Comprehensive Molecular Screening in Chinese Usher Syndrome Patients

Tengyang Sun, Ke Xu, Yanfan Ren, Yue Xie, Xiaohui Zhang, Lu Tian, and Yang Li

Beijing Institute of Ophthalmology, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Ophthalmology & Visual Sciences Key Lab, Beijing, China

Correspondence: Yang Li, Beijing Institute of Ophthalmology, Beijing Tongren Hospital, Houguo Lane 17, Chong Nei Street, Beijing, 100730, China; yanglibio@aliyun.com.

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Purpose. Usher syndrome (USH) refers to a group of autosomal recessive disorders causing deafness and blindness. The objectives of this study were to determine the mutation spectrum in a cohort of Chinese patients with USH and to describe the clinical features of the patients with mutations.

Methods. A total of 119 probands who were clinically diagnosed with USH were recruited for genetic analysis. All probands underwent ophthalmic examinations. A combination of molecular screening methods, including targeted next-generation sequencing, Sanger–DNA sequencing, and multiplex ligation probe amplification assay, was used to detect mutations.

Results. We found biallelic mutations in 92 probands (77.3%), monoallelic mutations in 5 patients (4.2%), and 1 hemizygous mutation in 1 patient (0.8%), resulting in an overall mutation detection rate of 78.2%. Overall, 132 distinct disease-causing mutations involving seven USH (ABHD12, CDH23, GPR98, MYO7A, PCDH15, USH1C, and USH2A) genes; 5 other retinal degeneration genes (CHM, CNGA1, EYS, PDE6B, and TULP1); and 1 nonsyndromic hearing loss gene (MYO15A) were identified, and 78 were novel. Mutations of MYO7A were responsible for 60% of USH1 families, followed by PCDH15 (20%) and USH1C (10%). Mutations of USH2A accounted for 67.7% of USH2 families, and mutation c.8559-2A>G was the most frequent one, accounting for 19.1% of the identified USH2A alleles.

Conclusions. Our results confirm that the mutation spectrum for each USH gene in Chinese patients differs from those of other populations. The formation of the mutation profile for the Chinese population will enable a precise genetic diagnosis for USH patients in the future.

Keywords: Usher syndrome, mutation screening, next generation sequencing
Mutations Spectrum of Chinese Patients With USH

SUBJECTS AND METHODS

Patients

In total, 119 unrelated individuals who were clinically diagnosed with USH1 (20 cases) and USH2 (99 cases) were enrolled at the Genetics Laboratory of the Beijing Institute of Ophthalmology, Beijing Tongren Ophthalmic Center. This cohort included 87 sporadic patients and 32 cases with family histories. All molecular screening procedures were prospectively reviewed and approved by the ethics committee of Beijing Tongren Hospital and carried out under the institutional instructions of the Beijing Tongren Hospital Joint Committee on Clinical Investigation and according to the tenets of the Declaration of Helsinki. Each patient underwent a standard ophthalmic evaluation consisting of best-corrected visual acuity (BCVA); slit-lamp biomicroscopy; and a fundus examination. Most of the patients also underwent optical coherence tomography (OCT); visual field; and electroretinogram (ERG) examinations. For those patients who had audiometric data, the severity of the hearing loss was divided by the pure-tone average (PTA) over 500, 1000, 2000, and 4000 Hz: normal hearing, <20 dB; mild, moderate, severe, and profound hearing defects corresponding to 21 to 40 dB, 41 to 70 dB, 71 to 90 dB, and >91 dB, respectively.1 Based on the previously reported clinical diagnostic criteria, the patients in the cohort were further classified into subtypes based on their clinical histories, ophthalmologic examinations, the severity of their hearing loss, and the vestibular function assessment (delay in motor development or unsteady gait). Peripheral blood samples of all probands and their relatives were collected, and genomic DNA was isolated from leukocytes by means of the genomic DNA extraction and purification kit (Vigorous Whole Blood Genomic DNA extraction Kit; Vigorous Technology Development Co. Ltd, Beijing, China), according to the manufacturer's protocol. The DNA was quantified with a spectrophotometer (Nanodrop 2000; Thermo Fisher Scientific, Waltham, MA, USA).

