Target-enriched massively parallel sequencing for genetic diagnosis of hereditary hearing loss in patients with normal array CGH result

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KEY MESSAGES

1. In our cohort, 15 common hearing-loss mutations with a high carrier frequency (15.9%) were screened; *GJB2* c.109G>A was the most common mutation (10.9%).
2. For patients with hearing loss who were negative for the 15 common mutations, our hearing-loss target capture panel combined with a massively parallel sequencing approach increased detection of pathogenic mutations or likely pathogenic variants by 21%.

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This summary is based on studies first reported in: (1) Chen Y, Cao Y, Li HB, et al. SNaPshot reveals high mutation and carrier frequencies of 15 common hearing loss mutants in a Chinese newborn cohort. Clin Genet 2015;87:467-72. (2) Lu Y, Zhou X, Jin Z, et al. Resolving the genetic heterogeneity of prelingual hearing loss within one family: Performance comparison and application of two targeted next generation sequencing approaches. J Hum Genet 2014;59:599-607.

Introduction

Hearing loss is defined as a partial or total inability to hear sound in one or both ears. It affects 278 million people worldwide and is the most common birth defect.[1] It has been estimated that the incidence of bilateral permanent sensorineural hearing loss (≥40 dB) is 1 per 500 infants at birth.[2] Genetic causes of hearing loss are estimated to account for as many as 68% of cases in newborns and 55% of cases by the age of 4 years. Up to 70% of hereditary hearing-loss cases are non-syndromic, whereas 30% are syndromic. To date, at least 75 non-syndromic deafness genes and more than 1000 discrete deafness-causing mutations have been described. For example, connexin 26 (encoded by *GJB2*) is responsible for more than half of the cases of hereditary pre-lingual sensorineural hearing loss in many populations, by virtue of hotspot mutations, as well as founder mutations among different ethnic groups. Mutations in pendrin (encoded by *SLC26A4*) can cause both non-syndromic deafness (DFNA4, MIM 600791) and syndromic deafness (Pendred syndrome, MIM 274600). Hereditary hearing loss is extremely heterogeneous.

In Hong Kong, the Department of Health offers genetic diagnosis of hearing loss for four genes only: *GJB2*, *GJB6*, mitochondrial 12S ribosomal RNA gene (1555A>G mutation), and *PAX3*. There is a strong need for a comprehensive, robust, and cost-effective method to enable genetic diagnosis of hearing loss. Children with hearing loss identified before 6 months of age who begin appropriate interventions demonstrate superior language skills to those identified after 6 months of age. Early identification of mutations can inform medical care and improve prognosis—for example, avoidance of ototoxity from aminoglycoside antibiotic therapy in the presence of the mitochondrial 1555A>G mutation.

Methods

This study aimed to estimate the frequency of 15 well-known pathogenic mutations in a sample of the Chinese population, based on the screening of a neonatal cohort in Suzhou, China. A total of 5800 newborns (3077 males and 2723 females) were enrolled between October 2011 and February 2012 at the Suzhou Hospital. To compare results with the general population of China, these blood samples were obtained from an established nationwide screening programme of inborn errors of metabolism, without special selection.

To screen for common hearing-loss mutations, 15 mutations were detected by massively parallel sequencing with the SNaPshot Multiplex System (Thermo Fisher Scientific, Waltham [MA], United States). The screening test covered the following mutations: 35delG, 109G>A, 176-191del16, 235delC, and 299-300delAT in *GJB2*; c.919-2A>G, 1174A>T, 1229C>T, 2027T>A, and 2168A>G in *SLC26A4*; and mt1494C>T, mt1555A>G, mt3243A>G, mt7444G>A, and mt7445A>G in the mitochondrial genome. In the target enrichment step, the targeted

regions of interest were designed to cover all the exons and flanking 15 bp of 261 human genes that are known to be causative hearing-loss genes or candidates. A solution-based capture approach was used (NimbleGen SeqCap EZ Choice kit; Roche, Basel, Switzerland).

