Identification of novel compound heterozygous mutations of the MYO15A gene with autosomal recessive non-syndromic hearing loss

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Abstract

Background: The most common inheritance pattern responsible for congenital deafness belongs to autosomal recessive non-syndromic hearing loss (ARNSHL) and mutations of the highly heterogeneous MYO15A locus are present in a large proportion of cases.

Methods: One Chinese family with ARNSHL was subjected to clinical evaluation and genetic analysis. We used targeted and whole exome sequencing with Sanger sequencing to identify and characterize mutations. Bioinformatics analysis was conducted to evaluate molecular functions.

Results: Three compound heterozygous MYO15A gene variants, including two novel variants, c.6804G>A (p.M2268I), and c.6188_6190delinsGTCA (p.F2063Cfs*60), responsible for deafness were identified. Pathogenicity was assessed by multiple bioinformatics analyses.

Conclusion: We identified novel mutations of the MYO15A locus associated with ARNSHL in a Chinese family. The current findings expand the MYO15A pathogenic mutation spectrum to assist with genetic counseling and prenatal diagnosis.

KEYWORDS
hearing loss, mutation, MYO15A, targeted exome sequencing, whole exome sequencing

1 | INTRODUCTION

Congenital deafness is a common otolaryngology genetic disease. This sensorineural disorder manifests as hearing loss (HL) which affects 1 in 1000 births. Over 50% cases may have a genetic basis and 224 deafness-associated genes have been identified (https://deafnessvariationdatabase.org). HL is classified as syndromic (30%) or non-syndromic (70%), according to its accompanying signs and symptoms. Non-syndromic hearing loss (NSHL) shows a quantity of different inheritance patterns, including dominant NSHL, recessive NSHL, X-linked NSHL, and other loci. The most common inheritance pattern is autosomal recessive non-syndromic hearing loss (ARNSHL), with the proportion approximately 70%-80% of NSHL. ARNSHL is a disease with a highly heterogeneous genetic background. In Hereditary Hearing Loss Homepage, a total of 108 ARNSHL-related loci have been summarized and 78 genes identified. MYO15A is commonly implicated in ARNSHL with only GJB2, SLC26A4, and OTOF showing more frequent involvement. It has been reported that MYO15A causes hearing loss through compound heterozygous or homozygous mutations inherited from the parents.
**MYO15A** is a 71 kb sequence encoding a protein called myosin XVA.\(^{11}\) This unconventional form of myosin has been shown by a mouse model with myosin XVA-deficient to be demanding for elongation and differentiation of the stereocilia and essential for mechanical conduction by cochlea hair cells (HCs).\(^{12,13}\) Cochlear HCs convert mechanical sound waves into electrical neural signals in a process fundamental for normal auditory function.\(^{14}\) Pathogenic mutations of MYO15A, of which 149 have been reported in ClinVar, can cause severe to profound hearing impairment, leading to autosomal recessive deafness 3 (DFNB3, MIM: 600316).\(^{15}\) However, many unidentified mutation sites in MYO15A remain and have varying frequency in different ethnic populations.

The current study focused on a Chinese family with ARNSHL and used targeted and whole exome sequencing to identify three compound heterozygous MYO15A gene variants. Among them, the two variants, c.6804G>A (p.M2268I) and c.6188_6190delinsGTCA (p.F2063Cfs*60), were novel. The current findings expand the MYO15A pathogenic mutation spectrum to assist with genetic counseling and prenatal diagnosis.

## 2 | MATERIALS AND METHODS

### 2.1 | Research subjects

One Chinese Han family, in which the father and the mother had congenital deafness, underwent clinical evaluation in Jiaxing Maternity and Child Health Care Hospital, Affiliated Women and Children's Hospital of Jiaxing University. Detailed medical and family histories were taken and a physical examination conducted. Written informed consent was given by all family members. This research was permitted by the Ethics Committee.