PCR-Based Sequencing of the USH2A Gene

Our previous study indicated that exons 43, 13, and 2 of USH2A were mutational hot spots, so these exons were first sequenced for all patients diagnosed with USH2. As long as a heterozygous pathogenic/likely pathogenic mutation was detected, the remaining 68 coding exons, including the intron-exon boundary of USH2A, were sequenced to screen for the other pathogenic mutations. Overall, coding regions (exons 2–72) of USH2A were amplified by PCR in 29 USH2 patients (including six previously described cases). The PCR amplifications were done with standard reaction mixtures, and purified amplicons were sequenced using a DNA sequencer (ABI Prism 373A; Applied Biosystems, Foster City, CA, USA). The sequencing results were compared with the published cDNA sequence of USH2A (GenBankNM_206933.2).

TES and Bioinformatics Analysis

A TES panel was developed to capture 188 known inherited retinal degeneration (IRD) genes using custom enrichment kits (GenCap; MyGenostics, Beijing, China) and following the manufacturer’s instructions, as described previously. The panel comprises 2894 exons with a total size of 550 kilobytes (kb). Of the 2894 exons, 410 belong to 11 USH genes and one possible USH modifier gene, which include ABHD12, CDH23, CIB2, CLRN1, DFNB31, GPR98, HARS, MYOTA, PCDH15, USH1C, USH2A, and PDZD7. A list of the target genes of our panel is summarized in Supplementary Table S1. Genomic DNA (1–3 μg) was fragmented into approximately 300–450 base pairs by endonuclease digestion and used to capture the targeted genomic sequences. Illumina library preparation and the capture experiments were performed as previously reported. The enrichment libraries were sequenced on a commercial device (NextSeq 500: Illumina, Inc., San Diego, CA, USA) as 100-bp paired end reads, according to the manufacturer’s protocol. Raw sequencing data were processed for subsequent bioinformatics analysis (Fig. 1A). First, the Illumina sequencing adapters and low-quality reads were removed by using fastq_mcf software. Then, the duplicated reads were removed by using Picard tools (http://broad institute.github.io/picard/, in the public domain), and the high-quality reads were aligned with the reference human genome (hg19) by the Burrows-Wheeler Aligner (http://bio-bwa.sour ceforge.net/, in the public domain). Finally, the single nucleotide polymorphisms (SNPs) and insertions or deletions (InDels) were called using the Genome Analysis Toolkit Haplotype Caller. The variants were further annotated using ANNOVAR and associated with multiple databases, including...
000 Genome (http://phase3browser.1000genomes.org/index.html, in the public domain); ESP6500 (http://evs.gs.washington.edu/EVS/, in the public domain); dbSNP (https://www.ncbi.nlm.nih.gov/snp, in the public domain); ExAC (http://exac.broadinstitute.org/, in the public domain); and an in-house variant database (My Genostics). The alternative alleles with less than five reads and/or a frequency less than 30% were removed to exclude likely false-positive variants. The pathogenicity of each variant was predicted by the PolyPhen2 (http://genetics.bwh.harvard.edu/pph/, in the public domain); Mutation Taster (http://www.mutationtaster.org/, in the public domain); and SIFT (http://sift.jcvi.org/, in the public domain) programs. Those variants that may involve the splicing effect were analyzed with the NetGene2 Server (http://www.cbs.dtu.dk/services/NetGene2/, in the public domain); Human Splice Finder (HSF, http://www.umd.be/HSF3/, in the public domain); and Berkeley Drosophila Genome Project (http://www.fruitfly.org/seq_tools/splice.html, in the public domain). Then, we carried out further analysis to identify the putative pathogenic mutations in each patient. Two databases, Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php, in the public domain) database and the Leiden Open (source) Variation database (http://www.lovd.nl/, in the public domain), were used to search for reported pathogenic mutations. Assessment of pathogenicity was based on the guideline reported by American College of Medical Genetics and Genomics.36 According to this guideline, we categorize variants into pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, or benign. Monoallelic mutations were considered only when they were classified as pathogenic or likely pathogenic variants. Presumed pathogenic or likely pathogenic variants and VUSs were verified by Sanger sequencing. Segregation analysis was performed in patients or likely pathogenic variants and VUSs were verified by Sanger sequencing. Segregation analysis was performed in patients with USH, or likely pathogenic variants. Presumed pathogenic or likely pathogenic mutations were considered only when they were classified as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, or benign. Monoallelic variants were categorized as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, or benign. Monoallelic variants were considered only when they were classified as pathogenic or likely pathogenic variants. Presumed pathogenic or likely pathogenic variants and VUSs were verified by Sanger sequencing.

