INFORMATION ON GENOME-WIDE GENETIC TESTING

WHAT IS A GENE, A GENOME, AN EXOME, A MENDELIOME?

Each human being is constituted out of billions of cells. Each cell contains genetic information, stored in 2 types of cellular parts: the nucleus (contains our genome, or chromosomes), and mitochondria (contain our mitochondrial DNA).

Genetic information is made of DNA. DNA is a double stranded chain of nucleic acids (or nucleotides, or bases). Four types of bases exist: A, T, G and C. It is the succession (or sequence) of those 4 bases that contains genetic information.

Different names are given to different aspects or parts of our DNA.

Chromosome is the name given to the DNA, when it is packed as the cell divides, and is visible under the microscope. We have 2 pairs of 23 chromosomes (so 46 in total). We receive 23 chromosomes from our mother, and 23 chromosomes from our father. The 23rd pair, the sex chromosomes, determines the gender. Indeed, women have 2 X chromosomes, while men have 1 X and 1 Y chromosome.

Genome is the name given to the entire DNA content within 23 chromosomes. We have two copies of the genome, (one received from our mother, one from our father). Each copy is made of 3 billions of base pairs (3Gb).

Gene is the name given to a small part of the genome, which contains the information to build proteins. Proteins are the key components for guidance of development and functioning of our body. Genes are composed of exons (which contain the crucial information to build the proteins) and introns (which are not coding for the proteins). We have about 20 000 genes in our genome.

Exome is the name given to the subset of our genome, composed of all the exons of our genes. Most of the genetic diseases can be explained by errors in the exome, although it represents only 1% of the total human genome.

Mitochondria can be considered as the energy factories of the cell. Mitochondria have their own DNA, which is approximately 16 500 bases long, and thus much smaller than the genome or exome. Nevertheless, errors in the mitochondrial DNA can give rise to complex disorders, which can vary from muscle to liver to brain abnormalities.

WHICH TYPES OF GENOMIC ALTERATIONS EXIST?

Our genomic and mitochondrial DNA contain ***variations***, which determines the looks (phenotype) of a person (e.g. colour of the eyes and hair), but also whether this person is more susceptible to some diseases or is affected or is a carrier of a genetic condition.

Point ***mutations*** are changes at one or a few specific base(s)
Deletions consist of the removal of 1 or more bases, or even larger fragments (exon(s)/gene(s)/chromosomal fragment(s))
Duplications consist of a few bases, or even larger fragments (exon(s)/gene(s)/chromosomal fragment(s)) that are duplicated
Deletions and duplications of exons, genes, or chromosomal fragments are called **Structural Variants** (SV)
Insertions consist of insertion of 1 or more bases, or even larger fragments (exon(s)/gene(s)/chromosomal fragment(s))
Inversions consist of the inversion of (mostly large) fragments in a chromosome
Repeats consist of repetitions of 2 or more bases; these are often seen in "repeats disorders"
Translocations consist of an exchange of genetic material between chromosomes not belonging to the same chromosome pair; translocations can be balanced (no net loss of DNA) of unbalanced (net loss of DNA)
Aneuploidy is the over- or underrepresentation of 1 or more (complete) chromosome(s)

HOW CAN WE EXPLORE OUR GENOMIC OR MITOCHONDRIAL DNA?

Various techniques have been developed to identify genetic anomalies, but no single technique can identify them all.

Comparative genomic hybridization array (CGH array)

CGH array allows the comparison of small chromosomal parts of the patient towards those of a control person. CGH array can identify variations in the "quantity" of DNA, such as deletion, or duplication, of at least a certain size, known as structural variants or SVs, which depend on the type of array used. As we can determine the exact position of each chromosomal part using known DNA fragments, we can deduce which regions (and thus genes) are over- or underrepresented. Deletion or duplication of (a) small chromosomal fragment(s) can be a cause of multiple congenital abnormalities and/or intellectual disability. Others can be risk factors, for example for the apparition of learning difficulties, or intellectual disability, and can be inherited from a normal parent. Most of the deletions and duplications identified are benign (and not reported), or of yet unknown clinical significance. In a prenatal setting, only the established, or very likely pathogenic variants are reported. CGH array cannot detect alterations in the sequence of nucleic acids.

Single nucleotide polymorphism array (SNP array)

A SNP array demonstrates several parallels with the CGH array technology. SNP arrays detect deletions and duplications of chromosomal parts, but also single base variations (SNP). It can be used to trace the transmission of small parts of chromosomes in a family. These are looked for in the context of recessive diseases, especially when there is parental consanguinity. It can be used to trace the association between a disease and regions in the genome in a specific family, also known as familial linkage analysis. In this case the more (affected) family members can be tested, the higher the success rate of the test. Linkage analysis also aids to the interpretation of other genome-wide test results. SNP arrays allow also for the identification of unequal inheritance of the parental chromosome set in the offspring called uniparental disomy, which can responsible for some rare diseases.

Single gene analysis

When it is known that a specific disease is often caused by mutations in a certain gene, that gene can be analysed first. Some genes show recurrent mutations, which we examine in first line. In other genes, mutations are wide-spread, in which case we analyse the complete sequence of the gene (gene sequencing).

