Screening Consanguineous Families for Hearing Loss Using the MiamiOtoGenes Panel

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Background: Hearing loss (HL) is one of the most common and genetically heterogeneous sensory disorders in humans. Genetic causes underlie 50–60% of all HL and the majority of these cases exhibit an autosomal recessive model of inheritance.

Methods: In our study, we used our targeted custom MiamiOtoGenes panel of 180 HL-associated genes to screen 23 unrelated consanguineous Iranian families with at least two affected children to identify potential causal variants for HL.

Results: We identified pathogenic variants in seven genes (*MYO7A*, *CDH23*, *GIPC3*, *USH1C*, *CAPB2*, *LOXHD1*, and *STRC*) in nine unrelated families with varying HL profiles. These include five reported and four novel mutations.

Conclusion: For small consanguineous families that were unsuitable for conventional linkage analysis the employment of the MiamiOtoGenes panel helped identify the genetic cause of HL in a cost-effective and timely manner. This rapid methodology provides for diagnoses of a significant fraction of HL patients, and identifies those who will need more extensive genetic analyses such as whole exome/genome sequencing.

Keywords: panel screening, hearing loss, consanguineous

Introduction

HEARING LOSS (HL) is a common sensorineural disorder in humans in the United States and ~70% is congenital with an incidence of 1.9 in 1000 or ~1 in 500 in newborns (Morton, 1991; Smith *et al.*, 2005). It is estimated that at least 50–60% of all cases are due to genetic causes in industrialized nations. Genetic non syndromic hearing loss (NSHL) can exhibit autosomal dominant, autosomal recessive (AR), X-linked, and mitochondrial inheritance. There is also a single report of a Y-linked locus (Wang *et al.*, 2013). More than 180 loci have been mapped for HL and genes have been identified for >100 of these. Approximately 63% of these genes cause autosomal recessive (AR) NSHL (Morton, 1991; Smith and Hone, 2013). With the development of next-generation sequencing (NGS), identifying genes underlying various genetic disorders has

become relatively easier, particularly for heterogeneous Mendelian conditions such as HL (Gilissen *et al.*, 2012). NGS has increased identification of genetic causes for small families where linkage significance is not attainable using conventional approaches and reduced the cost of sequencing the coding regions of the genome. In this study, we have utilized a time and cost-effective targeted sequencing panel (MiamiOtoGenes panel) consisting of 180 genes that have been reported to underlie syndromic and/or nonsyndromic forms of HL (Tekin *et al.*, 2016; Yan *et al.*, 2016). This is part of a powerful pipeline to screen for known variants or identify novel rare variants in patient cohorts and paves the way to molecular diagnostics. In this study, we were able to identify pathogenic variants in nine unrelated families with varying HL profiles in the following genes—*MYO7A* (OMIM#: 276903), *CDH23* (OMIM#: 605516), *GIPC3* (OMIM#: 608792), *USH1C*

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(OMIM#: 605242), *CAPB2* (OMIM#: 607314), *LOXHD1* (OMIM#: 613072), and *STRC* (OMIM#: 606440).

Materials and Methods

Ethical compliance

Written informed consent was obtained from participants of the 23 Iranian families ascertained (consent from parents was obtained in the case of minors under the age of 18). The study was approved by the local Institutional Review Board at the University of Miami and Kerman University of Medical Sciences, Kerman, Iran. Clinical evaluation and sample collection were done as previously described (Diaz-Horta *et al.*, 2014).

Clinical evaluation of subjects

Affected individuals were evaluated for their hearing by Pure-tone audiometry or auditory brainstem response at varying frequencies of 0.25, 0.5, 1, 2, 4, and 8 kHz. HL severity was categorized by decibel (dB) loss: normal (≤ 25 dB), mild (25–40 dB), moderate (40–60 dB), severe (60–80 dB), and profound (>80 dB). Affected members were examined for other phenotypes as previously described (Diaz-Horta *et al.*, 2014). Only families with individuals with clearly nonsyndromic HL were chosen for the study. The clinical information on probands (and available siblings) is summarized in Supplementary Table S1.

