Next-Generation Sequencing Facilitates Genetic Diagnosis And Improves The Management Of Patients With Hearing Loss In Clinical Practice

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Research Article

Keywords: hearing loss, next-generation sequencing, molecular analysis, clinical evaluation

Posted Date: June 24th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-546998/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at International Journal of Pediatric Otorhinolaryngology on October 1st, 2022. See the published version at https://doi.org/10.1016/j.ijporl.2022.111258.

Abstract

Background: Hearing loss (HL) is a prevalent sensorineural disorder, and is among the most etiologically heterogeneous disorders. With the advent of next-generation sequencing (NGS) technologies, hundreds of candidate genes can be analyzed simultaneously in a cost-effective manner.

Methods: 94 patients from 87 families diagnosed with non-syndromic or syndromic hearing loss were enrolled. A custom-designed HL panel and clinical exome sequencing (CES) were applied to explore molecular etiology in the cohort, and the efficacy of the two panels was examined.

Results: The etiologic diagnosis for hearing loss has been arrived at 40 out of 94 patients (42.6%), 28 with an autosomal recessive (AR) inheritance pattern and 12 with an autosomal dominant (AD) pattern. Candidate variants in 19 different genes were identified in the study cohort, 11 with AR inheritance pattern and 8 with AD pattern. 14 of the variants identified in the study were novel. Compared with CES, the custom-designed HL panel has comparatively higher diagnostic yield (61.5% vs. 29.1%), less expensive price, similar turn-around time, and can be used as an efficient diagnostic tool for hearing loss in the clinical practice.

Conclusions: Next-generation sequencing facilitates genetic diagnosis and improves the management of patients with hearing loss in the clinical practice.

Introduction

Hearing loss is a prevalent sensorineural disorder, and the reach of hearing loss extends far beyond sensory impairment.[1] According to the estimates of World Health Organization, approximately 466 million people worldwide suffer from disabling hearing loss, and 34 million of those are children under age of 15.[2] Lacking of adequate auditory stimulation or sufficient language exposure during early childhood may alter brain connectivity and processing, thus children who have not acquired adequate auditory stimulation may face a series of obstacles in their subsequent linguistic acquisition, cognitive development, and psychosocial functioning.[1,3,4] As a matter of fact, hearing loss can occur at any time during people's life, impair communication, affect social interaction and create difficulties in the workplace.[1] However, the complex etiology of hearing loss, combined with the highly variable and often overlapping presentations of different types of hearing loss, challenge the ability of traditional clinical diagnosis to arrive at.[5] As estimated, up to 60% of educationally significant congenital and early-onset hearing loss is associated with genetic factors.[5,6] The contribution of genetic causes to adult-onset hearing loss is less clear, but a growing number of susceptibility loci have been identified and a significant proportion of adult-onset hearing loss has been found to be associated with genetic factors.[5,7] Around 120 non-syndromic loci have been identified in humans (hereditaryhearingloss.org) and more than 400 genetic syndromes include hearing loss as a feature.[5,8] When the genetic etiology of hearing loss is indentified, the clinical genetics diagnosis can provide a number of potential benefits for the patients and their families, including improving the clinical management of patients, planning for their future medical and educational needs, and facilitating the estimation of likelihood of recurrence.[5] Traditional molecular diagnostic tests for hearing loss mainly involved genotyping or DNA sequencing to identify specific hearing loss variants or to screen a few genes associated with hearing loss. In the last decade, next generation sequencing (NGS) has revolutionized the genetic testing of diseases with high genetic and allelic heterogeneity, such as hearing loss, allowing hundreds of genes to be screened simultaneously.[8] In the present study, we examined the efficacy of a custom-designed panel (HL panel) and clinical exome sequencing (CES) as diagnostic tools in hearing loss, and improved the clinical genetics evaluations in the study cohort.

Materials And Methods

2.1. Ethics statement

The study has been approved by Medical Ethics Committee of Guangdong Women and Children Hospital. Written informed consent was obtained from all participants, their parents or legal guardians (in the case of children under 16). Authors had access to information that could identify individual participants, and the information was anonymized prior to submission. All the procedures performed in the study were in accordance with the Declaration of Helsinki and as we previously described.[9]

2.2. Patients and Samples

A total of 94 patients from 87 families diagnosed with non-syndromic or syndromic hearing loss were enrolled from January 2017 to December 2020 under an institutional review board-approved protocol of informed consent. A comprehensive history and physical examination record for each participant was obtained, including clinical history, severity of hearing loss, age and cause of onset, infection history, aminoglycoside antibiotics exposure, as well as genetic factors related to the hearing impairment.[10] All recruited patients presented with sensorineural or mixed hearing loss. Hearing loss severity was established as mild (hearing thresholds 26 to 40dB), moderate (hearing thresholds 41 to 60dB), severe (hearing thresholds 61 to 80dB) or profound (hearing thresholds more than 80dB).

