Putative Digenic *GJB2/MYO7A* Inheritance of Hearing Loss Detected in a Patient with 48, XXYY Klinefelter Syndrome

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Research

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Abstract

Background: 48, XXYY Klinefelter syndrome is a rare sex chromosome abnormality. Nonsyndromic hearing loss (NSHL) is the most frequent hereditary type of hearing impairment. There has been no report of NSHL combined with 48XXYY. The purpose of this study was to explore the underlying genetic cause in a three-generation family affected by NSHL. The proband had concomitant NSHL and 48, XXYY syndrome. The whole-exome sequencing was performed in the proband. The candidate pathogenic variants identified by whole-exome sequencing were then confirmed by Sanger sequencing and segregation analysis.

Results: The proband was identified to be compound heterozygous for c.109G>A (p.V37I) variant in the *GJB2* gene and additional heterozygous for the c.1039C>A (p.L347I) variants in the *MYO7A* gene. His mother had normal hearing and did not have any form of variant. His father and uncle, both had NSHL, were digenic compound heterozygote for the *GJB2* p.V37I and *MYO7A* p.L347I variants, thus suggesting a possible *GJB2/MYO7A* digenic inheritance of NSHL in this family consist with the clinical phenotype.

Conclusions: Our findings reported a putative *GJB2/MYO7A* digenic inheritance form of hearing loss, which expands the mutation spectrum of NSHL. This is also the first report of concomitant NSHL and 48, XXYY syndrome.

Background

48, XXYY syndrome is a rare type of Klinefelter syndrome, which was first described by Mulder et al. [1] in 1960. To date, more than 100 cases with 48, XXYY syndrome have been reported, with the incidence of 1/18,000–1/5,000 male births[2]. Compared with 47, XXY Klinefelter syndrome, 48, XXYY syndrome is typically associated with more severe neurodevelopmental and behavioral abnormalities[2–4]. Tartaglia et al.[2] found 48, XXYY syndrome patients had some facial dysmorphic features, such as ocular hypertelorism, pes planus, upward-slanting palpebral fissures, joint laxity, and dental problems. Other medical issues, including allergies and asthma, congenital heart defects, inguinal hernia, and cryptorchidism were also more common in these patients. However, there has been no report of congenital hearing loss combined with 48, XXYY syndrome.

Hearing loss (HL) is one of the most common prevalent sensory defects in humans with a frequency of every 1–3 per 1000 newborns and more than 50% of these cases are attributed to genetic factors [5]. There are two major types of hearing loss: syndromic hearing loss (SHL) and nonsyndromic hearing loss (NSHL). NSHL is genetically heterogeneous and has various modes of transmission, including autosomal recessive, autosomal dominant, X-linked and mitochondrial deafness[6]. To date, more than 100 genes are associated with NSHL (cf. Hereditary Hearing loss Homepage at <http://hereditaryhearingloss.org>). The frequently mutated gene in NSHL is *GJB2*, *SLC26A4*, *MYO7A*, *OTOF*, and *CDH23* genes. With the rapidly expanding accessibility of next-generation sequencing, the simultaneous detection of variants in

two or more genes in the NSHL patients is being discovered, paving a way to the possibility of digenic/oligogenic inheritance of the disease.

In the present study, we used whole-exome sequencing to identify a novel digenic *GJB2/MYO7A* inheritance of hearing loss in a patient with 48, XXYY Klinefelter syndrome.

Results

Clinical characteristics

The proband (Fig. 1A: III-1) was a 27-year-old male who was referred to our hospital due to the absence of pubertal development. Physical examination showed a height of 170 cm, a weight of 36 kg, and a Tanner developmental stage of G2P1. Laboratory tests showed low levels of testosterone (102.7 ng/d; reference range [RR]: 249–836 ng/dl), high level of follicle-stimulating hormone (FSH) (97.53 mIU/ml; RR: 1.7 ~ 8.6 mIU/ml) and luteinizing hormone (LH) (43.19 mIU/ml; RR: 1.7–8.6). Ultrasound examination showed small testicular volumes [15 × 7 mm (left); 15 × 8 mm (right)]. There were no dysmorphic facial abnormalities, such as ocular hypertelorism and pes planus. Delayed speaking and learning difficulties were found in the patient. His IQ report is only 70. Karyotype analysis showed a chromosomal composition of 48, XXYY (Fig. 1B), which indicated Klinefelter's syndrome. His parents denied consanguinity, and both had normal karyotypes.

The proband, his father (Fig. 1A: II-1), uncle (Fig. 1A: II-3), and grandfather (Fig. 1A: I-1) suffered from hearing loss since childhood, but all had never received hearing aids. The pure tone audiometry demonstrated bilateral sensorineural hearing loss of mild degree in the proband, and severe degree in his father (Fig. 1C). His sister (III-2), mother (II-2) and cousins (III-3,4) all had self-reported normal hearing. The audiological evaluation of his sister disclosed normal hearing (Fig. 1C). His uncle, mother and cousins declined the hearing test. No vestibular dysfunction or retinal defects were observed in the members of the family.

