Introduction

The National Cancer Institute estimates that 12% of all women (about 1 in 8) will be diagnosed with breast cancer and 3% will be diagnosed with ovarian cancer throughout their lifetime. 5-10% of all breast and ovarian cancers diagnosed are hereditary. Risk factors for hereditary breast and ovarian cancers include a family history with highly penetrant mutations in genes such as \textit{BRCA1} and \textit{BRCA2}, moderately penetrant mutations in other genes (such as \textit{ATM}, \textit{BRIP1}, \textit{CHEK2} and \textit{PALB2} among others), as well as more common genomic variants, including single nucleotide polymorphisms, associated with modest effect sizes. Novel biomarkers, associated with low and moderate penetrance genomic loci, have been revealed by Next Generation Sequencing (NGS). Therefore, testing multiple biomarkers simultaneously will allow clinicians to provide the most appropriate recommendations to risk reduction strategies, surveillance guidelines and family planning.
Penetrance/Cancer Risk Modifiers

The cumulative risks (lifetime risks) in female BRCA1-mutation carriers by age 70 are 50-80% for breast cancer and 20-45% for ovarian cancer. The corresponding estimates in female BRCA2 carriers are 40-85% and 10-25%. Male BRCA1-mutation carriers have a cumulative breast cancer risk of 1.2% by age 70 whereas BRCA2 carriers have a risk of 6.8%. In addition, men who carry germline mutations in the BRCA2 gene have up to 15% prostate cancer risk by age 65. Moreover, BRCA1- and BRCA2-mutation carriers are at an increased risk of developing melanoma, pancreatic, gall bladder, bile duct and stomach cancers. Carriers of moderate penetrance mutations in genes such as ATM, BRIP1, CHEK2 and PALB2 among others present a two-fold to four-fold increased risk for breast cancer, although the risk appears higher in the context of a family history. In particular, women with an abnormal PALB2 gene have a 33-58% lifetime risk of developing breast cancer.

Genetic Counselling and Testing

BRCA gene discovery opened the door to clinical benefits resulting from breast and ovarian deep genetic analysis. Since then, efforts have been made to develop effective screening methods for breast cancer detection. However, consensus methods for screening inherited predispositions to ovarian cancer are not yet established. Genetic counselling is a very important part of the genetic testing process to collect comprehensive information on family history, select the appropriate genetic test after obtaining informed consent, prior to communicating results on surveillance/prevention measures to patients as well as to at risk family members.

The identification of causative mutations as genomic biomarkers of inherited risk for breast and ovarian cancers is known to help decrease morbidity and mortality through most appropriate prevention. Testing for abnormal breast cancer genes is usually done on blood sample or saliva or cheek swab with DNA analysis of the entire genes. Until recently, individual genes were tested separately and sequentially by traditional Sanger sequencing technology. However, decreasing costs and improved efficiencies in high throughput Next Generation Sequencing have rendered full gene sequencing and multi-gene panels more cost-effective. In at risk families, identifying germline pathogenic mutations is a crucial component in the medical management of affected patients. The effect of genetic testing reaches beyond the index patient with cancer as there are implications for the entire family. Relatives of affected patients are then typically offered a genetic testing for a specific pathogenic mutation. Those who test positive for the germline pathogenic mutation can take appropriate actions to prevent cancer or have cancer diagnosed as early as possible for better treatment options.

On the other hand, at risk family members who are identified as non-carriers of the familial mutation can be reassured as they run a risk of breast and ovarian cancer similar to that of the general population (provided other relevant genes are also analyzed and found negative for a pathogenic mutation).

For the last two decades, genetic testing for hereditary breast and ovarian cancers looked for mutations mainly in BRCA1 and BRCA2 genes. BRCA1 and BRCA2 gene mutation testing have essentially three possible outcomes: a positive result, a negative result, or an ambiguous/uncertain result:

- A positive result indicates that a person has inherited a known harmful BRCA mutation and therefore, has an increased risk of developing breast and/or ovarian cancer. Although rare, BRCA1 and BRCA2 de novo mutations occasionally occur (mutation rate estimated at 0.3%) and give a positive result despite a negative family history. However, as mentioned above being at an increased risk doesn’t mean that the patient will always develop breast and/or ovarian cancer.

- A negative test indicates that the person doesn’t harbor a harmful BRCA mutation and therefore, has a risk of developing cancer closer to the one of the general population.

- An ambiguous result indicates that a person harbors a variant of unknown significance (VUS) in BRCA1 or BRCA2 genes that has not been previously associated with cancer. This type of test result is described as “ambiguous” because it isn’t known yet (to the current scientific knowledge) whether this specific gene change affects a person’s risk of developing cancer.