Peripheral blood samples were collected from patients and their lineal relatives. Genomic DNA was extracted using the SolPure Blood DNA kit (Magen, Shanghai, China) according to manufacturer's instructions and was fragmented by Q800R Sonicator (Qsonica, CT, USA). The paired-end libraries were prepared following Illumina library preparation protocol.

2.3. Custom-designed Panel for Hearing loss

39 patients received genetics evaluations by using a custom-designed panel as diagnostic tool for hearing loss. Custom-designed NimbleGen SeqCap probes (Roche NimbleGen, Madison, WI, USA) were used for in-solution hybridization to enrich targeted sequences, which included 190 genes associated with hereditary deafness based on databases of OMIM, HGMD, etc (Hearingloss Care Panel, AmCare Genomic Laboratory, Guangzhou, China). Captured DNA

samples were amplified by PCR with indexed primers and then sequenced on a NovaSeq 6000 sequencers (Illumina, San Diego, CA). NGS data processing and data quality control were carried out as previously described.[11] Raw-image data conversion and demultiplexing were performed following Illumina's primary data analysis pipeline using CASAVA version 2.0 (Illumina, San Diego, CA). Low-quality reads (Phred score < Q20) were removed before demultiplexing. Sequences were aligned to the hg19 reference genome by NextGENe software (SoftGenetics, State College, PA) using the recommended standard settings for single-nucleotide variant and insertion/ deletion discovery. The pathogenicity of the variants was estimated using the American College of Medical Genetics and Genomics (ACMG) guidelines.

2.4. Clinical Exome Sequencing

55 patients received genetics evaluations by using clinical exome sequencing as diagnostic tool for hearing loss. Custom-designed NimbleGen SeqCap probes (Roche NimbleGen, Madison, WI) were used for in-solution hybridization to enrich target sequences, which included ~ 5000 genes potentially associated with known Mendelian genetic diseases based on databases of OMIM, HGMD and new publications (AmCare Genomic Laboratory, Guangzhou, China). Captured DNA samples were amplified by PCR with indexed primers and then sequenced on a NovaSeq 6000 sequencers (Illumina, San Diego, CA). NGS data processing and data quality control were performed as previously described.[11] Raw-image data conversion and demultiplexing were performed following Illumina's primary data analysis pipeline using CASAVA version 2.0 (Illumina, San Diego, CA). Low-quality reads (Phred score < Q20) were removed before demultiplexing. Sequences were aligned to the hg19 reference genome by NextGENe software (SoftGenetics, State College, PA) using the recommended standard settings for single-nucleotide variant and insertion/deletion discovery. The pathogenicity of the variants was estimated according to the ACMG guidelines.

2.5. Data Analysis

The resulting sequencing data were analyzed with the NextGENe software in regard to the human assembly GRCh37/hg19. This software performs the alignment, variant calling and annotation of the variants. The annotated variants were filtered according to a minor allele frequency (MAF) value > 0.01 (the frequency of the variants was explored in the Exome Aggregation Consortium (ExAC) database, genomeAD (https://gnomad.broadinstitute.org/) and 1000 genomes (https://www.internationalgenome.org/). To classify the variants, we also took into account of their annotation in the dbSNP (www.ncbi.nlm.nih.gov/SNP/), their description in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Varsome (https://Varsome.com/), HGMD (http://www.hgmd.cf.ac.uk/), LOVD (https://www.lovd.nl/) and Deafness Variation Database (http://deafnessvariationdatabase.org/) and the variant type, as previously described.[8] Variants were evaluated with the predictors included in the Varsome website and SpliceAI software: BayesDel_addAF, DANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP, MVP, MutationAssessor, MutationTaster, REVEL, SIFT and GERP.

Sanger sequencing (BigDye Terminator kit v1.1, Applied Biosystems, Carlsbad, USA) was carried out to validate the pathogenic/ likely pathogenic point variants and to perform segregation analysis when patients' relatives were available.[8]

The performance evaluation of the custom-designed HL panel and CES was analyzed with chi-square text using SPSS 21.0 (SPSS Inc., Chicago, USA).

Results

A total of 94 patients were enrolled in the study, and the characteristics of the study cohort were demonstrated in Table 1. Eighty-three patients suffered from non-syndromic hearing loss (NSHL) and 11 from syndromic hearing loss (SHL). 84 had no family history of hearing loss, and 5 had multiple family members suffered from hearing impairments. The degree of hearing loss in the patients varied, and severe-to-profound hearing loss was observed in the majority (85.1%, 80/94). Besides, prelingual hearing loss was detected in 86.2% (81/94) patients.

