International Journal of Audiology

ISSN: 1499-2027 (Print) 1708-8186 (Online) Journal homepage: https://www.tandfonline.com/loi/iija20

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To cite this article: Azam Pourahmadiyan, Paria Alipour, Najmeh Fattahi, Mahbubeh Kasiri, Fateme Rezaeian, Afsaneh Taghipour-Sheshdeh, Javad Mohammadi-Asl, Mohammad Amin Tabatabaiefar & Morteza Hashemzadeh Chaleshtori (2019): A pathogenic variant in *SLC26A4* is associated with Pendred syndrome in a consanguineous Iranian family, International Journal of Audiology, DOI: 10.1080/14992027.2019.1619945

To link to this article: https://doi.org/10.1080/14992027.2019.1619945

Published online: 12 Jun 2019.

INTERNATIONAL JOURNAL OF AUDIOLOGY
https://doi.org/10.1080/14992027.2019.1619945

ORIGINAL ARTICLE

A pathogenic variant in *SLC26A4* is associated with Pendred syndrome in a consanguineous Iranian family

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ABSTRACT

Objective: Hearing loss (HL) is a common sensory deficit with high phenotypic and genotypic heterogeneity. A large Iranian family with HL was genetically assessed in this study.

Design: A proband from a consanguineous multiplex HL family from Iran was examined via Targeted Next-Generation Sequencing (TNGS). Sanger sequencing allowed the segregation analysis of the variant of interest and the investigation of its presence in a cohort of 50 ethnicity-matched healthy control individuals. The gene was previously associated with HL. Therefore, to determine whether the variant was specifically associated with Pendred Syndrome (PDS) or DFNB4, biochemical analyses, PTA, thyroid scans by Tc99m, perchlorate discharge test and high-resolution CT scan of the temporal bone were carried out on the affected family members.

Study sample: Ten members of a large multiplex Iranian family with HL were recruited in this study. In addition, 50 unrelated healthy controls of the same ethnic group were randomly selected to genotype the variant.

Results: A homozygous missense variant (NM_000441.1: c.1211C > T/p.Thr404Ile) in exon 10 was found segregating in the family. Based on the ACMG's guidelines, the variant was classified as pathogenic.

Conclusion: This study expands the spectrum of *SLC26A4* pathogenic variants in hearing loss.

ARTICLE HISTORY
Received 30 August 2017
Revised 5 May 2019
Accepted 9 May 2019

KEYWORDS
Hearing loss; *SLC26A4*; Missense variant; Iran; next-generation sequencing

1. Introduction

Hearing loss (HL) is the most common communication defect affecting about 360 million individuals worldwide (Organization 2009). The highest prevalence of HL is found in South Asia and sub-Saharan Africa (Stevens et al. 2013). Depending on the absence or presence of anomalies in other organs, such as the kidney, heart, or eyes, HL is categorised as nonsyndromic or syndromic. (Smith, Bale, and White 2005). Almost 70% of hereditary HL seems to be nonsyndromic (NSHL) (Van Camp, Willems, and Smith 1997). Syndromic hearing loss (SHL) accounts for the remaining 30% of genetic phenotypes in children (Gorlin, Toriello, and Cohen 1995). Some of the genes can cause both NSHL and SHL phenotypes (Friedman and Griffith 2003). For example, pathogenic variants in the *SLC26A4* gene cause both Pendred Syndrome (PDS) (OMIM 274600), characterised by sensorineural HL and goitre (Coyle et al. 1996), and DFNB4 (OMIM 600791), autosomal recessive NSHL without goitre (Li et al. 1998).

SLC26A4 (OMIM: 605646) was identified by Everett et al. (1997) after using positional cloning on chromosome 7q22-31 (Hilgert, Smith, and Van Camp 2009). This gene consists of 21 exons (Everett et al. 1997) and produces a 5 kb transcript and a 86 kDa protein with 780 amino acids, pendrin, which is a member of solute carrier family 26A (*SLC26A*) (Bizhanova and Kopp 2010; Campbell et al. 2001). Pendrin mediates the transport of Cl^-, $HCO3^-$, OH^-, I^- ions, as well as formate, nitrate and thiocyanate. Reduction of pendrin level functionally results in endolymph acidification, and is thought to be responsible for inhibition of Ca^{2+} re-absorption, leading to auditory sensory transduction defects (Wangemann et al. 2007).

The *SLC26A4* gene is mostly expressed in the inner ear, thyroid and kidney (Soleimani et al. 2001; Yoshida et al. 2002; Wangemann et al. 2007; Choi et al. 2011). In the inner ear, pendrin is found in endolymphatic sac and hair cells (Choi et al. 2011), where it is involved in pH homeostasis, acting as bicarbonate/chloride exchanger (Wangemann et al. 2007). In the thyroid, pendrin is expressed in follicular cells (Bidart et al. 2000) and is active as an electroneutral iodide/chloride exchanger, allowing iodide efflux from the cell to the follicular lumen (Yoshida et al. 2002). In the kidney, pendrin is found in both B and non-A, non-B cells of the cortical collecting duct (Royaux et al. 2001), either as a chloride/hydroxide or a chloride/bicarbonate exchanger (Soleimani et al. 2001).

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Supplemental data for this article can be accessed here.

