Molecular epidemiology of Chinese Han deaf patients with bi-allelic and mono-allelic GJB2 mutations

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Abstract

Background: Recessive mutations in GJB2 is the most common cause of genetic hearing loss worldwide. The aim of this study is to determine the spectrum and frequency of GJB2 variants in Chinese Han deaf patients and to investigate the underlying causative genes in patients with mono-allelic GJB2 mutations.

Methods: We analyzed the mutation screening results of GJB2 in 1852 Chinese Han probands with apparently autosomal-recessive hearing loss in our laboratory. Targeted next-generation sequencing of 139 known deafness-related genes were performed in 44 probands with mono-allelic GJB2 mutations.

Results: Bi-allelic GJB2 mutations was identified in 25.65% of patients, in which the c.235delC (p.L79Cfs*3) mutation is the most frequent cause for both severe-to-profound (84.93%) and mild-to-moderate hearing loss (54.05%), while the c.109G > A (p.V37I) mutation is another frequent cause for mild-to-moderate hearing loss (40.54%). In 3.89% of patients only one mutant allele can be identified in GJB2. Targeted next generation sequencing in 44 such probands revealed digenic heterozygous mutations in GJB2/GJB6 and GJB2/GJB3 as the likely pathogenic mechanism in three probands. In 13 probands, on the other hand, pathogenic mutations in other deafness-associated genes (STRC, EYA1, MITF, PCDH15, USH2A, MYO15A, CDH23, OTOF, SLC26A4, SMPX, and TIMM8A) can be identified as the independent genetic cause, suggesting that the mono-allelic GJB2 mutations in those probands is likely co-incidental.

Conclusions: Our results demonstrated that GJB2 should be a primary target for mutation screening in Chinese Han deaf patients, and those with mono-allelic GJB2 mutations should be further screened by next generation sequencing.

Keywords: Hearing loss, GJB2, Mutation screening, Epidemiology, Next generation sequencing

Introduction

Hearing loss is a heterogeneous disorder that affects language acquisition and social skill development in children. It is estimated that 50%~60% cases of hearing loss have a genetic etiology [1]. To date, there have been over 100 genes identified to cause non-syndromic hearing loss and over 700 genetic syndromes described with features of hearing loss. Despite this, mutations in a single gene GJB2 (OMIM 121011) account for a large proportion of non-syndromic hearing loss in most populations worldwide [2].

The GJB2 gene codes for a gap junction protein connexin-26 (Cx26), which is essential for the physiological function of supporting cells in the cochlea [3]. About 200 GJB2 pathogenic mutations have been reported so far [4]. A number of missense mutations may lead to autosomal dominant non-syndromic hearing loss DFNA3 and autosomal dominant syndromic hearing loss associated with hyperproliferative epidermal disorders [5, 6]. On the other hand, a majority of GJB2 mutations are inherited in a recessive form and lead to non-syndromic hearing loss DFNB1. The mutation spectrum of GJB2 and the frequencies of these mutations vary greatly across different ethnic groups [2, 7], and the Chinese population has a quite distinct spectrum of GJB2 mutations from other populations [8]. With China having approximately one fifth of the world’s population, evaluating the molecular epidemiology of GJB2
mutations in Chinese deaf patients has important implications in guiding genetic testing for deafness. In the present study, we analyzed the GJB2 mutation screening results and audiometric data of 1852 Chinese Han deaf probands to determine its GJB2 mutation spectrum and genotype-phenotype correlation.

In addition, previous mutation screening of GJB2 in deaf patients revealed that a substantial number of them carried only one mutant allele [2, 9, 10]. The allele frequency of GJB2 mutations in heterozygous patients was significantly higher than expected in the general population. Possibly other mutations, either within the DFNB1 locus or in other unlinked genes, could contribute to the hearing loss in patients with mono-allelic GJB2 mutations. To this end, this study also used targeted next-generation sequencing (NGS) to detect single nucleotide variants, small insertions and deletions (indels) and copy number variations (CNVs) of 139 known deafness-related genes in 44 patients with mono-allelic GJB2 mutations. The results would provide important information for genetic testing and counselling, especially for those with mono-allelic GJB2 mutations.

