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Novel Pathogenic Variants Underlie *SLC26A4*-related Hearing Loss in a Multiethnic Cohort

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Author Disclosure Statement

Authors declare that there is no conflict of interest to report.

7. WEB RESOURCES

Exome Aggregation Consortium (ExAC): <http://exac.broadinstitute.org/>

Polyphen2: <http://genetics.bwh.harvard.edu/pph2/>

SIFT: <http://sift.jcvi.org/>

ClinVar: <http://www.ncbi.nlm.nih.gov/clinvar/>

MutationTaster: <http://www.mutationtaster.org/>

GERP: <http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html>

PhastCons: <http://compugen.cshl.edu/phast>

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Abstract

Objectives—The genetics of sensorineural hearing loss is characterized by a high degree of heterogeneity. Despite this heterogeneity, DNA variants found within *SLC26A4* have been reported to be the second most common contributor after those of *GJB2* in many populations.

Methods—Whole exome sequencing and/or Sanger sequencing of *SLC26A4* in 117 individuals with sensorineural hearing loss with or without inner ear anomalies but not with goiter from Turkey, Iran, and Mexico were performed.

Results—We identified 27 unique *SLC26A4* variants in 31 probands. The variants c.1673A>G (p.N558S), c.1708-1G>A, c.1952C>T (p.P651L), and c.2090-1G>A have not been previously reported. The p.N558S variant was detected in two unrelated Mexican families.

Conclusion—A range of *SLC26A4* variants without a common recurrent mutation underlies *SLC26A4*-related hearing loss in Turkey, Iran, and Mexico.

Keywords

Hearing Loss; *SLC26A4*; Whole Exome Sequencing

1. INTRODUCTION

Hearing loss (HL) is the most common sensory disorder affecting 1.33 per 1,000 newborns [1]. Approximately half of HL is caused by genetic factors. More than 400 genetic syndromes have been reported to have HL as a finding. While mutations in over 100 genes have been identified to underlie HL, pathogenic variants in the *SLC26A4* gene (MIM 605646) have been reported by some studies as the most common cause after *GJB2* mutations in nonsyndromic HL [2]. *SLC26A4* is a member of the solute carrier 26 family and encodes pendrin that is an anion exchanger. [3]. *SLC26A4* is expressed in different tissues, including thyroid, kidney, and inner ear. In the cochlea, pendrin is found in the apical membrane of the outer sulcus and spiral prominence epithelial cells that border the endolymph, in the spiral ganglion and in supporting cells [3, 4].

Homozygous and compound heterozygous mutations in *SLC26A4* cause two different autosomal recessive disorders: 1) Pendred syndrome (PDS) (MIM 274600) which is associated with development abnormalities of the cochlea, sensorineural HL, and diffuse thyroid enlargement (goiter) [5] and 2) DFNB4 with enlarged vestibular aqueduct (EVA) (MIM 600791), which is characterized by prelingual, perilingual, or postlingual onset of sensorineural HL. The HL in DFNB4 is associated with inner ear anomalies, most commonly EVA with or without incomplete partition of cochlea type II, also referred to as Mondini dysplasia [6, 7].

In this study, we present *SLC26A4* variants detected in 31 unrelated families with nonsyndromic sensorineural HL with or without inner ear anomalies.

2. MATERIALS AND METHODS

2.1. Statement of Ethics

This study was approved by the University of Miami Institutional Review Board (USA), the Ankara University Medical School Ethics Committee (Turkey), the Growth and Development Research Ethics Committee (Iran), and the Ethics Committee of National Institute of Rehabilitation (Mexico). A signed informed-consent form was obtained from each participant or, in the case of a minor, from the parents.

