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PATIENT ID
(OR PATIENT NAME AND LOCATION)

NAME: .....

LOCATION: ..... **BATCH NUMBER:** .....

DATE: ...../...../..... **GENDER:**.....

INFORMED CONSENT ID: C-GEN-007	**MEDICAL AND MOLECULAR GENETICS INSTITUTE (INGEMM)**

PROCEDURE: GENETIC STUDY BY NEXT-GENERATION SEQUENCING (NGS)

WHAT ARE WE GOING TO DO?

1. Procedure description

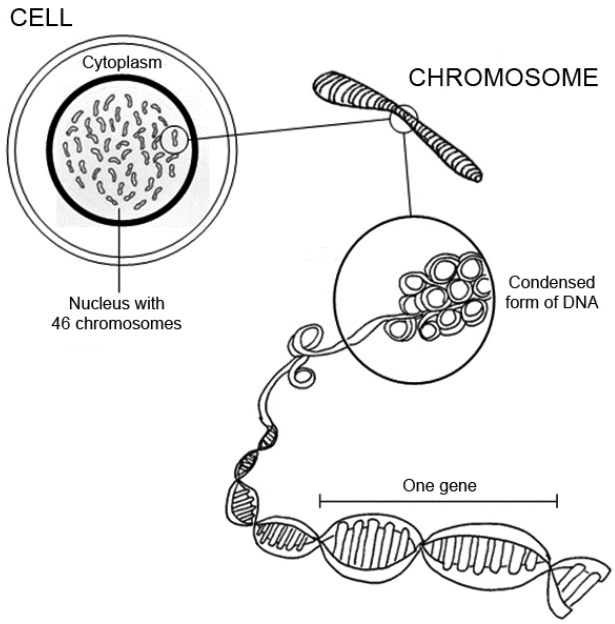
• What is Next-Generation Sequencing?

Next-Generation Sequencing (NGS) is a new type of genetic study that has been recently applied to genetic diagnosis. This informed consent reports the general aspects of NGS:

- What are chromosomes and genes?
- What is the utility of NGS?
- What genes are studied?
- What are the limitations of this technique?
- What are the possible results of a genetic study using NGS?
- What findings are reported and what are not?
- The need to regularly review the results of NGS studies according to advances in genetic knowledge.
- What is required to conduct a NGS study?
- The results obtained by NGS can be used in clinical genetics research.
- Contact data for further information.

➢ <u>What are chromosomes and genes?</u>

The genetic information is stored in each cell of our body, in an internal structure called nucleus. The genetic material that contains this information is **DNA**, compressed and packaged in the form of **chromosomes**, which are even visible using a microscope. **Genes** are pieces of DNA with specific instructions for the production of different proteins that control the development and function of our body. Every cell in our body contains 46 chromosomes grouped into 23 pairs of chromosomes, one coming from our mother and the other coming from our father. According to international guidelines, chromosomes are numbered from 1 to 22, from the largest to the smallest. The remaining pair is formed by the sexual chromosomes X and Y, that determine the sex of a person: females having two copies of the X chromosome (46, XX) whilst males carrying one of each (46, XY).



It is estimated that the 24 chromosomes (1-22, X and Y) comprise approximately 22,000 genes, with each chromosome containing between 700 and 3,000 genes. Each gene consists of fragments of DNA sequence called **exons**, which contain the information necessary for the synthesis of proteins, and **introns**, interspersed with exons, that carry out a different function. They can be represented like the following sentence, "bb**inf**bbcc**orm**ccbb**ación**gbbcc**enéc**cbb**tica**cc", in which the exons would be in bold. The entire DNA contained in the 46 chromosomes is called **genome**. The coding information (the information contained in the sequence of the exons of genes) is known as the **exome**. The exome represents 1-2% of the genome and contains 85% of disease-causing alterations of genetic disorders.

Each gene and encoded protein has a specific function, although this function is not still known in many cases. Diseases or genetic disorders may be due to one or more genes that carry alterations: there is a missing or an additional fragment of gene, or there is a single change in the DNA sequence of gene. Both situations trigger an alteration in the encoded protein.

- An alteration that affects the function of a gene, which is called **mutation**, can be inherited from one or both parents (familial inheritance), or can be generated for the first time in the egg or sperm that leads to that person (*de novo*).
- An alteration that does not affect the function of the gene or whose possible effects remain unknown, which is called a **variant**, can be benign or of uncertain significance, respectively. They can also follow the genetic inheritance within the family or the variant may appear as a *de novo* variant.

➢ <u>What is the utility of NGS?</u>

Until very recently, genetic studies consisted of the "one by one" sequencing of causal genes of a particular genetic disease using Sanger sequencing technology. The new technology of **NGS** allows us to determine simultaneously the DNA sequence of a variable number of genes. This is particularly useful in those diseases or genetic disorders caused by multiple different genes (e.g., it is known that more than 50 different genes can cause *retinitis pigmentosa*, a disease of the retina). In cases that clinical diagnosis is not so clear, the NGS may offer the possibility of studying the complete exome of the 22,000 genes of the genome, through a specific method that uses NGS technology called Whole-Exome Sequencing (WES).

