Breast Screen

What is a tumour?

Cancer nowadays is considered a pathology with a genetic component that occurs when cellular growth is out of control. Our body cells receive signals telling them when to grow and multiply and when such growth must stop. In a tumour, such cells do not respond to control signals and grow and multiply abnormally, spreading to different parts of the body, due to changes in their DNA.
The event leading to the alteration of genetic functions is called "**mutation**". When a gene is affected by mutation for different causes (biological, chemical and physical), information to the cell will not be adequate for its functions.

Is cancer hereditary?

Neoplasms are mostly pathologies caused by multiple factors generated by genetic and environmental risk factors. Most tumours are "**sporadic**", that is, they arise in the general population without any clear signs of a genetic susceptibility. In these types of tumours, **DNA alterations** (**mutations**) randomly develop in somatic cells, namely, those cells creating every organ and system of our body. These mutations develop in the DNA of a small group of cells and generate the genetic error that is perpetrated in the descendants of those cells. After having accumulated in an organ, they initially replace the healthy cells of that organ and then spread to other organs, both nearby and distant (metastasis).
There are forms of tumour called *"***familial***"*, when people affected by cancer in the family are close relatives. Cancer familiarity is a significant risk factor, especially when common environmental risk factors are also shared (lifestyles, diet, polluting agents, etc.) without a specific genetic alteration that makes the subject more susceptible to the disease.
Only a small, but significant, percentage of tumours are "**hereditary**". Estimates say that around **5-10% of breast cancers** and **10-25% of ovarian cancers** have a **hereditary component**. In these tumours, DNA mutations occur in germ or reproductive cell and, therefore, may be passed on to the person's offspring. At birth, the child will show the genetic defect in one or more genes in all cells of the body and, therefore, will be susceptible to the development of a neoplasm when, during his/her life, other mutations occur.

When a new person is conceived, s/he acquires two pairs of each gene, one pair from the father and one from the mother. Any genetic mutations in the DNA of the parents are, therefore, passed on to children. If one of the parents has a mutation in one of the cancer-generating genes (hereditary tumour), **the offspring has a 50% chance of inheriting that mutation.** People inheriting a germ cell mutation are born with a copy of the mutated gene. These people **do not inherit the tumour, but only the susceptibility to develop that type of cancer more easily,** compared to the general population.

The BreastScreen® test

BreastScreen® is a diagnostic test developed by GENOMA Group, which carries out a multiple genetic analysis **aimed at evaluating the susceptibility to the development of breast and ovarian cancer. Therefore, t**he test identifies patients with the chance of developing the aforesaid neoplasms by analysing their DNA.

Who should take the BreastScreen® test?

The genetic susceptibility test is addressed to those people who, from a thorough analysis of the family history, show a high and concrete incidence of neoplasms in previous generations and, therefore, have a high chance of carrying a germinal mutation.

A hereditary form of cancer may be present when the family has:

- Several individuals affected by the same type of cancer or correlated cancers,
- Individuals affected by multiple tumours,
- Tumours that arise at a young age.

The most significant signs are:

- Breast cancer diagnosed ≤ 45 years (especially when diagnosed ≤ 35 years);
- Primary breast tumours in the same patient;
- Bilateral breast cancer;
- Breast cancer in males (at any age);
- Ovarian cancer in the family;
- Breast and ovarian cancer diagnosed in the same patient;
- Three or more cases of breast cancer, ovarian cancer and/or pancreatic cancer in the family;
- Several members of the family (on the same side) with breast cancer or other types of cancer.
- Cancer in several members of the family in different generations.
- A known mutation in the family in one of the genes associated with breast/ovarian cancer susceptibility.

The geneticist, with the informed consensus of the person, will decide whether a DNA mutation diagnostic test is necessary.

What are the benefits of the BreastScreen® test?

The possibility to detect subjects at risk of developing a neoplasm is the best way for an early diagnosis of cancer and, therefore, to decrease mortality in that type of disease.
Members of families with high risks of inheriting cancer and especially those that were directly affected by a neoplasm may require genetic counselling and may discuss his/her clinical and genetic situation with the geneticist. This assessment may lead to a genetic test to evaluate whether the patient is a carrier of a mutation that increases susceptibility to the development of a specific tumour.
If the test result is positive, the analysis may be extended to the relatives of the patient, in order to find those at risk.

The information from the genetic test may offer several **benefits**, such as:

- Finding family members with a **high risk of developing cancer;**
- The development of an adequate **medical check plan** for high-risk subjects, in order to promote early diagnosis of cancer;
- Awareness that the **genetic mutations may be transferred to** the offspring and the detection of high-risk offspring with germinal genetic mutations;
- The possibility to undergo **prevention therapies.**
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How does the BreastScreen® test work?

The test is carried out with a blood sample. The DNA is isolated from the nucleated cells through a complex laboratory analysis **and amplified via PCR.** Then, with a state-of-the art technological **massive parallel sequencing** (**MPS**) process that uses ***Next Generation Sequencing*** (**NGS**) techniques with **ILLUMINA** sequencers, **12 genes** (exons and adjacent intrionic regions, ± 5 nucleotides) are completely sequenced at high reading depth (Table1). The chosen genes are often involved in hereditary susceptibility to the formation of breast and ovarian cancer;

The resulting genetic sequences are analysed with an **advanced bioinformatics analysis**, to find mutations of the examined genes, if present.

