidence interval = (1.47, 29.35); FET 2-tail p-value = 1.842E-2; affected individual count = 4; Note: Small number of affected individuals with variant.) (Strong)	PS4	Strong	3	Add
Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation (Supporting)	PP5	Supporting	2	Add

B. Bibliography for RAD51C p.Q33\*

Selected Clinical Cases All

Search Author, Title, Journal or PubMed ID. Use commas to separate terms.

Filter results by author Clear Filters

Refine references to Reported Not Reported Excluded Lab References Curated References

3 (of 3 total references with 4 unique variant findings) shown.

Report All Unreport All Sort By Date Add Reference Contact Us

Reporting Status	References	Actions
Reported	Carter NJ et al. (2018) Germline pathogenic variants identified in women with ovarian tumors. <i>Gynecol Oncol</i> 151(3):481-488. Epub 2018 Oct 12 (PMID: 30322717) Mutant human RAD51C protein (p.Gln33*, result of c.97C>T [germline]) is observed with breast and/or ovarian cancer in human.	
Reported	Sussex LR et al. (2015) Pathogenic and likely pathogenic variant prevalence among the first 10,000 patients referred for next-generation cancer panel testing. <i>Genet Med</i> 18(8):823-32. Epub 2015 Dec 17 (PMID: 26681312)	
Reported	Song H et al. (2015) Contribution of Germline Mutations in the RAD51B, RAD51C, and RAD51D Genes to Ovarian Cancer in the Population. <i>J Clin Oncol</i> 33(26):2901-7. Epub 2015 Aug 10 (PMID: 26261251)	

Figure 3. Clinical classification of variants using classification criteria and literature evidence. QCI Interpret provides classification criteria derived from the AMP/ACMG guidelines allowing evidence-based classification. A) Computed classification for the RAD51C p.Q33\* (loss) in the context of HBOC. The criteria PVS1, PS4 and PP5 are invoked and link to supporting evidence provided. B) Supporting evidence for the PS4 criteria. In addition, (data not shown) the variant is classified as pathogenic in ClinVar and "Damaging" in HGMD.

with cancer risks. Low-penetrance alleles comprise germline variants that are common in the general population. Genome-wide association studies (GWAS) in cancer, based on high-throughput sequencing approaches have identified many chromosomal regions associated with a small contribution to the cancer risk. Low-penetrance alleles are presented with relative risk around or smaller than 1.5 which means that carriers of these variants usually don't have higher risk for cancer than average. However, newly reported studies show that in cases when many of them are inherited together, low-penetrance alleles may increase cancer risks (8). Although there is some evidence showing that low-penetrance alleles might contribute to "missing heritability" in hereditary cancers (9), evidence-based guidelines for clinical management of carriers of low-penetrance variants still don't exist.

Intermediate-penetrance genes, on the other hand, carry moderate cancer risks (relative risk 1.5-5) but with the limited available data on the exact risk degree because they may be influenced by gene-gene or gene-environment interactions. For the carriers of mutations in intermediate-penetrance genes, guidelines for clinical management may exist for specific hereditary syndromes but in many cases the information from testing will not modify clinical management compared to the one based on family history alone.

Third category comprises genes that are associated with the cancer relative risk higher than 5. These genes cause familial cancer syndromes with high penetrance. For the deleterious mutations in high-penetrance genes genetic counseling and clinical management provide the greatest benefits and clinical guidelines are well established for most of the genes.

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It is important to note that a threshold for distinguishing high-penetrance from moderate-penetrance is actually arbitrary and that sometimes can be very difficult to make a distinction between these two categories. For example, some mutations within moderate-penetrance genes can, however, confer levels of risk that are similar to the ones associated with the high-penetrance gene mutation. Thus, some mutations detected in the otherwise characterized moderate-penetrance breast cancer gene, ATM, confer high risk for breast cancer. Conversely, certain mutations in high-penetrance genes might confer more modest degrees of risk.

## Conclusion

As the technology evolves, multi-gene panels will be more frequently used in the hereditary cancer testing. Since these tests will probably no longer be focused on clinical phenotypes, the potential of identifying VUS and incidental findings (IFs) will greatly increase. These issues will further emphasize the responsibilities of the providers to adequately interpret genomic results and to provide the patients with all information related to genomic testing. To work efficiently, clinicians will need reliable variant annotation systems that will help to collect and aggregate available data from various data sources acknowledging existing uncertainty. These systems should also include the pipelines for VUS reclassification and IFs management.

As we move forward with the advancement in genomic medicine, educational efforts and decision support tools will become critical in order to adequately implement and enhance proficiency in clinical genetics. The focus should particularly be on data sharing and distribution, protection of germline genetic data, return of the acquired knowledge and empowering patients and health care providers with information upon which to base genetic related health care decisions.

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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.

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