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ORIGINAL ARTICLE

The *OTOGL* p.Arg925* Variant is Associated with Moderate Hearing Loss in a Syrian Nonconsanguineous Family

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Aims: To screen for the genetic basis of congenital hearing loss in a Syrian family.
Methods: A Syrian patient living in Lebanon presented with moderate congenital hearing loss. The patient's large nonconsanguineous family was recruited. DNA was extracted from blood samples and sent for whole-exome sequencing. A detailed clinical examination along with audiograms was obtained for all subjects.
Results: Hearing loss was noted to be mild to moderate in the low and mid frequencies, sloping to moderate to severe in the high frequencies for all affected members. Results of DNA analysis showed the presence of a previously described p.Arg925* mutation in the *OTOGL* gene on both alleles in affected family members, whereas nonaffected members either had the wild type or one copy of the mutated allele.
Discussion: Mutations affecting the *OTOGL* gene have been recently connected with nonsyndromic sensorineural hearing loss. Seven such mutations have already been described. The p.Arg925* reported in this study has been found once in a French family. The current report is the first to describe this mutation in a Middle Eastern family.

Keywords: genetic testing, congenital hearing loss, OTOGL mutation, hearing screening

Introduction

Hearing loss or deafness is the most common sensory deficit in humans affecting 1.6 out of every 1000 infants screened in the United States (CDC, 2015) with around 60% of the cases tracing back to genetic causes. The remaining 40% of hearing loss cases are mainly due to environmental causes (Marazita *et al.*, 1993). Genetic etiology encompasses syndromic hearing loss with more than 400 forms described, and nonsyndromic hearing loss (NSHL) with more than 80 identified deafness genes to date (Van Camp and Smith, 2016). NSHL comprises 70% of deafness cases of which 75–80% follow an autosomal recessive inheritance pattern (Shearer and Smith, 2012). Genetic mapping and exome sequencing of families who suffer from the autosomal recessive form of the disease have led to the discoveries of more than 55 genes that when mutated could affect one of the different structure/function features in the cochlea (Van Camp and Smith, 2016). Interestingly, most of the studies were done on families living in the Middle East, where the high rate of consanguineous marriages is associated to the high prevalence of such genetic disorders (Najmabadi and Kahrizi, 2014). The most studied mutations are the ones affecting the *GJB2* gene, which encodes the gap junction protein connexin-26. Over 100 mutations have been identified in *GJB2* accounting for 50% of patients with autosomal recessive NSHL (Kenna *et al.*, 2007; Shearer *et al.*, 2011; Duman and Tekin, 2012; Tsukada *et al.*, 2015). The most common worldwide mutation is the c.35delG frameshift deletion variant that is found in 39 out of 52 countries with a predicted founder effect dating back to more than 10,000 years ago (Shearer *et al.*, 2011; Duman and Tekin, 2012).

In Lebanon the most common mutation described so far using Sanger sequencing is the c.35delG as is the case in Syria, although the number of patients and families who were screened do not reflect the exact prevalence of the disease in the two countries (Mustapha *et al.*, 2001; Belguith *et al.*, 2005; Al-Achkar *et al.*, 2011; Najmabadi and Kahrizi, 2014).

We, hereby, report the results of the first exome sequencing of a Syrian nonconsanguineous family with moderate hearing loss, living in Lebanon.

Materials and Methods

Subjects

A Syrian refugee with moderate congenital hearing loss living in Lebanon was referred to the Otolaryngology Department at the American University of Beirut (AUB). The

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patient's large nonconsanguineous family was recruited for genetic studies as part of an ongoing study on the genetic basis of hearing loss in Lebanon approved by the Institutional Review Board at AUB under protocol number (OTO.MB1.02). Informed consents were obtained from all adult members and parents, and informed assents in the case of minors. Affected members underwent extensive ear, nose, and throat exam as well as pure tone audiometry in soundproof rooms at the American University of Beirut Medical Center (AUBMC). They were referred to Ophthalmology, Cardiology and Nephrology for identification of possible other congenital abnormalities and ruling out syndromic hearing loss.