For bioinformatics analysis, data were processed in the following sequence. (1) Raw data were generated using the Illumina Pipeline (version 1.3.4; https://www.illumina.com/informatics/infrastructure-pipeline-setup.html). (2) Clean reads were selected and unqualified sequences were removed from the raw data using a local dynamic programming algorithm, and (3) reads were aligned against the reference human genome from the National Center for Biotechnology Information database (HomoloGene Build 37; https://www.ncbi.nlm.nih.gov/homologene) using the Burrows Wheeler Aligner Multi-Vision software package (http://bio-bwa.sourceforge.net/). (4) Single-nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs) were identified using the SOAPsnp (Short Oligonucleotide Analysis Package; http://soap.genomics.org.cn/soapsnp.html) and the GATK Indel Genotyper (Genome Analysis Toolkit; http://www.broadinstitute.org/gsa/wiki/index.php/), respectively. (5) Sequence variations were annotated using an in-house pipeline, consisting of the gene annotation software Reference Sequence (RefSeq; https://www.ncbi.nlm.nih.gov/RefSeq). (6) Known polymorphisms and minor alleles were identified with the dbSNP138 SNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi?view+summary=view+summary&build_id=138), HapMap database (https://www.ncbi.nlm.nih.gov/probe/docs/projhapmap/), 1000 Genomes database (http://www.internationalgenome.org/), and Exome Aggregation Consortium database (http://exac.broadinstitute.org/). (7) Variants (exonic, splice site, silent, missense) were then characterised. (8) Biological function was then predicted, including whether an amino acid substitution would affect protein function and thus be prioritised for further study, using tools for sorting intolerant from tolerant (SIFT; http://sift.jcvi.org/) and polymorphism phenotyping (PolyPhen; http://genetics.bwh.harvard.edu/pph2/), as well as other third-party software.

Results

In this cohort, 15.9% (923/5800) of newborns carried at least one of the 15 mutations of interest (Table 1), indicating that 1 in 6.3 newborns carried at least one mutant allele of a hearing-loss gene. The *GJB2* mutants accounted for up to 12.7% (735/5800) of cases. Additionally, 2.17% (126/5800) of newborns had at least one mutant allele of *SLC26A4*, and 1.07% (62/5800) were carriers of the mitochondrial mutant allele. The most prevalent mutated allele was *GJB2* c.109G>A, which had an allele frequency of 5.26% (610/11600), followed by *GJB2* c.235delC (0.94%, 109/11600) and *SLC26A* c.919-2A>G (0.84%, 98/11600). These alleles had a carrier frequency of approximately 10.29% (597/5800), 1.88% (109/5800), and 1.62% (94/5800), respectively.

TABLE 1. Carrier frequency of 15 common mutations

Mutations	No. of individuals (n=5800)	Carrier frequency (%)
Total	923	15.914
GJB2 c.109G>A	597	10.290
GJB2 c.235delC	109	1.879
SLC26A4 c.919-2A>G	94	1.621
mt7444G>A	41	0.707
SLC26A4 c.2168A>G	20	0.350
GJB2 c.299-300delAT	17	0.293
GJB2 c.176-191del16bp	11	0.190
SLC26A4 c.1174A>T	9	0.156
mt3243A>G	9	0.155
mt1555A>G	8	0.138
mt7445A>G	3	0.052
SLC26A4 c.2027T>A	2	0.034
GJB2 c.35delG	1	0.017
SLC26A4 c.1229C>T	1	0.017
mt1494C>T	1	0.017

In this cohort, 0.48% (28/5800) of newborns were genetically diagnosed with hearing loss because of mutations in *GJB2* and *SLC26A4*, whereas 19 newborns carried homozygous mutations in *GJB2* or *SLC26A4*. Nine newborns carried compound *GJB2* mutations, of whom eight harboured a *GJB2* c.109G>A mutation and a second mutation. Seven of these 28 newborns failed the otoacoustic emission (OAE) test for at least one ear. All other newborns passed either the initial OAE screening or the 1-month OAE follow-up test. No newborn carrying only one mutant allele failed both OAE tests. Genotypic and phenotypic information about these samples are provided in Table 2. Interestingly, newborns who carried homozygous *GJB2* c.109G>A mutations showed varied phenotypes, from a normal result to bilateral failure of the OAE test.