Materials and methods

Patients

We reviewed the records of patients with sensorineural hearing loss who received genetic testing for deafness in our laboratory in Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine. Included in this study were patients with bilateral, non-syndromic, sensorineural hearing loss. A total of 1852 unrelated deaf probands, 979 males and 873 females, were analyzed for GJB2 mutations. The familial cases were compatible with an autosomal-recessive inheritance and the rest cases were sporadic. The ages of the subjects ranged from 2 months to 68 years, with the median age of 12 years old. All subjects were of Chinese Han ethnicity. The severity of hearing loss was classified based on the better hearing ear as mild (21–40 dB), moderate (41–70 dB), severe (71–95 dB), and profound (>95 dB).

Ethics statement

A written informed consent was obtained from each subject or their guardians to participate in this study. This study was approved by the Ethics Committee of Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine.

Mutation analysis of the GJB2 gene

Genomic DNA was extracted from blood samples using the Genomic DNA Extraction Kit (Tiangen Biotech, Beijing, China). The GJB2 (NM_004004.5) coding exon (exon 2) and flanking regions as well as the non-coding exon 1 and its flanking splice sites were amplified by polymerase chain reaction (PCR), and the PCR product was then Sanger sequenced in both directions. Sequence data were analyzed using Sequencher 5.4.5, and primer sequences are provided in Additional file 1: Table S4.

Targeted next-generation sequencing

For library preparation, 2 μg genomic DNA was randomly fragmented to 150–200 bp fragments by ultrasound shearing. End-repair, adenylation, adapter ligation and PCR amplification were completed according to the standard Illumina protocol. The amplified DNA was captured with a Deafness-related Gene Panel (WuXi NextCODE, Shanghai, China) designed to capture all exons and splicing sites of 139 deafness genes. Sequencing of the enrichment libraries were then performed on the Illumina HiSeq high-throughput platform.

The raw reads were mapped to the human reference genome (UCSC hg19), and the Sentieon software suite was used to call Single Nucleotide Variants (SNVs) and small insertions or deletions (InDels). Copy number variation detection was carried out with CNVkit [11] and ExomeDepth [12] tools that detect copy number variations based on read depth. The SNVs and InDels were annotated with an in-house developed annotation pipeline developed by WuXi NextCODE using Variant Effect Predictor (VEP) software.

Variant filtering and interpretation

With the exception of three known common mutations in Chinese Hans, c.235delC and c.109G > A in GJB2 and c.919-2A > G in SLC26A4, an in-house Chinese Han allele frequency database was used to exclude variants with minor mutation frequency (MAF) higher than 0.005 in the general population. ClinVar, OMIM and HGMD database were used to annotate known pathogenic variants. In addition, multiple computational tools (SIFT, Polyphen2, PROVEAN, MutationTaster and PANTHER) were used to predict the functionality of nonsynonymous variants. Segregation analysis was performed when DNA samples from the family members were available. The reported variants and CNVs were validated by Sanger sequencing (primer sequences for PCR amplification are provided in Additional file 1: Table S4).