Panel sequencing and data analysis

The MiamiOtoGenes panel (Supplementary Table S2) and the data analysis pipeline to generate variant call format (VCF) files were designed as previously described (Yan *et al.*, 2016). VCF files were annotated using the program KggSeq (Li *et al.*, 2017). The resulting variants were primarily filtered based on homozygosity of the affected, minor allele frequencies, conservation, and pathogenicity; we also checked for heterozygous biallelic variants. Variant validation was done as described previously (Yan *et al.*, 2016). Sequence chromatogram analysis was performed using the Lasergene SeqMan Pro™ Version 7.1.0 software (DNAS-TAR, Madison, WI).

Results

Potential homozygous causal variants were identified in 9 of the 23 families (Table 1). The variants co-segregated (Fig. 1) with the HL phenotype under an AR mode of inheritance with affected individuals homozygous for the variant and parents heterozygous; unaffected siblings were either heterozygous or homozygous for the common allele. A pathogenic missense transition (c.5237G>A; p.R1746Q), previously reported in a Cuban family (Bolz *et al.*, 2001), was found in family KF-3 in *CDH23* (NM_022124.6; NP_071407.4). A homozygous variant (c.628A>T; p.K210X) in *USH1C* (NM_153676.4; NP_710142.1), previously reported in a Turkish proband (Bademci *et al.*, 2016), was identified in family KF-4. A previously reported stopgain variant (c.4936C>T; p.R1646X) (Wesdrop, 2017) in *LOXHD1* (NM_144612.6; NP_653213.6) was identified in family KF-18. A previously reported splice variant (Schrauwen *et al.*, 2012) c.637+1G>T in *CABP2* (NM_016366.3;

TABLE 1. LIST OF POTENTIAL CAUSAL VARIANTS IDENTIFIED IN NINE FAMILIES IN THIS STUDY

Family ID	Chromosome position	rsID	Gene	Exon position	CDS	AA change	MAF	SIFT	PolyPhen2	Pathogenicity	Novel/reported
KF-2	Chr11:76871318	.	<i>MYO7A</i>	Ex 11/49	c.1190C>T	p.A397V	0	0	0.989	Predicted pathogenic	Novel
KF-5	Chr11:76924081	.	<i>MYO7A</i>	Ex 47/49	c.6438+1G>T	Exon47GTdonor	0	-	-	Predicted pathogenic	Novel
KF-22	Chr11:76890169	.	<i>MYO7A</i>	Ex20/49	c.2361C>A	p.Y787X	3.2E-05	-	0.624	Pathogenic	Reported
KF-3	Chr10:73539073	rs111033270	<i>CDH23</i>	Ex 39/68	c.5237G>A	p.R1746Q	6.53E-05	-	-	Pathogenic	Reported
KF-4	Chr11:17547940	.	<i>USH1C</i>	Ex 8/27	c.628A>T	p.K210X	8.13E-06	-	0.965	Pathogenic	Reported
KF-15	Chr19:3586512	.	<i>GIPC3</i>	Ex 2/6	c.245A>G	p.N82S	4.07E-05	0.018	-	Predicted pathogenic	Novel
KF-16	Chr11:67287263	rs149712664	<i>CABP2</i>	Ex 6/7	c.637+1G>T	Exon6GTdonor	1.114 E-03	-	-	Predicted pathogenic	Reported
KF-18	Chr18:44102213	.	<i>LOXHD1</i>	Ex 32/40	c.4936C>T	p.R1646X	1.64E-05	-	-	Pathogenic	Reported
KF-TP	Chr15:43896963	.	<i>STRC</i>	Ex 20/29	c.4012C>T	p.R1338X	2.44-05	-	-	Predicted pathogenic	Novel

“.” indicates unavailable rsID and “-” indicates unavailable scores for SIFT and Polyphen2. CDS, coding sequence; MAF, minor allele frequency; SIFT, sorting intolerant from tolerant.