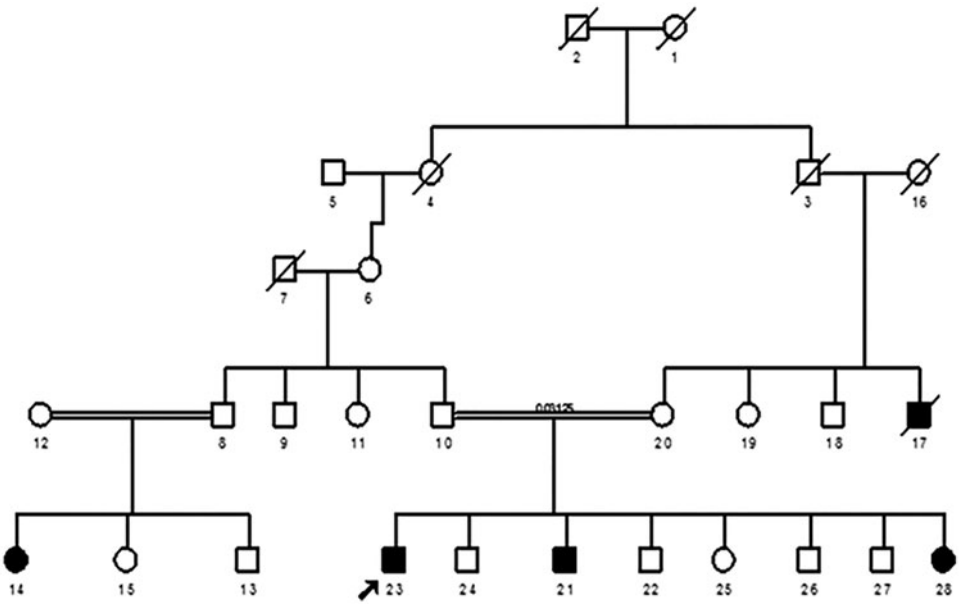

Figure 1. Family Y31 with four affected members, from Kohgiluyeh and Boyer-Ahmad.

In the past decade, next-generation sequencing (NGS) methods such as targeted next-generation sequencing (TNGS), whole exome sequencing (WES) and whole genome sequencing (WGS) have played a revolutionary role in both research and diagnostic areas (Vona et al. 2014; Shearer and Smith 2015; Goodwin, McPherson, and McCombie 2016). Recently, several TNGS panels, including some with known HL genes, have been developed (Shearer et al. 2010; De Keulenaer et al. 2012; Tang et al. 2012; Atik et al. 2015). In the present study, TNGS was performed on an Iranian family affected with HL. Large families with multiple HL-affected members seem appropriate for genetic studies (Saadat, Ansari-Lari, and Farhaud 2004), especially that Iran has a high frequency of consanguineous marriages (38%).

2. Materials and methods

2.1. Subjects and phenotype investigation

An Iranian consanguineous family (Y31) from the Kohgiluyeh and Boyer-Ahmad province, with three hearing-impaired siblings and one cousin was the subject of this study (Figure 1). Blood samples were obtained from all family members, along with 50 unaffected controls from the same province. The screening for *GJB2* pathogenic variants was done using previously described primers (Tabatabaiefar et al. 2010).

Written informed consent was obtained from family members or their proxy prior to sampling. Also, during the interview, a questionnaire was completed by the participants. The project was approved by the review boards of the Shahrekord University of Medical Sciences and the Ahvaz Jundishapur University of Medical Sciences.

2.2. Molecular study

2.2.1. DNA extraction, TNGS

DNA extraction was done using the Diatome kit (Ciulla, Sklar, and Hauser 1988). The quantity and the purity of the extracted DNA samples were measured by Nanodrop (Nanodrop 2000 Thermo Scientific, Waltham, MD). Prior to TNGS, the family was screened for *GJB2* mutations, as previously described (Chaleshtori, Farhaud, and Patton 2007) and no pathogenic variant was detected.

The American College of Medical Genetics and Genomic (ACMG) guidelines on deafness (Alford et al. 2014) recommends using NGS in *GJB2* mutation-negative probands. For TNGS of the family's proband, the Otogenetics Deafness Gene Panel (Otogenetics, Norcross, GA) (http://www.otogenetics.com/) was applied. It uses custom oligonucleotide-based target capture of the coding regions of 129 genes, followed by Illumina HiSeq 2000 sequencing (Illumina, San Diego, CA).

Briefly, genomic DNA was fragmented to 200–300 base pairs. Subsequently, amplification of the DNA library was done using ligation-mediated PCR (Oto-Enriched-001). The pooling of amplified multiplex DNA libraries was followed by hybridising samples to Oto-DA3-Probe. Untargeted DNA was washed, and captured DNA was recovered (Oto-Enriched-001) and amplified (Oto-Enriched-001). Finally, samples were sequenced on Illumina HiSeq/MiSeq with over 100× coverage for the Otogenetics DA3 gene panel. The targeted genes in this sequencing method are listed in Supplementary Table 1.

2.2.2. Bioinformatics analyses

After performing TNGS with the deafness panel, the data set was analysed using BWA mapping and the GATK SNP/Indel pipeline on www.DNAnexus.com. The snpEff annotation was used, and the data were filtered with Otogenetics advanced mutation screening to generate the final report. After all this filtration, the reported missense variant was evaluated by different *in silico* bioinformatic software tools, such as PROVEAN, PolyPhen-2, SNPs & GO, SIFT, PhD-SNP, PANTHER, Mutation Assessor, and FATHMM, to predict if the amino acid substitution had a deleterious effect on the protein, in terms of function and stability (Table 1). The level of conservation of the variant was also assessed using NCBI BLAST, which included five classes of vertebrates.

Table 1. List of *In silico* software tools and URLs.