Variants Identified by Whole Exome Sequencing

The proband with concomitant NSHL and 48, XXYY (Fig. 1A: III-1) was identified to be compound heterozygous for c.109G > A (p.V37I) variant in the *GJB2* gene and c.1039C > A (p.L347I) variants in the *MYO7A* gene (Fig. 1D).

His father (Fig. 1A: II-1) and uncle (Fig. 1A: II-3), who also had congenital deafness, were both digenic compound heterozygote for the c.109G > A (p.V37I) variant in the *GJB2* and c.1039C > A (p.L347I) variant in *MYO7A* gene. His mother (Fig. 1A: II-2) did not have any form of variant. His sister (Fig. 1A: III-2) and cousin (Fig. 1A: III-3) were both heterozygous for p.V37I mutation in *GJB2*. The hearing status of the proband's mother, cousin and sister were all normal.

In silico analysis

The *GJB2* missense variant p.V37I has been reported previously and was predicted to be damaging by PolyPhen-2, MutationTaster, tolerable by SIFT, and PROVEAN. The amino acid alignment of *GJB2* among different species revealed that the Valine mutated at position 37 was conserved among all species examined (Fig. 2A). According to ACMG guidelines, variant p.V37I of *GJB2* was interpreted as pathogenic since this variant has been reported as a pathogenic variant (PS1), with extremely low frequency (PM2) and was predicted to be damaging and disease-causing by PolyPhen-2, and MutationTaster (PP3).

The *MYO7A* variant p.L347I was predicted to be Disease_causing by SIFT, benign by PolyPhen-2, Tolerable by Mutation Taster and PROVEAN. The amino acid alignment of *MYO7A* among different species revealed that the leucine mutated at position 347 was conserved among species examined (Fig. 2B). According to ACMG guidelines, variant p.L347I of *MYO7A* was interpreted as VUS, since this mutation has an extremely low frequency (PM2) and was predicted to be damaging and disease-causing by SIFT (PP3).

The *MYO7A* p.L347I variant was not found in HGMD (The Human Gene Mutation Database, <http://www.hgmd.cf.ac.uk/>), dbSNP (<http://www.ncbi.nlm.nih.gov/snp>), and ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar>), indicating that it was a novel variants.

Discussion

Nonsyndromic genetic deafness is a genetically heterogeneous condition with over 100 genes that have been identified involved in its development[6]. In most cases, NSHL is the result of monogenic mutations. However, digenic mutations have also been reported recently[7–12]. In our cases, the compound heterozygous for *GJB2* variants (p.V37I) and *MYO7A* (p.L347I) variant were found in the proband using whole-exome sequencing. *GJB2* gene accounts for about 50% of nonsyndromic autosomal recessive hearing loss in different populations[13–14]. *GJB2* encodes the gap junction subunit protein connexin 26 (Cx26), a protein which plays a crucial role in intercellular communication by forming the cochlear gap junction. In the inner ear, *GJB2* plays a vital role in many auditory processes including potassium recycling, energy supply, and maintenance of the endolymphatic homeostasis[15–16]. To date, more than 300 variants within *GJB2* are associated with hearing loss (HGMD, <http://www.hgmd.cf.ac.uk/ac/>). The p.V37I variant of *GJB2* has a high allele frequency (up to 10%) in East Asians[13, 17–18]. However, the pathogenicity of the p.V37I has yet to be elucidated. early studies regarded the p.V37I as a benign polymorphism, as it was observed in unaffected heterozygous controls. However, research findings increasingly indicated that both homozygotes and compound heterozygous p.V37I variants were associated with mild to moderate hearing impairment [19–20].

MYO7A gene encodes the actin-based motor protein myosin-VIIa, which is especially crucial for the function of cochlear hair cells and eye development [21]. The myosin VIIa protein contains a conserved N-terminal actin-binding and ATPase domain (motor domain), a neck region containing five isoleucine-glutamine (IQ) motifs, and a short predicted coiled-coil domain, followed closely by two myosin tail homology 4 (MyTH4) domains, two-band 4.1-ezrin radixin-moesin (FERM) domains and an SH3 domain

[21]. *MYO7A* gene has long been associated with Usher Syndrome type 1B (USH1B), which is characterized by sensorineural hearing loss, retinitis pigmentosa, and vestibular dysfunction[21–22]. In 1997, Weil and Liu[23–24] identified that *MYO7A* gene mutations are associated with non-syndromic autosomal recessive hearing loss (DFNB2). So far, majority of the mutations identified in *MYO7A* are associated with USH1B, whereas only about 17 *MYO7A* mutations have been found to be responsible for DFNB2 [25–26]. The novel *MYO7A* missense p.L347I variant identified in our study located in the motor domain and was predicted to be damaging by in silico analysis. The absence of vestibular and retinal defects in the affected patients suggests that this family have isolated non-syndromic hearing loss presentation, instead of USH1B.