2.2. Subjects

Our study cohort consists of 45 probands from Turkey and 11 probands from Mexico with nonsyndromic sensorineural HL and inner ear anomalies, all diagnosed with either a high resolution temporal bone CT or MRI (Table 1). Additionally, 61 probands from unrelated Iranian families with nonsyndromic sensorineural HL who did not have an imaging study done were included. All probands were prescreened for *GJB2* variants. Sensorineural HL was diagnosed via standard audiometry in a soundproof room according to standard clinical practice. DNA was extracted from peripheral leukocytes of each member of the family by standard protocols. Probands underwent either whole exome sequencing (WES) or Sanger sequencing of *SLC26A4* to identify those with either homozygous or compound heterozygous mutations in *SLC26A4*.

2.3. DNA Sequencing

WES and Sanger sequencing was performed using our previously reported protocol [8, 9]. Polyphen2, SIFT and MutationTaster were used for *in silico* analysis, GERP and Phastcons scores were used to evaluate the conservation of the variants. The population frequency of each variation was evaluated using data from the ExAC database. ACMG guidelines were used for variant interpretation [10].

Probands who are either homozygous or compound heterozygous for *SLC26A4* variants via WES underwent Sanger sequencing for confirmation. Nine probands were only Sanger sequenced for *SLC26A4*.

3. RESULTS

There were 31 probands who are homozygous (Turkey: 15; Iran: 6; Mexico: 3) or compound heterozygous (Iran: 3; Mexico: 4) for variants in *SLC26A4* (NM_000441.1) (Table 2). 23 pathogenic variants had all been previously reported in patients with nonsyndromic HL or PDS and four novel variants were detected in this study (Table 2, Figure 1A & 1B). All variants co-segregated with HL in available family members. Six variants were found in multiple probands; three of these were present in probands from both Turkey and Iran. All fourteen probands with known consanguinity were homozygous, while there were 10 probands who were homozygous but were not known to be the product of a consanguineous mating.

Identified variants in *SLC26A4* were associated with EVA and/or IP2 in Turkish and Mexican families with inner ear anomalies (Figure 1B). *SLC26A4* variants were identified in 9 out of 61 Iranian families with nonsyndromic deafness without temporal bone imaging (15%).

4. DISCUSSION

Analysis of variants detected in our study suggests that certain variants are relatively common in certain geographical or ethnic groups. In our dataset, the most common pathogenic variants were c.1197delT (p.C400Vfs*32) and c.1226G>A (p.R409H). Tsukada et al. reported that p.R409H is the most common causative variant in Iran [11]. We found this variant in two families from Turkey (Family 834 and 836) and one from Iran (Family 1008). It is likely that this variant has a common founder in that region of the world. Similarly, p.C400Vfs*32 was reported to be the second most common pathogenic variation in the Iranian population [11]. Affected members of two Turkish families (Family 560 and 2136) were homozygous and probands from two Iranian families (Family 979 and 1040) were heterozygous for this variant. The most common pathogenic variants in *SLC26A4* found in Oriental populations are c.919-2A>G and c.2168A>G (p.H723R) [12]. In our cohort, we found each variant in two Turkish families, which might indicate common ancestors since historically Turks have migrated from Central Asia to Anatolia.

A review of the current literature revealed limited information about *SLC26A4* variants in the Mexican population. In this study, we present seven unrelated Mexican families with 10 different *SLC26A4* variants. We found a recurrent novel variant (c.1673A>G (p.N558S)) in two families (Family 1169 and 2215).

Previously published studies reported other founder variants not detected in this study. Borck et al. showed that p.V138F is a founder variant in German Pendred syndrome families [13]. Anwar et al. reported that p.V239D is a founder mutation that arose in a common ancestor in Pakistan [14]. While p.V239D has been reported in the Turkish population, we did not find this pathogenic variant in our current cohort. In addition, the p.Q514K variant was reported in Spain that derives from a common founder [15].

In conclusion, we found 27 different causative *SLC26A4* variants, four of which are novel (Table 2) in our cohort. In addition to two splice-site alterations, the novel variants included two missense mutations (Table 3) [10].

