Research Article

Targeted Next-Generation Sequencing Identified Compound Heterozygous Mutations in MYO15A as the Probable Cause of Nonsyndromic Deafness in a Chinese Han Family

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Hearing loss is a highly heterogeneous disorder, with more than 60% of congenital cases caused by genetic factors. This study is aimed at identifying the genetic cause of congenital hearing loss in a Chinese Han family. Auditory evaluation before and after cochlear implantation and targeted next-generation sequencing of 140 deafness-related genes were performed for the deaf proband. Compound heterozygous mutations c.3658_3662del (p. E1221Wfs*23) and c.6177+1G>T were identified in MYO15A as the only candidate pathogenic mutations cosegregated with the hearing loss in this family. These two variants were absent in 200 normal-hearing Chinese Hans and were classified as likely pathogenic and pathogenic, respectively, based on the ACMG guideline. Our study further expanded the mutation spectrum of MYO15A as the c.3658_3662del mutation is novel and confirmed that deaf patients with recessive MYO15A mutations have a good outcome for cochlear implantation.

1. Introduction

Approximately one in every 1000 newborns is affected by congenital hearing loss, and genetic factors account for more than 60% of them [1]. To date, more than 100 deafness-causative genes have been found. Among them, autosomal recessive nonsyndromic hearing loss (ARNSHL) accounts for up to 80% of nonsyndromic hearing loss [2], with more than 70 causative genes being identified (http://hereditaryhearingloss.org/).

Stereocilia is critical for the development and function of cochlear hair cells (HCs) [3–5]. The MYO15A gene contains 66 coding exons [6], which encode an unconventional myosin (myosin XVA) expressed at the tips of stereocilia in the cochlear HCs. Myosin XVA is essential for the mechanotransduction function of cochlear HCs. Myosin XVA interacts with the PDZ domain of whirlin and then delivers whirlin to the tips of stereocilia [7]. Myosin XVA-deficient mouse (shaker-2) shows abnormally short stereocilia bundles and diminished staircase [8–10]. In humans, mutations in MYO15A have been found to lead to recessive nonsyndromic deafness DFNB3 [11]. The prevalence of MYO15A mutations varies among different ethnic populations (3%-6.7%) and appears to be the third or fourth most frequent causes of ARNSHL [12–15].
Here, we report a nonconsanguineous Chinese Han family with profound ARNSHL, in which compound heterozygous mutations in MYO15A were identified as the probable cause of the deafness.

2. Materials and Methods

2.1. Subjects. A Chinese Han recessive deafness family (Figure 1) was enrolled in this study. All family members underwent clinical evaluation in the Department of Otolaryngology-Head and Neck Surgery, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine. The evaluation included a detailed clinical interview and physical examination. As shown in Figure 2(a), the proband had bilateral profound deafness. This study was approved by the ethic committee of Xinhua Hospital. Written informed consent was obtained for each participant.

2.2. Audiometric Evaluation. Audiometric assessments included otoscopic examination, pure tone audiometry (PTA), auditory brainstem response (ABR), and multiple steady-state responses (ASSR). Hearing level was assessed at 250, 500, 1000, 2000, 4000, and 8000 Hz. The hearing threshold was defined as the average of both sides. Inner-ear malformation and dysplasia of the auditory nerve related to the hearing loss were excluded by temporal bone Computerized Tomography (CT) scan and cranial Magnetic Resonance Imaging (MRI).