FIG. 1. Family pedigrees and sequencing results. This figure shows the pedigrees and sequence analysis in families of recessive inheritance with validated potential causal variants that segregates with HL (KF-2 through KF-TP). Family members whose DNA sample was available were sequenced (genotypes indicated). *Shaded squares or circles* indicate affected individuals; *unshaded* indicates unaffected individuals. The *dashed boxes* indicate the location of the variant. HL, hearing loss.

FIG. 1. (Continued)

NP_057450.2) co-segregated with HL in family KF-16. *MYO7A* (NM_000260.4; NP_000251) was found to be the genetic cause of HL in three families. A previously reported pathogenic variant in an Iranian family, *MYO7A* (c.2361C>A; p.Y787X), was identified in KF-22 (Shang *et al.*, 2018). A novel missense variant c.1190C>T (p.A397V), predicted to be pathogenic, was identified in *MYO7A* in KF-2. In family KF-5, a novel homozygous splice variant c.6438+1G>T in *MYO7A*, predicted to be pathogenic, was identified. In family KF-15, a novel homozygous missense variant (c.245A>G; p.N82S) in *GIPC3* (NM_133261.3; NP_573568.1), predicted to be pathogenic, was identified. In family KF-TP we identified a novel stopgain variant in *STRC* (c.4012C>T; p.R1338X) (NM_153700.2; NP_714544.1), which is predicted to be pathogenic.

Discussion

Deafness is a genetically heterogeneous sensory disorder as there are >180 genes that can result in nearly identical phenotypes. In addition, a gene may present as syndromic or nonsyndromic or may exhibit multiple modes of inheritance. Screening families for potentially pathogenic mutations can help identify carriers, assess and understand the risk for future generations and develop genotype–phenotype correlations (Chong *et al.*, 2015). Studying the functional impact of these pathogenic mutations could help develop personalized therapeutic treatment. In addition, discovering more mutations, particularly in understudied populations, helps to extend the genetic epidemiology of HL. Iran's second most prevalent disability is HL (Mahideh *et al.*, 2010). In this study, we have identified pathogenic/likely pathogenic variants as the cause of NSHL in 39% (9/23) of the Iranian families using the MiamiOtoGenes panel. In communities with a high rate of consanguineous marriages, there is a higher probability for homozygosity of rare pathogenic variants; 40% (Joghtaei *et al.*, 2004) of Iranian marriages are consanguineous and they are estimated to reach 65% in the near future (Amirsalari *et al.*, 2014). Three families in our study have homozygous variants in the *MYO7A* gene, a motor protein that plays an important role in differentiation, morphogenesis, and organization of cochlear hair cell bundles. The splice variant c.6438+1G>T in KF-5 located in the FERM domain of the protein that binds membranes or tails of integral membrane proteins is predicted to create an exonic splicing silencer (ESS) site in exon 47 causing alternative splicing. The missense variant c.1190C>T (p.A397V) in family KF-2 is located on the surface of the upper 50 kDa domain in the cleft of the motor domain that helps carry the protein along the actin filaments. Previously, a pathogenic change at the same site, c.1190C>A (p.A397D), has been shown to have a negative impact on the transduction pathway between the ATPase active site and actin-binding interface (Adato *et al.*, 1997; Watanabe *et al.*, 2008). In KF-22, a homozygous stopgain mutation, c.2361C>A (p.Y787X), is in the IQ motif that binds calmodulin. In KF-3, a missense variant (c.5237G>A; p.R1746Q) in *CDH23*, a cadherin protein required to maintain the proper organization of the stereocilia bundle, was identified; this variant causes an amino acid change between the EC16 and EC17 repeats in the linker domain of the protein. This has been reported in patients of Cuban,