Server name	Website URL	Basis
FATHMM	http://fathmm.biocompute.org.uk	Evolutionary conservation
MutationAssessor	http://mutationassessor.org	Evolutionary conservation
PANTHER	http://www.pantherdb.org/tools/csnpScoreForm.jsp	Evolutionary conservation
PhD-SNP	http://snps.biofold.org/phd-snp/phd-snp.html	Evolutionary conservation
SIFT	http://sift.jcvi.org	Evolutionary conservation
SNPs&GO	http://snps-and-go.biocomp.unibo.it/snps-and-go	Evolutionary conservation
PolyPhen-2	http://genetics.bwh.harvard.edu/pph2	Protein structure/function and evolutionary conservation
PROVEAN	http://provean.jcvi.org/index.php	Alignment and measurement of similarity between variant sequence and protein sequence homologue

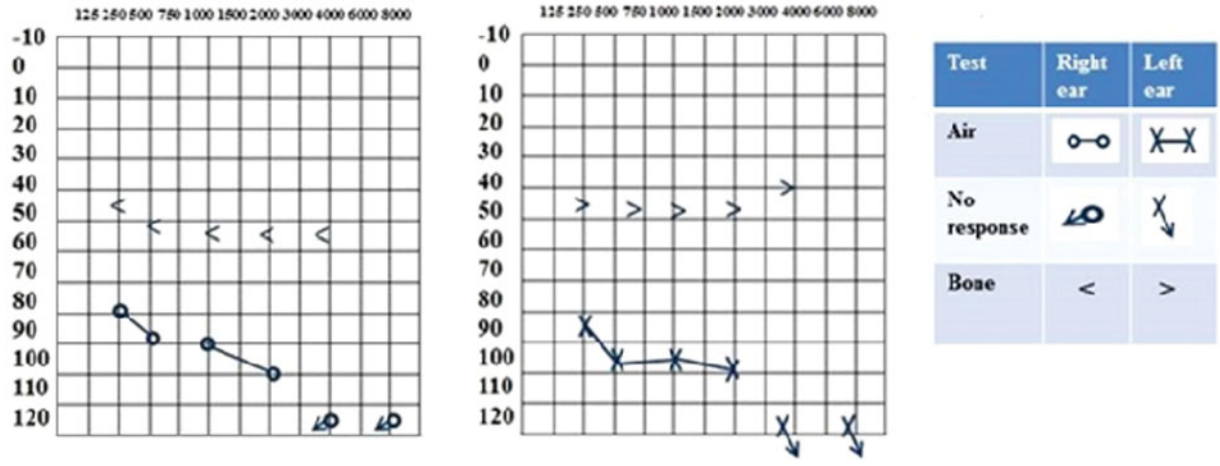

Figure 2. Phenotypical analysis of the family using PTA. Audiogram of an affected individual for both left ear and right ear.

2.3. Variant confirmation

Segregation analysis was performed for the candidate pathogenic variant for all members of the family through bi-directional Sanger sequencing, using an automated Genetic Analyser ABI 3130XL (Applied Biosystems, Foster City, CA). The forward and reverse primers (F: 5′-ACCACCACGCAGAGTAGG-3′, R: 5′-CTGTTGCCATTCCTCGACTTGTT-3′) were designed to amplify a 289 bp fragment, which included the missense variant NM_000441.1: c.1211C > T. Sequencher 5.4.5 (Gene Codes Corporation, Ann Arbor, MI) was used for sequence analysis. Sequences were compared to the reference sequence (NG_008489.1) to identify coding and intronic variants.

Similarly, the candidate variant was genotyped in 50 unrelated healthy controls of the same ethnic group randomly selected.

The pathogenicity of the *SLC26A4* variant was interpreted based on the ACMG guidelines (Richards et al. 2015), for which a given variant is categorised into one of five main classes, including Pathogenic, Likely pathogenic, Uncertain significance, Likely benign, or Benign.

2.4. Clinical investigation

Post-test genetic counselling and clinical examination were carried out for all affected members of the family, to determine whether the phenotype was related to DFNB4 (NSHL) or PDS (SHL). Pure Tone Audiometry (PTA) was performed for all patients in the range of 250–8000 Hz air conduction to measure hearing level. Based on the PTA average of five frequencies (250, 500, 1000, 2000, 4000, and 8000 Hz), the phenotype severity was defined as the following: mild for 26–40 dB, moderate for 41–55 dB, relatively severe 56–70 dB, severe 71–90, and profound >90 dB.

Function, structure, and size of the thyroid were evaluated through some tests, to determine the presence of goitre, associated with PDS. The levels of thyroid stimulating hormone (TSH), T3 and T4 were measured in all patients by means of Elecsys (Chemiluminescent Immunoassay) to evaluate the function of the thyroid. The size and structure of the thyroid were assessed by a Tc99m thyroid scan. The Perchlorate Discharge Test (PDT) was done in order to confirm the clinical features of PDS. One gram of perchlorate ($KClO_4$) was administered two hours after the administration of 131-iodine (50 mCi). Then, the discharge of iodide was measured. A discharge less than 10% of the incorporated iodide is expected in normal individuals (Wolff 1998; Reardon et al. 1999).

All three siblings also underwent a high-resolution computed tomography (CT) scan of the temporal bone, using a Somatom Sensation 16 (Siemens, Erlangen, Germany), to determine if there were alterations in the cochlea and vestibular aqueduct. When the diameter at the midway between the common crus and the external aperture was equal to or greater than 1.5 mm, it was described as Enlarged Vestibular Aqueduct (EVA) (Berrettini et al. 2005).