**CNVs Analysis and Validation**

The coverage of target regions was normalized and compared with the average normalized data of all other samples of the same run to obtain the ratio of the relative coverage in order to detect deletions and duplications in the patients’ genome sequences, when only monoallelic mutation or no mutation was identified by TES. The regions where the ratio was below 0.7 were considered putative deletions, while the regions where the ratio rose above 1.3 were speculated as putative duplications.10 Real-time q-PCR was carried out to validate putative CNVs of MYO7A, PCDH15, and USH1C in six patients. The q-PCR reactions were done on a commercial instrument (Rotor-Gene 6000; Corbett Research, Mortlake, NSW, Australia) in a 10-μl final volume, including 1-μl (100-ng) genomic DNA and 300-nM primers and a master mix (EvaGreen PCR; Bio-Rad Laboratories, Hercules, CA, USA), as we previously described.57 Each assay was performed in triplicate. The human GAPDH gene was used as an internal control. The relative quantification (RQ) of the target gene was accomplished by using the RQ manager software (Bio-Rad Laboratories) and was calculated with the 2^(-ΔΔCT) method. The threshold for the normal value was set at 0.8 to 1.3. The ranges of the RQ values for deletions and duplications were set at 0.45 to 0.74 and 1.6 to 1.8, respectively. The MLPA assay was performed to validate the putative CNVs of USH2A with a SALSA MLPA probe mix P361-A2/P362-A2 USH2A (Amsterdam, The Netherlands), following the manufacturer’s protocol; this kit contains one probe for each exon of the USH2A gene.

**Supplementary PCR-Based Sequencing**

We performed Sanger sequencing of the reported intronic mutation c.48G>A of MYO7A in USH1 patients6 and c.553-854A>G, c.7595-2144A>G, c.8845+628C>T, and c.9959-4159A>G of USH2A in patients with USH2A when only monoallelic mutation or no mutation was identified by TES.
Additionally, we carried out Sanger sequencing in insufficiently covered regions of MYO7A in four patients when only monoallelic mutation or no mutation was identified by TES.

**Targeted Exome Sequencing of Inherited Hearing Loss Genes**

We performed TES of inherited hearing loss genes in proband 019791 and his brother (019791 II:1). The panel includes 199 hereditary hearing loss genes and comprises 4688 exons with a total size of 641 kb. A list of the targeted genes is provided in the supporting material (Supplementary Table S2). The detailed procedure of library construction, capture, sequencing, and bioinformatics analysis was just the same as described above.

**Statistical Analysis**

Differences in the onset age of night blindness and hearing loss were compared with statistical software (SPSS version 20.0; SPSS, Inc., Chicago, IL, USA), and ANOVA was used to compare the differences among groups. A value of \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Characteristics of Target Gene Capture and Sequence Depth**

We applied our capture panel, which included 188 known retinal degeneration genes, to 90 USH patients in our study and achieved high-quality results. On average, the mean coverage of the target regions was 448.3x. For each sample, more than 99.5% of the target regions were covered, and 90.96% of the covered regions of MYO7A, GRP98, ABHD12, DFNB31, PCDH15, CIB2, CLRN1, and HARS).  

