

# Determinants of Test Selection for Hereditary Cancer Risk Assessment

## Introduction

The introduction of next-generation sequencing (NGS) technology in testing for hereditary cancer susceptibility allows testing of multiple cancer susceptibility genes simultaneously. While there are many potential benefits to utilizing this technology in the hereditary cancer clinic, including efficiency of time and cost, there are also important limitations that must be considered. The best panel for the given clinical situation should be selected to minimize the number of variants of unknown significance. In this application note, we review several determinants of test selection for assessing hereditary cancer risk.

## Determinants of test selection

### Family history

Assessing family history is an important component of the clinical evaluation that can help determine whether the patient or family members may benefit from genetic counseling and/or genetic testing. Sometimes, people would need more intensive follow-up care based on their family history even if they do not want or need genetic testing.

To properly analyze the need for genetic testing and to choose the right gene panel, the personal and family medical history is of crucial importance. Disease-related information is collected from at least three consecutive generations. Cancer histories of first-degree relatives (parents, children, and full siblings) and second-degree relatives (grandparents, aunts/uncles, nieces/nephews, grandchildren, and half siblings) are collected especially on the type of cancer(s), age at diagnosis, lineage (maternal or paternal side of the family tree), ethnicity, and the results of any previous cancer-related genetic testing.

Sometimes it is rather challenging to obtain detailed information on a patient's family history. Patients may not know details about their relatives and family history may be limited due to lack of information or death. Recent trends in reproduction, such as surrogates and sperm-donors, may also complicate family history gathering. The high population prevalence of sporadic cancers in older individuals can also obscure the patterns attributable to genetic factors which can make selection of the right panel even more difficult.



With rising trends in performing whole-exome sequencing (WES) and whole-genome sequencing (WGS), there is a question of whether family history should still be used as a tool for genetic test selection. While multi-gene panels can still be performed without looking at the family history, the information related to the burden of disease in a family is still very important since it can add important predictive information. In a study of women with pathogenic mutations in either BRCA1 or BRCA2, the existence of breast or ovarian cancer diagnosed before the age of 50 among close relatives significantly increase the risk of disease beyond the risk the mutation confers by itself (1). This and similar examples show that family history is still a major predictor of disease risk which cannot be fully replaced by the genotype itself.

### **Founder mutations**

Hundreds of mutations have been identified in various cancer predisposition genes, most of them being unique to the patient or to their families. However, frequent recurring mutations have been found in individuals from specific ethnic groups such as those of Ashkenazi Jewish descent, or persons from the Netherlands, Iceland, and Sweden. Mutations recur in these groups because of a founder's effect and are called founder mutations. The size of founder populations and reproductive isolation by geography or cultural practices are the reason for the high prevalence of specific mutations in many ethnic groups.

Two mutations in BRCA1 and one in BRCA2 account for the majority of BRCA mutations seen in people of Ashkenazi Jewish ancestry, with a carrier frequency of 2.5% (2). In Iceland, the most common founder mutation is 999del5 in the BRCA2 gene (3), which is found in 8.5% and 7.9%, of breast and ovarian cancer patients, respectively.

In Norway, there are four founder mutations in hereditary forms of breast and ovarian cancers that present 68% of BRCA1 mutations (4). Finland and Sweden report frequent mutations in their populations as well (5, 6). The large numbers of founder mutations that originate from France are reported in the population of Quebec (7). A study conducted in Southern California on high-risk families showed several recurrent mutations in BRCA1 that are present only in families of Latin-American, Caribbean, or Spanish origin (8).

The information related to the ethnic background has practical meaning when it comes to the choice of genetic tests because it might be reasonable to start testing with "ethnic-specific" mutation panels. Rather than performing comprehensive genomic screening laboratories could instead first look for specific mutations based on a person's ethnic background. Identification of founder mutations in the various ethnic groups will enable a more specific approach to molecular testing that would also be faster and cheaper. Also, a less expensive strategy might also allow extended testing and counseling to the families that otherwise do not fulfill stringent criteria for genetic testing and have low hereditary history.

