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RESEARCH NOTE

Single gene variants causing deafness in Asian Indians

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Abstract. Congenital deafness is one of the common disorders, with some common genes accounting for most of the cases. One in 1000 children are born with sensorineural hearing loss, and of that 50% are hereditary. In the Mediterranean Europeans, 80% of the nonsyndromic recessive deafness is due to homozygous mutation in *GJB2*, the 35del G allele. In Western population, the *GJB2* variation have been found in up to 30–40% cases. In Indians, the *GJB2* variants have been found in up to 20% cases, mostly from central and southern India. In the present study, DNA was extracted from blood using standard methods. This was used to perform targeted gene capture using a custom capture kit. Multiple genes causing deafness were sequenced by next-generation sequencing to mean > 80–100x coverage on Illumina sequencing platform. We found variants in *GJB2*, *WFS1*, *FGF3*, *EYA4*, *MYO7A* and *CHD7* genes. Most of these variants were pathogenic and novel, and possibly causative. Deafness is most commonly due to the autosomal dominant genes but in severe cases of early onset deafness, autosomal recessive genes may contribute in our population. In selected families of severe prelingual deafness, prenatal diagnosis can be done.

Keywords. *CHD7* gene; *GJB2* gene; hearing loss; next-generation sequencing; nonsyndromic hearing loss.

Introduction

Congenital deafness is one of the most common birth defects, with an incidence of one in 1000 newborns either presenting unilateral or bilateral sensorineural hearing loss. Most of these cases are attributed to genetic factors and the rest to acquired/environmental causes. Prelingual is the most severe form of deafness and autosomal recessive transmission accounts for over 50% of the cases (Petit 1996). Deafness can be of autosomal dominant, autosomal recessive, X-linked or even mitochondrial. The inheritance pattern among the disorders with prelingual nonsyndromic deafness is 80% autosomal recessive, 20% autosomal dominant, and 1–1.5% X-linked, or mitochondrial. Congenital deafness is most often autosomal recessive and nonsyndromic. The most common cause of severe-to-profound autosomal recessive nonsyndromic deafness in most populations is the mutation in *GJB2* gene (Maheshwari *et al.* 2003). However in Indians, *GJB2* variants have been found in around 20% cases (Mishra *et al.* 2018). Some mitochondrial gene variants have also been linked to nonsyndromic hearing loss (NSHL). The aetiology of deafness is genetically extremely heterogenous. Thus, it is important to establish a genetic diagnosis of deafness for the patients and their families. The advent of next-generation sequencing (NGS) technology is an emerging powerful application in discovery of new gene involved in congenital deafness and it is further characterized. A targeted genomic approach was used for diagnosis (Yan *et al.* 2016) to capture and sequence of all the coding exons and flanking introns of 120 known, and candidate genes for nonsyndromic and syndromic forms of deafness based on previous literature. NGS has the advantage of studying multiple genes by massive parallel sequencing. Hence, it is used mainly in disorders where multiple genes are involved in causation of similar phenotypes.

Materials and methods

Cases and clinical evaluation

Ten patients with deafness from unrelated families who came to the genetic clinic of a tertiary care centre for preconceptional genetic counselling were included in this study

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with appropriate informed consent. The clinical assessments of all the patients were accomplished by both an otolaryngologist and a clinical geneticist. The preliminary evaluations included a detailed physical examination; audiologic evaluation was performed including otoscope examination, tympanometry, and pure-tone audiometry (PTA). The temporal bone computed tomography (CT) scan was also performed for diagnosis of enlarged vestibular aqueduct (EVA) or inner ear malformation. The family members were also counselled for follow-up segregation analysis. Blood was collected from the patients and family members whenever available and genomic DNA was extracted from peripheral leukocytes by standard protocols using Qiagen kit method (Qiagen, The Netherlands).

Targeted sequencing and bioinformatics analysis

NGS was performed covering all possible target genes using capture system as per the manufacturer's standard protocols. The libraries were sequenced to mean >80–100x coverage on Illumina sequencing platform. The sequences obtained were aligned using BWA program to human reference genome and analysed using GATK v. 3.6 and Picard to identify variants significant to the clinical suggestion. The variants annotation was performed using VEP program alongside the Ensembl release 87 human gene model. Clinically significant variations were annotated comparing with published variants in literature and a set of disease or mutation databases: ClinVar, HGMD, OMIM and SwissVar (Smith and Jones 2016). Common variants were filtered based on allele frequency in 1000 Genome Phase 3, dbSNP, ExAC, 1000 Japanese Genome and our Indian population reference databases. Nonsynonymous variants effect was calculated using multiple algorithms such as SIFT, PolyPhen-2, Mutation Assessor MutationTaster2, and LRT. Only nonsynonymous and splice site variants found in the targeted panel were used for further clinical interpretation and subsequent segregation analysis. Consent was taken from the patients/guardian for mutation testing and the testing was done as per the declaration of Helsinki.

Results

In the patients, with syndromic and nonsyndromic deafness, we found a few pathogenic variants and some novel variants. It was interesting to note that some variants identified in genes were associated with syndromic hearing loss. The details of the variants identified in our study are provided in table 1. Patient 1 had deafness identified in later childhood (table 1).

Discussion

The common cause of autosomal recessive deafness is due to *GJB2* gene mutations. The carrier frequency of *GJB2* variants is 3% in Europeans and Americans, similar to that of cystic fibrosis (Cohn and Kelley 1999). However, *GJB2* (Connexin 26) gene mutations were seen only up to 20% of nonsyndromic deafness in the Indian population (Padma

Table 1. Details of the variants identified in our study through targeted next-generation sequencing.

