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Amino acid 118 in the Deafness Causing (DFNA20/26) *ACTG1* gene is a Mutational Hot Spot

Li Wang^{#1,2}, Denise Yan^{#2}, Litao Qin¹, Tao Li¹, Hongjian Liu³, Wan Li³, Rahul Mittal¹, Feng Yong⁴, Prem Chapagain^{5,6}, Shixiu Liao^{#1}, and Xuezhong Liu^{#2}

¹Institute of Medical Genetics, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou, China.

²Department of Otolaryngology, University of Miami Miller School of Medicine, Miami, USA.

³Department of Otorhinolaryngology, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou, China

⁴Department of Otolaryngology, Xiangya Hospital, Central South University, Changsha, China

⁵Department of Physics, Florida International University, Miami, Florida

⁶Biomolecular Sciences Institute, Florida International University, Miami, Florida

These authors contributed equally to this work.

Abstract

Background: Hearing loss is an economically and socially important cause of human morbidity, affecting 360 million people (over 5% of the world's population), of whom 32 million are children. Of the estimated minimum of 50% of hereditary hearing loss, non-syndromic hearing loss (NSHL) accounts for more than 70%. The autosomal dominant non-syndromic hearing loss (ADNSHL) is highly heterogeneous. To date, 67 ADNSHL loci (DFNA1–67) have been mapped; however, only 35 causative genes have been cloned since 1997 (<http://hereditaryhearingloss.org/>).

Methods: To identify the genetic basis of hereditary hearing loss in a Chinese family with ADNSHL, we undertook a targeted sequencing of 180 genes using a custom capture panel (MiamiOtoGenes).

Results: The onset of hearing loss in the family occurred between the ages of 15 and 18 years. Hearing loss was bilateral, started in the high frequency and progressed to lower frequencies. The c.353A>T (K118M) in the *ACTG1* gene was identified by panel and was confirmed by Sanger

Correspondence to: Dr. Xuezhong Liu Department of Otolaryngology (D-48), University of Miami 1666 NW 12th Avenue, Miami, Florida 33136, USA, xliu@med.miami.edu, Tel: 305 243 1484; Fax: 305 243 2009. Dr. Shixiu Liao, Medical Genetics Institute of Henan Province, the People's Hospital of Henan Province No.7 Weiwu Road, Zhengzhou, Henan 450003, P. R. China., Tel: 086-0371-65580927; Fax: 086-0371-65580463, henangenetics@hotmail.com.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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sequencing and was present in all affected family members. So far, five of the 23 DFNA20/26 families worldwide have been found to carry mutation involving the residue K118.

Conclusions: This is the first report of K118M mutation in the *ACTG1* gene causing hearing loss in the Chinese population. The present data are in line with previous evidence to suggest that codon K118 of *ACTG1* may represent a mutational hot spot that justifies a mutation screen for diagnostic purpose in the genetically heterogeneous group of DFNA20/26.

Keywords

Hearing loss; *ACTG1*; DFNA20/26; Heterogeneity; Next-generation sequencing

1 INTRODUCTION

Hereditary hearing loss is the most common sensory defect and shows the highest degree of genetic heterogeneity. Non-syndromic hearing loss (NSHL) accounts for more than 70% of genetic deafness and can follow a pattern of autosomal recessive, autosomal dominant, X-linked, and mitochondrial inheritance.¹

Autosomal dominant sensorineural hearing loss (ADSNHL) accounts for approximately 20% of hereditary hearing loss. In contrast to autosomal recessive hearing loss, most patients with ADSNHL present large variation in age of onset; hearing impairment often tends to start before the age of 20 years and progresses gradually. The extreme genetic heterogeneity of NSHL with more than 140 genetic loci currently mapped (Hereditary Hearing LossHomepage, <http://hereditaryhearingloss.org/>) has proven a major challenge for genetic testing and counseling. Although traditional genetic approaches, such as linkage analysis have predicted the presence of novel deafness associated genes, Sanger sequencing of genes one-by-one remains expensive and time consuming making thus difficult to analyze the hundreds of genes in the determined candidate intervals. Moreover, genome wide linkage study is unsuitable for small families with an inadequate number of affected individuals.