2.3. Mutation Identification. Blood samples were collected into an EDTA anticoagulant tube by venipuncture of the cubital vein. Extraction of genomic DNA was performed using a blood DNA extraction kit (QIAamp DNA Blood Mini Kit, Qiagen, Shanghai). As the first step, mutations in common deafness genes GJB2, SLC26A4, and MT-RNR1 were excluded by Sanger sequencing. Targeted next-generation sequencing was then performed in the proband as previously reported [16]. A total of 140 known deafness-related genes were captured by a customized capture assay (MyGenostics, Beijing, China) (Supplementary Table 1). The targeted region included exon, splicing sites, and flanking intron region. Then, potentially candidate variants such as missense, nonsense, and indel variants and the splice site were screened for quality, and variants with minor allele frequencies (MAFs) below 0.005 were further studied using public databases including dbSNP, 1000 Genomes Project, and Exome Aggregation Consortium (EXAC) and in-house data from 200 ethnically matched normal-hearing controls. Intrafamilial segregation of the candidate pathogenic mutations consistent with a presumable autosomal recessive inheritance. The mean depth of sequencing was 364.43X, and 98% of the targeted region was covered with at least 20X. Cosegregation of these two mutations with the hearing phenotype was confirmed within the family members (Figure 3). These two variants were not seen in public databases dbSNP, 1000 Genomes Project, and EXAC and the in-house databases of 200 Chinese Han normal-hearing controls. The frameshifting c.3658_3662del (p.E1221Wfs*23) mutation is located in exon 3, and it is novel and is predicted to result in a truncated protein after the motor domain (Figure 3). The c.6177+1G>T splice site mutation was previously reported in another Chinese Han family [18] and is predicted to result in an in-frame skipping of exon 26 and a protein product with 17-residue deletion in the first MyTH4 domain. Following the ACMG guideline in 2015 [17], the c.3658_3662del and c.6177+1>G>T mutations were classified as likely pathogenic (PVS2+PM2) and pathogenic (PVS1+PS1+PM2), respectively.

3. Results

3.1. Clinical Characterization. The proband was a 14-year-old male from Zhejiang Province, China. He had congenital, bilateral, profound hearing impairment with a threshold above 95 dBHL as revealed by the PTA (Figure 2(a)) and ABR tests. Hearing levels of this patient and his sister were normal. Otoacoustic emissions were absent for both ears. Temporal CT and cranial MRI showed no abnormalities (Figures 2(b) and 2(c)). No vestibular dysfunction was complained. No apparent syndromic features were found in the physical examination. The proband received unilateral cochlear implantation (Nucleus 5, Cochlear Corporation, Australia) through a typical round window route uneventfully at 12 years old. Hearing was markedly improved after cochlear implantation (Figure 2(a)).

3.2. Mutation Analysis. By targeted next-generation sequencing of 140 deafness-causative genes in the proband, compound heterozygous mutations c.3658_3662del and c.6177+1>G>T in MYO15A (NM_016239) were identified as the only candidate pathogenic mutations consistent with a presumably autosomal recessive inheritance. The mean depth of sequencing was 364.43X, and 98% of the targeted region was covered with at least 20X. Cosegregation of these two mutations with the hearing phenotype was confirmed within the family members (Figure 3). These two variants were not seen in public databases dbSNP, 1000 Genomes Project, and EXAC and the in-house databases of 200 Chinese Han normal-hearing controls. The frameshifting c.3658_3662del (p.E1221Wfs*23) mutation is located in exon 3, and it is novel and is predicted to result in a truncated protein after the motor domain (Figure 3). The c.6177+1G>T splice site mutation was previously reported in another Chinese Han family [18] and is predicted to result in an in-frame skipping of exon 26 and a protein product with 17-residue deletion in the first MyTH4 domain. Following the ACMG guideline in 2015 [17], the c.3658_3662del and c.6177+1>G>T mutations were classified as likely pathogenic (PVS2+PM2) and pathogenic (PVS1+PS1+PM2), respectively.
Figure 2: (a) Audiogram of the proband (II-1) before and after cochlear implantation and that of his unaffected sister (II-2). (b) Temporal bone Computerized Tomography (CT) scan of the proband (II-1). (c) Cranial Magnetic Resonance Imaging (MRI) of the proband (II-1).