**USH Mutation Detection Rates and Involving Disease-Causing Genes**

We identified biallelic disease-causing mutations in 92 probands (77.3%); monoallelic mutations in 5 probands (4.2%); one hemizygous mutation in one patient (0.8%); and no disease-causing allele in 21 unrelated patients (17.6%), achieving an overall mutation detection rate of 78.2% (93/119; Table 1). In total, we identified 132 distinct disease-causing mutations involving 13 genes in this cohort of patients (Supplementary Table S5). Mutations in USH1 genes were detected in 19.3% (23/119) of the probands in this cohort (Fig. 2A, Supplementary Table S4). Mutations in USH2 genes were identified in 58% (69/119) of the probands (Fig. 2A, Supplementary Table S4). Mutations in the atypical USH gene ABDH12 were identified in only 1% (1/119) of the probands (Fig. 2A, Supplementary Table S4). No mutation was detected in CIB2, DFNB31, and CLRN1. Mutations in non-USH IRD genes (CHM, CNGA1, and EYS) were detected in 3% (4/119) of the probands (Fig. 2A, Supplementary Table S4). Of the 152 putative disease-causing mutations, 78 were novel, including three CNVs (Fig. 3). Additionally, 11 VUSs involving five USH genes were detected in 10 patients (Supplementary Table S5).

**Molecular Diagnosis in USH1 Families and Clinical Profile**

Of the 20 unrelated patients who were clinically diagnosed with USH1, two disease-causing alleles were found in 17 patients and a single mutant allele was found in two probands. Cosegregation analyses were done in 16 of the 17 unrelated patients (94.1%) with biallelic mutations (Table 1 and Supplementary Table S4). Mutations of MYO7A were the most common in our cohort, accounting for 12 of the 20 probands with USH1 (60%), followed by mutations of PCDH15 and USH1C identified in 4 (20%) and 2 patients (10%), respectively (Fig. 2B). Table 2 summarizes the proportions of different kinds of mutations for the 36 mutant alleles. All USH1 patients had different extents of defects in visual acuity (0.1–0.9), profound hearing loss in their early childhood, and a history of motor developmental delay (Supplementary Table S4). The majority of the patients (14/19) underwent cochlear implantation in their early childhood. The mean onset age of the hearing defect of the patients was 0.5 years (range, 0–3 years), much earlier than the mean onset age of their visual defect, which was 5.07 years (range, 1–12 years; Table 3). Patient 019791 is worth describing in detail. This 7-year-old proband was diagnosed with USH1 based on her clinical phenotype and underwent cochlear implantation at around 3 years old. Her brother started to have a hearing impairment at around 11 years old. However, his audiograms showed a relatively mild downsloping hearing loss; he did not have any nyctalopia complaint, and his fundus was normal (Figs. 4A–C). We detected compound heterozygous VUSs (p.R356Q/p.R1604S) of PCDH15 and compound heterozygous mutations (p.R419Q/p.I407DfsTer3) of TULP1—an RP-causing gene in the proband. Cosegregation analysis showed that her brother (II:1) only carried one heterozygous mutation of TULP1 and one VUS of PCDH15. As the aforementioned results could not fully explain the hearing loss of the proband and her brother, we performed TES of inherited hearing loss in these two patients and detected compound heterozygous mutations (c.1128C>G; A, p.Y376X/c.6177+1G->T) of MYO15A in the proband and her brother (Fig. 4A, Supplementary Table S4).
FIGURE 2. Summary of proportion of patients with mutations of involved genes in this study. (A) Proportion of patients with mutations of each involved gene identified in all patients. (B) Proportion in USH1 patients. (C) Proportion in USH2 patients. The areas of slanted lines indicate patients with monoallelic mutations, and the grid areas indicate patients with mutations of two genes.