Results

Spectrum and genotype-phenotype correlation of GJB2 mutations

Sanger sequencing of both the coding and noncoding exon and flanking sites of GJB2 in 1852 Chinese Han deaf probands identified a total of 47 different mutations. The most frequent variants included four frameshift mutations c.235delC (p.L79Cfs*3, allele frequency 18.25%, 676/3704), c.299_300delAT (p.H100Rfs*14, 2.94%), c.507insAACG (p.A171Efs*40, 0.65%), c.36insG (p.V13Cfs*35, 0.24%) and three missense mutations c.109G > A (p.V37I, 7.88%), c.368C > A (p.T123N, 0.84%) and c.257C > G (p.T86R, 0.51%) (Table 1). In addition, four dominant mutations...
| Nucleotide Change | Effect on Protein | Allele counts | Allele Frequency in 1852 deaf patients (%,3704 allele) | Category |
|-------------------|-----------------|---------------|------------------------------------------------------|----------|
| Frameshift mutations |                 |               |                                                      |          |
| c.235delC         | frameshift      | 676           | 18.25                                                | pathogenic |
| c.299_300delAT    | frameshift      | 109           | 2.94                                                 | pathogenic |
| c.507insAAGC      | frameshift      | 24            | 0.65                                                 | pathogenic |
| c.36insG          | frameshift      | 9             | 0.24                                                 | pathogenic |
| c.176del16bp      | frameshift      | 7             | 0.19                                                 | pathogenic |
| c.605insA6        | frameshift      | 7             | 0.19                                                 | pathogenic |
| c.312del14        | frameshift      | 1             | 0.03                                                 | pathogenic |
| c.35delG          | frameshift      | 1             | 0.03                                                 | pathogenic |
| c.443delC         | frameshift      | 1             | 0.03                                                 | pathogenic |
| c.493insG         | frameshift      | 1             | 0.03                                                 | pathogenic |
| Nonsense mutation |                 |               |                                                      |          |
| c.9G > A          | p. W3*          | 4             | 0.11                                                 | pathogenic |
| c.139G > T        | p. E47*         | 5             | 0.13                                                 | pathogenic |
| c.231G > A        | p. W77*         | 2             | 0.05                                                 | pathogenic |
| Missense mutations |                 |               |                                                      |          |
| c.109G > A        | p. V37I         | 292           | 7.88                                                 | Pathogenic* |
| c.368C > A        | p. T123 N       | 31            | 0.84                                                 | unclassified |
| c.257C > G        | p. T86R         | 19            | 0.51                                                 | pathogenic |
| c.427C > T        | p. R143W        | 7             | 0.19                                                 | pathogenic |
| c.571T > C        | p. F191 L       | 5             | 0.13                                                 | unclassified |
| c.344T > G        | p. F115C        | 4             | 0.11                                                 | unclassified |
| c.11G > A         | p. G4D          | 3             | 0.08                                                 | pathogenic |
| c.224G > A        | p. R75Q         | 3             | 0.08                                                 | pathogenic |
| c.223C > T        | p. R75W         | 2             | 0.05                                                 | pathogenic |
| c.389G > T        | p. G130V        | 2             | 0.05                                                 | pathogenic |
| c.583A > G        | p. M195V        | 2             | 0.05                                                 | unclassified |
| c.164C > A        | p. T55 N        | 1             | 0.03                                                 | pathogenic |
| c.181A > C        | p. K61Q         | 1             | 0.03                                                 | pathogenic |
| c.187G > T        | p. V63 L        | 1             | 0.03                                                 | pathogenic |
| c.77C > T         | p. T26I         | 1             | 0.03                                                 | unclassified |
| c.95G > A         | p. R32H         | 1             | 0.03                                                 | pathogenic |
| c.232G > A        | p. A78T         | 1             | 0.03                                                 | unclassified |
| c.242T > G        | p. L81R         | 1             | 0.03                                                 | pathogenic |
| c.250G > A        | p. V84 M        | 1             | 0.03                                                 | pathogenic |
| c.253T > C        | p. S85P         | 1             | 0.03                                                 | pathogenic |
| c.257C > T        | p. T86 M        | 1             | 0.03                                                 | pathogenic |
| c.379C > T        | p. R127C        | 1             | 0.03                                                 | pathogenic |
| c.389G > C        | p. G130A        | 1             | 0.03                                                 | pathogenic |
| c.107T > C        | p. L36P         | 1             | 0.03                                                 | unclassified |
| c.398G > C        | p. W133S        | 1             | 0.03                                                 | unclassified |
| c.457G > A        | p. V153I        | 1             | 0.03                                                 | unclassified |
| c.478G > A        | p. G160S        | 1             | 0.03                                                 | unclassified |
| c.493C > T        | p. R165W        | 1             | 0.03                                                 | pathogenic |
c.164C > A (p. T55 N), c.224G > A (p. R75Q), c.223C > T (p. R75W) and c.551G > A (p. R184Q) were identified in seven subjects and fourteen uncategorized variants were detected in 51 subjects (Additional file 1: Tables S1& S3).

Overall, bi-allelic (homozygous and compound heterozygous) pathogenic mutations in \textit{GJB2} were identified in 475 probands (25.65%, Table 2). Among them, c.235delC/c.235delC (227, 47.79%), c.109G > A/c.109G > A (53, 11.16%), c.235delC/c.299_300delAT (86, 18.11%), c.235delC/c.507insAACG (18, 3.79%) and c.235delC/c.257C > G (11, 2.32%) were the most common pathogenic \textit{GJB2} genotypes. These six common genotypes were found in up to 87.37% probands with bi-allelic \textit{GJB2} mutations in our cohort (Table 2).