HEREDITARY BREST CANCER

Results of the BreastScreen® test

"POSITIVE" – Presence of one or more mutations: the result shows that there are one or more mutations in one (or more) genes leading to hereditary susceptibility to the development of breast and ovarian cancer; the test, therefore, shows a mutated copy of the gene. During a genetic consultancy session, our geneticist thoroughly explains the test results and calculates the likelihood of developing the specific cancer associated to that mutation detected in a specific gene.
A positive result does not necessarily mean that the patient with a mutation will develop a tumour; it only shows **susceptibility to developing that type of tumour** in the patient, or rather, the person has a **higher risk level** compared to a person without that specific mutation. In fact, not all people carrying mutations develop neoplasms. Although such mutations significantly increase the chance of developing a tumour, the cancer does not develop until the normal copy of the corresponding gene is mutated during the life of the person.
Since everyone inherits two pairs of the same gene, a mutation must occur in each pair to cancel the function of such gene. The acquisition of a new mutation may, therefore, directly lead to a tumour. Identifying cancer-susceptibility mutation allows us to develop an intense clinical check plan and evaluate preventive surgery. A mutation also leads to the examination of other relatives at risk if they want to check their situation. In relatives the test is a predictive analysis, because it detects, within these families, the carriers of the mutation and people that do not carry the mutation, finding exactly those people with a high chance of developing a tumour and those whose risk is at the same level as the general population. That way, carriers can start specific check protocols for early diagnosis or prevention, while the latter will be screened with the same protocols as the general population.
Mutations that may be detected with the **BreastScreen®** may be divided into the following prognosis categories:

- **With known prognostic outcome;**
- **With benign prognostic outcome,** since they may be found in healthy individuals and have no pathological significance;
- **With uncertain prognostic outcome** they are not not known or classified by the medical and scientific community. In this case further examinations are needed to clarify the significance f the variation.

"NEGATIVE" - No mutations: The results show no mutations in the examined genes. However, a negative result does not necessarily mean that the patient does not risk developing a tumour. These people have the same chance of developing cancer as the general population because most of these types of tumours are sporadic in nature.

RISK STATISTICS

Breast cancer
In-depth studies on families at risk show that women with hereditary mutation in BRCA1 or BRCA2 genes have an **87%** chance of developing breast cancer, compared to **10%** for women not carrying the mutation. Hereditary mutations at a genetic level lead to a sharp increase in the chance of developing breast cancer in young women (before the onset of menopause). Therefore, this is therefore a specific result of the hereditary susceptibility for cancer. Recent studies showed that

more than half of the women with mutations in BRCA genes develop breast cancer before their 50th birthday and the average age for the diagnosis of the tumour is 41.

Ovarian cancer
The risk of developing ovarian cancer in case of recurring mutations of the two genes mentioned above is between **44% and 60%** compared to the **1%** risk for non-carriers.

Recurring mutations
The genetic susceptibility test is very useful also for women who already developed breast cancer because, if they are carrying the BRCA gene, they have a high chance of developing a new tumour in the breast or the ovaries. For instance, studies show that BRCA1-mutation-carrying women surviving breast cancer have a **64%** chance of developing the new tumour. Ovarian cancer shows similar risk percentages.

Risk of developing other types of cancer
Recent studies show that hereditary mutations of BRCA1 or BRCA2 genes increase the risk of **prostate** cancer in men and colon cancer in both sexes. The studies show that the likelihood of prostate cancer is **3-4 times higher** compared to the general population in BRCA-mutation-carrying men, with a an **8%** cumulative risk, while, according to research, the risk of **colon** cancer is **4-5 times higher** both in women and in men, with a **6%** cumulative risk.

Possible preventive therapies to reduce the risk of developing cancer

* In contralateral breast cancer.

Parameters used for genetic variation reporting

Only the genes listed in Table 1 are analysed. Only mutations classified as "with known prognostic outcome" or "with uncertain prognostic outcome" according to the scientific literature and the classification in the Human Gene Mutation Database (HGMD) reference database updated on the day of the examination will be reported. Moreover, in line with American College of Medical Genetics (ACMG), only mutations with a Minor Allele Frequency (MAF) <5% (1000 Genomes Project), calculated as the frequency in which the least common allele in the population occurs, were considered as pathogenic or presumably pathogenic.

Target Coverage

Target Coverage is the average number of reads generated by the sequencing of each nucleotide base of the gene. Variations with a read depth (number of reads) lower than 30X are not highlighted by the bioinformatics analysis algorithm.

Accuracy of the BreastScreen® test

Present DNA sequencing techniques produce results with more than 99% accuracy. Even though this test is very accurate, the limits of the examination need to be considered.

Limitation of the BreastScreen® test

This examination analyses only genetic diseases and genes listed in Table 1 and cannot detect:

- Mutations positioned in the intrionic regions beyond ± 5 nucleotides from the breakpoints;
- Deletions, inversions, or duplications with a value of more than 20 bps;

- Germline mosaicism (i.e. mutations only in the gametes)

A **"NEGATIVE" - No mutations** result for the examined genes does not exclude the possibility that mutations are not present in a region of the genome that was not explored during the examination.

Some regions of our DNA may not be sequenced or have a lower coverage than the limitations set by GENOMA Group experts to guarantee an accurate examination of gene variations. These regions, therefore, are not included in the examination if they do not meet the requested qualitative standards.

In some cases the result of genome testing may reveal DNA variations or mutations with an unknown or unclassifiable clinical meaning within the current medical and scientific knowledge.

The interpretation of genetic variations is based upon the most updated knowledge available upon examination. Such interpretation may change in the future, when new scientific and medical information on the structure of the genome are acquired and may affect the evaluation of the genetic variations themselves.

Some pathologies may be caused or regulated by more than one or more variations in the DNA in one or more genes. Some of these variations may not be detected or validated by the scientific community and, therefore, may not be classified as pathogenic variations upon examination.

The intrinsic limitation of the NGS methodology is the lack of coverage uniformity of each examined genetic region. Due to this limitation, NGS tests may not detect specific genetic mutations in the selected genes.