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SHORT REPORT

Whole-exome sequencing identifies rare pathogenic and candidate variants in sporadic Chinese Han deaf patients

Songfeng Zou[1] | Xueshuang Mei[1] | Weiqiang Yang[1] | Rvfei Zhu[1] | Tao Yang[2,3,4] | Hongyi Hu[1]

[1]Department of Otorhinolaryngology, Peking University Shenzhen Hospital, Shenzhen, China

[2]Department of Otorhinolaryngology—Head and Neck Surgery, Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

[3]Ear Institute, Shanghai Jiaotong University School of Medicine, Shanghai, China

[4]Shanghai Key Laboratory of Translational Medicine on Ear and Nose Diseases, Shanghai, China

Correspondence
Hongyi Hu, Department of Otorhinolaryngology, Peking University Shenzhen Hospital, Shenzhen, China.
Email: hyihu@sina.com
Tao Yang, Department of Otolaryngology—Head and Neck Surgery, Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.
Email: yangtfxl@sina.com

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Abstract

Genetic causes of hearing loss are highly heterogeneous and often ethnically specific. In recent years, a variety of next-generation sequencing (NGS) panels have been developed to target deafness-causative genes. Whole-exome sequencing (WES), on the other hand, was rarely used for genetic testing for deafness. In this study, we performed WES in 38 sporadic Chinese Han deaf patients who have been pre-excluded for mutations in common deafness genes *GJB2*, *SLC26A4* and *MT-RNR1*. Non-synonymous variants have been filtered based on their minor allele frequencies in public databases and ethnically matched controls. Bi-allelic pathogenic mutations in eight deafness genes, *OTOF*, *TRIOBP*, *ESPN*, *HARS2*, *CDH23*, *MYO7A*, *USH1C* and *TJP2*, were identified in 10 patients, with 17 mutations identified in this study not being associated with deafness previously. For the rest 28 patients, possibly bi-allelic rare non-synonymous variants in an averaged 4.7 genes per patient were identified as candidate pathogenic causes for future analysis. Our study showed that WES may provide a unified platform for genetic testing of deafness and enables retro-analyzing when new causative genes are revealed.

KEYWORDS
deafness, recessive, sporadic, whole-exome sequencing

1 | INTRODUCTION

Hearing loss is the most common sensory disorder in humans, affecting approximately one in a thousand children at birth and another one in a thousand in childhood.[1] It was estimated that over half of the childhood hearing loss is caused by genetic mutations in a single causative gene, with 80% and 15% of cases were inherited in autosomal recessive and dominant forms, respectively.[2] To date, over 100 deafness-causative genes have been reported (updated in the Hereditary Hearing Loss Homepage, https://hereditaryhearingloss.org/). The molecular etiology of genetic hearing loss is highly heterogeneous and the mutation spectrum varies greatly in different ethnical groups. In previous

ABBREVIATIONS: CNV, copy number variation; MAF, minor allele frequency; NGS, next-generation sequencing; WES, whole-exome sequencing.

Songfeng Zou, Xueshuang Mei, and Weiqiang Yang contributed equally to this study.

[Correction added on 16 October 2019, after first online publication: The author name of Weiqiang Yang was previously missing from the footnote and has been added in this current version.

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studies of Chinese Han deaf patients, mutations in three common deafness genes *GJB2*, *SLC26A4* and *MT-RNR1* accounted for approximately 30% to 35% of the cases and were routinely screened by Sanger sequencing in China.[3,4] For the rest of the cases, causes of deafness are attributable to rare mutations in a vast variety of deafness genes and unknown etiologies.[3,5]

In the past decade, application of targeted next-generation sequencing (NGS) has rapidly changed the landscape of the genetic testing for rare diseases. This technology is especially suitable for genetically heterogeneous disorders such as hearing loss. Various panels targeting 60 to 200 deafness-associated genes have been developed and greatly improved comprehensive genetic diagnosis of deafness worldwide.[5-8] Those panels, however, differ in covered region, sequence-capturing methodology and data-analyzing pipeline, making the sequencing results generally not compatible for cross-platform re-analyzing and comparison. More importantly, for patients tested negative for a targeted NGS panel, their sequencing data do not support retro-analysis when new deafness genes are discovered.