The advent of next-generation sequencing (NGS) technologies can overcome these limitations through its high-throughput capacity to perform parallel sequencing of billions of DNA molecules at a low cost and high speed.² NGS technology has been applied to genetic diagnosis of nonsyndromic hearing loss and has accelerated the pace of deafness genes discovery.³

In this study, we ascertained a 5 generation Chinese family segregating progressive autosomal-dominant sensorineural nonsyndromic hearing loss. A targeted NGS panel composed of 180 known genes (MiamiOtoGenes)^{4,5} was used to identify the genetic etiology of deafness in this family.

2 MATERIALS AND METHODS

2.1 Family and clinical evaluation

We ascertained a five generation Chinese family with ADSNHL (Figure 1). Blood samples were collected and DNA was extracted from all available family members, of whom nine

were affected. Family medical history was gathered through a questionnaire that included degree of hearing loss, age at onset, evolution, symmetry of the hearing loss, presence of tinnitus, and other relevant clinical manifestations. None of the patients had any history of constant exposure to noise or to ototoxic drugs. The hearing levels of the participants were evaluated by audiological tests consisting of auditory brainstem response (ABR) and pure-tone audiometry (PTA). The study was approved by the ethics committee of Henan Province People's Hospital. Written informed consent was obtained from all adult subjects and guardians on behalf of the children prior to the clinical evaluation and blood sample collection.

2.2 Targeted genomic capture and next-generation sequencing

Genomic DNA was extracted from whole blood using the Gentra Puregene DNA isolation kit (QIAGEN, Hilden, Germany). Agarose gel and Qubit (Life technologies, CA, USA) were used for DNA quantity and quality assessment. All of the subjects were prescreened for mutation in the *GJB2*, *SLC26A4*, and *mtDNA12SrRNA* genes using a population-specific variant array and by Sanger sequencing and were found negative for these genes.

DNA samples from 2 affected individuals were subjected to a SureSelect custom NGS gene panel (MiamiOtoGenes) analysis for identification of the deafness causative gene.⁵ This 180-gene panel was designed to include all exons, 5' UTRs and 3' UTRs of the genes and covered a target size of approximately 1.158 MB encompassing 3494 regions. The targeted sequencing was processed at the Hussman Institute for Human Genomics (HIHG) Sequencing core, University of Miami. Approximately 3 µg of DNA was used to generate indexed Illumina libraries. The SureSelectTarget Enrichment (Agilent, Santa Clara, CA, USA) of coding exons and flanking intronic sequences in-solution hybridization capture system were used according to the manufacturer's standard protocol. Adapter sequences for the Illumina HiSeq 2000 were ligated, and the enriched DNA samples were prepared using the standard methods for the HiSeq 2000 instrument (Illumina, San Diego, CA, USA). Through the sample preparation, average insert size was 180 bp and paired-end sequencing reads were used.

2.3 Bioinformatics Analysis

The Illumina CASAVA v1.8 pipeline was used to assemble 99 bp sequence reads. Burrows-Wheeler Aligner (BWA) was applied for alignment of sequence reads to the human reference genome (hg19)⁶ and variants were called using FreeBayes⁷. Genesis 2.0 (<https://www.genesis-app.com/>) was then used for variant filtering based on quality/score read depth and minor allele frequency (MAF thresholds of 0.0005 for ADNSHL variants) as reported in dbSNP141, the National Heart, Lung, and Blood Institute Exome Sequencing Project Exome Variant Server, Seattle, WA Project, (Exome Variant Server, 2012). Exome Aggregation Consortium (ExAC) browser (<http://exac.broadinstitute.org/>), the 1000 Genome Project Database and our internal database of > 3,000 samples from European, Asian, and American ancestries. Variants meeting these criteria were further annotated based on their presence and pathogenicity information in Human Gene Mutation Database (HGMD; <http://www.hgmd.cf.ac.uk>), the Deafness Variation Database (DVD) (<http://deafnessvariationdatabase.org>) and ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>).

2.4 Confirmation and Segregation analysis

Samples from all available members from the family were subjected to Sanger sequencing to determine whether the variants in potential causative genes co-segregated with the disease phenotype. Specific exons containing candidate variations were amplified by polymerase chain reaction (PCR) using primer sets designed via Primer3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The PCR products were sequenced using BigDye Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, CA, USA) and analyzed using an ABI 3700XL Genetic Analyzer. Finally, the accurate genotype of the variations was confirmed by sequence analysis with the Sequencing Analysis software v5.2 (Applied Biosystems Corps., Foster City, CA, USA), and segregation analysis was then performed.

