Molecular etiology of hereditary deafness using next generation sequencing in northwest China

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Research

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Abstract

Objective: To analyze the molecular etiology of 92 hereditary deafness families and explore the genetic mechanism of newly identified genes in deafness heritability.

Methods: We analyzed the medical history, audiology, imaging, and physical examination data of 92 probands and their family members. Probands were selected from the hereditary deafness database; they did not have any of the common genetic mutation sites. Genomic DNA was extracted from blood samples and next generation sequencing was performed on an Illumina platform, followed by co-segregation analysis of family members. The control group included the clinical data and blood samples from 207 normal hearing people.

Results: Among the 92 samples, 30 homozygous variants were identified in 29 autosomal recessive hereditary deafness families, including 6 reported mutations and 26 novel mutations. Among them, *MYO15A* was the most frequently detected (6/92), followed by mutations in *CDH23*, *OTOF*, *FGF3* (3/92 each), *MYO7A*, *SLC26A4*, *MYO6* (2/92 each), *BSND*, *CLDN14*, *DFNB59*, *ILDR1*, *LHFPL5*, *LRTOMT*, *TMPRSS3*, *TPRN*, *USH1C*, and *LOXHD1 (1/92 each).*

Conclusion: In patients with autosomal recessive deafness, the *MYO15A*, *CDH23*, *OTOF*, and *FGF3* genes could be used as candidate genes for conventional genetic studies in northwest China.

1. Introduction

Hearing impairment is one of the most common sensory disorders, affecting 350 million people worldwide, equivalent to 5% of the world's population (WHO, 2017). It has been estimated that 50%-60% of deafness is related to hereditary causes[1]. For both congenital or prelingual deafness, genetic factors are considered to be the dominant etiology[2], especially for those with a family history. Approximately, 70% of the hereditary deafness cases can be classified as non-syndromic hearing loss (NSHL), for which hearing loss is the only phenotype[3]. In recent years, many gene variations have been confirmed to be related to deafness. The latest studies have shown that about 600 gene loci distributed on the 23 pairs of human chromosomes are related to the occurrence of hereditary deafness; among them, more than 150 loci and over 100 genes have been reported to be involved in NSHL (http://www.hereditaryhearingloss.org). In fact, deafness is a highly heterogeneous disorder and mutations in these genes do not occur at the same frequencies across ethnicities. Although significant progress has been made in the identification of specific genes and mutations that contribute to deafness, genetic variation hotspots and rates of appearance of common deaf-causing genes differ enormously from locus to locus as well as from population to population. Therefore, there is an urgent need for testing our population, searching for new loci and genes that might cause deafness.

Since the early 1990s, Sanger sequencing has been widely used to identify the etiology of deafness by testing known genes and mutations. But this method is cumbersome and inefficient; being its greatest hindrance that it analyses a limited number of sites. In contrast, next-generation sequencing (NGS), also known as high-throughput sequencing, is an innovative high-throughput DNA sequencing technology that was developed by optimizing Sanger sequencing. The global advantages of NGS, relative to Sanger sequencing, include the following[4]: (i) *in vitro* construction of a sequencing library, bypassing several bottlenecks that restrict the parallelism of conventional sequencing; (ii) compared with conventional capillary-based sequencing, array-based sequencing enables a much higher degree of parallelism; and (iii) array features are immobilized on a planar surface and can be enzymatically manipulated by a single reagent volume, reducing the cost of DNA sequence production.

Using NGS methods, it should be possible to find new genes related to deafness, providing with comprehensive genetic information about the disease and changing genetic counseling, while providing the basis for precision medicine of deafness. The emergence of NGS techniques has accelerated the discovery of new causative genes and illustrated the inheritance mechanisms of congenital deafness. However, NGS panels have a major limitation, that only known genes can be tested. Therefore, new disease-causing genes are constantly being identified and added to the panels to improve the analysis[5] .