Table 1
Characteristics of the study cohort

Characteristic	No.	%
All	94	100
Sex		
Male	51	54.3
Female	43	45.7
Family history		
Yes	10	10.6
No	84	89.4
Onset		
Prelingual (≤ 3 years)	81	86.2
Post-lingual (> 3 years)	13	13.8
Laterality		
Bilateral symmetric	69	73.4
Bilateral asymmetric	22	23.4
No record	3	3.2
Stability		
Stable	64	68.1
Fluctuating	24	25.5
No record	6	6.4
Aminoglycoside Exposure		
Yes	11	11.7
No	62	66.0
Uncertain	21	22.3
Severity		
Mild	2	2.1
Moderate	11	11.7
Severe	63	67.0
Profound	17	18.1
No record	1	1.1
Rehabilitation		
Hearing aid	39	41.5
Cochlear Implantation	47	50.0
Both	18	19.1
Not applied	11	11.7
Newborn hearing screening result		
Pass	19	20.2
Referral	39	41.5
Not applied/No record	36	38.3

The etiologic diagnosis for hearing loss has been arrived at 40 out of 94 patients (42.6%), 28 with an AR inheritance pattern and 12 with an AD pattern. Clinical data and genetics evaluations of the diagnosed patients are shown in Table 2. Candidate variants in 19 different genes were identified in the study cohort, 11 with AR inheritance pattern and 8 with AD pattern (Fig. 1). Among the 47 different candidate variants detected, 14 were frameshift, 13 missense, 7 nonsense, 2 in-frame ins/del, 3 were CNVs and 8 affect splice-sites. Fourteen out of 47 variants were novel (Tables 3).

Table 3
Classification of Novel Variants Identified in this Study

Variant				Frequency			Pathogenicity Scores	
Gene	Nucleotide	Protein	Classification	GnomAD Exomes	GnomAD Genomes	Deafness Variation Database	REVEL	Conservation Score (GERP)
MYO15A (NM_016239.4)	c.1203C > A	p.Tyr401Ter	P	NF	NF	NF	NA	5.45
SOX10 (NM_006941.4)	c.549_567del	p.Gln185ProfsTer95	LP	NF	NF	NF	NA	4.24
CDH23 (NM_022124.6)	c.1606C > T	p.Arg536Trp	VUS	0	NF	Unknown significance–Impact Moderate	0.6359	5.51
MYO15A (NM_016239.4)	c.5275A > C	p.Ser1759Arg	VUS	NF	NF	NF	0.4799	4.00
EYA1 (NM_000503.6)	c.473delC	p.Pro158LeufsTer83	LP	NF	NF	NF	NA	5.83
MYH14 (NM_001077186.2)	c.289_290insTGG	p.Gln97delinsLeuGlu	LP	NF	NF	NF	NA	4.44
LHFPL5 (NM_182548.4)	c.619delC	p.Leu207SerfsTer37	LP	0.00000398	NF	Unknown significance–Impact High	NA	5.7
MYO7A (NM_000260.4)	c.291T > G	p.Tyr97Ter	LP	NF	NF	NF	NA	4.88
MYO15A (NM_016239.4)	c.3866 + 5G > A	-	VUS	NF	NF	NF	NA	4.95
MYO15A (NM_016239.4)	c.5649 + 1G > T	-	P	NF	NF	NF	NA	5.77
CDH23 (NM_022124.6)	c.8371delC	p.Leu2791TrpfsTer46	LP	NF	NF	NF	NA	5.61
MYO15A (NM_016239.4)	c.8138T > G	p.Leu2713Arg	LP	NF	NF	NF	0.9179	5.19
MYO7A (NM_000260.4)	c.285 + 5G > C	-	VUS	NF	NF	NF	NA	4.86

3.1. Autosomal Recessive Hearing Loss

Twenty-eight probands carried biallelic pathogenic or likely pathogenic variants associated with an autosomal recessive pattern of inheritance (Table 2A). Twenty-seven cases presented with NSHL. These were linked to GJB2 (ten cases), SLC26A4 (five cases), MYO15A (five cases), MYO7A (two cases), LRTOMT (two cases), and one case in each of the CDH23, STRC, TMC1 and LHFPL5 genes (Fig. 1). One patient suffered from Usher syndrome was identified with likely pathogenic variants in CDH23 gene. Pedigree analysis for some representative cases is displayed in Fig. 2.

Ten of the AR likely pathogenic variants identified in the present study were novel. Four of them produced a premature stop codon: c.1203C > A (p.Tyr401Ter) in MYO15A gene, c.291T > G (p.Tyr97Ter) in MYO7A gene, c.8371delC (p.Leu2791TrpfsTer46) in CDH23 gene, and c.619delC (p.Leu207SerfsTer37) in LHFPL5 gene. Three novel splicing variants were identified in the study cohort: c.285 + 5G > C in MYO7A gene, c.3866 + 5G > A and c.5649 + 1G > T in MYO15A gene. Moreover, three novel missenses variants were detected: c.1606C > T (p.Arg536Trp) in CDH23 gene, c.5275A > C (p.Ser1759Arg) and c.8138T > G (p.Leu2713Arg) in MYO15A gene (Table 3).