Whole-exome sequencing (WES), on the other hand, virtually sequences all (>95%) exons of human genome and theoretically captures the majority (85%) of the genetic variants associated with human Mendelian disorders including deafness.[9] It provides a unified sequencing platform and allows retro-analysis for future-discovered causative genes at the same time. Despite WES has been extensively exploited to identify new deafness genes from large or multiplex families segregating with hearing loss, it has been rarely used for wide-spread genetic testing for the general deaf patients. In this study, we selected 38 sporadic Chinese Han deaf patients who have been previously excluded for mutations in common deafness genes *GJB2*, *SLC26A4* and *MT-RNR1* by Sanger sequencing. WES was performed in those patients and the analyzing approach and the screening results were summarized.

2 | MATERIALS AND METHODS

2.1 | Patients and clinical information

The 38 sporadic, unrelated deaf patients were consecutively recruited from Peking University Shenzhen Hospital and Shanghai Ninth People's Hospital for genetic testing from September 2018 to April 2019. All patients had: (a) congenital or childhood severe-to-profound sensorineural hearing loss, with the age of the original testing ranged from 10 months to 36 years; (b) no additional abnormalities other than deafness (non-syndromic); (c) no family history of deafness; and (d) no pathogenic mutations in common deafness genes *GJB2*, *SLC26A4 and MT-RNR1*. Written informed consent was obtained from the patients or the guardians to participating in this study. This study was approved by the Ethics Committee of Peking University Shenzhen Hospital.

2.2 | Whole-exome sequencing

Genomic DNA was extracted from peripheral blood DNA samples using the Genomic DNA Extraction Kit (Tiangen Biotech, China). The WES library was captured and constructed using the xGen Exome Research Panel v1.0 kit (Integrated DNA Technologies, Inc). Sequencing was performed on the Illumina HiSeq X-ten platform with a read depth over 100X and more than 95% of the targeted region covered over 20X.

The raw reads were aligned and mapped to the Genome Reference Consortium Human genome build 37 (GRCh37) by the Burrows-Wheeler Aligner software (v0.7-6a) and the Sequence Alignment/Map tools software (v0.1.18). The Picard software (v1.91) was used to remove the redundant sequence. SNVs and Indels were called using the Genome Analysis Toolkit software (v2.6-4) and functionally annotated using the Annovar software (2013Aug23 version).

2.3 | Variant filtering and prediction

Non-synonymous SNVs and Indels were filtered based on their minor allele frequencies (MAFs) in public databases gnomAD (http://gnomad.broadinstitute.org), 1000genomes (http://www.internationalgenome.org/) and an in-house WES database of 2114 Chinese Han control subjects. The MAF threshold for pathogenic mutations and candidate variants was set to less than 0.005 in all three databases as suggested for recessive mutations for deafness.[10] Based on the presumably recessive inheritance, only possibly bi-allelic variants (two different variants in a single gene) were considered to be candidate pathogenic variants. Mutation databases ClinVar (https://www.clinicalgenome.org/data-sharing/clinvar/), OMIM (https://omim.org/) and HGMD (http://www.hgmd.cf.ac.uk/ac/index.php) were used to annotate previously reported pathogenic mutations for deafness. The pathogenicity of the candidate variants was predicted by a variety of computational tools including SIFT (http://sift.jcvi.org), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org). Inner ear expression of the candidate genes was evaluated through the Shared Harvard Inner-Ear Laboratory Database (SHIELD, https://shield.hms.harvard.edu).

2.4 | Parental verification for bi-allelic mutations

Bi-allelic pathogenic mutations identified in the deafness genes were verified by Sanger sequencing. Parental genotyping was performed to verify that the mutations are bi-allelic.