➢ <u>What genes are studied?</u>

In most cases, the study is initially limited to the analysis of a group of genes (panel of genes) related to the disease or genetic disorder that has motivated the genetic study. In the event that the test does not identify any genetic alteration to explain the disease and there is still a medical indication, it may be possible to extend the study to include additional genes or even the complete exome (WES), if patient agrees.

In those cases that do not show any specific diagnosis, the application of WES could be directly chosen as the best strategy of genetic testing. The best strategy will depend on each case and will be explained in detail to the patient before starting the study.

Nevertheless, despite the comprehensiveness of these studies, their sensitivity does not reach 100%, and the cause of the disease or genetic disorder may not be found in every case.

➢ <u>What are the limitations of this technique?</u>

NGS studies generate a huge amount of data and it is mandatory to distinguish what is informative data and what isn´t. There are also some technical limitations that should be known to accurately interpret the results:

- There are some regions in genes that are difficult to analyze. If this is the case, it will be indicated in the report.
- When a study is limited to the analysis of a group of genes or a panel specific to a disease or group of diseases or genetic disorders, they can only detect mutations in these selected genes. Consequently, mutations in other genes, not included in this specific panel, will not be detected. The issued report includes the analyzed list of genes and the specific design of the NGS panel.

➢ What are the possible results of a NGS genetic study?

There are four possible scenarios of a NGS test:

1) *One or more alterations have been detected and they have been considered the **cause** of the disease or genetic disorder*, thus confirming or clarifying the suspected clinical diagnosis. In this situation the clinical geneticist will comment and discuss the clinical implications of these results with the patient .
2) *One or more variants of **uncertain significance** have been detected*. In this case, it may be necessary to request or consider the genetic study of other family members in order to confirm whether these findings are related or not with the disease or genetic disorder of patient.
3) *No alterations* to explain the disease or genetic disorder have been detected.
4) *In those cases that WES approach has been applied, the so-called **incidental findings**[1] can be detected.* These are alterations unrelated to the disease or genetic disorder that led the initial study, but that may give significant implications on the health of patient and/or family members.

➢ What findings are reported and what are not?

Reported findings:

- **Causal variants** (mutations) that explain the disease or genetic disorder that led the genetic study request.
- **Incidental findings** that are unrelated to the disease or genetic disorder that led the initial study, but that may have an important effect on the health of patients and/or family members (e.g., mutations in cardiovascular disease-causing genes that predispose to sudden death, or genes that give susceptibility to the development of hereditary cancers). This data is only reported when the patient or legal guardian has provided specific authorization.

Unreported findings:

- Findings of **uncertain significance** without apparent causal connection with the clinical diagnosis.
- Findings that confirm that the patient is a **healthy carrier** of a causative mutation of an autosomal recessive disease or genetic disorder (e.g., cystic fibrosis).

➢ The need to regularly review the results of NGS studies according to advances in genetic knowledge

The knowledge about the role of genes and their involvement in diseases and genetic disorders is constantly improving. A variant considered of uncertain significance today, may be considered as a pathological variant at a later date. Therefore, it is recommended that patients with uncertain significance results return to the clinical genetics department, 1-2 years later, to review the current situation and check if there is some new information that may modify the initial interpretation of their genetic findings.

➢ What is required to conduct a NGS study?

Type of sample:

- Peripheral blood sample (Adult: 3-6 ml, Child: 1-3 ml, Infant: 1 ml, in EDTA tubes) or other types of tissue from which a DNA sample can be extracted. Tubes must be clearly labelled. The blood samples should be sent in a temperature controlled container and should arrive at the INGEMM, Hospital La Paz within a maximum of 24 hours post extraction. However, if the blood sample is stored in a fridge or freezer, the delivery time can be increased to a maximum of 72 hours.
- Genomic DNA sample - DNA already extracted from a peripheral blood sample is recommended when sending samples from outside Spain. Minimum amount of 10 µg, minimum concentration of 50 ng/µl, A260/280 ratio near to 2.

In some cases, samples from parents or other relatives may be requested, in order to provide a better interpretation of the results.

➢ The results obtained by NGS can be used in clinical genetics research.

➢ Contact data for further information.

2. Objective of the study: Identify the genetic alteration responsible for the disease or genetic disorder of patient.

[1] Also known as casual, unforeseen, unexpected, unrequested, accidental, secondary, or complementary findings.

WHAT ARE THE RISKS OF THIS STUDY?

1. General risks:

Risks associated with venipuncture are mild and infrequent (hematoma in the puncture, fainting or dizziness).

2. Personal specific risks:

In addition to the specific risks due to the disease that patient is currently suffering, the patient may also suffer other complications.....