3. Results

3.1. Clinical testing results

The PTA assessment showed bilateral severe to profound HL in hearing-impaired individuals (Figure 2). Audiometry, which was obtained from family history and examination, demonstrated that HL was bilateral. The levels of thyroid hormones (TSH, T3, and T4) were normal, but thyroid scan detected enlarged multinodular goitre. There were multiple cold and functioning isoactive nodes on both lobes, which is strong evidence supporting

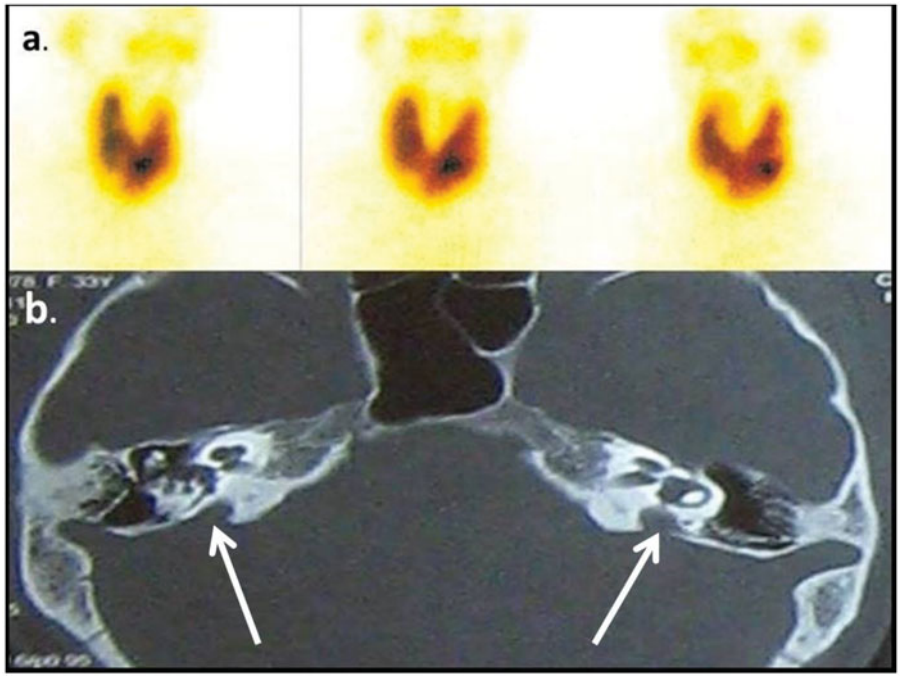

Figure 3. Paraclinical examination of the affected member. (a) Thyroid scans detected enlarged multi-nodular goitre, with multiple cold and functioning isoactive nodes in both lobes. (b) Temporal bone CT scan results of an affected member with EVA.

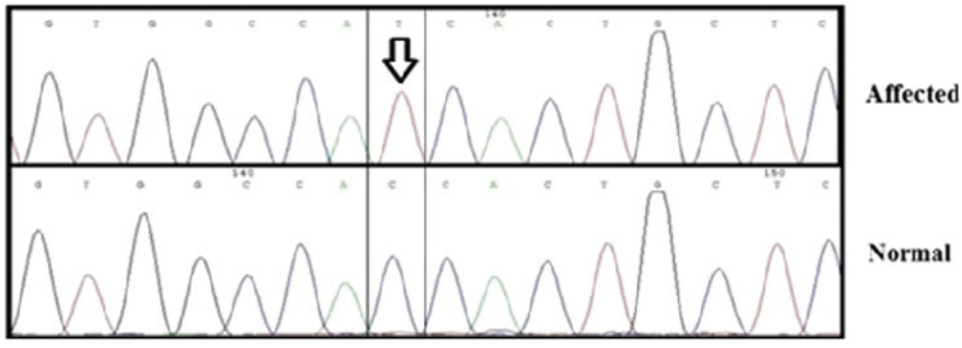

Figure 4. Genotype analysis of the family. Sequence chromatogram of the candidate variant (c.1211C>T) is shown. The arrowed site shows the region of the homozygous missense segregating with HL.

that this variant is associated with PDS (Figure 3(a)). Moreover, the PDT test showed 21% discharge of incorporated iodide in patients, which shows a defect in iodine organification into thyroglobulin. Lastly, CT scan showed EVA in the proband (Figure 3(b)).

3.2. Pathogenic variant identification

A missense variant in exon 10 of the *SLC26A4* gene (NM_000441.1: c.1211C > T/p.Thr404Ile, Figure 4) was detected in the proband through the TNGS method. Based on NCBI BLAST alignment with different species, the p.Thr404 residue of the pendrin protein was highly conserved (Figure 5). According to the following lines of evidence in the ACMG guidelines, we determined this candidate variant was pathogenic:

- We did not encounter the variant in 50 normal controls from the same ethnicity. Plus, the variant was not seen in the iranom project. For this project, 800 individuals from eight major ethnic groups in Iran went through whole exome sequencing. The eight groups comprised of 100 healthy individuals from each of the following ethnicities: Arabs, Azeris, Balochs, Kurds, Persians, Persian Gulf Islanders, Turkmen, and Lurs. The Iranian Lur people live in the mountainous areas in the southwest of Iran, occupying areas of northern Fars and southern Zagros. The territories occupied by Lurs are Lorestan, Bakhtiari, and Kohgiluyeh and Boyer-Ahmad (http://www.iranome.ir/). (PS4)
- Segregation with the disease was found in multiple affected family members. (PP1)
- The variant was located in a mutational hot spot and/or critical and well-established functional domain. (PM1)
- It was not found by the Exome Sequencing Project, the 1000 Genomes Project nor the Exome Aggregation Consortium. (PM2)
- The gene had a low rate of benign missense variations, and missense variants in the gene are a common disease mechanism. (PP2)

Figure 5. p.Thr404 is a highly conserved residue of *SLC26A4* in multiple-species alignment.

Table 2. Prediction of stability and function of the mutated protein (p.Thr404Ile) through different *In silico* software tools.