Nowadays, PALB2 is regarded as a bona fide breast cancer predisposition gene. This has led many nations, such as France, Germany and Belgium, to have best practice guidelines which include recommendations for PALB2 genetic testing and risk management.
Today, several options are offered in particular for pathogenic BRCA mutation carriers to manage cancer risk. These include regular surveillance, prophylactic mastectomy and salpingo-oophorectomies, which have been shown to reduce the risk of developing ovarian and breast cancer, and preventive hormonal therapy.

- **Regular surveillance**

  Women who test positive for BRCA1 or BRCA2 mutations start regular cancer screening at younger ages than the general population (between 25 and 30). Pregnant women and those who are treated for infertility are also monitored. Although enhanced screening may increase the chance of detecting a treatable breast cancer at early stage, 18% of breast cancers develop between two screening tests. 30% of these cancers are generally invasive with lymph nodes involvement (in 10% of cases) and may compromise patients’ lives. Recent studies demonstrated that MRI is more sensitive than mammography in detecting breast cancer at early stages. However, it is less specific (may lead to more false-positive results). Today, recommendations require an annual screening with a clinical breasts examination, mammography and MRI.6

  This standard has not yet been met for ovarian cancer screening. Still most centers recommend transvaginal ultrasound and blood testing for CA-125 tumor biomarker in women with harmful BRCA mutation, beginning at age 35. Men can also carry a harmful BRCA mutation and undergo mammography as well as prostate cancer testing.6

- **Prophylactic mastectomy and salpingo-oophorectomies**

  Bilateral prophylactic surgery involves removing both breasts to reduce by 90% the risk of developing breast cancer. Breast reconstruction usually takes place during the mastectomy.

  During reconstruction, the surgeon creates a breast shape using an artificial implant, a flap of tissue from another place of women’s body (autologous reconstruction), or both. Although reconstruction techniques have been improving, complications occur in 50% of the cases: wound infection, flap failure, hardening and changing shape of the implant, unequal breasts and leakage of the implant fluid.5,7

  Surgery to remove women’s ovaries and fallopian tubes (bilateral prophylactic salpingo-oophorectomy) can also be applied after parenting projects. It can help reduce the risk of not only ovarian cancer by 80%, but also breast cancer by 50% in premenopausal women. Breast cancer risk reduction is explained in part, by eliminating a source of hormones that can fuel the growth of hormone-dependent breast cancer.16,17

- **Preventive hormonal therapy**

  Two chemopreventive drugs - Tamoxifen and Raloxifene- have been approved by the U.S. Food and Drug Administration (FDA) to reduce breast cancer risk in women with a harmful BRCA mutation. Today there are no European recommendations regarding chemoprevention, although many studies have proved the effect of Tamoxifen, Raloxifene and also aromatase inhibitors on reducing breast cancer risk. This preventive therapy only concerns breast cancer.6,18,19,20

**Hereditary Cancer Solution by SOPHiA GENETICS**

Although mutations in the BRCA1 and BRCA2 genes by far represent the majority of causative inherited defects, mutations in additional genes have recently been identified as being also responsible for increased risk of breast and ovarian cancers. Thus extending the analysis to these genes is important in the prevention, diagnosis, and treatment of hereditary breast and ovarian cancers. The analysis of extended gene panels into clinical practice allows a growing number of genes to be routinely tested for cancer-related variants. Changes in gene patent laws and advances in sequencing technologies have resulted in rapid expansion of genetic testing. The Hereditary Cancer Solution (HCS) by SOPHiA GENETICS® bundles the analytical power of SOPHiA™ AI with a capture-based target enrichment kit and full access to SOPHiA DDM® platform. It represents a comprehensive solution with superior analytical performance for the detection of genomic variants associated with hereditary cancers. Moreover, the HCS obtained the CE-IVD mark for the risk assessment of hereditary breast, ovarian and digestive cancers. The gene panel covers the coding regions ± 25 bp of non-coding DNA in exon-flanking regions of 26 most clinically relevant genes associated with hereditary cancer syndromes, as shown in the table below.
**DISEASE** | **GENES**
--- | ---
Hereditary Breast & Ovarian Cancer | ATM, BARD1, BRCA1, BRCA2, BRIPI1, CDH1, CHEK2, FAM175A, MRE11A, NBN, PALB2, PIK3CA, RAD50, RAD51C, RAD51D, TP53 and XRCC2
Lynch Syndrome | EPCAM, MLH1, MSH2, MSH6, PMS2 and PMS2CL(*)
Intestinal Polyposis Syndrome | MUTYH, PTEN and STK11
Familial Adenomatous Polyposis | APC

* The pseudogene PMS2CL is part of the analysis but not a gene responsible for disease

In particular, it is very well suited for clinical routine diagnostics of hereditary breast and/or ovarian cancer, allowing variant detection in 17 disease-relevant genes, including BRCA1 and BRCA2, as well as PALB2 gene, mandatory to analyze since September 2015 in patients that are at high risk of developing hereditary breast cancer.