It is estimated that 6–20% of *GJB2* mutations in hearing loss subjects were monoallelic mutations[27]. Some researchers hypothesize that a single heterozygous *GJB2* mutant allele is possibly contributing to deafness via digenic inheritance. In 2009, Liu et al. [8] identified heterozygous *GJB2* and *GJB3* mutations in NSHL patients. Since then, many other digenic inheritance has been reported[7–12], such as *GJB2/GJB3*, *GJB2/MITF*, and *GJB2/ TMPRSS3* (Table 1). Although the digenic inheritance has been increasingly described in NSHL, the prevalence is unknown. Chen et al. [7] assess the contributions of variants in *GJB3* or *GJB6* in a cohort of 100 NSHL patients with likely pathogenic heterozygous *GJB2* mutations. Putatively causative *GJB3* variant were 1% (1/100) and no *GJB6* mutation was found in this cohort. Monika Oldak et al. [28] also screened the *GJB2* variants in 42 hearing loss patients with at least one *TMPRSS3* pathogenic variants and identified four individuals who were double heterozygous for pathogenic *GJB2* and *TMPRSS3* variants. They proposed that the contributions of *GJB2* digenic inheritance may not be predominant. In our case, we found the proband, his father and uncle; all were digenic compound heterozygote for the variant in *MYO7A* p.L347I and *GJB2* p.V37I variant. Based on the co-segregated analysis, we implied a possible *GJB2 /MYO7A* digenic inheritance of NSHL in this family. To the best of our knowledge, it is a novel *GJB2 /MYO7A* digenic combination involved in hearing loss development. However, our study has some weaknesses. First, it relies exclusively on data from only one family. Second, there is an absence of in vitro data that demonstrate a functional link between these two genes. Third, a dominant character of the identified *GJB2* p.V37I variant with a possible incomplete penetrance in the family can not be excluded. Further animal models or cell biology experiments and studies with a large sample size are still needed to clarify the role of digenic mutations involved.

In the present study, the proband was also diagnosed with 48, XXYY syndrome. 48, XXYY syndrome is a rare sex chromosome abnormality. Although the proband had some physical features similar to 47, XXY Klinefelter syndrome (tall stature, hypogonadism, and infertility), he had delayed speaking, learning difficulty and challenging IQ, which was consistent with previous studies that mental retardation is observed in 26% of the subjects with 48, XXYY syndrome and almost all have learning difficulty[2]. To the best of our knowledge, it is also the first case being reported in the literature with concomitant NSHL and 48, XXYY syndrome.

Conclusion

In conclusion, we reported a putatively *GJB2* /*MYO7A* digenic inheritance form of hearing loss in a 48, XXYY Klinefelter patient using whole-exome sequencing, which expands the genotypic spectrum of NSHL.

Methods

Family enrolment and clinical evaluation

We studied a three-generation Chinese family with four members affected by congenital hearing loss (Fig. 1A). 48, XXYY syndrome has been ascertained in the proband. Hearing status was determined by self-assessment or pure tone-audiometry. The pure-tone audiometry (PTA) was calculated as the average of the hearing level at 0.5, 1.0, 2.0, and 4.0 kHz. The level of hearing loss, in terms of PTA, was described as follows: normal hearing, < 25 dB; mild hearing impairment, 26–40 dB; moderate hearing impairment, 41–60 dB; severe hearing impairment, 61–80 dB; and profound hearing impairment, > 81 dB. Romberg test and tandem walking test were conducted to assess the vestibular function. The ocular examination included slit-lamp examination and stereoscopic fundoscopy.