To determine the clinical application of a target-enriched massively parallel sequencing system as a comprehensive, robust next-generation genetic test for hereditary hearing loss, a total of 100 patients with hearing loss (including 42 cases from 24 hearing-loss families) and with normal

TABLE 2. Genetically diagnosed cases of hearing loss

Mutation identified	Genotype	No. of cases	No. of cases failed otoacoustic emission test
Total		28	7 (25%)
GJB2 c.109G>A	Homozygous	13	4
GJB2 c.109G>A + *GJB2* c.176-191del16	Compound heterozygous	1	0
GJB2 c.109G>A + *GJB2* c.235delC	Compound heterozygous	6	2
GJB2 c.109G>A + *GJB2* c.299-300delAT	Compound heterozygous	1	0
GJB2 c.35delG + *GJB2* c.235delC	Compound heterozygous	1	0
SLC26A4 c.919-2A>G	Homozygous	4	1
SLC26A4 c.1174A>T	Homozygous	2	0

array CGH result were recruited, of whom 90% were referred because of congenital or early-onset hearing loss. Two families were suspected to have Pendred syndrome (Fig 1). Overall, read depth for each sample ranged from 86 to 352, with a target region of coverage of between 94.9% and 98.6%.

After primary bioinformatics analysis, an average of 6000 SNPs and INDELs were annotated. Data were filtered conservatively according to an established pipeline with consideration of (1) mean depth and read counts of ≥10, (2) removal of 3'UTR, 5'UTR, downstream, upstream, and non-coding exon transcript variants, and (3) removal of non-coding change types. We also compared the SNP allele frequency in an ethnically matched population in the 1000 Genomes or Exome Aggregation Consortium databases. Twenty-four patients were identified to carry at least one pathogenic or likely-pathogenic mutation in the known hearing-loss genes. Our data confirmed that *GJB2* c.109G>A is a common mutation in the Asian population, with a carrier frequency of as high as 10.9%. Among the 24 patients identified by target-enrichment sequencing, three patients who were known to be homozygous for *GJB2* c.109G>A were referred for further analysis, owing to severe or profound hearing loss. Two patients were found to carry other mutations: *BSND* c.10G>A in one, and *ATP6V0A4* c.1727T>A and *TMPRSS3* c.325C>T in the other (Fig 2).

