# Metabolic Diseases: A Differential Diagnosis of Primary Progressive Multiple Sclerosis

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## INTRODUCTION

Multiple sclerosis (MS) is a chronic demyelinating neurological disease primarily affecting young adults, with a prevalence of approximately 0.1% in the Caucasian population [1]. In recent years some studies have raised concern over the possibility of misdiagnosis in MS, which could be as high as 6%, particularly among patients with primary progressive MS [2]. Several single gene disorders share clinical and radiologic characteristics with MS, and have the potential to be overlooked in the differential diagnostic evaluation of both adult and pediatric patients [2]. Diagnosis of primary progressive MS has special challenges as there are no relapses and the MRI findings are different from those patients with relapsing onset MS. Clinically primary progressive MS is similar to spastic paraparesis like hereditary spastic paraparesis [3] or other metabolic disorders, such as lysosomal storage disorders [4], mitochondrial diseases [2] or neurometabolic disorders [5], presenting with this predominant symptom.

## OBJECTIVES

The overall aim of our research project is to develop a Next Generation Sequencing strategy to identify metabolic disorders in 104 patients with a presumptive diagnosis of primary progressive MS.

## MATERIAL AND METHODS

• Patients: We selected 104 patients with a presumptive diagnosis of primary progressive MS. Blood samples were collected from these patients, from several Portuguese hospitals, and all provided a written informed consent.

• NGS Panel Sequencing: We designed a custom panel (Suredesign - Agilent Technologies) including 250 nuclear genes involved in mitochondrial, lysosomal, peroxisomal and neurometabolic diseases. 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).

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

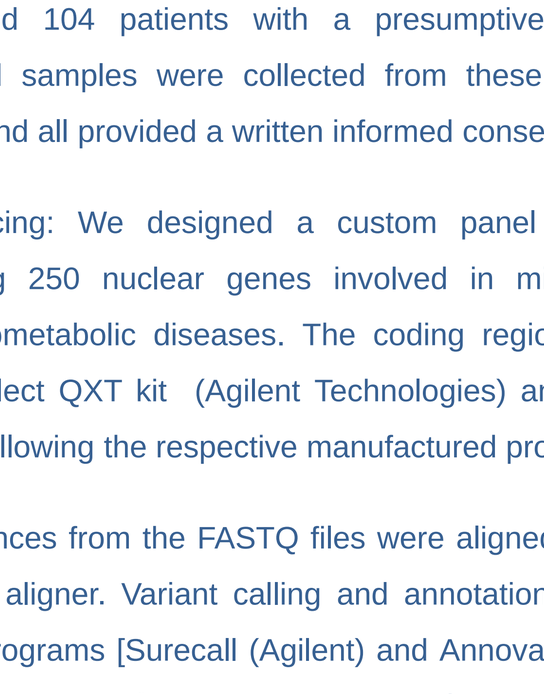


**Figure 1**: SureSelect Target Enrichment workflow (Agilent)

## RESULTS

For the 104 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.

Variants filtered as probably pathogenic mutations were detected in  of these patients (Table 1) and confirmed by Sanger sequencing. In the remaining 97 no probably pathogenic mutations were detected. Most of the variants found in this study were not described in the literature.

**Table 1:** Nuclear gene mutations identified in the studied patients.

| Patient | Age | Gene | Mutations | | References |
|---|---|---|---|---|---|
| | | | Allele 1 | Allele 2 | |
| 1 | 68Y | *GFAP** | p.Glu85Lys (c.253G>A) | -- | This study |
| 2 | 50Y | *CPT1C** | p.Glu237Lys (c.709G>A) | -- | This study |
| 3 | 42Y | *MFN2** | p.Leu753fs* (c. 2258dupT) | -- | [6] |
| 4 | 68Y | *SERAC1* | p.Phe47Ile (c.139T>A) | p.Phe47Ile (c.139T>A) | This study |
| 5 | 60Y | *IDUA* | p.Gly409Arg (c.1225G>C) | p.Asn297Asn (c.891C>T) | [7]; This study |
| 6 | 69Y | *WFS1** | p.Asp118Ala (c.353A>C) | -- | [8] |
| 7 | 60Y | *SPG7** | p.Val549Met (c.1645G>A) | -- | [9] |

*** Autosomal dominant inheritance**

## DISCUSSION AND CONCLUSION

This study contributed to identify pathogenic mutations in 7% of the studied patients in genes associated with hereditary spastic paraparesis and other metabolic disorders.

The uniqueness of this project is to bring NGS technology to the bedside in the management of MS-like conditions, helping clinicians who have patients with diseases for which a diagnosis has been elusive. Recognition of a single-gene disorder as causal for a patient's 'multiple sclerosis-like' phenotype is critically important for effective patient management, and has broad genetic counseling implications for affected families.

## REFERENCES

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## FUNDING SOURCES

We would like to thank to MERCK, SA and NORTE2020 (NORTE-01-0246-FEDER-000014) for funding this Project.