Reclassification of Whole Exome Sequencing-derived Genetic Variants in Pendred Syndrome with ACMG/AMP Standards

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Whole exome sequencing (WES) opens up an unbiased testing approach for genetic diagnosis of rare disorders compared with single-gene and multigene panel testing. Being a diagnostic tool for a wide range of genetic diseases, WES identifies causal variants in close to one-third of the referred cases, and variants of unknown significance (VUS) potentially responsible for the clinical presentations in another one quarter of index patients. Systematic guidelines from Human Genome Variation Society (HGVS), and American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) are currently available for standardizing the nomenclature and reporting the interpretation of genetic variants, respectively. Despite this, variant curation still remains a challenge in the face of advances in sequencing technologies, which may reveal large numbers of novel variants possibly associated with the clinical phenotype in the diagnostic setting. Depending on the disease context, reanalysis of WES-derived genetic variants can sometimes improve diagnostic yields or result in downgrading of the pathogenicity status of some previously reported variants.

Pendred syndrome (OMIM 274600) is an autosomal recessive disorder characterized by sensorineural hearing loss, goiter, and inner ear malformations. It is most commonly caused by biallelic loss-of-function variants in the SLC26A4 gene. Digenic inheritance of monoallelic loss-of-function variants in the SLC26A4 gene and another gene, for example, either KCNJ10, FOX1 or GJB2 have also been described. However, subsequent studies refuted the causal associations between KCNJ10 or FOX1 and SLC26A4 mutations in this syndrome.

Here, we demonstrated the reclassification of causative variants previously reported in a study in which WES was performed on a family with two daughters clinically diagnosed with Pendred syndrome and their unaffected parents. On the basis of possible autosomal recessive, digenic or oligogenic modes of inheritance, pathogenic variants were sought in the two sisters. Thyroid dysfunction in the two siblings was attributed to compound heterozygous variants (p.Lys350* and p.Arg1110Gln) in the DUOX2 gene. Interestingly, Chow et al proposed that the combination of heterozygous variants in three genes (SLC26A4 p.Ser448Leu, GJB2 p.Thr123Asn, and SCARB2 p.Thr305Met), which were found in both affected siblings, led to bilateral hearing loss.

Digenic inheritance usually involves pathogenic variants in genes encoding interacting proteins, and results in a “double hit” affecting the function or structure of the protein complex. Notably, the three genes reported by Chow et al, namely, SLC26A4, GJB2 and SCARB2, have no known protein interactions between their gene products. In Chow et al, the evidence of pathogenicity for the candidate variants in these genes were derived solely from in silico predictions, without evidence from functional analyses or gene-disease associations other than from the single family. This prompted us to further curate these variants according to the ACMG/AMP standards using VarSome. We also performed data mining in ClinVar, a database commonly referenced for germline variants. The pathogenicity of the variants identified in the two siblings with Pendred syndrome in Chow et al are shown in Table 1. After re-evaluation, the SLC26A4 NM_000441.2:c.1343C>T variant was classified as “pathogenic” and “likely pathogenic” by ACMG/AMP and ClinVar, respectively. Strikingly, the GJB2 NM_004004.6:c.368C>A and SCARB2 NM_005506.4:c.914C>T variants were classified as either benign or VUS. The GJB2 p.Thr123Asn variant has been detected at higher frequencies in controls compared with patients and identified in trans with pathogenic variants in the GJB2 gene in normal carriers. Taking these reevaluations into consideration, the GJB2 and SCARB2 variants are now “downgraded” in their pathogenicity status and unlikely to be associated with deafness in concert with the SLC26A4 variant.
Table 1 Classification of variants according to the ACMG/AMP standards and ClinVar

<table>
<thead>
<tr>
<th>Gene</th>
<th>mRNA level</th>
<th>Protein level</th>
<th>ACMG/AMP classification by VarSome version 8.4.7</th>
<th>ClinVar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Automated criteria</td>
<td>Short link</td>
</tr>
<tr>
<td>SLC26A4</td>
<td>NM_000441.2.c.1343C&gt;T</td>
<td>p.Ser448Leu</td>
<td>Pathogenic</td>
<td>PM1, PM2, PM3, PP2, PP3, PP5</td>
</tr>
<tr>
<td>GJB2</td>
<td>NM_004004.6.c.368C&gt;A</td>
<td>p.Thr123Asn</td>
<td>Benign</td>
<td>PM1, PM2, PP2, PP3, BP4</td>
</tr>
<tr>
<td>SCARB2</td>
<td>NM_005506.4.c.914C&gt;T</td>
<td>p.Thr305Met</td>
<td>Likely benign</td>
<td>PM1, PM2, BP1, BP4</td>
</tr>
<tr>
<td>DUOX2</td>
<td>NM_014080.4.c.1588A&gt;T</td>
<td>p.Lys530*</td>
<td>Pathogenic</td>
<td>PV51, PM2, PP3, PP5</td>
</tr>
<tr>
<td>DUOX2</td>
<td>NM_014080.4.c.3329G&gt;A</td>
<td>p.Arg1110Gln</td>
<td>Pathogenic</td>
<td>PM1, PM2, PP2, PP3, PP5</td>
</tr>
</tbody>
</table>

On the other hand, the two DUOX2 variants, namely, NM_014080.4.c.1588A>T and c.3329G>A were classified as “pathogenic” in the context of goiter by ACMG/AMP standards and in ClinVar. These classifications are consistent with Chow et al’s interpretations. The two pathogenic variants in the DUOX2 gene identified (p.Lys530* and p.Arg1110Gln) have been found in patients with congenital hypothyroidism.18–20

WES investigates all coding exons in a relatively time-efficient and cost-effective manner compared with the traditional single-gene or multigene panel testing. It is not unusual that many rare variants can be identified in multiple genes by WES. It remains a big challenge to firmly assign causal variants and genes when there is only one individual or a single family, especially in complex diseases with possibly digenic or polygenic inheritance. There are many essential points to consider when evaluating the pathogenicity of variants3 and also when conducting reevaluation and reanalysis of genomic test results.21 Having timely and accurately updated public databases of variants is essential to the research and clinical diagnostic communities for variant curation and clinical diagnosis.

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Conflict of Interest
None declared.

References