Information sheet

Genescreen is a carrier screening test for multiple genetic diseases and has been designed to assess the reproductive risk for monogenic inherited conditions. Genescreen offers comprehensive care and enables patients to make more informed reproductive decisions. Requesting Genescreen before pregnancy enables the future parents to gain knowledge about their reproductive health.

Several scientific organizations worldwide have published guidelines for carrier screening use, including benefits/limitations and technical and ethical foundations to consider. The American College of Obstetricians and Gynecologists (ACOG), the American College of Medical Genetics and Genomics (ACMG), the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) and the Italian Society of Human Genetics (SIGU) released official documents between 2017 and 2021, and these might be considered as representative guidelines.

How are genetic disorders inherited?

Expectant parents, or couples planning a family, may be at risk of passing on severe genetic diseases to their offspring due to the fact that they are carriers of genetic condition.

A **carrier** is a person who has a variation in his/her DNA that changes the sequence of a gene and impact on its function. These variations are thus called *pathogenic* (P) or *likely pathogenic* (LP) variants because they can cause a disease, and historically P and LP are known as "**mutations**".

Genetic condition may be transmitted from carrier parents to their offspring according to different patterns of inheritance. Genescreen detects genes **mutations** that cause **autosomal recessive** and **X-linked disorders**.

An **autosomal recessive disorder (AR)** occurs when an individual inherits two mutated copies of a gene - one from each carrier parent. If both partners are carriers of the same AR disorder, there is a **1 in 4 (25%) chance** that their child will be affected by that disorder.

X-linked (XL) conditions occur due to a mutated gene located on the X chromosome. Males have only one X chromosome, whereas females have two copies of the X chromosome. For this reason, males with a mutated gene on X chromosome are affected, while females usually are not, but they carry the disease and can transmit it. If a woman is carrier of a **XL** condition, she has a **chance of 1 in 2 (50%) to have an affected son** and a **chance of 1 in 2 (50%) to have a (healthy) carrier daughter**.

Genescreen **will only test females for XL conditions**, including the analysis of FMR1 gene to rule out the carrier status for Fragile X Syndrome.

Carriers of both AR and XL conditions are usually referred as "healthy" as they have no signs of the disease.

Other patterns of genetic inheritance are known, notably autosomal dominant conditions where the disease may be transmitted from a parent who is affected or can occur *de novo* as the result of a new mutation in the conception. Genescreen only focus on AR and XL conditions.

Who can Genescreen be offered to?

Genescreen is intended to be used as a family planning tool, allowing prospective parents to be tested individually or with their reproductive partner for their risk of having children with various genetic conditions. Research has shown that most people are carriers for at least one genetic disorder and usually they are not aware of their carrier status as they are healthy and may have no family history. This is why

screening may be offered before or during pregnancy to **all women of reproductive age** and their **reproductive partners**, as well as to **gamete (egg or sperm) donors**.

In some circumstances Genescreen is particularly indicated, notably when there is consanguinity (established or presumed), when the two partners come from a small region or belong to a specific genetic ancestry (i.e. Ashkenazi Jews, Finnish).

Positive family history is an indication to preconception/prenatal carrier screening; however, it is advisable to discuss with a healthcare provider or genetic counsellor the best options for testing.

Panels and auxiliary testing

Genescreen - Carrier screening

Genescreen is offered in different panels:

- Genescreen Focus includes **30 genes** associated to the most common inherited genetic conditions. The panel covers some of the genes that should be screened according to international guidelines.
- Genescreen Protect includes more than **120 genes** in which mutations may cause over **140 different AR and XL disorders**. The genes included are characterized by high carrier frequencies within general and specific populations. This panel **covers all Tier 3 AR genes included in the ACMG Practice Resource** for pregnancy and conception screenings.
- Genescreen Easy-Donor includes more than **400 genes** in which mutations may cause over **450 different AR and XL disorders**. This is a panethnic panel, and the genes included are not specific to any population, even if the related conditions may be more common in specific ethnic groups.
- Genescreen Complete is the most extensive panel that includes **more than 2000 genes**. This panel is recommended in specific circumstances (i.e. known family history of a disease caused by a gene included in the analysis, consanguinity), but may be requested for a more comprehensive reproductive risk assessment.

At the following link https://www.familyproject.it/genescreen-gene-list/ you can find the list of genes included in the different panels. Please consult with your doctor to determine which test is most appropriate for you.

Genescreen Matching

Genescreen Matching is a comparative study of the results of genetic carrier screenings performed in "reproductive partners" to assess the couple's reproductive genetic risk.

Most of the genetic conditions studied by Genescreen and other carrier screenings are inherited in autosomal recessive pattern, meaning that **the risk of affected offspring is present when both the "reproductive partners" (including gamete donors) are carriers of the same condition**.

Some of the genes included in genetic carrier screening **are located on X chromosome**, meaning that the risk of affected offspring is related to **female reproductive partner carrying a mutation in one of those genes**. Genescreen Matching can be requested for any couple undergoing genetic carrier screening. In the context of Assisted Reproductive Technologies, it may be required for couples undergoing homologous procedure as well as for those undergoing heterologous procedures (i.e. ART with gamete donors). Genescreen Matching is **available for any** Genescreen Panel and may also be applied to carrier screenings performed by third parties.