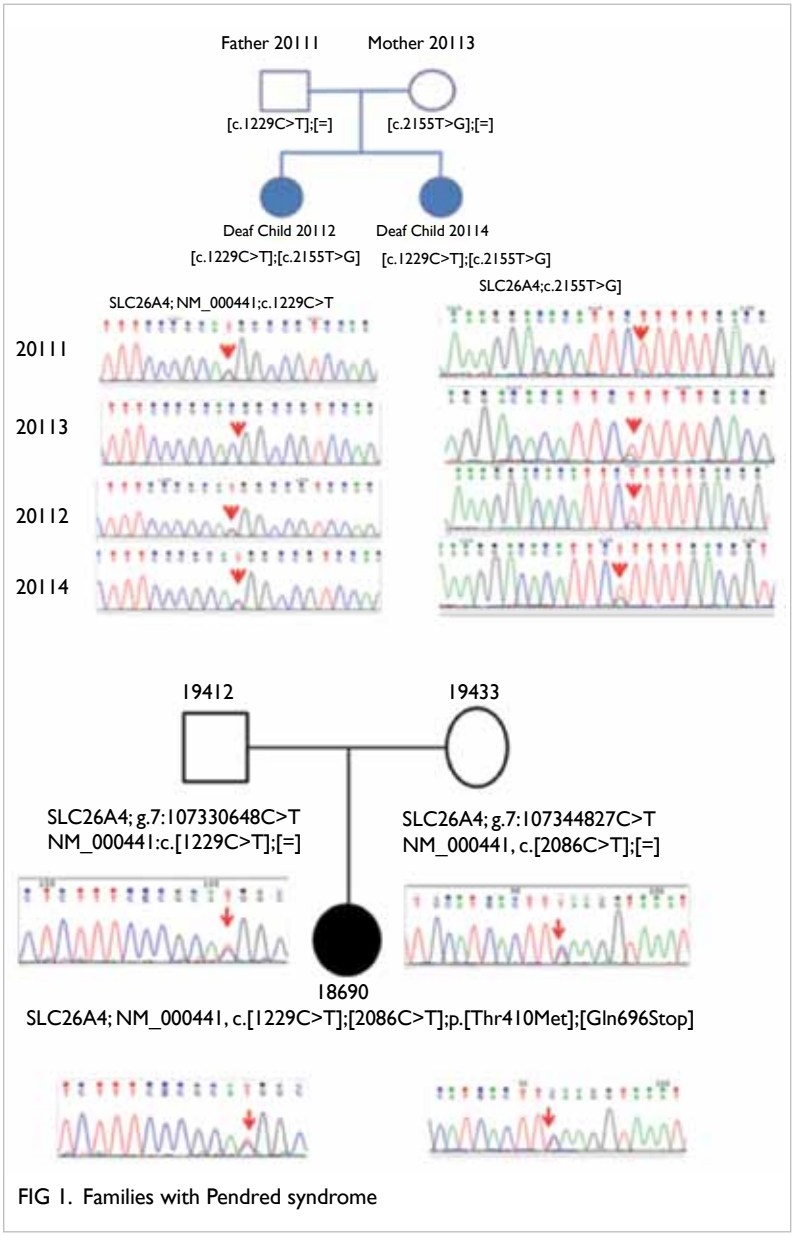
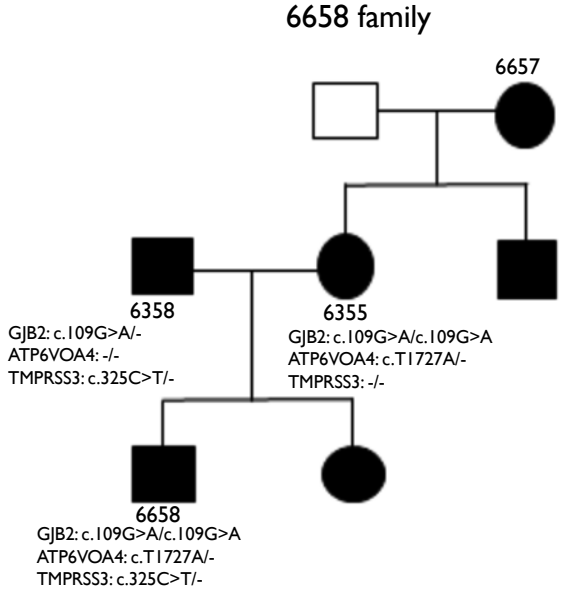


FIG 1. Families with Pendred syndrome

Discussion

Our study indicates a high carrier frequency of *GJB2* c.109G>A in a Chinese population. The total heterozygous and homozygous carrier frequencies of *GJB2* c.109G>A were as high as 10.29% and 0.22%, respectively; the heterozygous frequency of this mutant is comparable to the carrier frequency of 11.6% in a Taiwanese population. This finding indicates that *GJB2* c.109G>A is common in Asians



ATP6VOA4[NM_020632]: c.T1727A/, p.F576Y, Chr7:138417803(hg19), no report b4, in silico predicted as pathogenic
TMPRSS3: [NM_024022]: DFNBB/10, c.325C>T, p.Arg109Trp, Clinvar reported as pathogenic

FIG 2. A family with a neonate with severe hearing loss

but rare in Caucasians. Nonetheless, newborns who carry this homozygous mutation showed variable phenotypes, with a high OAE pass rate (9/13) in our cohort. This *GJB2* c.109G>A genotype was first reported as a polymorphism and has been suggested to be pathogenic and a cause of mild hearing loss. In our cohort, the carrier frequency of *GJB2* c.109G>A and *SLC26A4* c.1174A>T and c.2168A>G differed considerably from the global minor allele frequency calculated from the 1000 Genomes dataset. This difference suggests that the comparison of mutation frequency should be done among ethnically or geographically matched populations.

The use of the OAE test for early diagnosis is well established in Hong Kong. Considering its false-positive rate of 2.5% to 8%, follow-up auditory examinations to confirm the diagnosis are warranted. Our established test offers a genetic diagnosis and provides better sensitivity and specificity for managing hearing-loss patients and genetic counselling. Genetic diagnosis helps identify potential late-onset hearing loss and facilitate long-term follow-up of all pre-symptomatic cases.

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Ethical Approval

This study was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (CRE-2011.098).

Declaration

The authors have no conflicts of interest to disclose.

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