### 2.2 | Targeted exome sequencing

Biosan Inc. used whole-genome DNA fragments extracted from blood samples in the preparatory stage. Sequences of 122 HL-related genes in Table S1 for details were captured by a customized Agilent capture probe to enrich the target region through the multiple probe hybridization method. After construction and quantification of the library, targeted exome sequencing was performed on an Illumina HiSeq 2500 Sequencing System. Low-quality sequencing data were excluded, and the remaining sequences aligned to the hg19 using BWA. Mutations were identified by GATK and added to the public mutation database after annotation by ANNOVAR.

### 2.3 | Whole exome sequencing

BGI Inc. used genomic DNA from blood samples. Whole-genome DNA was interrupted for library preparation and sequences of target exons and adjacent splicing regions captured by the KAPA HyperExome Probes from Roche. Mutations were analyzed by MGISEQ-2000 sequencing platform. Sequenced fragments were mapped with hg19 by BWA to remove duplicates. GATK was used for base mass correction and SNV/INDEL detection. ExomeDepth\(^{16}\) was used to detect CNV at the exon level.

### 2.4 | Mutation identification and validation

Gene nomenclature was in accordance with HGNC, and the mutation was named according to HGVS. The subject's clinical information, population (1000 Genomes, ESP6500, ExAC, and gnomAD), and disease (OMIM, ClinVar and HGMD) databases were consulted over variant annotation and screening. Clinical manifestations were matched with phenotype standard terms by Human Phenotype Ontology (HPO). Classification of variant pathogenicity was according to ACMG standards and guidelines.\(^{17}\)

Sanger sequencing was applied to validate deafness-associated mutations. Primer5 was used to design PCR primers for amplification. SeqMan Pro was used to perform sequence alignment of MYO15A.

### 2.5 | Bioinformatics analysis

Missense mutations were predicted by PROVEAN and MutationTaster, splicing sites by SpliceAI and dbscSNV and nucleotide conservation by GERP\(^+\) and PhyloP.

The HomoloGene system was used to determine the degree of amino acid conservation of novel mutations across species. MYO15A sequences, including human and other species, were aligned by BioEdit.

Wild-type and mutated MYO15A protein were modeled by SWISS-MODEL and MODELLER software. The 3D structures of the modeling files were further generated and rendered by Chimera software.

## 3 | RESULTS

### 3.1 | Clinical Findings

The parents (II-2, II-3) and aunt (II-4) of the newborn child all had congenital deafness while the grandparents (I-1, I-2, I-3, I-4) and uncle (II-1) had unimpaired hearing (Figure 1). The hearing of a newborn child, III-1, was assessed by automated auditory brainstem response (ABR) revealing a severe abnormality in both ears (Figure 2).

### 3.2 | Identification and validation of novel mutations in MYO15A gene

Targeted exome sequencing was conducted for family members, II-2 and II-3; whole exome sequencing for III-1 and Sanger sequencing for I-3, I-4, II-2, II-3, and III-1. Four variants in
MYO15A were identified, as follows: NM_016239.4 as c.3742C>T (p.R1248W); c.6804G>A (p.M2268I); c.5964+3G>A (p.F2063Cfs*60). Compound heterozygous MYO15A variants, c.3742C>T & c.6804G>A and c.6188_6190delinsGTCA, were detected in the proband (II-3) and were inherited from his parents (I-3 & I-4), who were unaffected carriers. The proband’s wife (II-2) also had compound heterozygous variants, c.5964+3G>A & c.6188_6190delinsGTCA. Newborn (III-1) inherited a variant from his parents, respectively, and compound heterozygous variants, c.3742C>T & c.6188_6190delinsGTCA, were also shown to cause deafness (Table 1).

The c.5964+3G>A variant has been reported as rs530975087 with MAF 0.0003908 in GnomAD-EAS in the Chinese population. However, the other three mutations were not present in any population databases. Prediction results for candidate mutations are shown in Table 2 and pathogenicity was classified. The MYO15A c.3742C>T variant was classified to be likely pathogenic (PM2 + PM3 + PP1 + PP3) and the c.6804G>A variant to be uncertain significance (PM2). In addition, variant c.5964+3G>A had uncertain significance (PM2 + PM3), and c.6188_6190delinsGTCA was likely pathogenic (PVS1 + PM2).