3.2. Autosomal Dominant Hearing Loss

Twelve patients from nine families were identified with autosomal dominant hearing loss (Table 2B). Nine cases presented with NSHL. Three patients from two families present with variants associated with hearing loss in EYA1 gene, two patients from one family carry a likely pathogenic variant in TMC1gene, and another two patients from the same family carry a likely pathogenic variant in MYH14 gene. Pathogenic variants in GJB6 and GSDME gene were detected in

one patient each (Fig. 1). Moreover, three patients with syndromic hearing loss were identified with pathogenic or likely pathogenic variants. One patient suffered from Waardenburg syndrome was detected with a likely pathogenic variant in SOX10 gene, one patient with CHARGE syndrome was identified with a likely pathogenic variant in CHD7 gene, and another patient with LEOPARD syndrome carried a pathogenic variant in PTPN11 gene. Pedigree analysis for some representative cases is shown in Figs. 2 and 3.

Three of the AD likely pathogenic variants identified in the present study were novel. Two of them produced a premature stop codon: c.549_567del (p.Gln185ProfsTer95) in SOX10 gene, and c.473delC (p.Pro158LeufsTer83) in EYA1 gene. One in-frame novel insertion was found in MYH14 gene, c.289_290insTGG (p.Gln97delinsLeuGlu) (Table 3).

3.3. Performance evaluation of HL panel and CES

We examined the efficacy of the custom-designed HL panel and CES as diagnostic tools in hearing loss. The diagnostic yields were 61.5% (24 out of 39) for HL panel and 29.1% (16 out of 55) for CES ($\chi^2 = 8.5455$, P = 0.003464). The cost was 650 USD and 750 USD for HL panel and CES, respectively. The turn-around time for both assays was similar, 3–4 weeks.

Discussion

Hearing loss is among the most etiologically heterogeneous disorders, with more than 400 genetic syndromes that include hearing loss as a feature, more than 120 genes associated with non-syndromic genetic hearing loss, and a number of non-genetic causes.[5,12] With the advent of NGS technologies, hundreds of candidate HL genes can be analyzed simultaneously in a cost-effective manner. Even so, the diagnostic yield varies among different patient cohorts and depends on the detection methods used. The degree of hearing loss, the onset age of hearing loss, the existence of family history, the ethnic origin, and the number of genes contained in the NGS panel may affect the rate of genetic diagnosis.[8] In the present study, two NGS panels were applied to explore molecular etiology in a Chinese hearing loss cohort, and 40 out of 94 patients has been genetically diagnosed, rendering an overall yield of 42.6% in the study population. This is an acceptable yield, comparing to the 10–83% diagnostic rates reported in previous reports,[8,13–16] since a large number of hearing loss patients in our hospital choose a stepwise approach and be pre-excluded for mutations in common deafness genes GJB2, SLC26A4 and MT-RNR1 prior to NGS.[10] Therefore, it is an additional diagnostic rate. It also explains why the proportions of GJB2 and SLC26A4 gene variations are comparatively low in the study cohort (Fig. 1).

In the study, 39 patients received genetics evaluations by using the custom-designed HL panel and 24 were diagnosed (diagnostic rate was 61.5%); 55 patients received genetic testing by using clinical exome sequencing and 16 were diagnosed (diagnostic rate was 29.1%). Compared with CES, the custom-designed HL panel has comparatively higher diagnostic yield, less expensive price, and similar turn-around time. Besides, in this study cohort, all the genes detected by CES were included in the custom-designed HL panel. Thus, for patients with clinical diagnosed NSHL or SHL, the custom-designed HL panel can be an effective diagnostic tool, facilitates genetic diagnosis and improves the management of patients. However, due to the comparatively small sample size, the diagnostic yields of the two NGS tools may not adequately represent their diagnostic capacities. Besides, before the performance evaluation, doctors' choices between the two NGS tools were subjective, and bias can be induced in the process. CES may have better performance in cases when we cannot distinguish whether hearing loss is a feature of certain syndromes or in addition to multi-malformations, since it evaluate all exons in the genome for variations associated with clinical symptoms. Moreover, CES does not rely on a list of genes involved in a particular disease process, and may identify genetic defects that were not directly associated with hearing loss (not included in HL panels) but cause hearing loss through altering bone structure or affecting sound conduction. In one of our previous cases, the patient suffered from multiple enchondromatosis, atresia of the external auditory canal, abnormality of the middle ear and severe hearing loss on the right side, but normal hearing on the left side. CES detected an AD likely pathogenic variant in EXT1 gene, inherited from the patient's mother who had normal hearing but pelvic exostoses. EXT1 participate in forming a heterooligomeric complex, which is an essential factor in a signal transduction cascade for regulation of chondrocyte differentiation, ossification, and apoptosis.[17] Defects in EXT1 gene may affect its function and cause hearing loss through altering bone structure and affecting sound conduction in the patient.