In this study, we recruited 92 probands with similar phenotypes who had not been diagnosed by any common genetic test and performed NGS analyses to determine new candidate deafness-causing variants. Subsequently, we used Sanger sequencing to verify these variants.

2. Materials And Methods

2.1. Ethics approval and consent to participate: This study was approved by the Committee of Medical Ethics of Lanzhou University Second Hospital. Written informed consent was obtained from all the participants or the next of kin on behalf of the minors/children participants involved in this study.

2.2. consent for publication Consent for publication was obtained from all the participants or the next of kin on behalf of the minors/children participants involved in this study.

2.3. Subjects: From September 2013 to December 2016, we collected clinical data and peripheral blood samples from 92 probands with no detectable *GJB2*, *SLC26A4*, or mitochondrial DNA 1555 and 1494 mutation sites. Clinical evaluations were completed by both an otorhinolaryngologist and a clinical geneticist, including otoscopic examination, visual reinforcement audiometry, tympanometry, acoustic reflex, pure-tone audiometry or play audiometry, distortion product evoked otoacoustic emissions (DPOAEs), auditory brainstem responses (ABRs), and auditory steady-state response (ASSR). Bilateral air conduction (AC) thresholds were determined at octave frequencies of 0.25-8.0 kHz. The AC average thresholds at conversational frequencies of 0.5, 1, 2, and 4 kHz were measured and used to define the profoundness of the hearing loss. Hearing levels were classified as mild (26–40 dB), moderate (41–70 dB), severe (71–95 dB), or profound (95 dB). A total of 207 clinical data files and blood samples from a normal hearing population constituted the control group.

2.4. Targeted gene capture and high-throughput sequencing: Blood samples from the 92 probands were collected for genetic analysis. Genomic DNA was extracted from each sample using the Blood Genomic Kit, and 246 genes known to be related to deafness (Supplementary Table 1) were detected by targeted NGS using a GenCap kit (MyGenostics, Beijing, China), including 162 known nuclear genes associated with deafness —95 for non-syndromic and 67 for syndromic deafness— and 1 mitochondrial gene. The other 83 were genes associated with deafness that had not been reported in humans but that met the following three conditions: (i) they have been correlated with hearing in animal models; (ii) they are expressed in the inner ear; and (iii) they have been related to mitochondria. Sequencing was carried out on a NextSeq 500 system (Illumina Inc., San Diego, CA, USA) to generate paired-end reads (100 bps at each end), followed by bioinformatics analysis. Sequencing reads were aligned to the reference human genome (NCBI37/hg19) using BWA (Burrows Wheeler Aligner) software package. The SOAP alignment program and the GATK software were used to identify SNPs and InDels (insertions and deletions), respectively. Candidate variants were also evaluated using the NCBI dbSNP, 1000 Genomes, Ployphen, SIFT, and MutationTaster tools. To verify the results from gene chip capture and high-throughput sequencing, primers were designed upstream and downstream of all fragments containing sites of variation putatively responsible for pathogenesis. Then, PCR amplification was done to identify the corresponding genes and their mutations.

3. Results

A total of 92 deaf families were selected as the case group. The age of the probands ranged from 1 to 57 years old, with an average age of 20 years, whereas the onset age varied from 0 to 42 years old, with an average age of onset at 2 years. The sample included 43 male and 49 female patients, a male-to-female ratio of 0.88 to 1. According to the degree of hearing loss, there were 4 cases of moderate deafness, 6 cases of moderate to severe deafness, 7 cases of severe deafness, and 75 cases of profound deafness. According to age of onset, there were 85 cases of prelingual deafness and 7 cases of postlingual deafness. Among probands, 5 belonged to families with dominant deafness, while the remaining had at least one first-degree or second-degree deafness relative, which would be consistent with recessive inheritance. Patients' specific clinical data are shown in Table 1 and Supplementary Table 2.

Among the 207 healthy people constituting the control group, there were 85 males and 122 females.