Figure 3: Sanger sequencing results of the c.3658_3662del and c.6177+1G>T mutations in the family members.
4. Discussion

HCs in the cochlea play a critical role in converting mechanical sound waves into neural signals for hearing, and most of the hearing loss induced by gene mutation, noise, different ototoxic drugs, inflammation, or aging is caused by the HC malfunction [19–27]. The association between MYO15A mutations and recessive deafness DFNB3 was first discovered by Friedman et al. in Bali, Indonesia [28], in which two missense mutations and one nonsense mutation in MYO15A, all in a homozygous state, result in congenital, severe-to-profound hearing loss [11]. To date, more than 100 mutations in MYO15A have been reported, mostly reported in consanguineous families from the Middle East [27, 29–35]. In this study, two variants p.E1221Wfs*23 and c.6177+1G>T in MYO15A were identified. Like many previously reported truncating mutations in MYO15A, the p.E1221Wfs*23 variant is predicted to result in a truncated protein product without Motor, IQ, MyTH4, FERM, SH3, and PDZ domains (Figure 4). The c.6177+1G>T variant was previously reported in another Chinese Han family by Chen et al. [18], suggesting that this mutation may be either a founder mutation or a reoccurring hot spot. This mutation resides in the consensus splice acceptor site adjacent to exon 26 and is predicted to lead to an in-frame exon 26 skipping and a 17-amino acid residue deletion in the first myosin tail homology 4 (MyTH4) domain of myosin XVA. The MyTH4 domain provides a link between actin-based kinesin and the microtubule cytoskeleton. Mutation in this domain can disrupt the protein-protein interaction that is important for mechanotransduction of hearing [7].

Most recessive mutations in MYO15A are associated with congenital, severe-to-profound deafness [31, 33, 36], except for mutations affecting the N-terminal domain of MYOXA which may result in milder hearing loss with residual hearing of low frequency [37]. Both variants identified in our study are located outside of the N-terminal domain, and the associated profound hearing loss is consistent with the genotypphenotype correlation for DFNB3 deafness. Consistent with the specific role of MYO15A in the sensory HCs, the proband in our study had a marked improvement for hearing after cochlear implantation, showing a good prospective outcome for a similar procedure in other DFNB3 patients.

5. Conclusion

The p.E1221Wfs*23 and c.6177+1G>T compound heterozygous mutations in MYO15A are the probable cause of congenital, profound deafness in the Chinese Han family. Patients with recessive mutations in MYO15A may markedly benefit from cochlear implantation.

Data Availability

The data underlying the findings of this study is available upon request.

Conflicts of Interest

The authors declare no conflicts of interests.

Authors’ Contributions

Longhao Wang, Lin Zhao, and Hu Peng contributed equally to this work.
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Supplementary Materials

Supplementary Table 1: the 140 deafness-causative genes for targeted next-generation sequencing. (Supplementary Materials)