### **Costs, coverage, and insurance**

Insurance coverage for genetic testing varies based on the type of testing (single-gene or multi-gene), type of insurance, and whether the test has been ordered for diagnostic, preventive, or predictive purposes. Despite their increasing use, multi-gene panel tests are still unavailable for many people since many payers consider these tests investigative or experimental and do not cover their use. As the largest healthcare reimbursement system in the U.S., Medicare covers services that are deemed 'reasonable and

necessary for the diagnosis or treatment of illness or injury' excluding tests for screening 'that are performed in the absence of signs, symptoms, complaints, or personal history of disease or injury.' In contrast, Medicaid does not necessarily exclude predictive genetic testing coverage among asymptomatic individuals (9). Even so, state-level management has led to variation in coverage across the U.S.

Within the European countries, regulations on the provision of genetic services are differently organized and genetic testing is often considered directly related to health-care services (10). In order to make genetic testing available to as many people as possible, there is a need for harmonization of the rules involved in financial coverage of genetic testing.

Criteria for coverage by the private health insurance plans generally demand that testing have a direct influence on disease treatment management, diagnostic utility, or preventive measures for those at high-risk. The cost of testing has decreased dramatically enabling some patients to pay out-of-pocket for testing even when it's not covered by insurance. However, difficulties arise when recommended interventions are not accessible for some patients even if the initial testing is covered. Since benefits do not arise from the testing itself but rather from appropriately acting on the genetic information it is important to consider issues with the applicability of genetic tests in different settings and different circumstances.

#### **Laboratory differences, equipment, and technology**

Differences in hospital procedures and laboratory practices may impact genetic testing access and uptake. For example, not all laboratories that offer NGS testing will be able to perform screening for large genomic rearrangements (duplications/deletions of whole exons) because of their lack

of training or lack of equipment. This means that some laboratories will be limited for performing certain tests. Laboratories also differ in variant annotation approaches, variant reinterpretation policies, bioinformatics pipelines, and what types of variants they include in their test reports. Most of the large cancer genetic laboratories offer options for panels of various sizes and composition so the needs of the patients could be tailored according to the cancer type.

Also, some of them offer customized panels so specific genes could be selected for each patient. In addition, the variability in procedures used to follow-up with the patients after they are identified to be at risk and cascade testing of family members also exist. The time to result can differ significantly between the labs. Sometimes, complex multi-gene analysis can take many weeks and even months depending on the facilities available in the lab.

#### **Genetic risk assessment: Selection criteria, professional guidelines, and differences between geographic region**

The first genetic testing guidelines were established approximately 20 years ago with the aim to identify the patients with the highest likelihood of carrying pathogenic variants in hereditary cancers. The first guidelines were focused on Hereditary Breast and Ovarian Cancer syndrome (HBOC) and Lynch syndrome and were incorporated into the mutation predictors such as BRCAPro (11), Amsterdam (12), Bethesda (13) and modified Bethesda criteria (14). These tools are used to identify patients with a prior probability of at least 10% to carry an inherited cancer mutation.

Over the past 20 years, new genes with important implications for hereditary cancers were discovered, gene panels have been introduced, and the availability and cost of testing have significantly dropped.

New management guidelines have been proposed and the old ones have been constantly revised. Thus, all genetic testing criteria have been expanded over time to be more inclusive, and have been updated yearly following the new discoveries and published scientific data. The National Comprehensive Cancer Network (NCCN) offers hereditary cancer testing criteria that represent current standard for identifying persons at increased risk of hereditary cancers.

For example, NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian (15), and NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal (16) provide information surrounding cancer risks and management recommendations for a range of genes included on multi-gene panel tests. EU countries differ in criteria and guidelines for testing, but the access to genetic testing in most of the countries relies mostly on the family history of cancers. When specific threshold for mutation carriers is reached (10% or 20% depending on the country), genetic counseling and genetic testing are usually covered by the state health care system (UK, France, Netherlands, Germany) (17).

#### **Value and limitations of genetic testing guidelines**

Studies show that up to 50% of carriers of germline BRCA1 and BRCA2 pathogenic variants were not identified if persons underwent genetic testing based on the NCCN criteria marking these criteria as an obstacle rather than a useful tool (18). On this basis, the American Society of Breast Surgeons (ASBrS) recommended that germline genetic testing should be made available to all women with a personal history of breast cancer (19). It is expected that evidence to support testing will evolve and that therapeutic indications will play a major role in the incorporation of multi-gene genetic testing.