These four MYO15A mutations were confirmed by Sanger sequencing, indicating a co-segregation in Figure 3. Primer sequences are given in Table S2.

In addition, we identified several other variants in known deafness genes by targeted exome sequencing (II-2 and II-3) and whole exome sequencing (III-1). In II-2, we identified heterozygous variants c.595A>G (p.I199V) in GJB3 and c.5930G>A (p.R1977Q) in MYO7A. In II-3, we identified heterozygous variants c.109G>A (p.V37I) in GJB2 and c.772C>T (p.Q258*) in ILDR1. In III-1, we identified heterozygous variant c.546C>G (p.F182L) in KCNQ4. These variants were isolated and of uncertain significance, so we did not conduct further study.

### Table 1

| Family member | Variant 1     | Variant 2         | Zygosity          | Hearing loss |
|---------------|---------------|-------------------|-------------------|--------------|
| I-3           | c.3742C>T     | -                 | Heterozygous      | Unaffected   |
| I-4           | c.6804G>A     | -                 | Heterozygous      | Unaffected   |
| II-2          | c.5964+3G>A   | c.6188_6190delinsGTCA | Compound heterozygous | Affected     |
| II-3          | c.3742C>T     | c.6804G>A         | Compound heterozygous | Affected     |
| III-1         | c.3742C>T     | c.6188_6190delinsGTCA | Compound heterozygous | Affected     |
3.3 | Mutation analysis

The two novel variants, $\text{c.6804G}>\text{A (p.M2268I)}$ and $\text{c.6188}_6\text{190delinsGTCA (p.F2063Cfs*60)}$, were further analyzed. Alignment of $\text{MYO15A}$ sequences from human, chimpanzee, macaque, wolf, cattle, mouse, and rat showed a high degree of conservation of p.M2268 and p.F2063 (Figure 4), indicating that these residues are likely to be essential to normal protein function.

SWISS-MODEL was used to establish a 3D structural model of $\text{MYO15A}$ protein containing the variant, p.M2268I, in the first MyTH4-ERM domain, according to the crystal structure numbered

| Variant          | PROVEAN   | MutationTaster | SpliceAI | dbSNV  | GERP++ | PhyloP |
|------------------|-----------|----------------|----------|--------|--------|--------|
| $\text{c.3742C}>\text{T}$ | Deleterious | Deleterious | -        | -      | Conserved | Not conserved |
| $\text{c.6804G}>\text{A}$ | Neutral   | Deleterious   | -        | -      | Conserved | Conserved |
| $\text{c.5964+3G}>\text{A}$ | -         | -             | Tolerated| -      | Conserved | Not conserved |
| $\text{c.6188}_6\text{190delinsGTCA}$ | -         | -             | -        | -      | -      | -      |

**FIGURE 3** Sanger sequencing of the four mutations in five family members

**FIGURE 4** Multiple species sequence alignment of p.M2268 and p.F2063 variants of MYO15A

**TABLE 2** Software prediction of mutational effects
WANG et al. 5MV7 in the PDB database. The 3D simulative structure contained protein residues of myosin XVA, ranging from 2069 to 2269. The sequence similarity between target and template was 30.89%, meeting the requirement of average 30%. Chimera software was used to generate and render simulative structures of the MYO15A partial domain. The variant p.M2268I introduces an amino acid side chain which perturbs protein stability (Figure 5A). The variant p.F2063Cfs*60 was out of range, so we used MODELLER software to build the wild-type and mutant models. Comparison of these two simulative structures showed that the latter had an incomplete and dramatically altered structure (Figure 5B).

4 | DISCUSSION

The highly heterogeneous nature of the MYO15A mutation spectrum emphasizes the significance of regional variations and each country should construct an individual mutation spectrum for diagnosing hearing disorders. The current work reports a series of MYO15A mutations associated with ARNSHL in a Chinese family using NGS techniques. The proband and his wife had clear phenotypes of congenital deafness and targeted exome sequencing was employed to identify candidate mutations. Their newborn child was hospitalized with neonatal pneumonia and hypoglycemia and his hearing condition could not be determined at an early stage. Therefore, whole exome sequencing was used to detect candidate mutations. We identified four mutations that existing in compound heterozygous forms, including two missense variants (p.R1248W, p.M2268I), one splice site variant (c.5964+3G>A) and one frameshift variant (p.F2063Cfs*60).

