Putative Digenic GJB12/MYO7A Inheritance of Hearing Loss Detected in a Patient with 48, XXYY Klinefelter Syndrome

Tian-tian Qin  
Central South University Third Xiangya Hospital

Qin Zhang  
Central South University Third Xiangya Hospital

Wen-mu Hu  
Central South University Third Xiangya Hospital

Muhammad Usman Janjua  
Central South University Third Xiangya Hospital

Qin Long  
Central South University Third Xiangya Hospital

Ping Jin (جادل jping7676@hotmail.com)  
the third xiangya hospital of central south university  
https://orcid.org/0000-0002-7835-076X

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).


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
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 bee 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 NHSL 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. Base 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′-GAGCCTTGACGCTGAGCA-3’ and 5′-TGGCCTACCGAGACATGA-3’; the myosin VIIA (MYO7A) gene (NM_00112718) exon 10 were: 5′-TG CCTGCAGACTCATGGT-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 MOY7A 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.

**Acknowledgements**

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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.

**References**


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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, XXY (B). The different hearing levels and the genotype-phenotype relation in the family members were summarized (C). Sequencing chromatograms of the GJB2 p.V371 and MYO7A p.L347I variants (D).
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).
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.
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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