Exome sequencing

Blood samples were collected from all family members and blood DNA extraction was performed using the QIAamp DNA Blood Midi Kit (Qiagen Sciences, Inc., Germantown, MD), following the manufacturer's instructions. DNA quantification was performed using the NanoDrop (Thermo Fisher Scientific, Inc., Waltham, MA) at the molecular core facility at AUB. One microgram of coded DNA samples from both parents, the proband, four of her siblings, and three of her nephews were shipped to Macrogen (South Korea), where exome sequencing was performed using the V4 platform of the SureSelect Target Enrichment Capture system from Agilent on a HiSeq 2000 platform from Illumina (San Diego, CA).

Sanger sequencing

Exome sequencing results were validated by Sanger sequencing. Primers amplifying *OTOGL* exon 25 were designed: 5′-GAACTGGCAATATTCGTAAGGG-3′ (Forward), and 5′-CAGACCTTCCCAAGTACCCC-3′ (Reverse). The corresponding 440 bp polymerase chain reaction (PCR) products were resolved on agarose gels, and then purified using the QIAquick PCR Purification Kits (Qiagen Sciences, Inc.). Sanger sequencing was carried out on an ABI 3500 platform (Applied Biosystems, Foster City, CA) at the American University of Beirut Molecular Core Facility.