In silico server	Sift	Polyphen	Imutant	PHDSNP	Provean	Panther	Fathmm	Mutation assessor
PREDICTION (p.Thr404Ile / c.1211C > T)	DAMAGING / 0 / NOVEL	DAMAGING / 0.999	INCREASED STABILITY	DISEASE	DELETERIOUS:/ -4	DAMAGING / -4	DAMAGING / -3.42	MEDIUM

- Multiple lines of computational evidence supported the deleteriousness of the variant on the gene or gene product (conservation, evolution, splicing impact, etc.) (Table 2). (PP3)
- The patient's phenotype or family history was highly specific for a disease with a single genetic aetiology. (PP4)

4. Discussion

In the present study, TNGS followed by genotype-directed phenotyping were used to make an accurate genetic diagnosis of PDS. Pathogenic variants within the *SLC26A4* gene have been shown in different American, European and Asian populations such as Iranian (López-Bigas et al. 2002; Park et al. 2003). To date, more than 200 pathogenic variants within the *SLC26A4* gene have been detected (http://www.hgmd.cf.ac.uk). The most frequent pathogenic variants are missenses, each of which affecting different steps of transcription, translation, processing or protein function (Scott et al. 2000; Pryor et al. 2005).

Biallelic *SLC26A4* variants are thought to affect iodide efflux, promoting a localised defect of iodine organification. This defect is believed to be a cause of typical PDS, which is characterised by congenital fluctuating and progressive HL, associated with vertigo and/or goitre (Morgans and Trotter 1958). A number of variants within the same gene have been associated with ARNSHL and EVA (Dossena et al. 2011). Different studies have shown that pathogenic variants associated with PDS lead to deficiency in the transporting ability of the protein, but pathogenic variants associated with DFNB4 reduce the protein function (López-Bigas et al. 2002; Pryor et al. 2005).

The homozygous pathogenic variant identified in this study is in exon 10 of *SLC26A4*. Thus far, 24 missense pathogenic variants related to PDS and ARNSHL have been identified within this exon, eight of which have been experimentally tested (Bassot et al. 2017) (Table 3).

The variant, p.Thr404Ile, is located in the tenth transmembrane (TM) domain of the pendrin protein. In a model with 14 TM domains, the clusters of pathogenic variants were found in TM3, TM10, and at the C-terminus of TM11. These variants are suspected to promote disease onset by affecting anion transport between TM3 and TM10, two fundamental regions for the SLC26 protein family (Gorbunov et al. 2014; Geertsma et al. 2015). Specifically for *SLC26A4*, TM3 and TM10, as well as the SulP and Saier motifs, are involved in pendrin anion transport (Bassot et al. 2017). Of note, 35% of PDS-causative variants are localised within TM3 and TM10, emphasising that both regions are functionally relevant for pendrin activity. Moreover, the variant found in this study is rather conserved among different species, including five classes of vertebrates. Intriguingly, the residue is a serine in the zebra fish, suggesting that the OH group of serine and threonine might play a role in the function of the protein. Besides, the aforementioned variant is facing towards the protein interior, similar to other pathogenic variants. Importantly, a mutated residue has more pathological effect when altering the protein core than when contacting lipids. Essentially, lipid facing variants promote changes in solvation energy and have a disruptive effect on hydrophobic interactions with the lipid bilayer (Rotman-Pikielny et al. 2002; Taylor et al. 2002; Bassot et al. 2017).

Accessibility of TNGS-based gene panel enables a comprehensive genetic testing for all genes known to cause NSHL and SHL, which can manifest as non-syndromic like Usher and Pendred syndromes. As genomic information gets more affordable and readily available, it will have an extensive impact on the medical world. The inclusion of genetic information in healthcare has the possibility to provide patients with precise risk evaluations, based on their family history and genetic profile. Over the next decade, most of the variants responsible for HL have been identified, and such knowledge will result in the development of practical treatments (Ishihara et al. 2010).

In conclusion, in this study, TNGS was employed to clarify the etiology of HL in an Iranian multiplex PDS pedigree, and a pathogenic variant was identified in *SLC26A4*.

Table 3. Missense pathogenic variants in the exon 10 of the *SLC26A4* gene.

Nucleotide change	Residue change	Protein location	Pathogenic effect	Cellular localisation	Functionality	Predicted molecular effect
c.1151A > G[a]	p.E384G	TM9 (Inw)	PDS NSHL	ER Intracell	Loss of chloride and iodide uptake	Loss of H bond between E384 and Tyr127. Necessary for TM9 and TM2 interaction
c.1160C > T	p.A387V	TM 9 (Inw)	EVA	–	–	Larger side chain cause clashes with TM2
c.1165G > C	p.Gly389Arg	TM 9 (Inw)	EVA	–	–	Disruption of the interaction site between TM9 and TM4
c.1172G > A	p.S391R	TM 9 (Inw)	EVA	–	–	Charged residue in a hydrophobic pocket. Disruption of the arrangement of the central loops
c.1173C > A	p.S391N	TM 9 (Inw)	EVA	–	–	Formation of an H-bond with T126
c.1175A > G[a]	p.N392S	TM9 (Inw)	NSHL	Intracell	Reduction in the Cl-/I exchange	Clashes in the protein core
c.1174A > T	p.N392Y	TM9 (Inw)	EVA	–	–	Loss of H-bond with A403
c.1187G > A 10	p.G396E	TM9 (Inw)	EVA	–	–	Disruption of the interaction between TM9 and TM4
c.1195T > C	p.S399P	Extracell Loop	NSHL	–	–	Change of the local flexibility
c.1204G > A[a]	p.V402M	TM10 (Inw)	EVA	Intracell.	Loss of Cl-/I- and Cl-/HCO3 exchange activity	Clashes in the protein core.
c.1211C > T	p.T404I	TM10 (Inw)	PDS	–	–	Clashes in the protein core
c.1226G > A[a]	p.R409H	TM10 (Inw)	PDS	Partially PM	Reduction of chloride and iodide transport. Loss of iodide efflux	Loss of Arg409 putatively involved in anion binding
c.1226G > C	p.R409P	TM10 (Inw)	NSHL	–	–	Loss of Arg409 putatively involved in anion binding
c.1225C > T[a]	p.R409C	TM10 (Inw)	EVA	Intracell.	Loss of formate uptake	Loss of Arg409 putatively involved in anion binding
c.1229C > T[a]	p.T410M	TM10 (Inw)	PDS NSHL	–	Loss of iodide efflux	Alteration of the anion binding site
c.1231G > A	p.A411T	TM10 (Inw)	EVA	–	–	Formation of a new H-bond with Leu407
c.1231G > C	p.A411P	TM10 (Inw)	PDS	–	–	Insertion of a proline in the α helix
c.1238A > G	p.Q413R	TM10 (Inw)	NSHL	–	–	Loss of a H bond with TM1, Ser432 and TM12, Ser90. Disruption of local folding
c.1238A > C[a]	p.Q413P	TM10 (Inw)	PDS	–	Loss of cloride and iodide transport	Insertion of a proline in α helix
c.1240G > A	p.E414K	TM10 (Inw)		–	–	Charge inversion loss of salt bridge with Lys414
c.1245C > A	p.S415R	TM10 (Inw)	EVA	–	–	Charge insertion
c.1246A > C[a]	p.T416P	Cytosolic Interface	EVA	ER Intracell	Loss of chloride and iodide uptake	Insertion of a proline in loop
c.1259C > T	p.T420I	Cytosolic loop	NSHL	–	–	–
c.1261C > A	p.Q421K	TM11 (Inw)	PDS	–	–	Charge insertion
c.1262A > C	p.Q421P	TM11 (Inw)	EVA	–	–	Insertion of a proline in the α helix
c.1262A > G	p.Q421R	TM11 (Inw)	NSHL	–	–	Charge insertion
c.1262A > T	p.Q421L	TM11 (Inw)	EVA	–	–	–

