



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.¹ 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.^{2,3} 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 yields⁴ or result in downgrading of the pathogenicity status of some previously reported variants.⁵

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.⁶ Digenic inheritance of monoallelic loss-of-function variants in the *SLC26A4* gene and another gene, for example, either *KCNJ10*, *FOXI1* or *GJB2* have also been described.^{7–9} However, subsequent studies refuted the causal associations between *KCNJ10* or *FOXI1* mutations and *SLC26A4* mutations in this syndrome.^{10,11}

Here, we demonstrated the reclassification of causative variants previously reported in a study¹¹ in which WES was performed on a family with two daughters clinically diagnosed with Pendred syndrome and their unaffected parents.¹² 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.¹³ 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 standards³ using VarSome.¹⁴ We also performed data mining in ClinVar, a database commonly referenced for germline variants.¹⁵ 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 patients¹⁶ and identified in *trans* with pathogenic variants in the *GJB2* gene in normal carriers.¹⁷ 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.

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Table 1 Classification of variants according to the ACMG/AMP standards and ClinVar

Gene	Variant with HGVS nomenclature		ACMG/AMP classification by VarSome version 8.4.7			ClinVar	
	mRNA level	Protein level	Interpretation	Automated criteria	Short link	Interpretation	Variation ID
<i>SLC26A4</i>	NM_000441.2:c.1343C>T	p.Ser448Leu	Pathogenic	PM1, PM2, PM5, PP2, PP3, PP5.	http://varso.me/H0yK	Likely pathogenic	549979
<i>GJB2</i>	NM_004004.6:c.368C>A	p.Thr123Asn	Benign	PM1, PM2, PP2, BP4, BP6	http://varso.me/Fe5Y	Benign/Likely benign	44743
<i>SCARB2</i>	NM_005506.4:c.914C>T	p.Thr305Met	Likely benign	PM1, PM2, BP1, BP4	http://varso.me/OOe7	Uncertain significance	588761
<i>DUOX2</i>	NM_014080.4:c.1588A>T	p.Lys530*	Pathogenic	PV51, PM2, PP3, PP5	http://varso.me/EuGP	Pathogenic	287079
<i>DUOX2</i>	NM_014080.4:c.3329G>A	p.Arg1110Gln	Pathogenic	PM1, PM2, PP2, PP3, PP5.	http://varso.me/Eozb	Pathogenic	420157

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.Lys350* 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.

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