3.2. Variation identification and validation

The preliminary data obtained from the 246 deaf-causing genes from the 92 samples under study were sequenced using NGS. A total of 1087 variants of 178 genes were found, including 973 non-synonymous variants, 28 nonsense variants, and 86 types of frameshift InDel mutations. Of these, 126 genes are currently known to be associated with deafness in human beings, with a total of 903 mutations; the other 52 genes have been associated with deafness in other species or with mitochondrial function in other diseases. A total of 190 mutations were detected in these 52 genes. Detailed information is given in Supplementary Table 3.

Within the 92 samples, 30 homozygous variants were identified for 29 autosomal recessive hereditary deafness syndromes, 6 of which were already reported, including *CDH23*:c.C719T (p.Pro240Leu); *LHFPL5*:c.C494T (p.Thr165Met); *MYO7A*:c.3696_3706del (p.Arg1232fs); *OTOF*:c.2977_2978del (p.L937delinsFL); *SLC26A4*:c.T716A (p.Val239Asp); and TMPRSS3:c.G727A (p.Gly243Arg). Of the

24 newly discovered mutations, 13 were nonsense or frameshift InDel mutations, whereas 11 corresponded to missense mutations. All these mutations were found to be in protein functional conserved regions by homology alignment. At least one of the results obtained in the Mutation Taster, Polyphen2_HVAR, and SIFT prediction programs was pathogenic. Noteworthy, none of the mutations mentioned were found in any of the 207 normal hearing controls, suggesting that they may alter the structure or function of the encoded proteins, thereby causing deafness. Details are shown in Table 2.

In this study, no pathogenic mutations in the dominant hereditary deafness genes were found.

Overall, our targeted NGS screening identified 30 homozygous variants in 29 autosomal recessive hereditary deafness syndromes. Mutations in *MYO15A* were the most frequently detected (6/92), followed by mutations in *CDH23, OTOF*, *FGF3* (3/92 each), *MYO7A*, *SLC26A4*, *MYO6* (2/92 each), *BSND*, *CLDN14*, *DFNB59*, *ILDR1*, *LHFPL5*, *LRTOMT*, *TMPRSS3*, *TPRN*, *USH1C*, and *LOXHD1* (1/92 each).

4. Discussion

With the rapid development of biotechnology, more than 100 genes causing deafness have been identified and cloned, but there are still a large number of hereditary deafness patients with unknown etiologies. Therefore, it is important to determine the molecular causes of hereditary deafness and reduce the rate of births with this disease. The samples selected in this study were not screened for *GJB2, SLC26A4*, or the 12S rRNA mitochondrial gene (m.1494C>T and m.1555A>G) as in previous works. Here, target sequencing was carried out for those 246 genes that were known or suspected to be related to deafness in order to search for gene variations causing this phenotype. The deafness gene panel used in this study was designed and tested by Hangzhou Lianchuan Biotechnology Co., LTD (LC-Bio); it includes 162 nuclear and mitochondrial genes that have been reported to be associated with deafness and 83 genes that have not yet been reported but may be associated with this disorder. Standard bioinformatics analysis procedures were applied to our results to identify possible gene pathogenic variants, including those rare variants where the candidates were limited to the minor allele frequency (MAF) < 0.5%. Based on the internal data from 207 normal hearing controls, no candidate dominant variant was found. Homozygous variants of alleles were identified only as candidate recessive variants, genotype-phenotype coisolation of family members was performed, and correlation analysis procedures were used to predict the effect of candidate variants on the corresponding proteins.

In this study, our 92 samples were found to carry a total of 1087 variants —including 973 non-synonymous variants, 28 nonsense variants, and 86 types of frameshift InDel mutations— of 178 different genes. From these, 126 genes and a total of 903 mutations were already associated with deafness in human beings. The other 52 genes, with 190 different mutations, have been associated with deafness in other species and with mitochondrial function in other diseases.