[a]Variants are tested experimentally.
Inw: facing the inside of the protein; Lip: lipid-exposed; PM: plasma membrane; ER: endoplasmic reticulum; Intracell: intracellular.

Acknowledgements

Hereby, we would like to express our special thanks to our patients for their collaboration.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The project was supported by deputies of research of Shahrekord University of Medical Sciences [1600, 1497], Isfahan University of Medical Sciences [194068], and Ahvaz Jundishapur University of Medical Sciences [U-91074].

References

Alford, R. L., K. S. Arnos, M. Fox, J. W. Lin, C. G. Palmer, A. Pandya, H. L. Rehm, et al. 2014. "American College of Medical Genetics and Genomics Guideline for the Clinical Evaluation and Etiologic Diagnosis of Hearing Loss." *Genetics in Medicine* 16: 347–355.

Atik, T., H. Onay, A. Aykut, G. Bademci, T. Kirazli, M. Tekin, F. Ozkinay. 2015. "Comprehensive Analysis of Deafness Genes in Families with Autosomal Recessive Nonsyndromic Hearing Loss." *PLoS One* 10 (11): e0142154. doi:10.1371/journal.pone.0142154.

Bassot, C., G. Minervini, E. Leonardi, and S. C. E. Tosatto. 2017. "Mapping Pathogenic Mutations Suggests an Innovative Structural Model for the Pendrin (SLC26A4) Transmembrane Domain." *Biochimie* 132: 109–120. doi:10.1016/j.biochi.2016.10.002.

Berrettini, S., F. Forli, F. Bogazzi, E. Neri, L. Salvatori, A. P. Casani, S. S. Franceschini. 2005. "Large Vestibular Aqueduct Syndrome: Audiological, Radiological, Clinical, and Genetic Features." *American Journal of Otolaryngology* 26 (6): 363–371. doi:10.1016/j.amjoto.2005.02.013.

Bidart, J.-M., C. Mian, V. Lazar, D. Russo, S. Filetti, B. Caillou, and M. Schlumberger. 2000. "Expression of Pendrin and the Pendred Syndrome (PDS) Gene in Human Thyroid Tissues." *Journal of Clinical Endocrinology & Metabolism* 85: 2028–2033. doi:10.1210/jc.85.5.2028.

Bizhanova, A., and P. Kopp. 2010. "Genetics and Phenomics of Pendred Syndrome." *Molecular and Cellular Endocrinology* 322 (1–2): 83–90. doi:10.1016/j.mce.2010.03.006.

Campbell, C., R. A. Cucci, S. Prasad, G. E. Green, J. B. Edeal, C. E. Galer, L. P. Karniski, V. C. Sheefield, and R. J. Smith. 2001. "Pendred Syndrome, DFNB4, and PDS/SLC26A4 Identification of Eight Novel Mutations and Possible Genotype–Phenotype Correlations." *Human Mutation* 17 (5): 403–411. doi:10.1002/humu.1116.

Chaleshtori, M. H., D. Farhud, and M. Patton. 2007. "Congratulation to Margaret Chan Familial and Sporadic GJB2-Related Deafness in Iran: Review of Gene Mutations." *Iranian Journal of Public Health* 36: 1–14.

Choi, B. Y., H. M. Kim, T. Ito, K. Y. Lee, X. Li, K. Monahan, Y. Wen, et al. 2011. "Mouse Model of Enlarged Vestibular Aqueducts Defines Temporal Requirement of Slc26a4 Expression for Hearing Acquisition." *The Journal of Clinical Investigation* 121 (11): 4516–4525. doi:10.1172/JCI59353.

Ciulla, T. A., R. M. Sklar, and S. L. Hauser. 1988. "A Simple Method for DNA Purification from Peripheral Blood." *Analytical Biochemistry* 174 (2): 485–488. doi:10.1016/0003-2697(88)90047-4.

Coyle, B., R. Coffey, J. A. L. Armour, E. Gausden, Z. Hochberg, A. Grossman, K. Britton, M. Pembrey, W. Reardon, and R. Trembath. 1996. "Pendred Syndrome (Goitre and Sensorineural Hearing Loss) Maps to Chromosome 7 in the Region Containing the Nonsyndromic Deafness Gene DFNB4." *Nature Genetics* 12 (4): 421. doi:10.1038/ng0496-421.