In the present study, a number of novel variants have been detected and the pathogenicity of them has been estimated according to the ACMG guidelines. As shown in Table 3, six variants predicted to generate direct stop codons were classified as pathogenic or likely pathogenic. One variant located at a canonical splice site (+/−1 and +/−2 positions of an intron) in MYO15A gene was classified as pathogenic for which loss of function is reported as cause of the disease. Another two variants predicted to affect splice-sites (c.3866 + 5G > A in MYO15A gene and c.285 + 5G > C in MYO7A gene) were classified as variants of uncertain clinical significance (VUS). Missense variant c.8138T > G (p.Leu2713Arg) found in trans with another previously described likely pathogenic variant in MYO15A gene was not found in gnomAD exomes / genomes and predicted as damaging by multi computational programs in Varsome. The variants c.1606C > T (p.Arg536Trp) in CDH23 gene and c.5275A > C (p.Ser1759Arg) in MYO15A gene located at highly conserved regions, were predicted to alter the structure / function of the proteins by bioinformatics predictions and segregation analyses, but remain classified as VUS according to the ACMG guidelines. The in-frame insertion c.289_290insTGG (p.Gln97delinsLeuGlu) in MYH14 gene was found in a patient and his affected father with an AD pattern of inheritance. The variant was not found in healthy control population databases, and was predicted as conserved by bioinformatic tool of PhyloP100way. The detection and evaluation of novel variants is of importance in expanding the spectrum of variants associated with hearing loss.

Furthermore, NGS panels allow the simultaneous analysis of hundreds of genes, and sometimes pathogenic variants in different genes can be found in one patient.[8] In the study cohort, seven cases harbored pathogenic variants in different genes or multi pathogenic variants in the same genes. The patient from Case 21980 carried homozygous c.235delC (p.Leu79CysfsTer3) variant in GJB2 gene in addition to the heterozygous c.5217delT (p.Val1740LeufsTer4) AR variant in PTPRQ gene. The patient from Case 23697 harbored homozygous c.109G > A (p.Val37Ile) variant in GJB2 gene in addition to exon 16 deletion in STRC gene. The patient from Case 25257 carried homozygous c.453 + 2T > C variant in TMC1 gene in addition to the heterozygous c.109G > A (p.Val37Ile) variant in GJB2 gene, was born to a consanguineous family with another hearing loss child. The patient from Case 65920 carried c.291T > G (p.Tyr97Ter) and

c.285 + 5G > C variants in TMC1 gene in compound heterozygous state, in addition to the heterozygous c.109G > A (p.Val37Ile) variant in GJB2 gene. The patient from Case 51456 carried homozygous c.109G > A (p.Val37Ile) variant in GJB2 gene, in addition to a de novel variant c.4353G > C (p.Gly1451=) in CHD7 gene, which may be responsible for CHARGE syndrome the patient presented with. The patient from Case 39130 carried c.919-2A > G and c.589G > A (p.Gly197Arg) variants in SLC26A4 gene, which may be responsible for hearing loss and enlarged vestibular aqueduct (EVA) in the patient. Besides, the patient carried whole gene deletion (17p12) of PMP22, which may be the cause of hereditary neuropathy with liability to pressure (HNLP) in the patient. The patient from Case 4377 presented with hearing loss and EVA has been detected with three heterozygous LP variants in SLC26A4 gene, c.1547dupC (p.Ser517PhefsTer10) inherited from her father, c.563T > C (p.Ile188Thr) and c.1746delG (p.Ala584ArgfsTer2) from her healthy mother. These findings have important implications for reproductive genetic counseling.

Conclusions

NGS facilitates genetic diagnosis and improves the management of patients with hearing loss in the clinical practice. The custom-designed HL panel include almost all known genes associated with hearing loss, and can be used as an effective diagnostic tool in the clinical practice. CES evaluate all exons in the genome for variations associated with clinical symptoms, with not much higher price and similar turn-around time, can also be efficient for the clinical genetic diagnosis of hearing loss. Identifying the etiology of hearing loss may improve the management of patients with hearing loss, refine genetic counseling and facilitate the estimation of likelihood of recurrence.[5]

Abbreviations

HL: Hearing loss

NGS: Next-generation sequencing

CES: Clinical exome sequencing

AR: Autosomal recessive

AD: Autosomal dominant

ACMG: American College of Medical Genetics and Genomics

MAF: Minor allele frequency

ExAC: Exome Aggregation Consortium

NSHL: Non-syndromic hearing loss

SHL: Syndromic hearing loss

Declarations

Ethics approval and consent to participate

The study has been approved by the Institutional Review Board/ Medical Ethics Committee of Guangdong Women and Children Hospital (IRB reference number: 201711085). Written informed consent was obtained from all participants, their parents or legal guardians (in the case of children under 16).

Consent for publication

Written informed consent for publication was obtained from all participants, their parents or legal guardians (in the case of children under 16). Authors had access to information that could identify individual participants, and the information was anonymized prior to submission.

Availability of data and material

The datasets generated and/or analyzed during the current study are not publicly available due individual privacy but are available from the corresponding author (Aihua Yin, E-mail: yinaihua0131@163.com) on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the Medical Science and Technology Research Project of Guangdong Province (Grant No. A2020302). The funding did not play role in the design of the study, collection, analysis, interpretation of data, or in writing the manuscript.

