Title: Re-classification of a variant identified in ARSB gene using biochemical, genetic and bioinformatics approaches

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Introduction

Arylsulfatase B is an enzyme that is present in the lysosomes and involves in the breakdown of large sugar molecules called glycosaminoglycans (GAGs)[1]. The enzyme chemically modifies two GAGs dermatan sulfate and chondroitin sulfate by removal of the sulfate group Figure 1.0. Mutation in the gene that encodes the enzyme, leads to lysosomal storage disorder, mucopolysaccharidosis type VI (MPS VI): OMIM:253200 [2]. In ClinVar database for genetical mutations Over 450 mutations have been identified and reported in ARSB gene. MPS VI an autosomal recessive disorder also known as Maroteaux-Lamy syndrome, is a progressive condition that causes many tissues and organs to enlarge and become inflamed or scarred. Skeletal abnormalities are also common in this condition. The rate at which symptoms worsen varies among affected individuals [3].

Figure 1.0: Shows the pathway for arysulfetase enzyme (ARSB) in metabolisim of chondrion sulfate and dermant sulfate.

Diagnosis of the disease is carried through a series of biochemical, genetical and bioinformatical analysis.

Aim and objective

Aim of the paper is to confirm the diagnosis of patient with hearing loss and MPS VI using various diagnosis tests designed for MPS VI.
Methods and Materials

1.0 Ethical consideration
This study was approved by the Twam Human Research Ethics Committee (T-HREC), reference number AA/AJ/584 as per national regulations.

2.0 Methods for biochemical tests

2.1 Total Glycosaminoglycans (GAGs) quantification
GAGs MPS serum was sent to Mayo clinic. Using liquid chromatography Tandem mass Spectrometry serum GAGs were quantified.

2.2 Serum MPS
Serum MPS including dermatan sulfate, heparan sulfate and Keratan sulfate were measured using liquid chromatography tandem mass spectrometry. The assay was conducted at myoclonic

2.3 Enzymatic assay
Levels of N-acetylgalactosamine-sulphatase were measured by enzymatic assay. It was conducted on the probands skin fibroblast. Assay was performed at SA pathology laboratory in Adelaide, Australia.

3.0 Whole exome sequencing (WES)

3.1 In silico protein analysis
Centogene used different in silico tools to report the variant effect on the encoded protein. They used Polyphen, Align-GVGD and mutation Taster.
Results

Three years old boy was diagnosed with hearing loss at birth. The patient had ear tube surgery and a conchlear implant at 10 months of age. Patient has a family history of Ehlers-Danlo syndromes (EDS) and a strong family history of sudden death for several family members see Figure 2.0.

Figure2.0: Family pedigree for the index patient diagnosed with hearing loss

1.0 Molecular analysis

Whole exome sequencing was ordered from Centogene. Three variants were reported and classified according to the ACMG guideline.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant coordinates</th>
<th>Amino acid change</th>
<th>Zygosity</th>
<th>Types</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDH23</td>
<td>NM_022124.5:c.5237G&gt;A</td>
<td>p.(Arg1746Gln)</td>
<td>Homozygous</td>
<td>Missense</td>
<td>Pathogenic (class I)</td>
</tr>
<tr>
<td>ARSB</td>
<td>NM_000046.3:c.475C&lt;T</td>
<td>p.(Arg159Cys)</td>
<td>Homozygous</td>
<td>Missense</td>
<td>Likely pathogenic (class II)</td>
</tr>
<tr>
<td>SMARCC2</td>
<td>NM_003075.3:c.1063G&gt;A</td>
<td>p.(Val355Met)</td>
<td>Homozygous</td>
<td>Missense</td>
<td>Uncertain significance (class 3)</td>
</tr>
</tbody>
</table>

A homozygous pathogenic variant was identified in the CDH23 gene c.5237G>A p.(Arg1746Gln). The genetic diagnosis of autosomal recessive CDH23 gene hearing impairment disorder is confirmed. A homozygous likely pathogenic variant was identified in the ARSB. In silico toll classified the detected variants in CDH23 as pathogenic and ARSB as likely pathogenic.

2.0 Biochemical analysis and findings

First assay which was conducted as part of the clinical investigation to confirm MPS VI diagnosis was GAGs MPS serum and it was normal.

<table>
<thead>
<tr>
<th>Mucopolysaccharides (Quant m)</th>
<th>Concentration (ng/ml)</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatan sulfate</td>
<td>61.16</td>
<td>&lt;300</td>
</tr>
<tr>
<td>Heparan sulfate</td>
<td>16.52</td>
<td>&lt;55</td>
</tr>
<tr>
<td>Total Keratan Sulfate</td>
<td>1078.87</td>
<td>&lt;1800</td>
</tr>
</tbody>
</table>

Serum MPS done at Mayo Clinic, dermatan sulfate, heparan sulfate and Keratan sulfate were normal. Enzymatic assay was ordered using skin fibroblast that is the gold standard in genetical testing was ordered from SA pathology laboratory in Adelaide. The assay result showed N-actylgalactosamine sulphatase was normal, hence the patient is negative for MPS VI.
Whole exome sequencing results identified missense variants, p. Arg159Cys and p. Arg1746Gln in ARSB and CDH23 genes, respectively. Bioinformatics tools predicted these variants as pathogenic (p. Arg1746Gln) and likely pathogenic (p. Arg159Cys). Several biochemical assays were performed to confirm the differential diagnosis of MPS VI, Urine glycosaminoglycans (GAGs) and serum mucopolysaccharides results were normal. Arylsulfatase B enzyme activity on DBS was low (this is screening test) does not confirm the diagnosis. The detected mutation is classified as likely pathogenic, based on another affected patient but enzyme activity was not done for this patient. The levels of N-acetylgalactosamine-4-sulphatase was also observed normal. Based on clinical and biochemical findings, variant p. Arg159Cys of ARSB is likely benign and did not support the diagnosis of MPS VI. However, pathogenic variant (Gln1746) of CDH23 support the underlie cause of hearing loss. The initial in silico genetic finding is inconsistent with the patient’s phenotype. Thus, the reported variant should be reclassified from likely pathogenic (class 2) to benign.

Conclusion

This study highlights the importance of robust correlation of genetic results with functional and biochemical analysis prior to a differential diagnosis. Moreover, clinical experience and careful interpretation of WES data is essential to start any therapy based on genetics data.

References


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