FIGURE 3. Colored fundus (CF) photographs and results of CNVs of the probands in families 019101, 019248, and 019511. (A-C, left) CF photographs show attenuation of retinal blood vessels and pigment proliferation. (A) Bar chart of readcount (middle) of proband 019101 showing two suspicious areas of CNVs (red squares) of USH1C. The real-time quantitative PCR result (right) verified the deletion of exon 7 (red square). (B) Electroretinogram (middle) of proband 019101 showing no responses in all five standard reactions. The real-time quantitative PCR result (right) show the deletion of exon 19 of MYO7A. (C) Bar chart of readcount (right superior) showing a suspicious area of CNV (red square) of USH2A identified in proband 019511. The result of multiplex ligation dependent probe amplification verified the gross deletion from exon 47 to exon 51 (red square).
Table 2. Number and Percentage of the Mutant Alleles Identified in Each USH Gene*

<table>
<thead>
<tr>
<th></th>
<th>ABHD12, n</th>
<th>CDH23, n</th>
<th>GPR98, n</th>
<th>MYO7A, n (%)</th>
<th>PCDH15, n (%)</th>
<th>USH1C, n</th>
<th>USH2A, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mis</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>8 (36)</td>
<td>5 (85)</td>
<td>2 (25)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Non</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>4 (18)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spl</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>7 (32)</td>
<td>0</td>
<td>1 (12.5)</td>
<td>0</td>
</tr>
<tr>
<td>Fs</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2 (9)</td>
<td>1 (17)</td>
<td>5 (62.5)</td>
<td>0</td>
</tr>
<tr>
<td>CNV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (5)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>22</td>
<td>6</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

Values in bold represent the number and percentage of the mutant alleles identified in the USH1 patients. Values with no bold represent the number and percentage of the mutant alleles identified in the USH2 patients. Fs, frameshift mutations; Mis, missense mutations; Non, nonsense mutations; Spl, splicing mutations.

Molecular Diagnosis in USH2 Families and Clinical Profile

Of the 99 unrelated patients who were clinically diagnosed with USH2, two disease-causing alleles were revealed in 75 patients, a single mutant allele in 3 probands, and a hemizygous mutation in one patient (Fig 2C). Cosegregation analyses were done in 59 of the 75 unrelated patients (78.6%; Supplementary Table S4). Mutations of USH2A were the most frequent in our cohort, representing 67 of the 99 USH2 patients (36%). The most frequent missense mutation (p.R5143C, and p.R626X (each 2.2%, 3/136; Supplementary Table S4). The most frequent missense mutations, one reported mutation (p.D428N of CDH23) was relatively weak and was predicted to be disease causing only by the Mutation Taster; the other three mutations were predicted to be probably damaging or disease causing by all three programs (PolyPhen2, Mutation Taster, and SIFT). All USH2 patients carrying the mutations of USH1 genes experienced night blindness, progressive visual defect, and different degrees of hearing loss. For the patients who carried USH2A mutations, excluding the four patients with a history of ototoxic drug usage (two patients with a history of streptomycin injection and the other two patients with a history of gentamycin injection), the mean onset age of the hearing defect of the patients carrying deleterious mutations (either compound heterozygous or homozygous) was statistically younger than that of the patients harboring two missense alleles (ANOVA P = 0.019) or the patients with one missense coupled with one deleterious allele (ANOVA P = 0.024; Table 3). In contrast, no statistically significant difference was observed in the onset age of the visual defect among the patients with different kinds of mutations (Table 3). The mean onset age (of both visual and hearing defects) of the four patients with the mutations of USH1 genes was earlier than that of the patients with USH2A mutations (Table 3). Patient 019691, who carried two compound mutations of CDH23 (p.D428N/p.G2190D) and PDE6B (p.H557Y/p.G576S), was a 45-year-old male who had suffered from night blindness since he was 25 years old. He had complained of a mild hearing defect over the past 4 years.