3 | RESULTS

3.1 | Bi-allelic pathogenic mutations identified in known deafness genes

The 38 sporadic Chinese Han deaf patients have been previously excluded for mutations in common deafness genes *GJB2*, *SLC26A4*

TABLE 1 Bi-allelic mutations in known deafness-associated genes identified by WES

Patient ID	Gene	Reference sequence	Mutation	Zygosity	MAF- Chinese	MAF-1000G	MAF-GnomAD	SIFT	POLYPHEN-2	Mutation taster	References
D2036	*TJP2*	NM_004817	c.2044C > T:p.Arg682Trp	Hetero	0	0	0.0000159	D (0.0)	D (1.0)	D (1.0)	Novel
			c.3262A > T:p.Met1088Leu	Hetero	0	0	0	D (0.011)	P (0.91)	D (1.0)	Novel
D2053	*TRIOBP*	NM_001039141	c.3193A > G:p.Ile1065Val	Hetero	0	0	0.00001206	D (0.0)	D (0.984)	N (0.995)	Novel
			c.5945G > A:p.Arg1982His	Hetero	0	0	0.00006480	D (0.001)	D (1.0)	D (1.0)	Novel
D2072	*OTOF*	NM_001287489	c.1194 T > A:p.Asp398Glu	Hetero	0.00425733	0	0.00021965	T (0.366)	B (0.027)	D (0.998)	[11]
			c.2567A > T:p.Asn856Ile	Hetero	0.00236519	0	0.00006893	D (0.003)	P (0.949)	D (1.0)	Novel
D2090	*ESPN*	NM_031475	c.1306_1310del:p.Ser436Profs	Hetero	0.00047304	0	0.00209827	-	-	-	Novel
			c.2224A > C:p.Thr742Pro	Hetero	0	0	0.00000422	D (0.001)	D (0.999)	D (0.761)	Novel
D2092	*HARS2*	NM_001278731	c.572G > A:p.Arg191Gln	Hetero	0.00023652	0	0.00000797	T (0.146)	D (0.989)	D (1.0)	Novel
			c.622C > T:p.Arg208Cys	Hetero	0	0	0.00001593	D (0.0)	D (0.999)	D (1.0)	Novel
D2113	*CDH23*	NM_001171931	c.1205C > T:p.Pro402Leu	Hetero	0	0	0.00003214	D (0.0)	D (1.0)	D (0.993)	Novel
			c.1616G > A:p.Gly539Asp	Hetero	0	0	0.00006147	D (0.010)	P (0.886)	D (1.0)	[12]
D2129	*MYO7A*	NM_000260	c.3603 T > A:p.Cys1201Ter	Hetero	0	0	0	-	-	D (1.0)	Novel
			c.6062A > C:p.Lys2021Thr	Hetero	0	0	0	D (0.001)	D (1.0)	D (1.0)	[13]
D2135	*OTOF*	NM_001287489	c.77G > A:p.Arg26Gln	Hetero	0	0	0.00000796	D (0.0)	D (0.984)	D (0.999)	Novel
			c.3572A > G:p.Asp1191Gly	Hetero	0	0	0	D (0.003)	D (1.0)	D (1.0)	Novel
D2138	*USH1C*	NM_153676	c.503G > C:p.Gly168Ala	Hetero	0.00023652	0	0.00001838	D (0.002)	D (1.0)	D (1.0)	Novel
			c.2551 T > G:p.Ser851Ala	Hetero	0.00260171	0.00019968	0.00016305	T (0.078)	B (0.0)	D (1.0)	Novel
D2144	*OTOF*	NM_001287489	c.5320C > A:p.Gln1774Lys	Hetero	0	0	0	D (0.0)	P (0.872)	D (1.0)	Novel
			c.2215-1G > C; exon skipping?	Hetero	0	0	0	D (0.0)	P (0.733)	D (0.815)	Novel

Abbreviations: B, benign; D, deleterious; Hetero, heterozygous; N, neutral; P, possibly damaging; T, tolerated.

and *MT-RNR1* by Sanger sequencing. WES was performed in all patients. The averaged depth of the exome was 137.4X with 97.8% of the regions covered by more than 20X. For deafness-causative genes, all patients have >99% of the coding regions covered by more than 20X.