The myosin XVA encoded by MYO15A contains three relatively conserved regions.21 The forehead encompasses a long N-terminal part and an ATPase motor domain. The neck encodes IQ motifs. The long tail encodes two MyTH4-FERM domains with a SH3 domain between and a C terminal PDZ domain. In addition, alternative splicing generates a transcript in which exon 2 is present (class I) or an alternative isoform where it is absent (class II). Exon 2 encodes the N-terminal region, the presence of which enables myosin XVA to maintain mechanically transduced static cilia and is essential for hearing.22,23 The binding of ATP and actin by the motor domain generates the force required to move the actin filaments.24 The IQ motifs constitute the binding sites for calmodulin light chains. The MyTH4-FERM domain binds microtubules and actin25 and localizes myosin XVA to the correct location.26 The locations of four MYO15A mutations found in the current research are shown in Figure 6. Variants c.3742C>T (p.R1248W) and c.5964+3G>A28-30 had been reported previously, whereas c.6804G>A (p.M2268I) and c.6188_6190delinsGTCA (p.F2063Cfs*60) were novels. The p.M2268I variant in exon 33 is located in the first MyTH4-FERM domains and may influence the assembly of myosin XVA into complex with whirlin and Eps8 and prevent myosin XVA from being transported correctly on actin polymerization during static cilia elongation. Conservation and 3D modeling analyses indicate the potential pathogenicity of p.M2268I. The p.F2063Cfs*60 variant in exon 29 is located at the beginning of the first MyTH4 domain. This frameshift mutation may lead to premature termination, reducing the number of amino acid residues from 3530 to 2121. The loss of the MYO15A tail region would result in loss of function. The ABR result of the newborn child with the p.F2063Cfs*60 variant indicates profound deafness, consistent
with the prelingual deafness phenotype associated with MYO15A mutations.

The spatial structure of the mutated protein MYO15A formed by p.M2268I and p.F2063Cfs*60 may be fatally altered, disrupting the binding to whirlin and Eps8. This affects the formation of tip complex density (TCD)-like condensates, impairing actin bundling and leading to HL, which may be the underlying regulatory mechanism of the mutated protein MYO15A.

The c.3742C>T variant was first reported in a study in 2019 as a compound heterozygous mutation c.3742C>T & c.10251_10253delCTT. The corresponding clinical symptom was severe deafness, which was similar to the results of this study. The c.5964+3G>A variant was first reported in two studies in 2013. One was presented as a compound heterozygous mutation c.IVS25+3G>A & c.8357T>C. Another was documented in the supplementary from variants identified in simplex or recessive multiplex probands. This locus was reported again in 2019 as a compound heterozygous mutation c.3742C>T & c.8791del with symptoms of profound deafness. According to expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss, combined with the references, based on the clinical symptoms of probands II-3 and II-2 and the genetic testing results of this study, MYO15A: c.3742C>T and c.5964+3G>A mutations were likely to be pathogenic.

The disadvantage of this study is that it is only limited to in silico analysis, there is no expression level study in cells, and no animal model is established to verify the impact of the identified variants on function. In addition, this study could further research drug-gene interactions through bioinformatics to find associated drugs on hearing loss patients.

5 | CONCLUSION

In summary, we identified novel mutations of the MYO15A locus associated with ARNSHL in a Chinese family. The current findings expand the MYO15A pathogenic mutation spectrum to assist with genetic counseling and prenatal diagnosis.

AUTHOR CONTRIBUTIONS

Conceived and designed the study: Luming Wang, Yue Zhang, Xiaodan Liu. Recruited the family and collected samples: Pinghua Huang. Performed the clinical evaluation: Yue Zhang, Xiaodan Liu. Performed the experiments: Luming Wang, Qiuxia Xue. Analyzed the data: Luming Wang, Qiuxia Xue. Wrote the paper: Luming Wang. Critical reading and discussion of manuscript: Yue Zhang, Xiaodan Liu. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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