Patient	Age/sex	Gene/zygosity	Variant	Interpretation
1	24/M	*GJB2* heterozygous	Exon1 Gln124* c.370C>T	Damaging by LRT and Mutation Taster 2
2	5/M	*FGF3* homozygous	c.534C>G p.Phe178Leu	Michel aplasia (inner ear) likely pathogenic
3	31/F	*EYA4* heterozygous	Intron 3 c.84-2A>G	Splice site variant deafness 10
4	29/F	*MY07A* heterozygous	Exon16 delGCT p.Leu621del (Inframe deletions)	Usher syndrome type1
5	26/M	*CHD7* heterozygous	c.6163C>G p.Leu2055Val	Likely pathogenic CHARGE syndrome
6	36/F	*ESPN* heterozygous	c.205A>C p.Asn69His	VUS neurosensory deafness
7	26/M	*MARVLED2* homozygous	1331+2T>C splice junction variants	Pathogenic nonsyndromic hearing loss
8	30/F	*WFS1* heterozygous	c.577A>C p.Lys193Gln	VUS congenital deafness
9	30/M	*WFS1* heterozygous	c.1228-1231del CTCT p.Val412Serfs*29	Congenital deafness
10	28/M	*GJB2* homozygous	Exon2 c.231G>A p.Trp77*	Pathogenic variant deafness 1A

et al. 2009; Mishra *et al.* 2018). In the present study, one patient showed a heterozygous missense variation in the *GJB2* gene c.370C>T. In heterozygous state, *GJB2* variants were also found in late onset deafness with some preserved speech. In one of the patients (table 1) the Gln124* variant in the *GJB2* gene had a minor allele frequency of 0.02% and 0.01% in the 1000 genomes and the ExAC databases, respectively. The *in silico* prediction of the variant was damaging by LRT and Mutation Taster2.

The other three gene variants identified in the present study were present in *FGF3*, *EYA4* and *CHD7* genes. *FGF3* gene mutations are associated with Michel aplasia. The *FGF3* variant has not been reported in the 1000 genome database and has an allele frequency of < 0.002% in ExAC database. The variant has been reported earlier in one Indian patient, 13 years old, diagnosed with LAMM syndrome (Singh *et al.* 2014). On *in silico* analysis, the *FGF3* variant c.534C>G (p.Phe178Leu) was found damaging on SIFT, LRT and disease causing on MutationTaster2. Some patients with their gene mutations in homozygous state can also have microtia and microdontia. Microdeletion in chromosome 11q13 involving the *FGF3* gene also causes deafness and dental dysplasia. Another gene variant which we have found in one patient was *CHD7:*c.6163C>G (p.Leu2055Val) which is associated to coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities and ear abnormalities syndrome (Hale *et al.* 2016). The patient with this syndrome manifest with cardiac defect, renal anomalies and hearing loss. The patient 5 with this *CHD7* variant also had hypogonadotropic hypogonadism. Thus, in some patients, we may get milder phenotypes in case of syndromic deafness.

The two variants in the *MYO7A* gene reported here are not described in the 1000 genome and ExAC databases. The termination mutation in exon 4 leads to premature truncation of the protein and we found damaging by LRT and disease causing via Mutation Taster 2. The exon 16 variant leads to in-frame deletion and is in the motor domain of the protein. The region is conserved across species and predicted to be damaging on Mutation Taster2. The myosins are actin proteins which have been classified as class II MYO which are conventional myosins and unconventional myosins which include the other class of myosins. They have a critical role in production of adenosine triphosphate for cytoskeleton movement and are important in the hearing process especially in cochlea in human ear; and are associated with NSHL (Jajjo *et al.* 2007). The *MARVELD2* variant found in one of our patients −1331+2T>C has been previously reported in nonsyndromic deafness (Nayak *et al.* 2015). A recurrent allele c.1331+2T>C has been found to cause autosomal recessive deafness in Pakistani families. The human *MARVELD2* gene is located on chromosome 5q13.2 and is one of the integral membrane proteins, concentrated in TJ strands of inner-ear epithelia and has an important role in the formation of epithelial barrier.

The heterozygous splice site variation in the E*YA4* gene c.84-2A>G affects the AG acceptor splice site of exon 4 of the gene on *in silico* analysis. *EYA4* gene variants are associated with postlingual sensorineural hearing loss inherited in an autosomal dominant manner. In some patients, *EYA4* gene variants may also lead to cardiomyopathy (Liu *et al.* 2015). We found *WFS1* variants in two patients with deafness in the targeted exome panel. *WFS1* variation in heterozygous state cause congenital deafness; but in homozygous state it leads to Wolfram syndrome (Cryns *et al.* 2002). Thus, in severe cases of hearing loss or early onset deafness, genetic factors are likely and should be evaluated by next-generation sequencing. In families with severe prelingual deafness, especially likely syndromic forms, prenatal diagnosis can be planned in next pregnancy.

Since *GJB2* and *GJB6* variants are less common in Indians, further testing of other genes is necessary for planning in family (Padma *et al.* 2009; Adhikary *et al.* 2015). Identification of several pathogenic variations and some novel variations specifies that customized gene targeted sequencing is highly effective for clinical and population based genetic studies of heterogeneous malformations. This can further provide genotype–phenotype correlation and may enable the severity of disease prediction and identify additional genes and phenotypes. Hence, it is imperative to diagnose and manage in a well-timed and proper manner.

In conclusion, early identification of the cause of hearing loss can help in planning appropriate interventions for improving the hearing outcomes. In some countries, new born screening (NBS) is done for congenital deafness. With advancement in genetic technologies and identification of common variants in different genes in the population, DNA based screening may be possible for severe prelingual deafness. Additionally, our findings in the patients add to the mutation spectrum in the background population and provide more information on planning prenatal diagnosis and better patient counselling as well.

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