In the first step of the data analysis, non-synonymous variants in 159 deafness-associated genes (Table S1) was interrogated. In 10 (26%) of the 38 patients, bi-allelic mutations were identified in *OTOF* (n = 3), *TRIOBP*, *ESPN*, *HARS2*, *CDH23*, *MYO7A*, *USH1C* and *TJP2* (n = 1 each, Table 1). All 20 mutations have MAFs less than 0.005 in databases gnomAD, 1000genomes and 2114 Chinese Han controls. Parental genotyping confirmed that those bi-allelic mutations were indeed in separate alleles. Two mutations c.1306_1310del in *ESPN* and p.Cys1201Ter in *MYO7A* were predicted to lead to prematurely stopped protein products. All 18 missense mutations were predicted to be deleterious by at least one computational program. Three mutations were previously reported as pathogenic mutations for deafness.[6,11,13] Seventeen mutations identified in this study have not been associated with deafness in previous reports.

3.2 | Candidate variants in all genes

In the second step of the data analysis, non-synonymous variants in all genes were interrogated in the rest 28 deaf patients. Candidate variants were determined by their possibly compound heterozygosity (two non-synonymous variants in each candidate gene) and low allele frequencies in the ethnically matched controls (MAF < 0.005). Without parental genotyping for all candidate variants, possibly bi-allelic variants were identified in 4.7 genes per patient on average (Data S1). The data containing full annotation of the candidate variants are available upon request.

4 | DISCUSSION

In this study, we performed comprehensive genetic testing in 38 sporadic, non-syndromic Chinese Han deaf patients by WES. Our recruiting criteria represent the majority of clinical testing cases in China. Most affected children do not have siblings due to China's 'one-child' policy in the past decades. A total of 20 pathogenic mutations, including 17 novel mutations, were identified in nine deafness genes. Our results expanded the mutation spectrum of deafness genes in Chinese Hans.

One major aim of this study is to test the capability using WES in genetic testing for deafness. On average, WES in this study had >20X sequence depth in >99% of coding regions of the 159 deafness genes, indicating good overall capturing and sequencing quality. The percentage of patients with bi-allelic pathogenic mutations in the present study was 26% (10/38). Similarly, in our previous study of 44 sporadic Chinese Han deaf patients, targeted NGS detected 30% (13/44) of patients with bi-allelic mutations[14] (note: mutations in *GJB2*, *SLC26A4* and *MT-RNR1* was excluded in both studies). The comparable detection rate suggested that WES may serve as an alternative approach for genetic testing for deafness.

The major advantage of using WES for genetic testing lies in the ability to screen virtually all genes for potential pathogenic causes. This is especially important for patients with no pathogenic mutations identified in known deafness genes. In this study, a large number of candidate genes and possibly bi-allelic variants can be pulled from the WES data of 28 patients with negative testing results (Data S1). Among them, several candidate genes including *MYO3B*, *GPR179*, *COL7A1* and *COL19A1* were interesting, as members of the same or similar gene family, namely *MYO3A*, *GPR98*, *COL11A1*, *COL11A2*, *COL1A1*, *COL1A2*, *COL2A1*, *COL4A3*, *COL4A4*, *COL4A5*, *COL4A6*, *COL9A1* and *COL9A2*, have been associated with various forms of syndromic and non-syndromic hearing loss. By searching through the inner ear expression database SHIELD, a number of candidate genes with significant (FDR < 0.05) hair cell enrichment, such as *DNAH5*, *FSTL4*, *MYO3B* and *KIF27*, were identified and annotated in the Data S1. Accumulation and cross-study of such WES datasets for deaf patients, in combination with genetic analysis in human pedigrees and functional studies in animal models, may facilitate discovery of new deafness-causative genes in future. Patients with WES sequencing results, in return, may also benefit from retro-analysis of the updated deafness genes when available.

In this study, we also noted several limitations for using WES to perform genetic testing on deafness: (a) WES has lower sequencing depth (averaged 137.4X in this study) in comparison with targeted NGS for deafness genes (generally >300X), considerably attenuating its ability to detect copy number variants (CNVs); (b) Although the overall coverage for the deafness genes was good (>99% of the region were covered by 20X or higher), we do observed low coverage rates (<90% by 20X or higher) for several deafness genes including *PTPRQ*, *STRC* and *OTOA*; and (c) In clinical application, ethical concerns may rise when massive genetic information could be revealed irrelevant to deafness.