Genescreen Extension

Genescreen Focus, Protect, Easy-Donor may be upgraded to higher level of testing or may be integrated by the specific screening of other genes included in the Genescreen Complete panel.

Turn Around Time

20 working days from the date the sample is received in the laboratory.

Documents required for testing

For the execution of Genescreen it is necessary that the Test Requisition Form (TRF) is filled out properly. If any required information is missing, the laboratory will contact the referral clinician or the patient to obtain this information. This may cause a delay in processing the sample and issuing the report.

Technical summary

Genescreen combines different genetic technologies and bioinformatics pipeline to provide a wide carrier screening detecting pathogenic variants in hundreds of genes.

Next Generation Sequencing

The analysis performed by Next Generation Sequencing (NGS) is designed to examine coding regions and splicing junctions of several genes. Sequencing analysis allows to detected single nucleotide variants (SNV) as well as gross deletion/duplication of the analyzed genes.

Variants are classified according to ACMG/AMP standards of interpretation using publicly available databases including but not limited to dbSNP-NCBI, ClinVar-NCBI, gnomAD.

Only pathogenic or likely pathogenic variants are reported.

Benign, likely benign, and variants of uncertain significance are not reported, but may be reported in certain circumstances. Variant classification is based on our current understanding of genes and variants at the time of reporting, knowledge acquired in the future may lead to variant re-classification.

Although NGS technologies and bioinformatics analysis significantly reduce the contribution of pseudogene sequences or other highly homologous sequences, these may still occasionally interfere with the technical ability of the assay to identify pathogenic variants in both sequencing and deletion/duplication analyses. Orthogonal methods may be used to confirm variants with low quality scores and to meet coverage standards. This assay will not detect certain types of genomic alterations that may cause disease, including but not limited to, translocations and inversions, repeat expansions (i.e. trinucleotides or hexanucleotides), alterations in most regulatory regions (promoter regions) or deep intronic regions (more than 20 bp from an exon). This assay is not designed or validated for the detection of somatic mosaicism or somatic mutations.

Fluorescent PCR

Fragile-X carrier testing provides information about the carrier status of Fragile X syndrome, which is caused, in most cases, by expansions in a (CGG)n repeat sequence. The test is performed by fluorescent PCR amplification of the trinucleotide repeated region located in the 5' UTR region of the FMR1 gene. The method does not allow to detect single nucleotide variants (also known as point mutations), gene deletions and/or duplications, or the methylation status of genes.

Detection Rate and Residual Risk

Genescreen analytical detection rate is high and varies by disease. Residual risk is the chance that the patient screened is a carrier even after a negative screening test result. Residual risk is strictly related to the carrier frequency of the disease and the detection rate of the test for that condition. Carrier frequency may vary widely among different populations/ethnic group. Genescreen provides residual risk calculated by an in-house algorithm.

Genescreen limitations

All laboratory tests have limitations. **A positive result does not imply that there are no other variants in the patient's genome, and negative results do not eliminate the risk for the patient's children to be affected by a**

genetic disorder. Even when one or both members of a couple screen negative for pathogenic variants in a specific gene, the risk to be carriers and the disease risk for their offspring is reduced but it is not completely ruled out.

Carrier rates before and after testing may vary by ethnicity. These rates assume a negative family history for each disease screened and the absence of clinical symptoms in the patient. If a condition is suspected in a patient or there is a positive family history, it is strongly suggested to contact the laboratory or a genetic professional to evaluate diagnostic testing for that disorder. In patients with a positive family history of a specific genetic disease, there is an increased carrier risk prior to testing and if the disease-causing variant in their family is not included in the test, the carrier risk remains unchanged. Genetic counseling is recommended for patients with a family history of genetic disease to determine and discuss risk figures based on their actual family history, along with potential implications for reproduction.

Since genetic variation, as well as systematic and technical factors, can affect the accuracy of testing, the results of testing should always be interpreted in the context of clinical and familial data. It is recommended that patients receive appropriate genetic counseling to explain implications of the test result, including residual risks, uncertainties and reproductive or medical options.

Extensive genetic analysis by NGS has a potential to identify and report incidental or secondary findings that are unrelated to the indication for ordering the sequencing but may be medically valuable for patient care. The American College of Medical Genetics and Genomics (ACMG) published and updated different policy statements on clinical sequencing that emphasized the importance of alerting the patient to the possibility of such results. Although Genescreen is intended to assess the risk to be carrier of genetic condition, it can highlight mutations that may influence the individual personal risk to be affected by a genetic condition as it may identify variants in genes included in the ACMG statement list. The list of genes included is available upon request. Variants included in the genes listed will not be reported unless specifically required.

Even if carrier of AR and XL conditions are usually healthy and they are unlikely to be affected, there is a risk that they have signs of the disease or will in the future. This may be explained differently according to the inheritance pattern, residual risk and type of condition. Genetic counselling is recommended after positive results when the carrier subject have sign that can be ascribed to the condition.

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