When analyzing the hearing loss levels in these subgroups, we found that 92.21% (438/475) of patients with bi-allelic \textit{GJB2} mutations exhibited severe-to-profound hearing loss (Table 2). The c.235delC mutation alone, was identified in 372 (84.93%) such probands in at least one allele. On the contrary, the c.235delC and c.109G > A mutations are the major causes for the remaining 37 probands with mild-to-moderate hearing loss, accounting for 54.05% (20/37) and 40.54% (15/37) of probands in at least one allele, respectively.

**Table 1** Pathogenic or unclassified variants in \textit{GJB2} among 1852 deaf patients (Continued)

| Nucleotide Change | Effect on Protein | Allele counts | Allele Frequency in 1852 deaf patients (%|3704 allele) | Category |
|-------------------|------------------|---------------|------------------------------------------|------------|
| c.551G > A        | p. R184Q         | 1             | 0.03                                     | pathogenic |
| c.551G > C        | p. R184P         | 1             | 0.03                                     | pathogenic |
| c.586A > G        | p. I196V         | 1             | 0.03                                     | pathogenic |
| c.587T > C        | p. I196T         | 1             | 0.03                                     | pathogenic |
| c.598G > A        | p. G200R         | 1             | 0.03                                     | pathogenic |

Splice site mutation

| Nucleotide Change | Allele Frequency in 1852 deaf patients (%|3704 allele) | Category |
|-------------------|------------------------------------------|------------|
| c.-3170G > A      | 0.03                                     | pathogenic |

*The p.V37I variant is a pathogenic variant with variable expressivity and incomplete penetrance [13].

Despite that genomic deletions containing \textit{GJB6} and upstream regions of \textit{GJB2} was frequently detected in several ethnic groups [14, 15], such genomic deletion was not detected in our CNV analysis based on read depth of the NGS. Instead, in three probands (D592, C290 and D1028) with mono-allelic c.235delC mutation in \textit{GJB2}, we identified an additional heterozygous mutation c.538C > T (p. R180*) in \textit{GJB3}, c.547G > A (p.E183K) in \textit{GJB3} and c.228delG (p. L79Cfs*3) in \textit{GJB6}, respectively (Table 3). These \textit{GJB2}/\textit{GJB3} and \textit{GJB2}/\textit{GJB6} mutations may combine to cause hearing loss in a digenic inheritance pattern as previously reported [15, 16].

Our targeted NGS also identified a variety of independent pathogenic mutations in 13 (29.55%) probands (Table 3, Additional file 1: Table S3), indicating that they were simply coincidental carriers of the \textit{GJB2} mutations. Among them, probands D908 and D2002 were found to carry homozygous deletions of the entire \textit{STRC} gene (Additional file 1: Figure S1) and proband D1857 has a heterozygous deletion and a nonsense mutation c.3696G > A (p. W1232*) in \textit{STRC}. Consistent with previous studies [17], all three probands with \textit{STRC} homozygous or compound heterozygous deletions have moderate hearing loss (PTAs of 40-50 dB HL). Fourteen of the sixteen other independent mutations identified in this study have been reported to be associated with hearing loss in previous studies, including dominant mutations \textit{EYA1} c.1276G > A (p. G426S) [18] and \textit{MITF} c.877C > T (p. R293*) [19], recessive mutations \textit{PCDH15} c.4133C > T (p. T1378I) and c.1453delT (p. S485Rfs*2) [20], \textit{USH2A} c.10904C > A (p.T3635N) [21], \textit{MYO15A} c.8158G > A (p. D2720N) and c.10258_10260delTTC (p.F3420-) [22], \textit{CDH23} c.7630T > G (p. L2544V) and c.8257G > A (p.A2753T) [20], \textit{OTOF} c.2122C > T (p.R708*) and c.1194T > A (p.D398*) [23, 24], \textit{SLC26A4} c.1174A > T (p. N392Y) and c.1975G > C (p.V659L) [25], and \textit{SMPX} c.55A > G (p. N19D) [26]. One novel hemizygous mutation, c.201delT (p.E685fs*11) in \textit{TIMM8A}, was identified in a male proband D211 as a likely pathogenic mutation since similar
### Table 2: Genotypes and phenotypes of 475 deaf probands with bi-allelic GJB2 mutations