Nevertheless, our study indicated that WES can serve as an alternative approach for comprehensive genetic testing for deafness. Similarly, two previous studies also utilized WES in genetic diagnosis of multiplex deaf families of various ethnicities.[15,16] With further technical fine-tuning for deafness gene coverage and continuing decrease of the sequencing cost, gaining extra exome sequencing data may benefit the patients in the long run.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Tao Yang https://orcid.org/0000-0001-6011-987X

REFERENCES

1. Morton NE. Genetic epidemiology of hearing impairment. *Ann N Y Acad Sci*. 1991;630:16-31.
2. Morton CC, Nance WE. Newborn hearing screening—a silent revolution. *N Engl J Med*. 2006;354:2151-2164.
3. Yang T, Wei X, Chai Y, Li L, Wu H. Genetic etiology study of the non-syndromic deafness in Chinese Hans by targeted next-generation sequencing. *Orphanet J Rare Dis*. 2013;8:85.
4. Yuan Y, You Y, Huang D, et al. Comprehensive molecular etiology analysis of nonsyndromic hearing impairment from typical areas in China. *J Transl Med*. 2009;7:79.
5. Gu X, Guo L, Ji H, et al. Genetic testing for sporadic hearing loss using targeted massively parallel sequencing identifies 10 novel mutations. *Clin Genet*. 2015;87:588-593.
6. Mutai H, Suzuki N, Shimizu A, et al. Diverse spectrum of rare deafness genes underlies early-childhood hearing loss in Japanese patients: a cross-sectional, multi-center next-generation sequencing study. *Orphanet J Rare Dis*. 2013;8:172.
7. Park JH, Kim NK, Kim AR, et al. Exploration of molecular genetic etiology for Korean cochlear implantees with severe to profound hearing loss and its implication. *Orphanet J Rare Dis*. 2014;9:167.
8. Shearer AE, DeLuca AP, Hildebrand MS, et al. Comprehensive genetic testing for hereditary hearing loss using massively parallel sequencing. *Proc Natl Acad Sci USA*. 2010;107:21104-21109.
9. Rabbani B, Tekin M, Mahdieh N. The promise of whole-exome sequencing in medical genetics. *J Hum Genet*. 2014;59:5-15.
10. Shearer AE, Eppsteiner RW, Booth KT, et al. Utilizing ethnic-specific differences in minor allele frequency to recategorize reported pathogenic deafness variants. *Am J Hum Genet*. 2014;95:445-453.
11. Wang DY, Wang YC, Weil D, et al. Screening mutations of OTOF gene in Chinese patients with auditory neuropathy, including a familial case of temperature-sensitive auditory neuropathy. *BMC Med Genet*. 2010;11:79.
12. Mizutari K, Mutai H, Namba K, et al. High prevalence of CDH23 mutations in patients with congenital high-frequency sporadic or recessively inherited hearing loss. *Orphanet J Rare Dis*. 2015;10:60.
13. Roux AF, Faugere V, Vache C, et al. Four-year follow-up of diagnostic service in USH1 patients. *Invest Ophthalmol Vis Sci*. 2011;52:4063-4071.
14. He L, Pang X, Liu H, Chai Y, Wu H, Yang T. Targeted next-generation sequencing and parental genotyping in sporadic Chinese Han deaf patients. *Clin Genet*. 2018;93:899-904.
15. Bademci G, Foster J 2nd, Mahdieh N, et al. Comprehensive analysis via exome sequencing uncovers genetic etiology in autosomal recessive nonsyndromic deafness in a large multiethnic cohort. *Genet Med*. 2016;18:364-371.
16. Lewis MA, Nolan LS, Cadge BA, et al. Whole exome sequencing in adult-onset hearing loss reveals a high load of predicted pathogenic variants in known deafness-associated genes and identifies new candidate genes. *BMC Med Genomics*. 2018;11:77.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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