3 RESULTS

3.1 Clinical features of the Chinese family

The family exhibited a typical autosomal dominant inheritance pattern of hearing loss (Figure 1). None of vestibular dysfunction or other clinical abnormalities indicating syndromic hearing loss; both the auricle and external auditory meatus of patients developed normally, and the tympanic membranes were intact. The age of affected individuals at the time of diagnosis ranged from 22 (IV: 14) to 75 (II: 1) (Table 1), and each displayed approximately the same age of onset (ages 15 ~ 18 years). All of the patients had normal auditory senses and verbal function before the onset of hearing loss. Audiological assessments of the affected subjects revealed bilateral, progressive, sensorineural hearing loss that affected both genders. During the early stages of disease, the primary symptoms were tinnitus and high frequency hearing loss, yielding an audiogram with a “slope or gentle slope” configuration in both ears (Figure 2A-B). The patients present with a gradual deterioration of hearing, with all frequencies become involved as age increases, yielding an audiogram with a “flat” configuration. Patients III1 and III6 started to wear hearing aids as their hearing loss became severe in their 40’s.

3.2 Mutations Detection

In the present study, using a targeted gene panel, we identified a previously reported missense variant (c.353A >T; p.K118M) in exon3 of *ACTG1* in a heterozygous state in the affected individuals subjected to analysis (Figure 3A). This mutation results in a lysine to methionine substitution at position 118 in ACTG1. Sanger sequencing revealed that all of the affected family members were heterozygous for this mutation, while the variant was not observed in family members with normal hearing (Figure 3B) and is highly conserved in different species (Figure 3C).

4 DISCUSSION

The ultimate goal of genetic disease research is to build genetic databases used for clinical diagnosis, treatment and prevention. Therefore, accurate understanding of the genetic background of the disease is of vital importance. Traditional molecular testing that analyzes a single gene at a time is labor-intensive and not cost-effective. This targeted genetic

screening focuses on identifying allele variants of only few major genes, such as *GJB2*, *SLC26A4*, *GJB3* and mitochondrial genes⁸. With the advent of sequence capture enrichment strategies and massively parallel sequencing, NGS technology is bringing the significant improvement of mutational studies for hearing loss at low cost. The capacity to simultaneously screen hundreds of target genes makes this technique an especially powerful tool for detecting pathogenic mutations that cause hereditary hearing loss. In the present study we ascertained a family with ADNSHL and after exclusion of the most common deafness-related mutations in *GJB2*, *SLC26A4*, and *mtDNA12SrRNA* in Chinese population, we identified a mutation in *ACTG1* c.353A>T (K118M) segregating with autosomal-dominant hearing impairment in the family by gene panel analysis. Segregation analysis confirmed that the mutation was causative mutation for deafness. This is the first report of K118M mutation of *ACTG1* causing hearing loss in the Chinese population.

ACTG1 (NM_001199954) has been reported as one of the causative genes for ADSNHL and is linked to the DFNA20/26 locus (OMIM #604717) on chromosome 17q25.3^{9–11}. Some defects in this gene are also associated with Baraitser-Winter syndrome (OMIM 102560), which is a rare developmental disorder characterized by facial dysmorphologies, brain malformations, colobomas and variable hearing loss.^{12,13} *ACTG1* encodes γ -actin, that is predominantly expressed in cochlear hair cells and contributes to the structural maintenance of stereocilia, cuticular plates and adherens junctions.^{11,14} Through polymerization and depolymerization of fibrils, the protein has an important role in hair cell shape, motility, contraction and growth. *In vivo* and *in vitro* studies indicate that the γ -actin protein is required for long-term stability of actin filamentous structures of stereocilia, but not for auditory hair cell development, which is consistent with the post-lingual and progressive character of the hearing loss due to *ACTG1* mutations.^{15,16}