Next Generation Sequencing (NGS) is successfully replacing much less cost-effective Sanger-based sequencing methods. However, the current limitation of NGS is related to its failure to detect large genomic rearrangements or Copy Number Variations (CNVs) typically affecting several hundreds of base pairs of genomic DNA.

Up to now, algorithm limitations for detecting and quantifying CNVs have systematically precluded the simultaneous analysis of all most relevant BRCA variants (as well as other gene variants involved in breast and ovarian cancer) in a one shot experiment.

Overcoming algorithm limitations has been possible today, thanks to SOPHiA AI, which accurately detects, annotates and pre-classifies CNVs to help clinicians better diagnose their patients.

Thus, by detecting all BRCA variants (as well as all other genes of the HCS panel) in a single NGS experiment, the HCS allows sparing the extra cost and time associated with additional experiments.

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**Reference Partners**

**CHU de Nantes - Laboratory of Medical Genetics**

Back in 2010, the laboratory of Medical Genetics at the CHU de Nantes was using a panel for BRCA1 and BRCA2 mutation detection, based on Multiplex PCR amplification for DNA enrichment, to diagnose patients that were at high risk of developing breast and ovarian cancers. This PCR was completed by MLPA technology for BRCA1 and BRCA2 in order to detect large deletions/rearrangements.

In 2016, the laboratory decided to implement the HCS by SOPHiA GENETICS in routine diagnostics, and to adopt SOPHiA DDM, the SaaS Analytical Platform.

Thanks to the SOPHiA GENETICS’ Validation Program, the laboratory rapidly adopted the HCS based on capture technology for DNA enrichment.

“SOPHiA GENETICS offers today a complete, integrated and validated technology ready to use in routine clinical diagnostics including DNA sequencing, data analysis and patients’ data storage in secured servers. Only accessible on SOPHiA DDM platform, SOPHiA AI allows a robust and accurate CNV detection for BRCA1 and BRCA2 genes”

Pr. Stéphane Bézieau, Head of Medical Genetics Department at the CHU de Nantes.
IPG - Institute of Pathology and Genetics in Gosselies

The Institute of Pathology and Genetics (IPG) is heir to a long tradition of diagnostics and clinical analysis, bringing together under one roof all activities and analyses in the fields of anatomical pathology, human genetics and molecular biology.

IPG was using a panel detecting mutations in BRCA1 and BRCA2 genes since 2014, but CNV detection was not optimal. Two years later, the laboratory decided to switch to an extended panel and chose the HCS by SOPHiA GENETICS. It was the first site ever to adopt the HCS extended panel in routine diagnostics.

“You have found everything there was to find. I am impressed that SOPHiA got the analysis right the first time and could find all the variants and CNVs in a selection of difficult and diverse patients’ samples. Thanks to SOPHiA, we are now able to detect the ALU insertion in the exon 3 of BRCA2 gene”

Dr. Pascale Hilbert, Director of Molecular Biology Department.

“I have to say, I am impressed by the performance of SOPHiA GENETICS’ solution, and I am someone who is not easily impressed”

Mr. Nicolas Simonis, Lead Bioinformatician at IPG

Conclusion

The Hereditary Cancer Solution by SOPHiA GENETICS, in combination with the Illumina MiSeq® instrument, lead to an excellent performance (see table on the right). Hospitals and laboratories can now make use of their NGS technologies and better diagnose their patients who are at high risk of developing breast and ovarian cancers. This should ultimately enable the prescription of adapted drugs.

<table>
<thead>
<tr>
<th>PERFORMANCE MEASUREMENT</th>
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<td>Sensitivity</td>
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A total of 386 samples were processed to obtain the above-mentioned metrics

About Us

Global leader in Data-Driven Medicine, SOPHiA GENETICS is a healthtech company which has developed SOPHiA AI, the most advanced technology for clinical genomics, helping healthcare professionals better diagnose and treat patients.

The global network of hundreds of institutions worldwide that use SOPHiA DDM® analytical platform powered by SOPHiA, form the world’s largest clinical genomics community.

By enabling the rapid adoption of genomic testing, turning data into actionable insights, and sharing knowledge through its community, SOPHiA GENETICS is democratizing Data-Driven Medicine to save lives.

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