Authors' contributions

CL, YH and AY conceived and designed the study. CL, YH, YZ, HD, AW, LY, YZ, LL, YL, YQ, FL, JW, LD, YW, FM, QZ and XW performed the genetic diagnosis. CL, YH and YH analyzed and interpreted data. CL, YH drafted the manuscript and AY revised it. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

References

1. Wilson BS, Tucci DL, Merson MH, O'Donoghue GM. Global hearing health care: new findings and perspectives. Lancet. 2017;390(10111):2503–2515.
2. World Health Organization. Addressing the rising prevalence of hearing loss. https://apps.who.int/iris/bitstream/handle/10665/260336/9789241550260-eng.pdf?sequence=1&ua=1. Published 2018. Accessed April 8, 2021.
3. Kral A, O'Donoghue GM. Profound deafness in childhood. N Engl J Med. 2010; 363: 1438–1450.
4. Lieu JEC, Kenna M, Anne S, Davidson L. Hearing Loss in Children: A Review. JAMA. 2020;324(21):2195–2205.
5. Alford RL, Arnos KS, Fox M, et al. American College of Medical Genetics and Genomics guideline for the clinical evaluation and etiologic diagnosis of hearing loss. Genet Med. 2014;16(4):347–355.
6. Schimmenti LA, Martinez A, Fox M, et al. Genetic testing as part of the early hearing detection and intervention (EHDI) process. Genet Med. 2004;6(6):521–525.
7. Michels TC, Duffy MT, Rogers DJ. Hearing Loss in Adults: Differential Diagnosis and Treatment. Am Fam Physician. 2019;100(2):98–108.
8. García-García G, Berzal-Serrano A, García-Díaz P, et al. Improving the Management of Patients with Hearing Loss by the Implementation of an NGS Panel in Clinical Practice. Genes (Basel). 2020;11(12):1467.
9. Liu C, Zhang X, Wang J, et al. Genetic testing for Prader-Willi syndrome and Angelman syndrome in the clinical practice of Guangdong Province, China. Mol Cytogenet. 2019;12:7.
10. Yin A, Liu C, Zhang Y, et al. Genetic counseling and prenatal diagnosis for hereditary hearing loss in high-risk families. Int J Pediatr Otorhinolaryngol.2014;78(8):1356–1359.
11. Feng Y, Ge X, Meng L, et al. The next generation of population-based spinal muscular atrophy carrier screening: comprehensive pan-ethnic SMN1 copy-number and sequence variant analysis by massively parallel sequencing. Genet Med. 2017;19(8):936–944.
12. Hereditary Hearing Loss and Its Syndromes, 3rd edn. Toriello?HV, Smith?S (eds). Oxford University Press: New York, NY, 2013.
13. Sloan-Heggen CM, Bierer AO, Shearer AE, et al. Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. Hum Genet. 2016;135(4):441–450.
14. Cabanillas R, Diñeiro M, Cifuentes GA, et al. Comprehensive genomic diagnosis of non-syndromic and syndromic hereditary hearing loss in Spanish patients. BMC Med Genomics. 2018;11(1):58.
15. Zou S, Mei X, Yang W, Zhu R, Yang T, Hu H. Whole-exome sequencing identifies rare pathogenic and candidate variants in sporadic Chinese Han deaf patients. Clin Genet. 2020;97(2):352–356.
16. Shearer AE, Smith RJ. Massively Parallel Sequencing for Genetic Diagnosis of Hearing Loss: The New Standard of Care. Otolaryngol Head Neck Surg. 2015;153(2):175–182.
17. Heinritz W, Hüffmeier U, Strenge S, et al. New mutations of EXT1 and EXT2 genes in German patients with Multiple Osteochondromas. Ann Hum Genet. 2009;73(Pt 3):283–291.

Tables

Due to technical limitations, table 2 is only available as a download in the Supplemental Files section.