γ -Actin is one of the most highly conserved proteins known, with >90% homology between the single yeast actin (*ACT1*) gene and human gene. Therefore, mutations that change the amino acid structure of actin are generally not tolerated. To date, a total of 18 mutations in *ACTG1* gene associated with DFNA20/26 have been reported (Figure 4).^{11,17–28} These mutations are located in various binding domain, but so far only missense mutations have been identified. Three DFNA20/26 associated mutations (K118M, E241K and L299V) were identified in at least two families, whereas the remainder are “private” as they have been found in only one family. The K118M mutation in *ACTG1* has been reported in four families and is thus the most common mutation. In addition, a mutation involving the same residue (K118N) has also been identified in a Spanish family¹⁹. So far, five of the 23 (21%) DFNA20/26 families worldwide have been found to carry mutation involving the residue K118, suggesting that codon K118 of *ACTG1* may represent a mutational hot spot which can be used as the key screening site of the *ACTG1* gene detection and the target of gene therapy in the future. Previous biochemical studies provide convincing evidence that K118M and K118N have pathogenic effects on the normal functions of γ -actin in the hair cells. γ -actin encoded by *ACTG1* consists of 4 sub-domains (sub-domains 1–4) (Figure 5A–D). K118M and K118N are located in subdomain 1 of the protein (Figure 5A–D) near a region of the actin monomer that is considered to be an important binding interface for many of actin’s regulatory actin-binding proteins (ABPs)²⁹. Furthermore, the side chain of K118 is outward-facing and solvent-exposed, creating a prime site for interactions with other

proteins. Both of the mutations at the 118 site significantly alter the biochemistry of this residue by preventing it from salt bridges and ionic contacts with other proteins.^{19,30}

Based on previous reports, most patients with DFNA20/26 displayed progressive, bilateral, sensorineural hearing loss that begins in the high frequency, particularly for the frequencies from 6 to 8 kHz.³¹ As age increases, the degree of hearing loss increases with threshold shifts observed at all frequencies. As described by Teig, 1968³², the HL first affects the highest frequencies, and progresses to 60–80 dB HL for frequencies >1000 Hz. It then progresses to a 40–60 dB HL for frequencies <1000 Hz. The audiological features of the affected patients found in this study showed a similar tendency. De Heer et al (2009)¹⁸ reported that the rate of deterioration varied across the different mutations from 2 dB to 6 dB/year. A detailed long-term follow-up of the Chinese family is currently under way to observe the changes of hearing status in these patients. The age of onset of hearing loss caused by *ACTG1* differs among the different patients, which ranges from the first to third decade of life. There is no enough evidence of the correlation between genotypes and phenotype. The clinical features of the five families with K118 mutation are listed in Table 2. Most cases of DFNA20/26 present with a gradual deterioration of hearing into severe to profound before 45 years old. The typical age of onset of hearing loss in the present DFNA20/26 Chinese family was the second decade of life, thus similar to that of two of the 4 previously published families with the same segregating K118 mutation. However, one family described by Miyagawa et al (2015)¹⁷ displayed a different phenotype. The patient with a history of hearing loss from 26 years seemed to develop rapid further deterioration of the hearing, which required hearing aids use at 28 years of age. That is consistent with the Spanish patients with K118N mutation¹⁹. We speculate that the difference between these patients with the same mutation may be the results of the modification effect by other genes and environmental factors, like noise and ototoxic drugs. The additive of other gene might play a role in the phenotype, such as the variants of the *Atp2b2* gene that have been reported to modulate the severity of hearing loss in individuals with *CDH23* mutation.³³ Furthermore, tinnitus has been reported in three families with K118 mutation, including the current Chinese family.

This is the first report of K118M mutation of the *ACTG1* gene causing ADNSHL in the Chinese population. The information derived from this study significantly enhances our understanding about the prevalence of K118M mutation in diverse ethnic population. The results of this study will pave the way to design targeted gene chip panels for screening hearing loss in Chinese ethnic groups with the inclusion of K118M mutation.

Our present data also corroborate previous findings that indicate that codon K118 of *ACTG1* may represent a mutational hot spot for severe to profound late-onset hearing loss.