| Genotype | Severity of Hearing Loss | Total |
|----------|--------------------------|-------|
|          | Mild | Moderate | Severe | Profound |       |
| c.235delC /c.235delC | 0 | 11 | 77 | 139 | 227 |
| c.109G > A / c.109G > A | 7 | 8 | 20 | 18 | 53 |
| c.299_300delAT / c.299_300delAT | 0 | 0 | 3 | 0 | 3 |
| c.507insAACG / c.507insAACG | 0 | 0 | 1 | 0 | 1 |
| c.235delC / c.299_300delAT | 0 | 2 | 34 | 50 | 86 |
| c.257C > G / c.257C > G | 0 | 0 | 1 | 0 | 1 |
| c.257C > G / c.257C > G | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.257C > G | 0 | 0 | 1 | 0 | 1 |
| c.235delC / c.257C > G | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.257C > G | 0 | 0 | 0 | 2 | 2 |
| c.299_300delAT / c.176del16bp | 0 | 0 | 0 | 2 | 2 |
| c.36insG / c.176del16bp | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.223C > T | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.223C > T | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.231G > A | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.242 T > G | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.257C > T | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.257C > T | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.257C > T | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.35delG | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.35delG | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.257C > T | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.257C > T | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.312del14 | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.312del14 | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.11G > A | 0 | 0 | 0 | 2 | 2 |
| c.235delC / c.11G > A | 0 | 0 | 0 | 2 | 2 |
| c.299_300delAT / c.3170G > A | 0 | 0 | 0 | 2 | 2 |
| c.257C > G / c.299_300delAT | 0 | 0 | 0 | 2 | 2 |
| c.299_300delAT / c.257C > G | 0 | 0 | 0 | 2 | 2 |
| c.299_300delAT / c.427C > T | 0 | 0 | 0 | 2 | 2 |
| c.299_300delAT / c.443delC | 0 | 0 | 0 | 2 | 2 |
| c.299_300delAT / c.605insG46 | 0 | 0 | 0 | 2 | 2 |
| c.36insG / c.109G > A | 0 | 0 | 0 | 2 | 2 |
| c.36insG / c.427C > T | 0 | 0 | 0 | 2 | 2 |
| c.36insG / c.507insAACG | 0 | 0 | 0 | 2 | 2 |
| c.9G > A / c.231G > A | 0 | 0 | 0 | 2 | 2 |
| c.257C > G / c.493insG | 0 | 0 | 0 | 2 | 2 |
| c.257C > G / c.299_300delAT | 0 | 0 | 0 | 2 | 2 |
| c.379C > T / c.478G > A | 0 | 0 | 0 | 2 | 2 |
| c.109G > A / c.605insG46 | 0 | 0 | 0 | 2 | 2 |
| c.109G > A / c.299_300delAT | 0 | 0 | 0 | 2 | 2 |
| c.109G > A / c.181A > C | 0 | 0 | 0 | 2 | 2 |
truncating mutations p.E24* and p.R80* in TIMM8A have been reported to cause hearing loss associated with Mohr-Tranebjaerg syndrome [27]. The novel c.392A > G (p.N131S) in USH2A identified in compound heterozygosity with the known c.10904C > A (p.T3635 N) mutation is a variant of uncertain significance (VUS).

Based on the new genetic diagnosis, we revisited the clinical aspects of proband D289 and D554. Proband D289 with SLC26A4 c.1174A > T (p.N392Y) and c.1975G > C (p.V659L) mutations had profound hearing loss and bilateral enlarged vestibular aqueduct, which is characteristic of biallelic SLC26A4 mutations. Proband D554 with USH2A c.10904C > A (p.T3635 N) and c.392A > G (p.N131S) mutations was two years old and had no signs of retinitis pigmentosa so far. As retinitis pigmentosa may develop after puberty for patients with USH2A mutations, we recommended that visual acuity and visual fields of the patient be monitored by an ophthalmologist at older age.

Discussion
In this study, we presented an overview on the mutation spectrum of GJB2 in a large cohort (n = 1852) of patients with hearing loss in Chinese Hans. Biallelic mutations in GJB2 are responsible for up to 25.65% of patients, representing the most frequent cause for genetic hearing loss in our cohort. The most prevalent GJB2 mutations identified in this study were c.235delC and c.109G > A, accounting for 65.16 and 11.79% of the mutant alleles. Most (92.21%) of the patients with bi-allelic GJB2 mutations had severe-to-profound hearing loss, in which the c.235delC is the predominant causes (84.93%). Interestingly, our results showed that c.235delC also contributes to mild-to-moderate hearing loss in a significant percentage (54.05%) of such patients, with c.109G > A being another major contributor (40.54%, Table 2). In comparison with previous studies of other Chinese ethnicities such as the Uyghur population [28], the mutation spectrum of GJB2 is considerably different in Chinese Hans, as c.35delG, a common GJB2 mutation in both Uyghurs and Caucasians, was detected in only one proband in our cohort.