In this study, 30 homozygous variants were identified in 29 autosomal recessive hereditary deafness syndromes. In previous studies, 6 of these mutations have been proven to encode for pathogenic variations. We identified 26 novel mutations, from which 15 were either nonsense mutations or frameshift InDel mutations and 11 were missense mutations. Hilgert et al.[6] revealed that the most common deafening genes among European and American populations were *GJB2*, *SLC26A4*, *OTOF*, *MYO15A*, *CDH23*, and *TMC1*. After excluding the three most common genes causing deafness (i.e., *GJB2*, *SLC26A4*and *mtDNA*), Yang et al.[7], analyzing 28 deafness probands in 2013, found that the most frequent variations occurred in the *MYO15A, GPR98, TMC1, USH2A,* and *PCDH15* genes. In 2019, Liu et al.[8] analyzed the incidence of deafness genes in 41 children with non-syndromic deafness who had cochlear implants in Taiwan and found that variations in the *MYO15A*, *TMC1*, *MYH14*, *MYO3A*, *ACTG1*, *COL11A2*, *DSPP*, *GRHL2*, and *WFS1* genes were the most frequent. Our results showed that, in addition to the three common genes causing deafness, the *MYO15A* mutation (6/92) was the most commonly found, followed by *CDH23* (3/92), *OTOF* (3/92), *FGF3* (3/92), *MYO7A* (2/92), and *SLC26A4* (2/92).

The *MYO15A* gene is located in chromosome 17p11.2; it encodes for XVA myosin, a protein with 3530 aa and a molecular weight of 39.5 kDa [9]. The *MYO15A* gene is usually associated with autosomal recessive deafness type 3. After comparing different ethnic groups, *MYO15A* has been considered to be the third or fourth most common deafness gene. Deafness caused by mutations in *MYO15A* are manifested as a congenital profound non-syndromic sensorineural deafness. Studies have shown that *MYO15A* plays an important role in the elongation and development of static cilia and actin filaments, the cohesiveness of the stereocilia also results from the interaction between the *whirlin* and *MYO15A* genes[10]. When a pathogenic mutation occurs in the *MYO15A* gene, ciliary dyskinesia can cause deafness[11]. Rehman Au et al. [12] reported 192 recessive mutations in 49 out of the 67 exons of *MYO15A*. Recently, more than 200 mutations in the *MYO15A* gene were identified. In this study, we found a total of 6 cases of homozygous variation (probands Jx81, Sf61, Sf67, Sf40, Sf88, and Jx50) that were all present in congenital profound deafness, which further confirmed the pathogenicity of *MYO15A* in the population.

CDH23 is located in the long arm of chromosome 10 (10q22.1) (National Center for Biotechnology Information NCBI, gene ID 64072). Some variations in this gene are the pathogenic cause of both autosomal recessive non-syndromic hearing loss (DFNB12) and Usher syndrome 1D (USH1D). It has been reported that *CDH23* is expressed in the stereocilia of the hair cells of the inner ear at the region of the connecting cilia at the junction between the inner and outer segments[13]. Besides, *CDH23* is also harbored in the retina[14] within the calyceal processes of the cone photoreceptors[15]. *CDH23* contains 70 exons that encode for approximately 3354 amino acids and has a cDNA size of 10 kb (https://www.uniprot.org/uniprot/Q9H251). The exons 2-64 encode extracellular repeat (EC) domains, while exon 65 and exons 66-70 encode single-pass transmembrane and cytoplasmic domains, respectively[16]. The main clinical characteristics of DFNB12 are profound sensorineural hearing loss and no retinitis pigmentosa. In turn, USH1D is associated with severe manifestations, including visual problems due to retinitis pigmentosa, congenital profound deafness, and vestibular areflexia[17]. In this study, we identified three novel homozygous candidate mutations c.393G>T (p.Leu131Phe), c.490dupG (p.Ala163fs), and c.1271_1273del (p.424_425del) of *CDH23* in probands Sf48, Jx58, and Sf87. Sanger sequencing of available family members revealed that these mutations were present in all affected family individuals but not in normal individuals. All three patients manifested a congenital profound deafness with no ophthalmic diseases. Therefore, based on a clinical phenotypic characterization combined with each individual's genotype, patients were diagnosed with autosomal recessive non-syndromic hearing loss (DFNB12) caused by *CDH23* mutations.