Figures

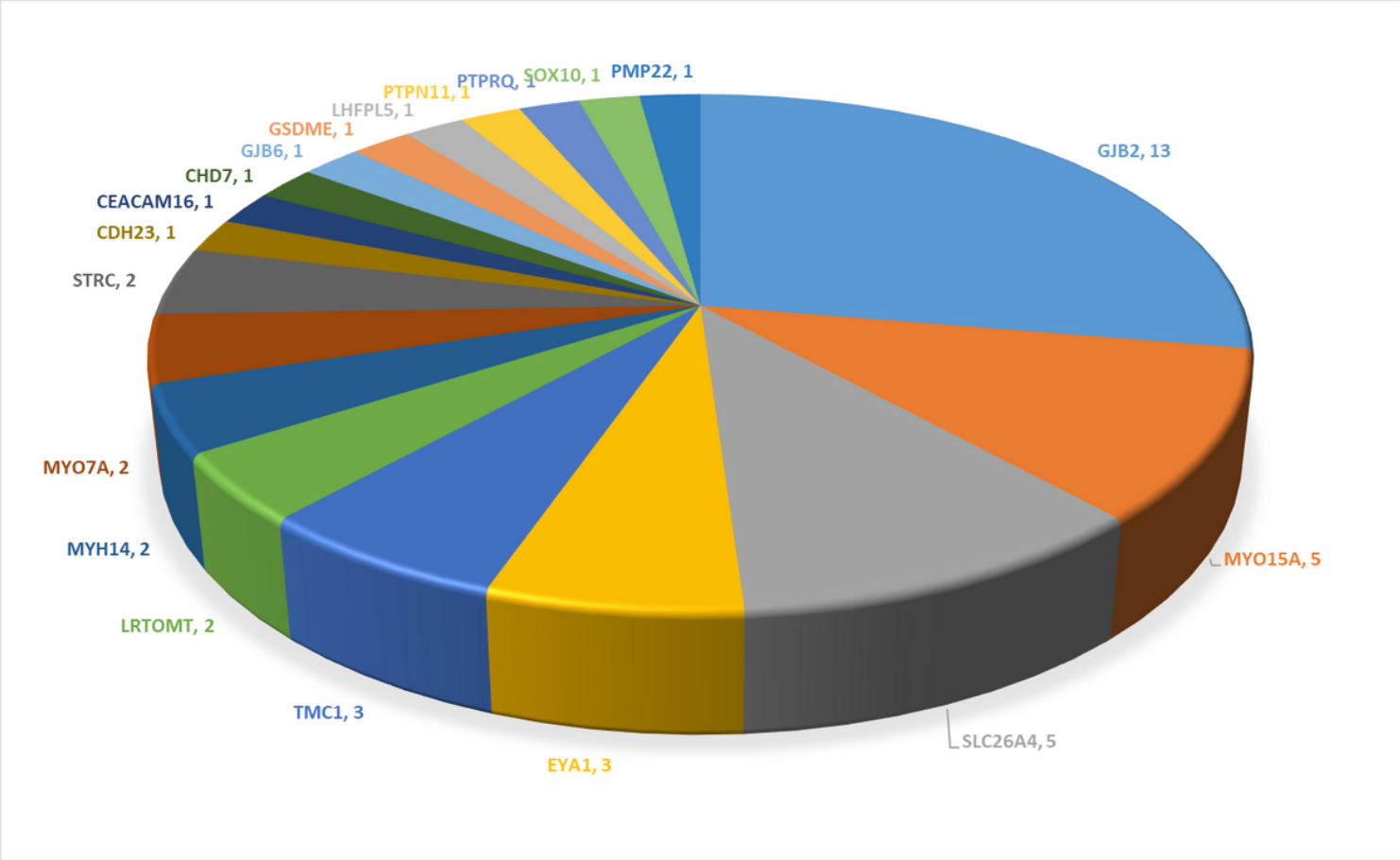

Figure 1

Genetic spectrum of diagnosed patients with hearing loss

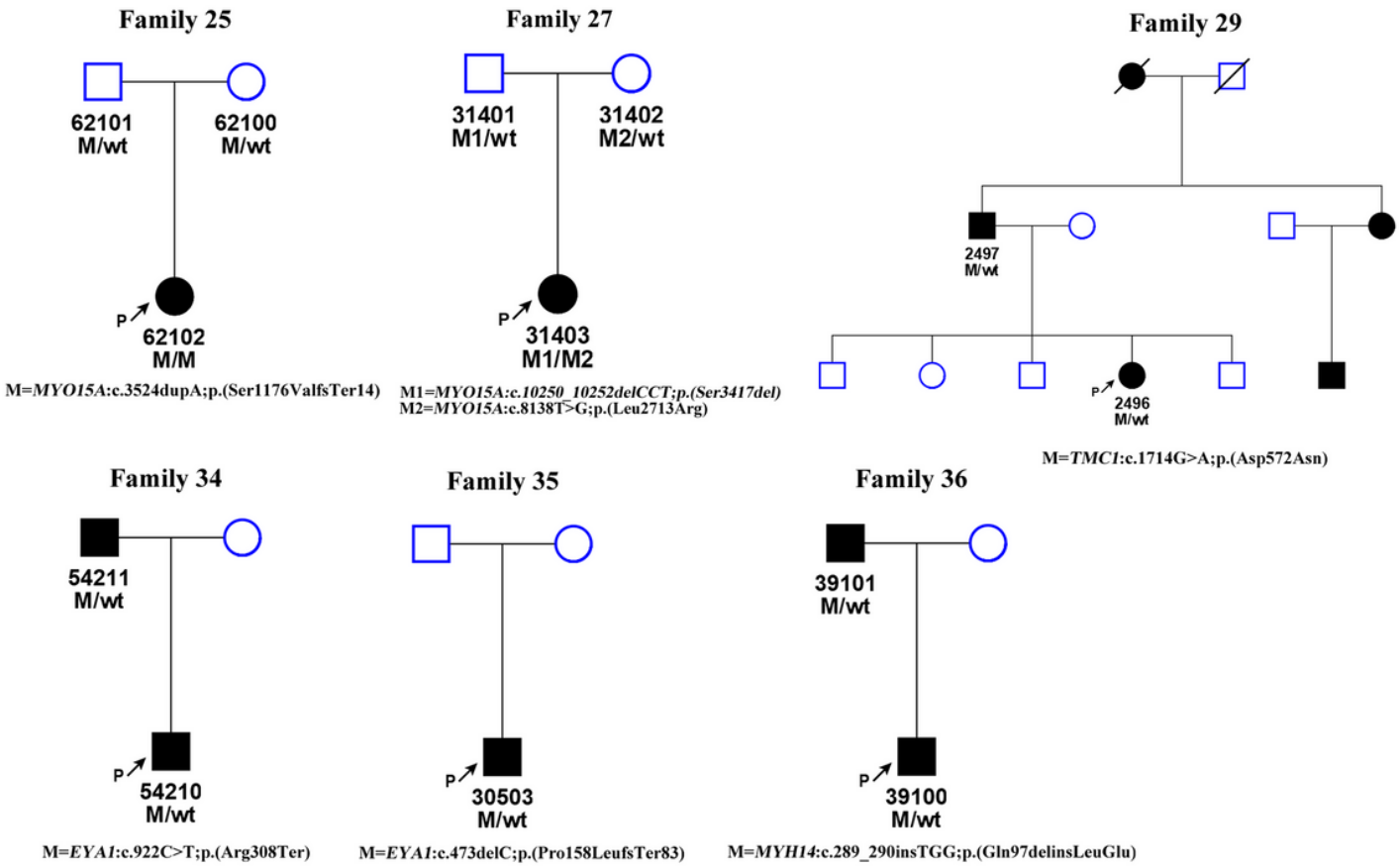

Figure 2

Pedigree analysis for some representative cases Figure legend: Arrow indicates the proband, M indicates the pathogenic or likely pathogenic variant and wt indicates wild type sequence.