3. Benefits of the procedure in the short and medium term:

The identification of the genetic alteration responsible for a disease or a genetic disorder may not only confirm the diagnosis, but also provide useful information for the patient´s prognosis and treatment, allow prenatal diagnosis to be undertaken and provide relevant information to other family members.

WHAT OTHER ALTERNATIVES ARE THERE?

There are no other alternatives for this type of analysis.

DO YOU AUTHORIZE US?

By signing this document, we ask for permission to perform the procedure and/or test explained above, and to strictly use images or other clinical information recorded at the medical history of patient only for educational or scientific purposes, as the patient is being diagnosed/treated in a university teaching hospital. Anonymity will be respected at all times.
All information related to the study is strictly confidential and will be treated in accordance with the Constitutional Personal Data Protection Law 15/1999, with the rights of access, cancellation, rectification and opposition.
The NGS results will be stored in the INGEMM and may be shared, in an anonymous way, without any personal identification, with other research groups in order to improve the knowledge of the genetic diseases.

STATEMENTS AND SIGNATURES

Please do not be afraid to ask any questions before signing this document, whether in reference to this procedure or to request more information. Please note that you have the right to revoke your decision and withdraw your consent for this test at any time, upon written request.

I declare my agreement with the following points

Regarding the scope of the test and the information of the findings:

I agree that the DNA extracted from me/my child/the person under my guardianship can be analyzed by NGS techniques for the following health problems, and to carry out the following analyses:

- Medical problem: .....……

- Type of analyses:

  ☐ Option 1: Analysis of a group of genes or panel of genes
  This analysis is exclusively performed on a list of known genes related to a disease or a genetic disorder.

  ☐ Option 2: Extended analysis
  This expanded analysis provides the analysis of additional genes related to a disease or a genetic disorder, or even the full exome (WES), in case of medical indication.

  I understand that following any of the two options of NGS analysis annotated above it is possible to get uncertain significance findings.

If I have chosen the option 2 (extended analysis), I understand that there is a possibility of detecting incidental findings unrelated to the disease or genetic disorder that led the initial study, but which may have an important effect on the health of me and/or my family members.

☐ I agree ☐ I do not agree to be informed of any incidental finding that affects a gene unrelated to the disease or genetic disorder that led to the initial study, but that as clinical relevance and may have significant consequences for my health or that of my family.

Regarding the use of the DNA sample:

☐ I wish ☐ I do not wish my DNA to be destroyed after the study has been performed

☐ I authorize ☐ I do not authorize for the excess DNA sample to be stored in the INGEMM DNA bank

☐ I authorize ☐ I do not authorize for my anonymized DNA sample or NGS data to be shared between other research groups in order to improve the kknowege of genetic disorders.

☐ I authorize ☐ I do not authorize for my anonymized DNA sample to be used in the process of optimization and quality control of genetic tests.

I understand that medical knowledge is constantly improving and it is recommended to periodically came back to clinical genetics department in order to review the current situation and check if there is some new information that may modify the initial interpretation of the genetic findings.

1. Patient signature:

Mr./Miss/Mrs/Ms. .....

I have been properly informed of the genetic study that will be performed with my DNA sample. I have understood the risks, complications and alternatives, and I have had enough time to evaluate my decision. Subsequently, I am satisfied with the given information. Therefore, I give my consent for the performance of this genetic test by a specialist and to be informed of the results in another clinical genetics consultation. This is a voluntary acceptance and I can withdraw this consent whenever I consider appropriate, upon written request, and without any impact in my later healthcare attendance.

Patient signature Date: ...../...../.....

2. Legal guardian signature:

Patient Mr./Mrs/Miss/Ms. ..... has no ability to decide by him/herself at this time.

Mr./Mrs/Miss/Ms. ..... legal guardian of patient, has been properly informed of the genetic study that will be performed with the DNA sample of the patient. Therefore, I give my consent. This is a voluntary acceptance and I can withdraw this consent whenever I consider appropriate, upon written request.

Legal guardian signature Date: ..... /...../ .....

3. Requesting physician:

Dr. ..... I have informed the patient and/or legal guardian about goals and technical details of the genetic study that will be performed, and explained the risks, complications and alternatives.

Requesting physician Date: ...../...../.....

4. Regarding the rejection (REVOCATION) of the Informed Consent:

Mr./Mrs/Miss/Ms……

I have been informed that I can revoke this document before the performance of the genetic study explained above, so **I DO NOT** give my consent to perform this test. I also want to make the following comments.....

Patient signature Date: ...../...../.....

5. Regarding the DISCLAIM of the right to be informed:

Mr./Mrs/Miss/Ms. ……..

I manifest that, due to personal reasons, I disclaim the right to be informed that I own as being a patient. I report my desire not to receive information about the progression of my disease by now. This situation does not imply that I can not give my consent to the performance of this genetic study, as I have given and signed in point 1.

Patient signature

Date: ...../ ...../ .....