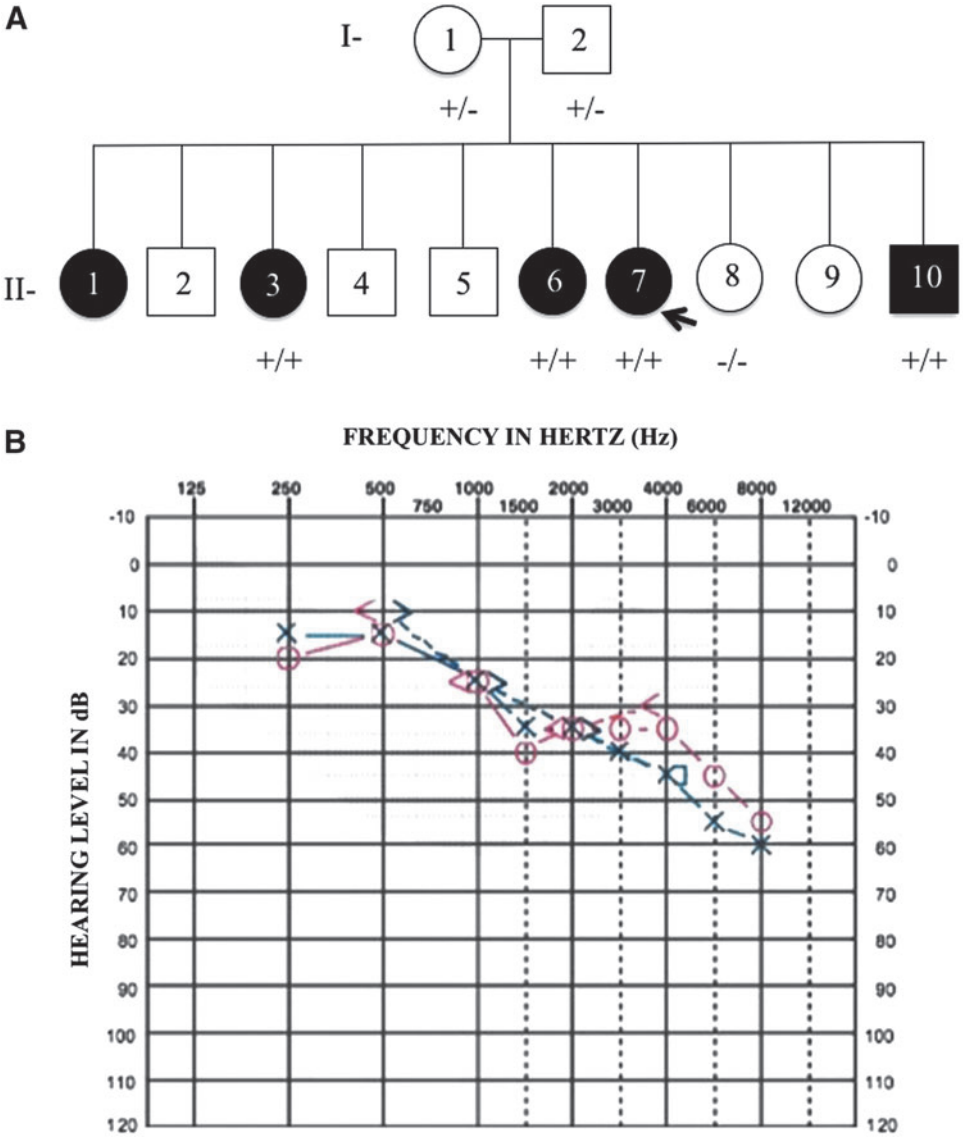

FIG. 1. Family pedigree **(A)** with the audiogram of the affected proband **(B)**. Affected individuals are marked with *black*. Proband is marked with the *arrow*.

FIG. 2. Confirmation of the *OTOGL* variant by Sanger sequencing.

Data analysis

Primary analysis was done at Macrogen. Generated FASTQ files were mapped to the reference genome using the Burrows-Wheeler Alignment program. The Genome Analysis Toolkit was used for variant calls, whereas the SnpEff software was used to annotate the variants.

Results

The family consists of nonconsanguineous parents with normal hearing and 10 children, 5 of them having hearing impairment diagnosed in childhood (Fig. 1). The proband presenting to the clinic was a 19-year-old girl with an audiogram showing bilateral moderate sloping to severe sensorineural hearing loss at high frequencies (Fig. 1). Hearing loss was nonprogressive. Audiometric analysis of all affected individuals showed a mild-to-moderate hearing sensitivity in the low frequencies sloping to moderate-to-severe sensorineural hearing loss in the high frequencies.

Exome sequencing for individuals I-1, I-2, II-3, II-6, II-7, II-8, and II-10 yielded an average total yield of $\sim 6 \times 10^9$ bp reads, with an average throughput depth of 131. From a total number of around 70,000 single-nucleotide polymorphisms and 6000 insertions/deletions (Indels), we only analyzed those occurring in the coding regions of the genes. We started by filtering out all variants with a minor allele frequency >0.05, and we focused on those 87 genes used nowadays for clinical diagnosis of CHL (OtoGenome, 2016). The results did show that a previously described *OTOGL* variant segregates with the phenotype in the family. The chr12:80672818 C > T variant leading to a premature termination of protein synthesis p.Arg925* (NM_173591), was found on one allele in the parents, and on both alleles in the affected patients. The results were confirmed by Sanger sequencing of exon 25 of *OTOGL*. All affected individuals bear the homozygous mutation, whereas healthy individuals are either heterozygous or do not carry the mutated allele (Fig. 2).

Discussion

Over the past two decades, tremendous success has been achieved in the search of pathogenic genes causing NSHL. More than 60% of NSHL cases occurring before childhood have been correlated with at least one genetic mutation (Morton and Nance, 2006). NSHL is a clinically heterogeneous disorder, involving different patterns of inheritance, including autosomal dominant, autosomal recessive, X-linked, and mitochondrial. The majority of cases follow an autosomal recessive pattern and a total of 80 loci have already been mapped and 55 genes identified (Gu *et al.*, 2015).