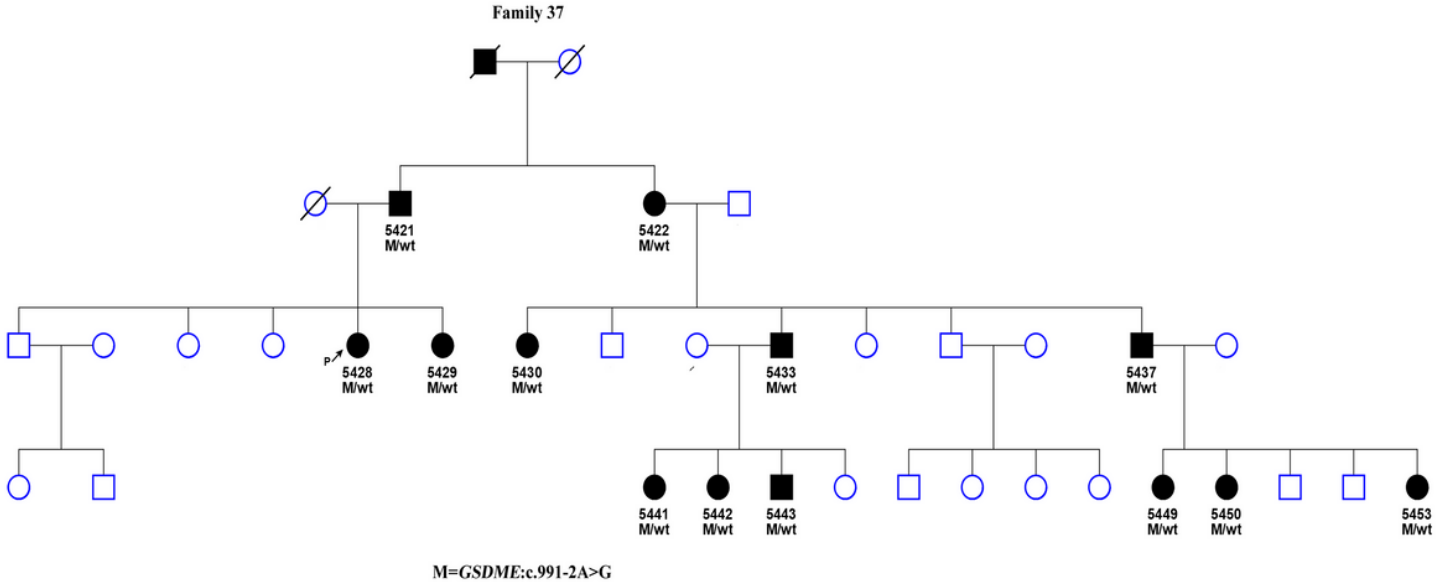

Figure 3

Pedigree analysis for Family 27 with multiple members suffered from hearing loss Figure legend: Arrow indicates the proband, M indicates the likely pathogenic variant and wt indicates wild type sequence.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2.docx