De Keulenaer, S., J. Hellemans, S. Lefever, J. P. Renard, J. De Schriver, H. Van de Voorde, M. A. Tabatabaiefar, et al. 2012. "Molecular Diagnostics for Congenital Hearing Loss Including 15 Deafness Genes Using a Next Generation Sequencing Platform." *BMC Medical Genomics* 5: 17. doi:10.1186/1755-8794-5-17.

Dossena, S., C. Nofziger, G. Tamma, E. Bernardinelli, S. Vanoni, C. Nowak, E. Grabmayer., et al. 2011. "Molecular and Functional Characterization of Human Pendrin and Its Allelic Variants." *Cellular Physiology and Biochemistry* 28 (3): 451–466. doi:10.1159/000335107.

Everett, L. A., B. Glaser, J. C. Beck, J. R. Idol, A. Buchs, M. Heyman, F. Adawi., et al. 1997. "Pendred Syndrome Is Caused by Mutations in a Putative Sulphate Transporter Gene (PDS)." *Nature Genetics* 17 (4): 411. doi:10.1038/ng1297-411.

Friedman, T. B., and A. J. Griffith. 2003. "Human Nonsyndromic Sensorineural Deafness." *Annual Review of Genomics and Human Genetics* 4 (1): 341–402. doi:10.1146/annurev.genom.4.070802.110347.

Geertsma, E. R., Y. N. Chang, F. R. Shaik, Y. Neldner, E. Pardon, J. Steyaert, and R. Dutzler. 2015. "Structure of a Prokaryotic Fumarate Transporter Reveals the Architecture of the SLC26 Family." *Nature Structural & Molecular Biology* 22 (10): 803–808. doi:10.1038/nsmb.3091.

Goodwin, S., J. D. McPherson, and W. R. McCombie. 2016. "Coming of Age: ten Years of Next-Generation Sequencing Technologies." *Nature Reviews Genetics* 17 (6): 333. doi:10.1038/nrg.2016.49.

Gorbunov, D., M. Sturlese, F. Nies, M. Kluge, M. Bellanda, R. Battistutta, and D. Oliver. 2014. "Molecular Architecture and the Structural Basis for Anion Interaction in Prestin and SLC26 Transporters." *Nature Communications* 5: 3622. doi:10.1038/ncomms4622.

Gorlin, R. J., H. V. Toriello, and M. M. Cohen. 1995. *Hereditary Hearing Loss and Its Syndromes*. New York: Oxford University Press.

Hilgert, N., R. J. Smith, and G. Van Camp. 2009. "Forty-Six Genes Causing Nonsyndromic Hearing Impairment: which Ones Should Be Analyzed in DNA Diagnostics?" *Mutation Research/Reviews in Mutation Research* 681 (2–3): 189–196. doi:10.1016/j.mrrev.2008.08.002.

Ishihara, K., S. Okuyama, S. Kumano, K. Iida, H. Hamana, M. Murakoshi, T. Kobayashi., et al. 2010. "Salicylate Restores Transport Function and Anion Exchanger Activity of Missense Pendrin Mutations." *Hearing Research* 270 (1–2): 110–118. doi:10.1016/j.heares.2010.08.015.

Li Xc, E. L., A. K. Lalwani, D. Desmukh, T. B. Friedman, and E. D. Green. 1998. "A Mutation in PDS Causes Nonsyndromic Recessive Deafness." Nature Genetics 18: 215–217. doi:10.1038/ng0398-215.

López-Bigas, N., S. Melchionda, R. di Cid, A. Grifa, M. L. Arbones, P. Gasparini, X. Estivill. 2002. "Erratum: Identification of Five New Mutations of PDS/SLC26A4 in Mediterranean Families with Hearing Impairment." *Human Mutation* 20: 77–78. doi:10.1002/humu.9043.

Morgans, M., and W. Trotter. 1958. "Association of Congenital Deafness with Goitre the Nature of the Thyroid Defect." *Lancet (London, England)* 1 (7021): 607–609.

Park, H., S. Shaukat, X. Z. Liu, S. H. Han, S. Naz, M. Ghosh, H. N. Kim, et al. 2003. "Origins and Frequencies of SLC26A4 (PDS) Mutations in East and South Asians: global Implications for the Epidemiology of Deafness." *Journal of Medical Genetics* 40 (4): 242–248. doi:10.1136/jmg.40.4.242.

Pryor, S., A. C. Madeo, J. C. Reynolds, N. J. Sarlis, K. S. Arnos, W. E. Nance, Y. Yang, et al. 2005. "SLC26A4/PDS Genotype-Phenotype Correlation in Hearing Loss with Enlargement of the Vestibular Aqueduct (EVA): Evidence That Pendred Syndrome and Non-Syndromic EVA Are Distinct Clinical and Genetic Entities." *Journal of Medical Genetics* 42 (2): 159–165. doi:10.1136/jmg.2004.024208.

Reardon, W., R. Coffey, T. Chowdhury, A. Grossman, H. Jan, K. Britton, P. Kendall-Taylor, and R. Trembath. 1999. "Prevalence, Age of Onset, and Natural History of Thyroid Disease in Pendred Syndrome." *Journal of Medical Genetics* 36: 595–598.

Richards, S., N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W. W. Grody., et al. 2015. "Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology." *Genetics in Medicine* 17 (5): 405–423. doi:10.1038/gim.2015.30.

Rotman-Pikielny, P., K. Hirschberg, P. Maruvada, K. Suzuki, I. E. Royaux, E. D. Green, L. D. Kohn, et al. 2002. "Retention of Pendrin in the Endoplasmic Reticulum Is a Major Mechanism for Pendred Syndrome." *Human Molecular Genetics* 11 (21): 2625–2633. doi:10.1093/hmg/11.21.2625.