References

[1] C. C. Morton and W. E. Nance, “Newborn hearing screening—a silent revolution,” vol. 354, pp. 2151–2164, 2006.
[2] G. Van Camp, P. J. Willems, and R. J. Smith, “Nonsyndromic hearing impairment: unparalleled heterogeneity,” American Journal of Human Genetics, vol. 60, no. 4, pp. 758–764, 1997.
[3] J. Qi, Y. Liu, C. Chu et al., “A cytoskeleton structure revealed by super-resolution fluorescence imaging in inner ear hair cells,” Cell Discovery, vol. 5, no. 1, 2019.
[4] Y. Liu, J. Qi, X. Chen et al., “Critical role of spectrin in hearing development and deafness,” Science Advances, vol. 5, no. 4, p. eaav7803, 2019.
[5] J. Qi, L. Zhang, F. Tan et al., “Espin distribution as revealed by super-resolution microscopy of stereocilia,” American Journal of Translational Research, vol. 12, pp. 130–141, 2020.
[6] Y. Liang, A. Wang, I. A. Belyantseva et al., “Characterization of the Human and Mouse Unconventional Myosin XV Genes Responsible for Hereditary Deafness DFNB3 and Shaker 2,” Genomics, vol. 61, no. 3, pp. 243–258, 1999.
[7] I. A. Belyantseva, E. T. Boger, S. Naz et al., “Myosin-XVa is required for tip localization of whirlin and differential elongation of hair-cell stereocilia,” Nature Cell Biology, vol. 7, no. 2, pp. 148–156, 2005.
[8] D. W. Anderson, F. J. Probst, I. A. Belyantseva et al., “The motor and tail regions of myosin XV are critical for normal structure and function of auditory and vestibular hair cells,” Human Molecular Genetics, vol. 9, no. 12, pp. 1729–1738, 2000.
[9] I. J. Karolyi, F. J. Probst, L. Beyer et al., “Myo15 function is distinct from Myo6, Myo7a and pirouette genes in development of cochlear stereocilia,” Human Molecular Genetics, vol. 12, no. 21, pp. 2797–2805, 2003.
[10] F. J. Probst, R. A. Fridell, Y. Raphael et al., “Correction of deafness in shaker-2 mice by an unconventional myosin in a BAC transgene,” Science, vol. 280, no. 5368, pp. 1444–1447, 1998.
[11] A. Wang, Y. Liang, R. A. Fridell et al., “Association of unconventional myosin MYO15 mutations with human nonsyndromic deafness DFNB3,” Science, vol. 280, no. 5368, pp. 1447–1451, 1998.
[12] M. Miyagawa, S.-y. Nishio, M. Hattori et al., “Mutations in theMYO15AGene are a significant cause of nonsyndromic hearing loss,” Annals of Otology, Rhinology & Laryngology, vol. 124, 1_suppl, pp. 1585–1685, 2015.
[13] Y. Li, A. W. Lin, X. Zhang, Y. Wang, X. Wang, and D. W. Goodrich, “Cancer cells and normal cells differ in their requirements for Thoc1,” Cancer Research, vol. 67, no. 14, pp. 6657–6664, 2007.
[14] T. Yang, X. Wei, Y. Chai, L. Li, and H. Wu, “Genetic etiology study of the non-syndromic deafness in Chinese Hans by targeted next-generation sequencing,” Orphanet Journal of Rare Diseases, vol. 8, no. 1, p. 85, 2013.
[15] Z. Fattahi, A. E. Shearer, M. Babanejad et al., “Screening for MYO15A gene mutations in autosomal recessive nonsyndromic, GJB2 negative Iranian deaf population,” American Journal of Medical Genetics Part A, vol. 158A, no. 8, pp. 1857–1864, 2012.
[16] X. Wang, L. Wang, H. Peng, T. Yang, and H. Wu, “A Novel p.G141R Mutation in ILDR1 Leads to Recessive Nonsyndromic Deafness DFNB42 in Two Chinese Han Families,” Neural Plasticity, vol. 2018, Article ID 7272308, 6 pages, 2018.
[17] ACMG Working Group on Update of Genetics Evaluation Guidelines for the Etiologic Diagnosis of Congenital Hearing Loss; for the Professional Practice and Guidelines Committee, “American College of Medical Genetics and Genomics guideline for the clinical evaluation and etiologic diagnosis of hearing loss,” Genetics in Medicine, vol. 16, no. 4, pp. 347–355, 2014.
[18] S. Chen, C. Dong, Q. Wang et al., “Targeted next-generation sequencing successfully detects causative genes in Chinese patients with hereditary hearing loss,” Genetic Testing and Molecular Biomarkers, vol. 20, no. 11, pp. 660–665, 2016.
[19] H. Li, Y. Song, Z. He et al., “Meclofenamic acid reduces reactive oxygen species accumulation and apoptosis, inhibits excessive autophagy, and protects hair cell-like HEI-OC1 cells from cisplatin-induced damage,” Frontiers in Cellular Neuroscience, vol. 12, 2018.
[20] L. Liu, Y. Chen, J. Qi et al., “Wnt activation protects against neomycin-induced hair cell damage in the mouse cochlea,” Cell Death & Disease, vol. 7, no. 3, article e2136, 2016.
[21] S. Gao, C. Cheng, M. Wang et al., “Blebbistatin inhibits neomycin-induced apoptosis in hair cell-like HEI-OC1 cells and in cochlear hair cells,” Frontiers in Cellular Neuroscience, vol. 13, p. 590, 2020.
[22] S. Zhang, Y. Zhang, Y. Dong et al., “Knockdown of Foxg1 in supporting cells increases the trans-differentiation of supporting cells into hair cells in the neonatal mouse cochlea,” Cellular and Molecular Life Sciences, vol. 77, no. 7, pp. 1401–1419, 2020.
[23] Z. He, L. Guo, Y. Shu et al., “Autophagy protects auditory hair cells against neomycin-induced damage,” Autophagy, vol. 13, no. 11, pp. 1884–1904, 2017.
[24] Z. He, Q. Fang, H. Li et al., “The role of FOXG1 in the postnatal development and survival of mouse cochlear hair cells,” Neuropharmacology, vol. 144, pp. 43–57, 2019.
[25] Z.-h. He, S.-y. Zou, M. Li et al., “The nuclear transcription factor FoxG1 affects the sensitivity of mimetic aging hair cells to inflammation by regulating autophagy pathways,” Redox Biology, vol. 28, article 101364, 2020.
[27] X. Yu, Y. Lin, J. Xu et al., “Molecular epidemiology of Chinese Han deaf patients with bi-allelic and mono-allelic GJB2 mutations,” *Orphanet Journal of Rare Diseases*, vol. 15, no. 1, p. 29, 2020.

