NGS AND LYSOSOMAL DYSFUNCTION
NOVEL MUTATIONS ASSOCIATED WITH NEURODEGENERATIVE DISORDERS

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LSD diagnosis can be difficult due to considerable clinical overlap and variability.

Multiple samples and test are often required before a diagnosis is reached (is time consuming).

High-throughput sequencing is cost and time effective.

Custom-targeted panel for LSD genes

Identification of disease-causing mutations in LSD

Unidade de Rastreio Neonatal Porto
Sample Preparation and Sequencing (Illumina MiSeq) (2-3 days)

Analysis Software (1-2 days)

Clinical and/or biochemical diagnosis of LSD

14 Patients

Composed of genes envolved in LSD

NGS PANEL

VARIANTS FILTERING

X-linked hypothesis

Recessive hypothesis

One homozygous

Compound heterozygous

Genotype/Phenotype Concordance

If negative

UNSOLVED

No variants

Coverage analysis

uncovered regions - Sanger
<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Impact on Protein</th>
<th>Molecular Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFSD8</td>
<td>p.G455R/G455P</td>
<td>YES</td>
<td>Neuronal Ceroid Lipofuscinosis 7</td>
</tr>
<tr>
<td>NAGLU</td>
<td>p.D312N/D312N</td>
<td>YES</td>
<td>Mucopolysaccharidosis type IIIB</td>
</tr>
<tr>
<td>GM2A</td>
<td>p.G104Gfs<em>14/G104Gfs</em>14</td>
<td>YES</td>
<td>GM2 Gangliosidosis AB variant*</td>
</tr>
<tr>
<td>GALC</td>
<td>p.Y205X/Y205X</td>
<td>YES</td>
<td>Krabbe</td>
</tr>
<tr>
<td>NPC1</td>
<td>p.V505G/V562V</td>
<td>Unclear</td>
<td></td>
</tr>
<tr>
<td>NPC1</td>
<td>p.A3T/N961S</td>
<td>YES</td>
<td>Niemann-Pick type C</td>
</tr>
<tr>
<td>MAN2B1</td>
<td>p.802Qfs<em>128/802Qfs</em>128</td>
<td>YES</td>
<td>Alpha-mannosidosis</td>
</tr>
</tbody>
</table>
**Mutation** p.G455R in *MFSD8*

CLN7 protein (unclear function)
Lysosome membrane mainly

**Mutation** p.G104Gfs14 in *GM2A*

GM2 activator protein (necessary for substrate solubilisation)
Lysosome lumen

**CLN7**- Late infantile form of NCL

*There are at least 14 different genes associated with NCL*
Patients with Neurodegenerative LSD

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**GM2 Gangliosidosis** - AB variant

*Clinically and biochemically undistinguishable of the other two variants (Sandhoff and Tay-Sachs)*

Our NGS approached allowed a rapid and precise diagnosis in these patients
CLN7 protein

- p.Gly455Arg
- p.Gly52Arg
- p.Tyr121Cys
- p.Arg139His
- p.Gly429Asp
- p.Asp368His
- p.Gly310Asp
- p.Gly158Ser
- p.Arg233Gly
- p.Ala157Pro
- p.Thr160Asn
- p.Thr160Ile
- p.Glu336Gln
- p.Pro447Leu
- p.Pro412Leu
- p.Pro465Trp
- p.Arg465Gln
- p.Arg465Gln
- p.Trp407Arg
- p.Gly480Val
- p.Gly480Val
- p.Arg458Thr
- Lumen
- Lysosomal membrane
- Cytoplasm
Future perspectives

- Expand the present NGS panel in order to include more genes involved in the Lysosomal Function.

- For the cases where a diagnostic is not reached even after using the extended panel perform other NGS analysis (WES and WGS).

For further informations about these two customized gene panels, please contact Dr. Sandra Alves: sandra.alves@insa.min-saude.pt
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