TABLE 1. THE DIFFERENT MUTATIONS IN *OTOGL* ASSOCIATED WITH CONGENITAL HEARING LOSS

Mutation	*Protein change*	*Exon*	*Age of onset*	*Hearing loss severity*	*Ethnicity*	*References*
c.1430delT	p.Val477Glufs*25	15	Unknown	Moderate	Turkish	Yariz *et al.* (2012)
c.547C>T	p.Arg183*	7	Congenital	Moderate	Dutch	
c.5238 + 5G>A		IVS43				
c.6467C>A	p.Ser2156*	53	7 and 9	Moderate	Chinese	Gu *et al.* (2015)
c.6474dupA	p.Ser2159Metfs*2	53	7 and 9			
c.1558C>T	p.Gln520*	15	5	Mild to moderate	French	Bonnet *et al.* (2013)
c.2773C>T	p.Arg925*	25	Unknown	Mild to moderate	Syrian	This study

Several extracellular matrix proteins encoded by *TECTA*, *COL11A2*, *COCH*, *OTOA*, *OTOG*, *OTOGL*, and *STRC* have been recently studied and associated with different degrees of hearing loss and various clinical scenarios (Hilgert *et al.*, 2009).

In this study, we have identified a specific mutation, p.Arg925*, of the *OTOGL* gene causing mild-to-moderate hearing loss in a nonconsanguineous Syrian family.

Shahin *et al.* first identified *OTOGL* in 2010 as a homologous gene to *OTOG* (Shahin *et al.*, 2010). With 58 exons on 12q21.31, the gene encodes a 2344 amino acid protein, called otogelin-like, which comprises an epidermal growth factor (EGF)-like domain, four von Willebrand factor (VWF) domains, four cysteine-rich domains (C8), four trypsin inhibitor-like (TIL) domains, an alpha-L-arabinofuranosidase B domain, and a C-terminal cysteine-knot domain (Yariz *et al.*, 2012; Bonnet *et al.*, 2013; Gu *et al.*, 2015). Otogelin-like and otogelin, the protein products of OTOG, have been found to share 33.3% homology in their respective amino acid sequences. Otogelin also contains four VWF and C8 domains, and a C-terminal cysteine-knot domain, but has only one TIL domain (Cohen-Salmon *et al.*, 1999).

Both proteins have been recently implicated in the development of NSHL, hence identifying *OTOG* and *OTOGL* as novel human deafness genes (Simmler *et al.*, 2000a; Yariz *et al.*, 2012; Schraders *et al.*, 2012; Oonk *et al.*, 2014).

Otogelin and otogelin-like proteins are both detected by immunolocalization in all three acellular membranes of the inner ear, including the otoconial membrane and cupulae in the vestibular end organs, and the tectorial membrane in the cochlea (Yariz *et al.*, 2012). Their exact functions are still equivocal, but they have been found to contribute to the structural stability and strength of the extracellular matrix whose role is essential for the propagation and detection of sound within the cochlea (Gu *et al.*, 2015). Loss of function of *OTOGL* in zebrafish leads to sensorineural hearing loss and anatomical changes within the inner ear, but we still do not have a mouse model to recapitulate the human phenotype (Yariz *et al.*, 2012).

To date, seven *OTOGL* mutations in four families with different ethnicities have been identified in the literature (Table 1). All seven known mutations followed an autosomal recessive pattern resulting in a nonsense mutation that presumably led to a total loss of expression of the protein. Interestingly, phenotypic characteristics of all seven mutations shared a moderate severity of sensorineural hearing loss, at different ages of onset, as is our case with relatively a homogenous pattern among all members of the family. Bonnet *et al.* (2013) first identified the p.Arg925* mutation in a French family and, in their study, the proband had a mild-to-moderate hearing loss. They believed that was the result of a biallelic nonsense mutation since they found two different mutations in the *OTOGL* gene sequence: a nonsense mutation in exon 15 (c.1558C>T; p.Gln520*) and another one in exon 25 (c.2773C>T; p.Arg925*). The father and the mother were both heterozygous for the p.Arg925* and p.Gln520* mutations, respectively (Bonnet *et al.*, 2013). In this report, we document the first biallelic mutation for the p.Arg925* variant with a similar phenotype to the previously published finding confirming the deleterious effect of both mutations and arguing for a common pathway that involves messenger RNA decay due to a premature stop codon.

In conclusion, we present the first case of an *OTOGL* variant as responsible for a familial nonconsanguineous hearing loss case in the Middle East, and we propose to include this gene in any diagnostic targeted exome screen.

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Author Disclosure Statement

No competing financial interests exist.

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