Whole exome sequencing

The whole-exome sequencing was performed in the proband. Proband DNA was isolated from peripheral blood by standard phenol-chloroform procedures. The isolated DNA was sheared on a Bioruptor UCD-200 (Diagenode). For all samples, shearing worked very consistently and the size distribution peak was around 200 bp. DNA libraries were prepared with the KAPA Library Preparation Kit (Kapa Biosystems, KR0453). The whole exome sequencing was performed using Agilent SureSelectXT2 Target Enrichment System. Sample dilution, flowcell loading, and sequencing, were performed according to Illumina's specifications. DNA libraries were sequenced on the HiSeq2500 platform (Illumina, San Diego, CA) as paired-end 200-bp reads. Raw data were filtered and aligned against the human reference genome (hg19) using the BWA Aligner (<http://bio-bwa.sourceforge.net/>). Single-nucleotide polymorphisms (SNPs) were identified using Genome Analysis Toolkit software (www.broadinstitute.org/gatk). Variants were annotated using ANNOVAR (annovar.openbioinformatics.org/en/latest/). We anticipated that a causative variant would be missense, or gene-disrupting, and would be rare in the overall population. Selected variants were checked in relevant variant frequency databases (ESP, dbSNP, 1000Genomes, ClinVar and HGMD). We applied filtering criteria that required a variant to have a frequency that was < 1% in the 1000 Genomes, ExAC, and gnomAD databases. In addition, variations that can cause clinical symptoms were further analyzed. Finally, the specific mutations were confirmed by designing specific primers and performing Sanger sequencing. Sanger sequencing of specific regions of the gene was performed in family members to confirm the presence of co-segregation of the disease. All variants were interpreted according to the standards set out by the American College of Medical Genetics (ACMG), and categorized as pathogenic, likely to be pathogenic, variants of uncertain significance (VUS), likely to be benign, or benign.

Sanger sequencing

The candidate genes identified by whole-exome sequencing were then confirmed by polymerase chain reaction (PCR). Primer sequences used for the PCR amplification and DNA sequencing of the gap junction protein beta 2 (*GJB2*) gene (NM_004004) exon 2 were: 5'-GAGCCTTGACAGCTGAGCA-3' and 5'-TGGCCTACCGGAGACATGA-3'; the myosin VIIA (*MYO7A*) gene (NM_00112718) exon 10 were: 5'-TGCCTGCAGACTCATGGT-3' and 5'-CACAGGACCTGCACATGGT-3'. Variants were identified by direct sequencing of PCR products bidirectionally using an ABI 3730xl automated sequencer (Applied Biosystems, USA). Segregation analysis of the respective *MYO7A* and *GJB2* pathogenic variants were performed within the families.

Abbreviations

DFNB2 : Non-syndromic autosomal recessive hearing loss

GJB2: Gap junction protein beta 2

MYO7A: Myosin VIIA

NSHL Nonsyndromic hearing loss

NSHL : Nonsyndromic hearing loss

PCR: Polymerase chain reaction

SHL: Syndromic hearing loss

USH1B: Usher Syndrome type 1B

Declarations

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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Authors' contributions

All authors participate in data collection, data analysis and manuscript preparation. The authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

Peripheral blood samples were collected from the three-generation family. DNA was isolated from peripheral blood by phenol-chloroform procedures. The Institutional Ethics Committee of The Third Xiangya Hospital approved this study. Written informed consent was obtained from all subjects enrolled in this study. All procedures were in accordance with the World Medical Association's Declaration of Helsinki.

Consent for publication

All the authors have consented for the publication.

Competing interests

The authors declare that they have no competing interests.

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Table

Table 1
Reported digenic and trigenic inheritance in NSHL

Patient no.	Variant 1	Variant 2	Variant 3	Reference
1	<i>GJB2</i> c.109G > A	<i>GJB2</i> c.638T > C	<i>GJB3</i> c.250G > A	Chen et al[7]
2	<i>GJB2</i> c.638T > C	<i>GJB3</i> c.250G > A		Chen et al[7]
3	<i>GJB2</i> c.235delC	<i>GJB3</i> c.497G > A		Liu et al[8]
4	<i>GJB2</i> c.235delC	<i>GJB3</i> c.580G > A.		Liu et al[8]
5	<i>GJB2</i> c.299delAT	<i>GJB3</i> c.580G > A		Liu et al[8]
6	<i>GJB2</i> c.109G > A	<i>GJB3</i> c.580G > A		Chen et al [9]
7	<i>GJB2</i> c.578T > A	<i>GJB3</i> c.580G > A		Kim et al[10]
8	<i>GJB2</i> c.235delC	<i>MITF</i> c.1021C > T		Kim et al[10]
9	<i>GJB2</i> c.487A > G	<i>TMPRSS3</i> c.895G > A		Leone et al[11]
11	<i>GJB2</i> c.35delG	<i>TMPRSS3</i> c.208delC	<i>TMPRSS3</i> c.579dupA	Battelino et al[12]
12	<i>GJB2</i> c.109G > A	<i>MYO7A</i> c.1039C > A		Present study
NSHL:nonsyndromic hearing loss				

Figures

Figure 1

Illustration of the family with digenic GJB2/MYO7A variants. The pedigree showed mutations in GJB2/MYO7A genes (A). Chromosome analyses of the proband revealed 48, XXYY (B). The different hearing levels and the genotype-phenotype relation in the family members were summarized (C). Sequencing chromatograms of the GJB2 p.V37I and MYO7A p.L347I variants (D).

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Figure 2

Multiple alignment of the GJB2, MYO7A protein sequence in different species, indicating conservation of the residues GJB2 p.Val37 (A), and MYO7A p. Leu347(B) affected by these mutations.

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