Table 3. Correlations Between Onset Age of Patients With USH and Their Carrying Mutations

<table>
<thead>
<tr>
<th>Patients</th>
<th>Number</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total USH1*</td>
<td>17</td>
<td>4.87 ± 3.81</td>
<td>1–12</td>
<td>0.47 ± 0.92</td>
<td>0–3</td>
</tr>
<tr>
<td>With MYO7A mutations*</td>
<td>10</td>
<td>3.65 ± 2.20</td>
<td>2–8</td>
<td>0.33 ± 1.00</td>
<td>0–3</td>
</tr>
<tr>
<td>With other USH gene mutations</td>
<td>6</td>
<td>6 ± 4.43</td>
<td>2–12</td>
<td>0.5 ± 0.84</td>
<td>0–2</td>
</tr>
<tr>
<td>With multiple genes mutations</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total USH2*</td>
<td>71</td>
<td>17.21 ± 9.47</td>
<td>2–49</td>
<td>17.56 ± 15.00</td>
<td>0–59</td>
</tr>
<tr>
<td>With USH2A mutations*</td>
<td>60</td>
<td>18.1 ± 9.28</td>
<td>2–49</td>
<td>17.6 ± 14.78</td>
<td>0–59</td>
</tr>
<tr>
<td>Three mutations</td>
<td>4</td>
<td>16.75 ± 3.59</td>
<td>14–22</td>
<td>11.75 ± 8.30</td>
<td>5–22</td>
</tr>
<tr>
<td>Mis/Mis mutations</td>
<td>12</td>
<td>21.42 ± 9.98</td>
<td>5–38</td>
<td>23.83 ± 15.83‡</td>
<td>3–56</td>
</tr>
<tr>
<td>Mis/Del mutations</td>
<td>20</td>
<td>17.6 ± 7.08</td>
<td>3–30</td>
<td>21.75 ± 16.50‡</td>
<td>0–59</td>
</tr>
<tr>
<td>Del/Del mutations</td>
<td>24</td>
<td>17.08 ± 11.08</td>
<td>2–49</td>
<td>12.80 ± 11.52‡</td>
<td>0–45</td>
</tr>
<tr>
<td>With GPR98 mutations</td>
<td>2</td>
<td>16 ± 8.49</td>
<td>10–22</td>
<td>6.5 ± 3.54</td>
<td>4–9</td>
</tr>
<tr>
<td>With USH1 gene mutations</td>
<td>4</td>
<td>9.67 ± 5.86</td>
<td>3–14</td>
<td>3.67 ± 3.06</td>
<td>1–7</td>
</tr>
<tr>
<td>With multiple genes mutations</td>
<td>1</td>
<td>25</td>
<td>-</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>With non-USH gene mutations*</td>
<td>3</td>
<td>13.3 ± 14.57</td>
<td>3–30</td>
<td>37.33 ± 6.03</td>
<td>31–43</td>
</tr>
</tbody>
</table>

|          |      |           |       |           |       |
|-----------------------------------------------------------|
| Patients with non-USH gene mutations*                      | 28   | 56 ± 10.5 | 30–70 | 23.9 ± 7.6 | 15–38 |

Del, deleterious mutations (including frameshift mutations, splicing mutations, nonsense mutations, and gross deletion); HL, hearing loss; NB, night blindness.

* Exclude patients with history of ototoxic drugs usage.
† P = 0.024.
‡ P = 0.019.
His fundus showed a typical RP appearance (Fig. 4D); unfortunately, the patient was unwilling to undergo a pure-tone audiometry examination. Of the four patients carrying mutations of other IRD-causing genes, patients 019512 and 019650 harbored the same homozygous mutation (p.L89FfsTer4 of CNGA1); patient 019415 carried mutations of EYS; and proband 019524 had a mutation of CHM. In our further medical history review and cosegregation analysis, we found that patient 019650 had a history of plural streptomycin injection when he was 1 year old, while his twin brother (without the usage of ototoxic drugs) who carried the same mutations did not complain of any hearing defect (Figs. 4A, 4E). Patients 019512, 019415, and 019524 all complained about hearing loss since their middle age, but patient 019415 pure-tone audiometry examination showed normal results after his molecular testing.