Guidelines will be updated and will be more inclusive but it is critical to support them by evidence and to work on strategies for screening, medical, and/or surgical care. Based on this assumption, according to ACMG, neither BRCA1 or BRCA2 nor multi-gene panel testing is recommended for all breast cancer patients because there is not enough evidence to support this decision (20).

Risk assessment tools are still useful to estimate the likelihood that a patient may carry a mutation but there is no absolute threshold that must be met to consider genetic testing. The clinical judgment of the providers should determine the appropriateness of genetic testing in a particular situation. This is very important when considering testing for non-white populations as most risk assessment models were validated in the populations with European ancestry.

#### **Somatic testing and germline genetic status**

Identification of somatic mutations through tumor mutation profiling is important for guiding personalized treatment and targeted therapies for cancer patients. However, data from somatic mutation analyses may also reveal important germline findings unrelated to the indication for performing somatic testing. Studies show that 5-15% of patients unselected for family history harbor deleterious mutations in hereditary cancer predisposition genes (21).

#### **"On-tumor"/"off-tumor" association of gene with tumor type**

Since there is overlap between cancer genes at the somatic and germline levels, patients should be aware of this possibility before they undergo genetic testing. 'On-tumor' and 'off-tumor' associations of gene with tumor type should be distinguished. It is not the same when a BRCA1 mutation is detected in a breast tumor, which would be described as 'on-tumor', or when it is detected in bladder cancer, which would

be defined as 'off-tumor' as presence of a germline BRCA1 mutation does not confer elevated risk of bladder cancer. In fact, 70-80% of detected tumor BRCA mutations in ovarian cancer is of a germline origin which has further implications to the medical management of the patient and has implications for family members (22). On the other hand, the probability of detecting TP53 germline mutation during somatic genetic testing is very low since TP53 is the most frequently mutated gene in human tumors. The overall germline conversion rate for TP53 is 1%, with modestly higher rate in the 'on-tumor' (2%) than 'off-tumor' (0.7%) setting (23). The only way to truly determine if the detected variation is tissue-specific or is in fact germline is to simultaneously analyze tumor and normal DNA. This means that the results from the somatic mutation profiling might lead the decision on the choice of panel for germline genetic testing.

#### **Guidelines for germline-focused tumor analysis**

The American College of Medical genetics and Genomics (ACMG) published a policy statement on clinical sequencing recommending that constitutional mutations from a panel of 59 disease associated genes should be reported to the ordering clinicians regardless of indication for which the testing has been ordered (24). This specific list has been chosen due to the proven clinical utility of these genes with available preventive strategies and/or treatment for mutation carriers. Half of these genes are associated with high-risk cancer syndromes.

The American Society for Clinical Oncology (ASCO) policy statement on genetic and genomic testing for cancer susceptibility includes germline implications of somatic mutation profiling. ASCO recommends that the possibility of identifying incidental germline mutations and the implic-

ations of these findings should be discussed with all patients before testing. ASCO endorses respect for patients' decision if they chose not to receive incidental germline information (25). European Society for Medical Oncology (ESMO) Precision Medicine Working Group recommends that germline-focused tumor analysis should be carried out in all laboratories as part of the routine analysis of a large tumor panel, but to be restricted to variants that have variant allelic frequency higher than 30% (23).

#### **Conclusion**

As the costs continue to drop, WES and WGS will be more frequently used in hereditary cancer testing. However, these tests have a higher chance of identifying VUS and incidental findings (IFs). These issues will further emphasize the responsibilities of the providers to adequately interpret genomic results and provide patients with all information related to genomic testing.

The pipelines for VUS reclassification and IFs management should be developed in clinical setting. In the light of the rising complexity of genetic data the need for close collaboration between primary care providers, various health care specialties and genetics experts will be further emphasized. Germline and somatic testing will continue to overlap indicating the need for multidisciplinary tumor boards. Possibly, variations in moderate/low risk genes will be incorporated into polygenic risk scores to provide more detailed and comprehensive risk stratification. Population based screening of unaffected individuals will become more tempting especially if it can be cost-effective. 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.



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