American, and Irish origin presenting with atypical Usher syndrome phenotypes with profound congenital HL (Astuto *et al.*, 2002; Bademci *et al.*, 2016). The affected members of KF-3 also presented with congenital HL but at the time of examination exhibited no features suggesting Usher syndrome. Patients homozygous for R1746Q have been reported show symptoms of “atypical Usher syndrome” with variable retinal phenotypes (Bolz *et al.*, 2001; Astuto *et al.*, 2002). In the Iranian population, 13 missense and 2 splice *CDH23* mutations have been identified (Sloan-Heggen *et al.*, 2015; Bademci *et al.*, 2016; Ghasemnejad *et al.*, 2017; Shang *et al.*, 2018). The stopgain variant *USH1C* c.628A>T (p.K210X) in KF-4 has been previously observed only in a Turkish family (Bademci *et al.*, 2016). *USH1C* encodes a PDZ domain-containing protein, harmonin, that participates in the organization of macromolecular complexes associated with specific domains of the plasma membrane (Ouyang *et al.*, 2002). Three *USH1C* mutations have been reported in the Iranian population (Sloan-Heggen *et al.*, 2015). In family KF-16, we found a reported splice variant (c.637+1G>T) in *CABP2*, which belongs to a family of Ca²⁺ binding proteins (Schrauwen *et al.*, 2012). It is known that CaBPs modulate cell signaling through Ca²⁺ gated channels. Three other mutations in this gene have been reported in the Iranian deaf population (Sloan-Heggen *et al.*, 2015). Although this mutation has been shown to cause skipping of exon 6 by an exon trapping study (Schrauwen *et al.*, 2012), another study has called into question the clinical significance of this variant (DiStefano *et al.*, 2018). Hence, further functional studies need to be done to confirm the variant's predicted pathogenicity. In KF-18, we found a reported variant in *LOXHD1* (c.4936C>T; p.R1646X) in the catalase core domain, which functions to break down hydrogen peroxide to water and oxygen molecules to protect cells from toxic effects. This gene has been associated with DFNB77 (Wesdrop, 2017). In family KF-15, a novel homozygous missense variant (c.245A>G; p.N82S) in *GIPC3*, required for postnatal maturation of the hair bundle and long-term survival of hair cells and spiral ganglion in the ear, was identified. Four other *GIPC3* mutations have been previously reported in the Iranian population (Sloan-Heggen *et al.*, 2015; Asgharzade *et al.*, 2018). Family KF-TP has a stopgain mutation in the *STRC* (c.4012C>T; p.R1338X) gene that encodes for stereocilin, an extracellular protein that localizes in the outer and inner hair cell stereocilia and is necessary for the positioning of the stereociliary tips (Verpy *et al.*, 2011). To confirm that the mutation was indeed located in the *STRC* gene and not the pseudogene *STRCP1*, we followed a previously described strategy (Francey *et al.*, 2012) and compared the genomic sequences. Indeed, the sequences do not show 100% homology between exons 16 and 29, the region where the variant was detected in this study (exon 20), confirming that the change is in the actual gene.

In this study, we identified potential causal variants, four novel and five reported, in 9 out of the 23 unrelated families we ascertained. This suggests that the panel is a helpful screening tool for identifying known mutations, even when studying new populations. Despite no significant differences in sequencing coverage (Supplementary Fig. S1) or the bioinformatics pipeline, we had an ~40% success rate in identifying causal variants, whereas ~60%

of our cases remain unsolved. The mating structure of the population (frequent inbreeding) makes it possible for HL to be the result of very rare variants. In the past, a comprehensive study to detect the cause of HL in the Iranian population has also shown a success rate of ~40%; the study showed the genetic diversity associated with a high rate of inbreeding in the population (Sloan-Heggen *et al.*, 2016). Twenty-six genes that had not been previously reported as a cause of ARNSHL in Iran were also identified, suggesting a need to expand the genetic search area beyond the known and associated HL genes, especially considering the ethnic diversity (Sloan-Heggen *et al.*, 2015). This opens an avenue to discover numerous undiscovered HL genes within the Iranian population. Therefore, our next step will be to investigate those families using whole exome sequencing.

Authors' Contributions

Abhiraami Kannan-Sundhari participated in the design of the study, data generation and analysis, interpretation, and validation, and is also responsible for the preparation of this original article. Denise Yan participated in revising the article critically for important intellectual content. Xue Zhong Liu and Susan H. Blanton participated in the design, interpretation and overall supervision of the study as well as critical revision of the article. Drs. Saeidi and Sahebzamani are from Kerman Institute of Medical Sciences in Iran, where research and education are the primary functions.

Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Figure S1

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