It has long been puzzling that mutation screening of GJB2 in a large proportion (6–15%) of patients with autosomal recessive hearing loss would identify only one pathogenic mutant allele [9, 29, 30]. In our cohort, we also identified 72 (3.89%) subjects carrying only a single recessive pathogenic mutation in GJB2, and that excludes those carrying the incompletely penetrant c.109G > A variant, which has a carrier frequency of 12.2% in Chinese Han normal hearing controls [31]. In our cohort, the carrier rate of mono-allelic mutations in GJB2 (3.89% overall, 2.97% for c.235delC) is higher than that previously reported in the Chinese Han general population (2.45% overall, 1.78% for c.235delC) [32], suggesting that at least in some cases a second unidentified pathogenic mutation may act either in cis or in trans to the GJB2 mutation to lead to the hearing loss. This hypothesis has subsequently been proved by our targeted NGS in 44 probands with mono-allelic GJB2 mutations. In three probands, digenic inheritance of GJB2/GJB3 and GJB2/GJB6 mutations was identified as the likely pathogenic cause for their hearing loss (Table 3). On the other hand, two dominant and a series of recessive mutations in 11 deafness-associated genes were also identified as the independent pathogenic causes in 13 additional probands, suggesting that those probands are coincidental carriers of the GJB2 mutations.

Overall our targeted NGS resolved the pathogenic cause in 16 (36.36%) probands with mono-allelic GJB2 mutations, validating the importance of high-throughput sequencing in such patients. For the remaining unresolved cases, possible pathogenic causes may include: 1) a second mutant allele in GJB2 may exist deeply in the introns or the non-coding regulatory regions uncovered by the targeted NGS; 2) mutation in a yet unknown deafness-associated gene may lead to the hearing loss in coordination with or independent to the GJB2 mutation; and 3) in some sporadic cases environmental factors may contribute to the hearing loss.

Table 2 Genotypes and phenotypes of 475 deaf probands with bi-allelic GJB2 mutations (Continued)

| Genotype                        | Severity of Hearing Loss | Total |
|---------------------------------|--------------------------|-------|
|                                 | Mild | Moderate | Severe | Profound |
| c.109G > A / c.427C > T        | 0    | 0        | 1      | 0        | 1     |
| c.176del16bp /c.389G > T       | 0    | 0        | 0      | 1        | 1     |
| c.176del16bp /c. 95G > A       | 0    | 0        | 0      | 1        | 1     |
| Total                           | 7    | 30       | 170    | 268      | 475   |

Yu et al. Orphanet Journal of Rare Diseases (2020) 15:29
**Table 3** Pathogenic mutations identified by targeted NGS in probands with GJB2 mono-allelic mutations