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Figure 1. Representation of electropherograms and phenotypes of the families with *SLC26A4*. **(A)** Shows electropherograms of the novel c.1673A>G (p.N558S), c.1952C>T (p.P651L), c.1708-1G>A and c.2090-1G>A variants in Families 987, 2214, 1393, 1169 and 2215. **(B)** Shows ethnicity, inner ear anomaly type and *SLC26A4* information of the families.

Table 1

Ethnicity and inner ear anomaly type of the Mexican and Turkish families.

Inner Ear Anomaly Type	# of Families from Turkey	# of Families from Mexico	Total
EVA/IP2	26	9	35
Cochlear Aplasia	7	0	7
Common Cavity	3	2	5
IP1	5	0	5
Michel Aplasia	4	0	4
Total	45	11	56

EVA: Enlarged Vestibular Aqueduct, **IP1:** Incomplete Partition 1, **IP2:** Incomplete Partition 2

Table 2

Causative *SLC26A4* variants identified in our cohort.

Family ID	Country of Origin	Simplex/Multiplex	Parental consanguinity	Results of Temporal Bone Imaging		Genotype	cDNA	Protein	Reference	Method
				Left Ear	Right Ear					
834	Turkey	Simplex	Yes	EVA	IP2	HM	c.1226G>A	p.R409H	van Hauwe et al. [16]	Sanger
836	Turkey	Simplex	No	EVA	EVA	HM	c.1226G>A	p.R409H	van Hauwe et al. [16]	Sanger
857	Turkey	Multiplex	Yes	IP2	IP2	HM	c.2168A>G	p.H723R	van Hauwe et al. [16]	Sanger
1558	Turkey	Simplex	No	EVA	EVA	HM	c.2168A>G	p.H723R	van Hauwe et al. [16]	WES
1555-1001	Turkey	Simplex	Yes	EVA	EVA	HM	c.919-2A>G	Splice	Yong et al. [17]	WES
2137	Turkey	Simplex	No	EVA	EVA	HM	c.919-2A>G	Splice	Yong et al. [17]	Sanger
587	Turkey	Simplex	Yes	IP2	IP2	HM	c.1147C>G	p.Q383E	Everett et al. [5]	WES
704	Turkey	Simplex	Yes	EVA	EVA	HM	c.1341delG	p.K447Nfs*8	Everett et al. [5]	WES
1555-1000	Turkey	Simplex	Yes	EVA	EVA	HM	c.1262A>C	p.Q421P	Wang et al. [18]	WES
1556	Turkey	Simplex	Yes	EVA	EVA	HM	c.1229C>T	p.T410M	Coyle et al. [19]	WES
1561	Turkey	Simplex	Yes	EVA	EVA	HM	c.578C>T	p.T193I	Adato et al. [20]	WES
1564	Turkey	Simplex	Yes	EVA	EVA	HM	c.1489G>A	p.G497S	Li et al. [21]	WES
2138	Turkey	Simplex	No	EVA	EVA	HM	c.170C>G	p.S57*	Park et al. [22]	Sanger
560	Turkey	Multiplex	Yes	EVA	EVA	HM	c.1198delT	p.C400Vfs*32	Everett et al. [5]	Sanger
2136	Turkey	Multiplex	Yes	EVA	EVA	HM	c.1198delT	p.C400Vfs*32	Everett et al. [5]	Sanger
979	Iran	Multiplex	No	N/A	N/A	HT	c.1198delT	p.C400Vfs*32	Everett et al. [5]	WES
				N/A	N/A		c.1574C>T	p.P525L	Rendtorff et al. [23]	
1040	Iran	Multiplex	No	N/A	N/A	HT	c.1198delT	p.C400Vfs*32	Everett et al. [5]	WES
				N/A	N/A		c.1262A>C	p.Q421P	Wang et al. [18]	
987	Iran	Multiplex	No	N/A	N/A	HT	c.890delC	p.P297Qfs*6	Gillam et al. [24]	WES
				N/A	N/A		c.1952C>T	p.P651L	This study	
989	Iran	Multiplex	Yes	N/A	N/A	HM	c.1546C>T	p.P516S	Zhao et al. [25]	WES