Massive Parallel sequencing

Massive Parallel Sequencing (MPS) technology allows us to determine simultaneously (a part of) the complete genome. MPS can look at a set of genes (this is called a gene panel), or at all the genes (this is called an exome, around 20.000 protein-coding genes), or at a well-studied subset of the exome called the mendeliome (around 3500 genes well-known to be implicated in genetic disorders) or even at the total genome (called whole genome sequencing), or at the mitochondrial DNA. MPS is offered as the technology of choice for disorders for which different genes might be responsible for the phenotype.

In this case, MPS might greatly accelerate the genetic diagnosis, as in previous years only one single gene analysis at the time could be offered, while now all genes of interest can be analysed simultaneously in one experiment. It is important to note that gene panels, exome, mendeliome, genome or mitochondrial DNA analyses are all different types of MPS tests. The clinical geneticist will, depending on your phenotype and disease, determine which test is most appropriate. MPS will only be offered when no simpler or cheaper test is available to assess the genetic diagnosis. MPS delivers big amounts of data necessitating bioinformatic tools to analyse them. The analysis can be restricted to a limited number of genes, even if more data are available (such as in mendeliome, exome, or genome sequencing), which is called targeted gene *analysis*. If no result can be obtained with the targeted analysis, the search can (depending on the type of MPS test used) be extended to the whole mendeliome, exome or genome.

Functional analysis

This type of analysis is not part of routine lab procedures. Yet, it might be necessary to analyse the consequences of the variations that are identified. This is only possible via dedicated (long) research projects. This can be done in many ways.

WHAT KIND OF RESULTS CAN BE EXPECTED?

- **No** anomaly is identified, this **does not** rule out the possibility of a genetic condition.
- **One or more** variations are identified that **explain** your disorder.
- **One or more** variations are identified that **might** explain your disorder; however additional research is required to determine the causality. This might be facilitated through analysis of the candidate causal variants in samples of family members and/or by other type of research.
- When a genome wide (or "unselected") analysis is performed, one or more variations might be identified that are associated with another disease than the one you were genetically counselled for. Some of these "incidental" findings (also called secondary variants) can cause diseases for which prevention and treatment is available (e.g. cancer, heart disease or other "actionable" genetic disorders). In these diseases, specific, defined medical recommendation(s) are expected to improve the outcome(s) in terms of disease severity and/or mortality. Incidental finding of mutations in these so-called "actionable" genes can - with your prior consent - be reported to you. The list of "actionable" genes responsible for these diseases can be consulted on the website of the Center for Medical Genetics.

In case of MPS, with which no variation explaining the condition could be observed, a temporary result will be reported. Periodic re-analysis is possible (see follow-up of your file).

HOW DO WE MANAGE YOUR TEST RESULTS?

Given the fact that test results not only have an impact on the patient, but also on the family members, the law on confidentiality of genetic (and medical) information is applicable.
However, when genome wide analysis is performed, given the complexity of the analysis, we might request advice of external experts that also respect the genetic/medical confidentiality. This external expert will thus get access to the complete or partial dataset (inclusive of clinical information but exclusive of personal information). When sharing data, your results will be coded (pseudonymised), so that they cannot be directly coupled to your personal data or identity.
Your pseudonymised or anonymised data (depending of the nature of the collaboration) might be shared with (external) scientific experts/collaborators and/or used to simplify future analyses for similar disorders to improve in general the health care.

WHAT ARE THE LIMITATIONS & RISKS?

Due to the complexity of genome wide tests and their bioinformatic analyses, limitations are present: not all genetic variations can be picked up. Moreover, the diagnostic yield is influenced by the sensitivity of the specific array or the massive parallel sequencing technology used. In general, the cause of the disorder can be elucidated in 5% to 40% of cases, depending on the type of disorder, the mode of inheritance, the familial history, etc.
Be aware that these tests can result in secondary findings (not related to the disorder). You can opt in or out to be informed about these findings.

HOW DO WE COMMUNICATE TEST RESULTS?

Once the diagnostic test results are available, you will be invited to the certified university genetic center for a genetic counselling to discuss the test result and its possible impact on your life and that of your family. Be aware that you might be informed stepwise in case of interim test results, taking into account the complex nature of these types of tests with its advantages, but also with its limitations and risks.

WHAT DO WE NEED FROM YOU?

To start with CGH array, SNP array or MPS technology, we need minimally the following items:
- A blood sample (10ml ETDA tube) of you and preferentially your 1st degree relatives (e.g. parents and/or siblings)
- A completed and signed informed consent

To validate results in a research setting, other samples such as biopsies, may be asked later, upon your consent.

WHAT ARE THE COSTS?

Genome-wide tests are expensive. However, some of these tests and the genetic counselling are reimbursed by the national health care system (for Belgium RIZIV/INAMI) in such a way that only a limited fee needs to be contributed by you. Expenses related to genome-wide tests performed in research will be supported financially by the investigator's research funds.

IS FOLLOW-UP POSSIBLE?

Genetics is a fast evolving domain, with regular novel discoveries on gene and protein function and the impact of specific variants/mutations.
In case of inconclusive results at the time of the availability of the first test results, it is possible that we re-analyse the data at a later stage in a scientific context to re-evaluate your results when more and updated data resources are available. Relevant novel information will then be communicated to you upon your consent.

QUESTIONS?

Do you want to receive additional information on genome wide testing after reading this information leaflet?
Do you feel unsure about the informed consent and the use of your test results?
Would you just like to exchange opinions or ideas?
You are welcome to discuss your questions with your referring physician or you can make an appointment for a genetic consultation.