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Abbreviations

ACTG1	Actin gamma 1
DFNA	Autosomal dominant
NSHL	Non-syndromic hearing loss
ADSNHL	Autosomal dominant sensorineural non syndromic hearing loss
NGS	Next-generation sequencing
ABR	Auditory brainstem response
PTA	Pure-tone audiometry
MAF	Minor allele frequency

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Highlights

- > Extreme genetic heterogeneity of non-syndromic hearing loss has proven a major challenge for genetic testing and counseling.
- > The c.353A>T (K118M) in the *ACTG1* gene was identified by panel in a Chinese family and confirmed by Sanger sequencing.
- > The codon K118 of ACTG1 may represent a mutational hot spot for severe to profound late-onset hearing loss.

Figure 1. Pedigree of the family with ADSNHL.

The pedigree of the five generation Chinese family carrying the mutation in the *ACTG1* gene. Filled symbols represent affected individuals.

Figure 2. Audiograms of 4 affected family members.

The audiometric features of the affected subjects, performed when the subjects were respectively: III: 1 53 years old (y.o.), VI: 1 29 y.o., VI: 3 24 y.o., IV: 8 22 y.o. The audiograms showing a typical pattern found in DFNA20/26 with a moderate downward slope into higher frequencies with a gradual worsening over time affecting all frequencies.

Figure 3. DNA sequence chromatograms.
DNA sequence chromatograms show the c.353A>T (K118M) variant in *ACTG1* at the heterozygous state identified in III: 1 (A), while normal hearing member (III: 5) has wild-type allele (B). The residue K (highlighted in red box) is perfectly preserved across the species (Figure 3C).

Figure 4. Mutations in the *ACTG1* gene.

Schematic representation of the full-length *ACTG1* gene showing the different exons and locations of the reported mutations.

Figure 5. Three dimensional representation of the human cytoplasmic actomyosin complex based on the cryo-EM structure:

Side view (A) and bottom view (B) with γ -Actin rendered in surface representation, α -Actinin (Myosin-14) rendered as cylinders, and Tropomyosin α -3 helices as ribbon. Ribbon representation of the five γ -Actin molecules, with K118 residue highlighted as spheres (C). γ -Actin molecules showing the location of K118 (D). Images were obtained from Protein Data Bank (PDB ID: 5j1h).List of Table Captions

Table 1.

Phenotypes and genotypes of the current DFNA20/26 Chinese family members.

Patient	Sex	Age (Yrs)		PTA (dBHL)		Audiogram Shape	Level of hearing loss	Tinnitus	Genotype in <i>ACTG1</i>
		At Testing	At Onset	Left ear	Right ear				
III1	F	75	16	110	110	Flat	profound	+	c.353A>T
III1	M	53	18	105	110	Flat	profound	+	c.353A>T
III3	F	51	18	85	90	Gentle sloping	severe	+	c.353A>T
III5	M	49	–	25	25	Normal	Normal	–	N
III6	F	45	17	80	75	Gentle sloping	severe	+	c.353A>T
III8	M	42	18	80	85	Gentle sloping	severe	+	c.353A>T
IV1	F	29	15	55	60	Gentle sloping	moderate	+	c.353A>T
IV3	M	24	16	45	45	Gentle sloping	moderate	+	c.353A>T
IV4	F	22	–	20	20	Normal	Normal	–	N
IV5	M	20	15	50	50	Gentle sloping	moderate	–	c.353A>T
IV6	M	20	–	10	10	Normal	Normal	–	N
IV7	M	9	–	10	20	Normal	Normal	–	N
IV8	M	22	17	45	45	Gentle sloping	moderate	–	c.353A>T
IV9	M	16	–	20	20	Normal	Normal	–	N

+: existing symptoms;

–: without symptoms;

N: normal

Table 2.

Clinical features of patients with amino acid 118 mutation in ACTG1.

No.	Nucleotide Change	Amino acid change	Family nationality	Number of patients	Age at onset	Severity	Progression	Tinnitus	Method of detection (References)
1	c.353A>T	K118M	American	8	14	Profound (48y) *	++	Unknown	Linkage analysis/ Sanger sequencing (11)
2	c.353A>T	K118M	Japanese	3	26	Moderate (26y)	++	+	NGS (17)
3	c.353A>T	K118M	Japanese	2	17	Severe (37y)	+	+	NGS (17)
4	c.353A>T	K118M	Chinese (present study)	9	15	Severe (42y)	+	+	NGS
5	c.354G>C	K118N	Spanish	2	30	Profound (36y)	++	Unknown	Sanger sequencing (19)

++: rapidly progressive,

* age at the time of testing