[28] T. B. Friedman, Y. Liang, J. L. Weber et al., “A gene for congenital, recessive deafness DFNB3 maps to the pericentromeric region of chromosome 17,” *Nature Genetics*, vol. 9, no. 1, pp. 86–91, 1995.

[29] C.-C. Wu, C.-Y. Tsai, Y.-H. Lin et al., “Genetic epidemiology and clinical features of hereditary hearing impairment in the Taiwanese population,” *Genes*, vol. 10, no. 10, p. 772, 2019.

[30] S. S. Di Ma, H. Gao, H. Guo et al., “A novel nonsense mutation in MYO15A is associated with non-syndromic hearing loss: a case report,” *BMC Medical Genetics*, vol. 19, no. 1, p. 133, 2018.

[31] H. Zhou, A. Kuermanhan, Z. Zhang et al., “Identification of a novel homozygous mutation in the MYO15A gene in a Kazakh family with non-syndromic hearing loss,” *International Journal of Pediatric Otorhinolaryngology*, vol. 125, pp. 128–132, 2019.

[32] N. Danial-Farran, Z. Brownstein, S. Gulsuner et al., “Genetics of hearing loss in the Arab population of Northern Israel,” *European Journal of Human Genetics*, vol. 26, no. 12, pp. 1840–1847, 2018.

[33] N. Zarepour, M. Koohiyan, A. Taghipour-Sheshdeh et al., “Identification and clinical implications of a novel MYO15A variant in a consanguineous Iranian family by targeted exome sequencing,” *Audiology & Neurotology*, vol. 24, no. 1, pp. 25–31, 2019.

[34] R. Cabanillas, M. Diñeiro, G. A. Cifuentes et al., “Comprehensive genomic diagnosis of non-syndromic and syndromic hereditary hearing loss in Spanish patients,” *BMC Medical Genomics*, vol. 11, no. 1, p. 58, 2018.

[35] Z. Brownstein, L. M. Friedman, H. Shahin et al., “Targeted genomic capture and massively parallel sequencing to identify genes for hereditary hearing loss in Middle Eastern families,” *Genome Biology*, vol. 12, no. 9, p. R89, 2011.

[36] J. Zhang, J. Guan, H. Wang et al., “Genotype-phenotype correlation analysis of MYO15A variants in autosomal recessive non-syndromic hearing loss,” *BMC Medical Genetics*, vol. 20, no. 1, p. 60, 2019.

[37] N. Nal, Z. M. Ahmed, E. Erkal et al., “Mutational spectrum of MYO15A: the large N-terminal extension of myosin XVA is required for hearing,” *Human Mutation*, vol. 28, no. 10, pp. 1014–1019, 2007.