The *OTOF* gene, encoding otoferlin, is associated with recessive non-syndromic deafness DFNB9 and non-syndromic recessive acoustic neuropathy (NSRAN)[18]. Otoferlin, located in the basolateral region, is mainly expressed in the inner hair cells of the adult mammalian cochlea, participating in afferent synaptic contacts[19]. It was reported that otoferlin is involved in the docking of synaptic vesicles to the plasma membrane, mediating their fusion and exocytosis in a Ca^{2+} - dependent manner[20]. To date, more than 160 mutations in *OTOF* have been reported, and most of the patients with *OTOF* mutations have prelingual severe to profound hearing loss. In this study, the hearing levels in three patients with biallelic *OTOF* mutations were found to be mostly severe to profound, and according to the clinical manifestations and CT examination, the possibility of an auditory neuropathy was not diagnosed. The *OTOF*:c.2977_2978del variation has been reported in a Chinese patient with temperature-sensitive non-syndromic acoustic neuropathy[21]; besides; the Sf21 proband in this study was homozygous for this variant. This variant, which has not yet been identified in large population studies, is expected to cause transcoding, alter the amino acid sequence of the protein (starting at codon 994), and cause premature termination to produce truncated or missing proteins. c.2093delC, c.2807G>T, and c.2808_2809insTTC are novel mutations identified in this study. SF93 proband carried compound homozygous mutations of c.2807G>T and c.2808_2809insTTC, and had profound congenital prelingual sensorineural hearing loss. Sanger sequencing of available family members revealed that these mutations were not found in normal individuals.

5. Conclusion

In conclusion, we suggest that *MYO15A*, *CDH23*, *OTOF*, and *FGF3* could be used for conventional genetic tests, increasing the options for a clinical diagnosis of deafness and providing with theoretical support for the design of useful genetic markers for neonatal deafness in northwest China.

Abbreviations

NSHL: non-syndromic hearing loss; NGS: Next-generation sequencing; DPOAE: evoked otoacoustic emission; ABR: auditory brainstem responses; ASSR: auditory steady-state response; AC: air conduction; SNPs: single nucleotide polymorphisms; InDels: inserts and deletions; PCR: polymerase chain reaction.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YG, XL, BX, and YZ designed the project. SL, SW, WD, and LD performed patient workup. XL, BX, SW, and YZ involved genetic analysis. SW, YZ, and XL drafted the manuscript. YG, XL, and BX approved the final version to be published and agreement to be accountable for all aspects of the work.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1. Deaf probands (n=92) categorized by their clinical characteristics

	Sporadic cases	probands with family history	
		Recessive	Dominant
	(n=69)	(n=18)	(n=5)
Sex			
Male	32	7	4
Female	37	11	1
Age at the test			
0-6 years	5	2	0
6-18 years	44	5	3
18-35years	10	6	1
> 35 years	10	5	1
Age of onset			
Prelingual or early onset (onset≤6 years	65	16	4
Late onset(> 6 years)	4	2	1
Severity of hearing impairment			
Mild	0	0	0
Moderate	5	0	1
Severe	7	1	3
Profound	57	17	1

Table 2. Homozygous candidate variation in a recessive inherited deafness proband