Royaux, I. E., S. M. Wall, L. P. Karniski, L. A. Everett, K. Suzuki, M. A. Knepper, E. D. Green., et al. 2001. "Pendrin, Encoded by the Pendred Syndrome Gene, Resides in the Apical Region of Renal Intercalated Cells and Mediates Bicarbonate Secretion." *Proceedings of the National Academy of Sciences* 98 (7): 4221–4226. doi:10.1073/pnas.071516798.

Saadat, M., M. Ansari-Lari, and D. Farhud. 2004. "Short Report Consanguineous Marriage in Iran." *Annals of Human Biology* 31 (2): 263–269. doi:10.1080/03014460310001652211.

Scott, D. A., R. Wang, T. M. Kreman, M. Andrews, J. M. McDonald, J. R. Bishop, R. J. Smith, et al. 2000. "Functional Differences of the PDS Gene Product Are Associated with Phenotypic Variation in Patients with Pendred Syndrome and Non-Syndromic Hearing Loss (DFNB4)." *Human Molecular Genetics* 9 (11): 1709–1715. doi:10.1093/hmg/9.11.1709.

Shearer, A. E., and R. J. Smith. 2015. "Massively Parallel Sequencing for Genetic Diagnosis of Hearing Loss: The New Standard of Care." *Otolaryngology–Head and Neck Surgery* 153 (2): 175–182. doi:10.1177/0194599815591156.

Shearer, A. E., A. P. DeLuca, M. S. Hildebrand, K. R. Taylor, J. Gurrola, S. Scherer, T. E. Scheetz., et al. 2010. "Comprehensive Genetic Testing for Hereditary Hearing Loss Using Massively Parallel Sequencing." *Proceedings of the National Academy of Sciences* 107 (49): 21104–21109. doi:10.1073/pnas.1012989107.

Smith, R. J., J. F. Bale, and K. R. White. 2005. "Sensorineural Hearing Loss in Children." *Lancet (London, England)* 365 (9462): 879–890. doi:10.1016/S0140-6736(05)71047-3.

Soleimani, M., T. Greeley, S. Petrovic, Z. Wang, H. Amlal, P. Kopp, C. E. Burnham., et al. 2001. "Pendrin: An Apical Cl-/OH-/HCO3- exchanger in the kidney cortex." *American Journal of Physiology. Renal Physiology* 280 (2): F356–F364. doi:10.1152/ajprenal.2001.280.2.F356.

Stevens, G., S. Flaxman, E. Brunskill, M. Mascarenhas, C. D. Mathers, and M. Finucane. 2013. "Global and Regional Hearing Impairment Prevalence: An Analysis of 42 Studies in 29 Countries." European Journal of Public Health 23 (1): 146–152. doi:10.1093/eurpub/ckr176.

Tabatabaiefar, M. A., M. M. Zohour, L. Sahriati, J. Saffari-Chalestori, K. Ashrafi, A. Gholami, E. Farrokhi, et al. 2010. "Mutation Analysis of GJB2 and GJB6 Genes and the Genetic Linkage Analysis of Five Common DFNB Loci in the Iranian Families with Autosomal Recessive Non-Syndrom." *Journal of Sciences Islamic Republic Of Iran* 21 (2): 105–112.

Tang, W., D. Qian, S. Ahmad, D. Mattox, N. W. Todd, H. Han, S. Huang., et al. 2012. "A Low-Cost Exon Capture Method Suitable for Large-Scale Screening of Genetic Deafness by the Massively-Parallel Sequencing Approach." *Genetic Testing and Molecular Biomarkers* 16 (6): 536–542. doi:10.1089/gtmb.2011.0187.

Taylor, J. P., R. A. Metcalfe, P. F. Watson, A. P. Weetman, and R. C. Trembath. 2002. "Mutations of the PDS Gene, Encoding Pendrin, Are Associated with Protein Mislocalization and Loss of Iodide Efflux: implications for Thyroid Dysfunction in Pendred Syndrome." *The Journal of Clinical Endocrinology & Metabolism* 87: 1778–1784. doi:10.1210/jc.87.4.1778.

Van Camp, G., P. J. Willems, and R. Smith. 1997. "Nonsyndromic Hearing Impairment: unparalleled Heterogeneity." *American Journal of Human Genetics* 60 (4): 758–764.

Vona, B., T. Müller, I. Nanda, C. Neuner, M. A. H. Hofrichter, J. Schröder, O. Bartsch., et al. 2014. "Targeted Next-Generation Sequencing of Deafness Genes in Hearing-Impaired Individuals Uncovers Informative Mutations." *Genetics in Medicine* 16 (12): 945. doi:10.1038/gim.2014.65.

Wangemann, P., K. Nakaya, T. Wu, R. J. Maganti, E. M. Itza, J. D. Sanneman, D. G. Harbidge., et al. 2007. "Loss of Cochlear HCO3− Secretion Causes Deafness via Endolymphatic Acidification and Inhibition of Ca2+ Reabsorption in a Pendred Syndrome Mouse Model." *American Journal of Physiology-Renal Physiology* 292 (5): F1345–F1353. doi:10.1152/ajprenal.00487.2006.

Wolff, J. 1998. "Perchlorate and the Thyroid Gland." *Pharmacological Reviews* 50 (1): 89–106.

World Health Organization. 2009. Global health risks: mortality and burden of disease attributable to selected major risks. World Health Organization.

Yoshida, A., S. Taniguchi, I. Hisatome, I. E. Royaux, E. D. Green, L. D. Kohn, and K. Suzuki. 2002. "Pendrin Is an Iodide-Specific Apical Porter Responsible for Iodide Efflux from Thyroid Cells." *The Journal of Clinical Endocrinology & Metabolism* 87: 3356–3361. doi:10.1210/jc.87.7.3356.