# Molecular Diagnosis of Mitochondrial Disease with Targeted Next Generation Sequencing: a Cohort of 250 Patients

**Nogueira C[1,2], Pereira C[2], Silva L[1], Leão Teles E[3], Rodrigues E[3], Campos T[3], Cardoso T[3], Chaves P[3], Janeiro P[4], Gaspar A[4], Dupont J[4], Bandeira A[5], Martins E[5], Soares G[5], Magalhães M[5], Sequeira S[6], Vieira JP[6], Jacinto S[6], Ferreira AC[6], Santos H[7], Vilarinho L[1,2]**

[1]Unidade de Investigação e Desenvolvimento, Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge - INSA, Porto, Portugal; [2]Unidade de Rastreio Neonatal Metabolismo e Genética, Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge - INSA, Porto, Portugal; [3]Centro Hospitalar São João, EPE, Porto, Portugal; [4]Centro Hospitalar Lisboa Norte, EPE, Lisboa, Portugal; [5]Centro Hospitalar e Universitário do Porto, EPE, Porto, Portugal; [6]Centro Hospitalar Lisboa Central, EPE, Lisboa, Portugal; [7]Centro Hospitalar de Vila Nova de Gaia, EPE, Porto, Portugal.

## INTRODUCTION

Mitochondrial diseases are a group of rare inherited disorders characterized by extreme phenotypic heterogeneity that can be transmitted by any mode of inheritance, with hitherto no effective therapy options. Defects in mitochondrial energy production often have multisystemic effects and tend to preferentially affect organs with high energy requirements, such as the central nervous system, cardiovascular system, and skeletal muscle [1]. It is estimated that 1:5,000 individuals will develop a mitochondrial disease [2]. The molecular diagnosis in mitochondrial disorders is a great challenge and compared with traditional approaches, the recent evaluations of Next Generation Sequencing (NGS) have shown that this technology is more likely to provide a diagnosis for these diseases. Moreover, it is quicker and cheaper as the amount of genetic information that can be obtained in a single test previously required several different analyses to be made. Many of the newly nuclear gene loci linked to mitochondrial diseases have been discovered with NGS methods as well as novel phenotypes associated to genes previously linked to mitochondrial diseases [3,4].

## OBJECTIVES

The purpose of our project* was to develop a NGS strategy to identify the genetic defects in 250 patients suspicious of mitochondrial disorders, to confirm the clinical and biochemical diagnosis of the disease.

## MATERIAL AND METHODS

• **Patients**: We selected 250 patients from several Portuguese hospitals suspected of mitochondrial diseases, through the clinical and laboratory investigations, but with no molecular etiology known. Blood samples from these patients were collected and all provided a written informed consent.

• **NGS Panel Sequencing**: We designed a custom panel (Suredesign - Agilent Technologies) including 209 nuclear genes involved in several pathways of mitochondrial metabolism. The coding region of these genes was captured using SureSelect QXT kit (Agilent Technologies) and sequenced in MiSeq Sequencer (Illumina), following the respective manufactured protocols (Figure 1).

• **NGS Whole Mitochondrial DNA Sequencing**: The entire human mtDNA was enriched by a single amplicon, using back to back primers, by long-range PCR [5]. Indexed paired-end DNA libraries were prepared using NexteraXT kit (Illumina), according to the manufacturer instructions, and sequenced in MiSeq Sequencer (Illumina).

• **Data Analysis**: Sequences from the FASTQ files were aligned to the human genome (hg19) using the BWA aligner and to mtDNA reference sequence using SeqMan Ngen (DNAStar). Variant calling and annotation were performed using available commercial programs [Surecall (Agilent), Annovar and SeqMan Pro (Agilent)]. Variants were filtered taking into account the type of mutation, the population frequency, presence in databases (dbSNP, HGMD, ClinVar, Mitomap, etc), *in silico* predictors, etc. After filtered, the variants already classified as pathogenic mutations or possibly damaging by *in silico* predictions, were confirmed by Sanger sequencing. When DNA from other family members was available, co-segregation studies were also done (Figure 1).

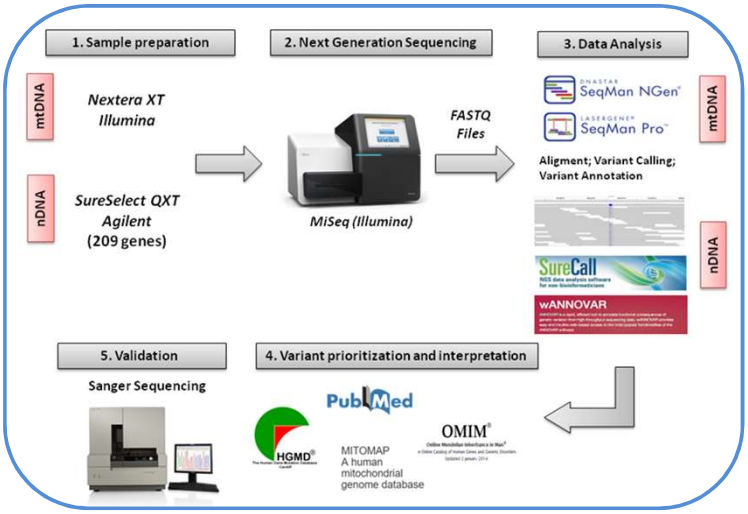


**Figure 1 –** Schematic representation of NGS workflow of mitochondrial DNA and nuclear DNA

## RESULTS

For the 250 analysed samples an average of 1,484,317 reads/sample of the covered regions was obtained by the nuclear panel and an mean read depth of 261X per sample. On average 364 variants were detected per sample.

A molecular diagnosis was attained in 62/250 (25%) of the studied patients that harbored 21 pathogenic variants, previously reported in the literature, 11 novel variants probably pathogenic and 54 novel variants of unknown significance (VUS). These mutations were confirmed by Sanger sequencing in the index cases and in their relatives. In the remaining 188 studied patients no probably pathogenic mutations were detected (Figure 2).

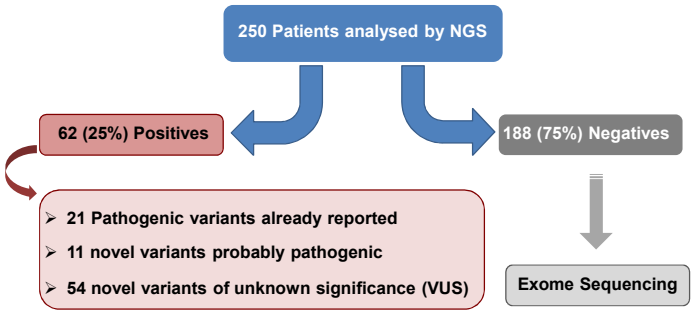


**Figure 2 -** Results of NGS analysis of 250 patients.

## DISCUSSION AND CONCLUSION

Our NGS approach revealed to be an useful strategy to provide a molecular diagnosis in a substantial fraction of patients (25%) with mitochondrial diseases of unclear etiology, expanding the mutational spectrum of these disorders. Undiagnosed patients will be selected for Exome Sequencing in order to find mutations in new genes that until now were unknown to be associated with these diseases. These technologies transfer from research to clinical practice is accelerating the diagnosis of mitochondrial disorders in families and is improving its genetic counseling [6].

## FUNDING SOURCES

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## REFERENCES

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