Sample ID	Gene	Type of variant	Nucleotide change	Amino acid change	Phylop score	SIFT (score)	Polyphen2_HVAR	Mutation Taster	Allele frequency in controls	Novelty
Jx81	*MYO15A*	Stop gain	c.2482C>T	p.Arg828Ter	-0.367	T(1)	-	N(1)	0	Novel
Sf61,Sf67	*MYO15A*	Stop gain	c.9514C>T	p.Gln3172Ter	7.229	D(0.04)	-	A	0	Novel
Sf40	*MYO15A*	Stop gain	c.7015G>T	p.Glu2339Ter	1.57	T(0.89)	-	N	0	Novel
Sf88	*MYO15A*	Frame-shift indel	c.10251_10253del	p.3417_3418del	-	-	-	-	0	Novel
Jx50	*MYO15A*	Stop gain	c.4176C>A	p.Tyr1392Ter	1.885	T(1.00)	-	D	0	Novel
Jx58	*CDH23*	Frame-shift indel	c.490dupG	p.Ala163fs	-	-	-	-	0	Novel
Sf87	*CDH23*	Frame-shift indel	c.1271_1273del	p.424_425del	-	-	-	-	0	Novel
Jx48	*CDH23*	Missense	c.719C>T	p.Pro240Leu	7.222	T(0.23)	D(0.999)	A	0	Reported
Sf24	*OTOF*	Frame-shift indel	c.2093delC	p.Pro698fs	-	-	-	-	0	Novel
Sf21	*OTOF*	Frame-shift indel	c.2977_2978del	p.Ser993fs	-	-	-	-	0	Reported
Sf93	*OTOF*	Missense	c.2807G>T	p.Arg936Leu	4.881	T(0.06)	D(0.915)	D	0	Novel
		Frame-shift indel	c.2808_2809insTTC	p.Leu937delinsPheLeu	-	-	-	-	0	Novel
Jx86,Sf104	FGF3	Missense	c.137G>C	p.Arg46Pro	6.648	-	B(0.073)	D	0	Novel
Sf30	FGF3	Frame-shift indel	c.63delT	p.Pro21fs	-	-	-	-	0	Novel
Sf96	MYO7A	Frame-shift indel	c.3696_3706del	p.Arg1232fs	-	-	-	-	0	Reported
Sf90	MYO7A	Frame-shift indel	c.5142_5144del	p.1714_1715del	-	-	-	-	0	Novel
Sf62	*SLC26A4*	Stopgain	c.170C>A	p.Ser57Ter	5.926	T□1.00□	-	D	0	Novel
Sf53	*SLC26A4*	Missense	c.716T>A	p.Val239Asp	7.373	D□0□	P□0.892□	D	0	Reported
Jx80	MYO6	Missense	c.1801T>A	p.Leu601Ile	2.907	D(0.02)	D(0.998)	D	0	Novel
		Missense	c.1802T>C	p.Leu601Ser	7.632	D(0)	D(1)	D	0	Novel
	LOXHD1	Missense	c.1481T>C	p.Met494Thr	2.983	T(0.32)	P(0.884)	D	0	Novel
Sf48	BSND	Missense	c.393G>T	p.Leu131Phe	0.036	D(0.02)	D(0.998)	D	0	Novel
Sf22	CLDN14	Frame-shift indel	c.355_361del	p.Ile119fs	-	-	-	-	0	Novel
Sf105	DFNB59	Missense	c. 1031T>C	p.Phe344Ser	8.04	D(0)	D(0.995)	D	0	Novel
Sf15	ILDR1	stopgain	c.1117C>T	p.Arg373Ter	3.507	T(0.87)	-	A	0	Novel
Sf63	*LHFPL5*	Missense	c.494C>T	p.T165Met	7.818	D(0)	D(0.999)	A	0	Reported
Jx55	LRTOMT	Missense	c.555G>C	p.Met185Ile	2.717	T(0.47)	B(0.023)	D	0	Novel
Sf59	TMPRSS3	Missense	c.727G>A	p.Gly243Arg	7.091	-	D(1)	D	0	Reported
Sf103	TPRN	Missense	c.499C>A	p.Pro167Thr	1.794	-	B□0.003□	N	0	Novel
sf75	USH1C	Stopgain	c.1134G>A	p.Trp378Ter	6.409	T(1.00)	-	A	0	Novel

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable2.doc
- SupplementaryTable3.doc
- SupplementaryTable1.doc