**DISCUSSION**

In this study, we performed comprehensive disease-causing mutation screening in 119 Chinese USH patients. Combined TES and Sanger–DNA direct sequencing determined that our overall mutation detection rate for the current cohort was 78.2%. This solving proportion is compatible with the reported rates in several previous studies using TES or Sanger–DNA direct sequencing; however, it is still about 15% lower than the 92.7% rate reported recently by Bonnet et al. The mutation detection rate is related to the accuracy of the patients’ clinical diagnoses. In our study, the mutation detection rate (85%) for USH1 patients was higher than that (76.8%) for USH2 patients. The reason might be that the USH1 patients’ relatively distinct phenotype makes it easier to achieve a precise clinical diagnose. In contrast, the clinical diagnosis of USH2 is relatively challenging due to different

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**FIGURE 4.** Pedigrees, ocular examinations, and PTA results of probands from families 019691, 019791, and 019650. (A) Pedigree and segregation analysis of families 019791, 019691, and 019650. The solid symbols indicate patients with both nyctalopia and hearing loss; the half-solid symbols indicate patients with only hearing loss or only visual defect. (B) CF photographs of proband 019791 showing waxy pallor of optic discs and attenuation of retinal blood vessels. The auditory steady state response showed profound hearing loss in both ears. (C) CF of the brother (019791, II:1) showing normal fundus. The PTA shows severe bilateral sensorineural hearing impairments. (D) Photographs of proband 019691 showing waxy pallor of optic discs, attenuation of retinal blood vessels, and obvious choroidal atrophy. (E) CF of proband 019650 presenting RP appearance. The PTA shows bilateral profound sensorineural hearing impairments.
degrees of hearing loss (from mild to severe). Hearing impairment may be related to many nongenetic factors, such as excessive noise exposure in the working or living environment, trauma, the usage of otoxic drugs, and a combination with other diseases. In this cohort, more than half of the USH2 patients did not undergo a pure-tone audiometry examination, leading to the possibility of a misdiagnosis for some of these patients, just like patient 019415. This issue is one of the limitations of the current study.

Consistent with the previous study, MYO7A and USH2A were the most common mutated genes in the USH1 and USH2 patients, accounting for 60% and 68% of mutations, respectively. However, the mutation spectrum observed in the current study differed from that found in the Caucasian population. More than half of the mutations of these two genes were first identified in the current study. Consistent with previous studies, the most common mutation of USH2A was c.8559-2A→G, which was only detected in Chinese and Japanese patients. In contrast, the most frequent mutations (p.Glu767Serfs*21, p.C3267R, and p.T3571M) in European patients were not detected in the current study. Targeted NGS can detect a large genomic DNA rearrangement, which could not exclude the previously mentioned misdiagnosis possibility. The other possibility is that they carried some mutations of other genes that were not included in our panel.

In conclusion, our results suggest that Chinese patients appear to have a different mutation spectrum for each USH gene. The formation of the mutation profile for the Chinese population will enable precise genetic diagnoses for USH patients, especially for atypical clinical cases in the future.

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References


In this cohort, five patients had only one mutation detected in USH2A or MYO7A; other unidentified mutations may be in the promoter region or in some deep intronic regions. Given the different mutation spectrum in Chinese patients, it is no surprise that the five mutations in the deep intronic regions of USH2A and MYO7A that were reported in European patients were not found in the current cohort. Of the 21 patients with no identified mutation, five patients were found carrying heterozygous variants of uncertain of the 5 USH genes (Supplementary Tables S4, S5); therefore, they might carry the second missing mutant allele in some deep intron regions or some CNVs of these five genes. For the remaining 16 patients, most acquired a hearing impairment after reaching 30 years of age and did not undergo a pure-tone audiometry examination, which could not exclude the previously mentioned misdiagnosis possibility. The other possibility is that they carried some mutations of other genes that were not included in our panel.

In conclusion, our results suggest that Chinese patients appear to have a different mutation spectrum for each USH gene. The formation of the mutation profile for the Chinese population will enable precise genetic diagnoses for USH patients, especially for atypical clinical cases in the future.


