

# NEXT GENERATION SEQUENCING (NGS) AN OPPORTUNITY FOR PATIENTS NON-DIAGNOSTIC IN LYSOSOMAL STORAGE DISEASE

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## Introduction

Diagnostic procedures based on new technologies allow mass population studies to be carried out with greater sensitivity and precision and with fast and acceptable costs (3). Lysosomal storage diseases (LSD) are a heterogeneous group (around 70) of rare, multisystem diseases, characterized by substrate accumulation due to an enzymatic deficiency in the majority of cases. Until now, the enzymatic analysis was the gold standard LSD screening programs (2). However, difficulty to identify patients can appear and therefore causing an uncompleted diagnosis (5). The introduction of new sequencing techniques permits the simultaneous study of different gene variants and enables more precise identification (4). In the last decades, the use of Next-Generation Sequencing (NGS) has allowed the sequencing of massive groups of genes in a particular experiment and has allowed the discovery of new genes associated with different genetic diseases.

## Objectives

- ✓ Design a panel based to in the causal gene variants of LSD focused in the disorders with an approved treatment or with new therapies in clinical trials.
- ✓ Validate panel NGS with samples previously analyzed in laboratory.

## Methods

### 1. Disorders with an approved treatment or with new therapies in clinical trials

- Review list of LSD from LDN.
- Use LSD classification from Orphanet.
- Evaluation criteria publication include key words with lysosomal disease, treatment, clinical trial.

- Pathways affected ORPHA: 68366.
- Publication review about the genes associated with LSD:
  - <https://rarediseases.org/>
  - <https://www.nlm.nih.gov/>
  - <https://www.ncbi.nlm.nih.gov/pubmed/>
  - ClinicalTrials.gov

- 5' and 3' UTR
- Exons
- Exon-intron boundaries
- Deep intron with damaging genetic variants
- Promoter

**2. Sample preparation**

>11 Dried Blood Spot (DBS) of patients that diagnosed in our laboratory.

>DNA extraction: PrepFiler™ Kit (1)



## Results

PATIENT	SAMPLE STATUS	GENE	DNA NUCLEOTIDE CHANGE		REFERENCE SEQUENCES
			Allele 1	Allele 2	
1	CARRIER	<i>GLB1</i>	c.918C>A	~	NM_000404.3
2	AFFECTED	<i>SMPD1</i>	c.1829_1831delGCC	c.1829_1831delGCC	NM_000543.5
3			c.1458T>A	c.1458T>A	
4	AFFECTED ♂	<i>GLA</i>	c.1046G>A	~	NM_000169.3
5	CARRIER ♀		c.155G>A	~	
6	AFFECTED	<i>NPC1</i>	c.2567T>C	c.3182T>C	NM_000271.5
7			c.1757delA	c.2746_2748del	
8	AFFECTED	<i>LIPA</i>	c.894G>A	c.894G>A	NM_000235.4
9			c.111+5G>C	c.894G>A	
10	AFFECTED	<i>GBA</i>	c.1226A>G	c.1448T>C	NM_000157.4
11			c.1226A>G	c.42_65del	

Table 1: Patients detected for NGS.

NGS method detected all variants in 11 previously studied patients (Table 1).

### • VALIDATION

Despite the pseudogenes existence, damaging variants detection was correct.

### • PSEUDOGEN INTERFERENCE

## Conclusions

- Our NGS panel detect properly damaging variants on included genes.
- Variants were properly detected even when pseudogenes existed.
- The design of an NGS panel covering the treatable LSD diseases is a good approach to improve its diagnosis.

## What is next?

The next step is to analyze neonatal sample of DBS collected

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