Family ID	Country of Origin	Simplex/Multiplex	Parental consanguinity	Results of Temporal Bone Imaging		Genotype	cDNA	Protein	Reference	Method
				Left Ear	Right Ear					
998	Iran	Multiplex	Yes	N/A	N/A	HM	c.1334T>G	p.L445W	van Hauwe et al. [16]	WES
1008	Iran	Multiplex	No	N/A	N/A	HM	c.1226G>A	p.R409H	van Hauwe et al. [16]	WES
1013	Iran	Multiplex	No	N/A	N/A	HM	c.304G>A	p.G102R	Taylor et al. [26]	WES
1038	Iran	Multiplex	No	N/A	N/A	HM	c.1001G>T	p.G334V	Kahrizi et al. [27]	WES
1039	Iran	Simplex	Yes	N/A	N/A	HM	c.1334T>G	p.L445W	van Hauwe et al. [16]	WES
2215	Mexico	Multiplex	No	IP2	IP2	HM	c.1673A>G	p.N558S	This study	WES
1169	Mexico	Simplex	No	EVA	EVA	HT	c.1673A>G	p.N558S	This study	Sanger
							c.918+2T>C	Splice	Rendtorff et al. [23]	
1393	Mexico	Multiplex	No	EVA	EVA	HT	c.170C>G	p.S57*	Park et al. [22]	WES
							c.1708-1G>A	Splice	This study	
2211	Mexico	Simplex	No	EVA	EVA	HT	c.165-1G>A	Splice	Gonzalez et al. [28]	WES
							c.626G>T	p.G209V	van Hauwe et al. [16]	
2214	Mexico	Multiplex	No	EVA	EVA	HT	c.1489G>A	p.G497S	Li et al. [21]	WES
							c.2090-1G>A	Splice	This study	
1180	Mexico	Simplex	No	EVA	EVA	HM	c.1149+3A>G	Splice	Park et al. [29]	Sanger
1318	Mexico	Multiplex	No	EVA	EVA	HM	c.2162C>T	p.T721M	Usami et al. [30]	WES

EVA: Enlarged Vestibular Aqueduct, **IP2:** Incomplete Partition 2, **N/A:** Not available, **WES:** Whole Exome Sequencing, **HM:** Homozygous, **HT:** Heterozygous.

Table 3

Characteristics of novel causative variants.

Family ID	cDNA	Protein	ACMG	ExAC	Clin Var	SIFT score	SIFT pred	Polyphen2 HDIV score	Polyphen2 HDIV pred	Polyphen2 HVAR score	Polyphen2 HVAR pred	Mutation Taster score	Mutation Taster pred	GERP++ RS	phastCons 7way vertebrate	phastCons 20way mammalian
987	c.1952C>T	p.P651L	VUS	-	-	0.001	DL	1	PD	1	PD	1	DC	5.89	0.965	0.994
1393	c.1708-1G>A	Splice	P	-	-	.	.	.	.	.	.	1	DC	5.72	1	1
2214	c.2090-1G>A	Splice	P	-	-	.	.	.	.	.	.	1	DC	5.51	0.975	1
1169 and 2215	c.1673A>G	p.N558S	VUS	0.00002492	-	0	DL	1	PD	0.995	PD	0.9996	DC	5.51	1	0.9769

P: Pathogenic, **VUS:** Variant Unknown Significance, **DL:** Deleterious, **PD:** Probably Damaging, **DC:** Disease Causing, **ExAC:** Exome Aggregation Consortium, **ACMG:** American College of Medical Genetics (Richards et al., 2015)