| Patient ID | Gene a | Type of variation | Nucleotide change | Amino acid change | Zygosity | Segregating with HL^b | The GJB2 mutant allele |
|------------|--------|-------------------|-------------------|-------------------|-----------|----------------------|-----------------------|
| D592       | GJB2   | Frameshift indel  | c.235delC         | p. L79Cfs*3       | het       | ND                   | c.235delC             |
|            | GJB3   | Stop_gained       | c.538C > T        | p. R180*          | het       |                      |                       |
| C290       | GJB2   | Frameshift indel  | c.235delC         | p. L79Cfs*3       | het       | Yes                  | c.235delC             |
|            | GJB3   | Missense          | c.547G > A        | p. E183K          | het       |                      |                       |
| D1028      | GJB2   | Frameshift indel  | c.235delC         | p. L79Cfs*3       | het       | Yes                  | c.235delC             |
|            | GJB6   | Frameshift indel  | c.228delG         | p. W77Gfs*5       | het       |                      |                       |
| D908       | STRC   | Whole gene deletion | –                | –                 | hom       | Yes                  | c.235delC             |
|            | STRC   | Whole gene deletion | –                | –                 | hom       |                      |                       |
| D2002      | STRC   | Whole gene deletion | –                | –                 | hom       | Yes                  | c.235delC             |
|            | STRC   | Whole gene deletion | –                | –                 | hom       |                      |                       |
| D1857      | STRC   | Partial gene deletion | –                | –                 | het       | Yes                  | c.235delC             |
|            | STRC   | Stop_gained       | c.3696G > A       | p. W1232*         | het       |                      |                       |
| D1807      | EYA1   | Missense          | c.1276G > A       | p. G426S          | het       | De novo              | c.235delC             |
| D281       | MITF   | Stop_gained       | c.877C > T        | p. R293*          | het       | De novo              | c.299_300delAT        |
| D349       | PCDH15 | Missense          | c.4133C > T       | p. T1378I         | compound | Yes                  | c.235delC             |
|            | PCDH15 | Frameshift indel  | c.1453delT        | p. S485Rfs*2      | compound |                       |                       |
| D554       | USH2A  | Missense          | c.10904C > A      | p. T3635 N        | compound | het                   | Yes                   |
|            | USH2A  | Missense          | c.392A > G        | p. N131S          | compound | het                   |                       |
| D822       | MYO15A | Missense          | c.8158G > A       | p. D2720N         | compound | Yes                  | c.235delC             |
|            | MYO15A | In-frame del      | c.10258_10260delTTC | p. F3420         | compound |                       |                       |
| C649       | CDH23  | Missense          | c.7630T > G       | p. L2544 V        | compound | Yes                  | c.235delC             |
|            | CDH23  | Missense          | c.8257G > A       | p. A2753T         | compound |                       |                       |
| D463       | OTOF   | Stop_gained       | c.2122C > T       | p. R708*          | compound | Yes                  | c.235delC             |
|            | OTOF   | Missense          | c.1194T > A       | p. D398E          | compound |                       |                       |
| D289       | SLC26A4| Missense          | c.1174A > T       | p. N392Y          | compound | Yes                  | c.235delC             |
|            | Missense| Missense          | c.1975G > C       | p. V659 L         | compound |                       |                       |
| D237       | SMPX   | Missense          | c.55A > G         | p. N19D           | hemi      | ND                   | c.235delC             |
| D211       | TIMM8A | Frameshift indel  | c.201delT         | p. E685S*11       | hemi      | ND                   | c.235delC             |

Abbreviations: HL hearing loss, het heterozygous, hom homozygous, hemi hemizygous, ND not determined or not conclusive

^aThe reference sequence transcript IDs for each gene were GJB2 NM_004004.5, GJB3 NM_024009.2, GJB6 NM_006783.4, STRC NM_153700.2, EYA1 NM_000503.5, MITF NM_000248.3, PCDH15 NM_001142771.1, USH2A NM_206933.2, MYO15A NM_016239.3, CDH23 NM_022124.5, OTOF NM_194248.2, SLC26A4 NM_000441.1, SMPX NM_014332.2, TIMM8A NM_004085.3

^bSegregating with HL, determined on basis of segregation analysis in affected and/or unaffected family members

Yu et al. Orphanet Journal of Rare Diseases (2020) 15:29
Conclusions
Our results showed that mutations in GJB2 account for over 25% of pathogenic causes in Chinese Han deaf patients, with expanded screening in other deafness-associated genes may help to further resolve cases with mono-allelic GJB2 mutations. The sequential Sanger sequencing and targeted next generation sequencing may be an efficient approach for genetic diagnosis of deafness in Chinese Hans.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s13023-020-1311-2.

Additional file 1: Table S1. Phenotype and GJB2-associated genotypes of 1852 Chinese Han deaf patients. Table S2. 139 deafness-related genes sequenced in the targeted panel. Table S3. Function prediction of missense mutations using multiple computational tools. Table S4. Primers used in this study. Figure S1. Ratio plots showing homozygous deletion of STRC in patient D908, D2002 and heterozygous deletion of STRC in patient D1857.

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Authors’ contributions
Conceptualization, TY and HW; Formal analysis, X-YY and TY; Funding acquisition, TY and HW; Investigation, X-YY, YL and JX; Methodology, T-JC and LL; Project administration, TY and HW; Supervision, HW; Writing — original draft, X-YY; Writing — review & editing, TY. All authors read and approved the final manuscript.

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Availability of data and materials
The data that support the findings of this study are openly available in SRA database at https://www.ncbi.nlm.nih.gov/sra, reference number PRJNA560673.

Ethics approval and consent to participate
A written informed consent was obtained from each subject or their guardians to participate in this study. This study was approved by the Ethics Committee of Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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