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Early Detection of Common Deafness Gene Mutations Associated with Diagnosis and Treatment

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Abstract: Approximately, there are tens of thousands hearing-impaired newborns in Vietnam annually. Early detection of common deafness mutations is a key factor in diagnosing, treatments, helping the hearing-impaired children to develop their language and with their social inclusion. The objective of our research is to identify the incidence of hearing-impaired mutations with Next Generation Sequencing (NGS) and evaluate the roles of mutations in deciding treatment methods. 100 diagnosed hearing-impaired children from Hanoi medical university hospital from 3/2017 till 10/2019 and their families took part in the research. Next Generation Sequencing (NGS) was applied to inspect 100 mutations on 18 genes, which are the most common in hearing-impaired patients worldwide. During the research 30 mutations in 100 children (30%) were identified, including both homozygous and heterozygous mutations of 6 genes: GJB2, SLC26A4, TMC1, 12S-rARN, MT-TH, MT-TL1. The incidence of deafness mutations was 30%. Mutations of gene GJB2 had the largest proportion (16%) among 6 genes indentified. All the mutations above showed positive results with cochlear implant.

Keywords
Next Generation Sequencing; NGS; Hearing-impairment; GJB2; SLC26A4; TMC1; 12SrARN; MT-TH; MT-TL1

INTRODUCTION

Congenital hearing-impaired (hearing loss present at birth) is a condition that causes a wide range of impairment or a complete loss of hearing from newborn period. According to 2014 statistics of the Hanoi Obstetrics and Gynecology Hospital, a screening of 38331 infants found 688 cases with hearing loss (1.5%) [1]. In a study of Shierer et al., the rate of congenital defects due to genetic causes is 80% [2]. Currently, in Vietnam, the methods used in diagnosing congenital hearing loss are mostly physical such as OAE (Otoacoustic Emissions), ABR (Auditory Brainstem Response).

However, with the increasing need for early and accurate mutations detection, molecular genetics techniques have shown many advantages.

Nowadays, much molecular biology techniques have been used to diagnose genetic causes of deafness, but most of these methods are not suitable for multi-gene diagnosis. Next Generation Sequencing (NGS) is one of the most efficient methods in investigating nucleotide sequences, with the ability to detect mutations quickly, accurately, save costs and labor, diagnose multi-mutations, multi-genes and many patients at the same time; especially in prenatal or neonatal diagnosis, from which interventions or early access to special education programs for the children to develop normally. Still, there are only a few studies on the application of NGS to early diagnosis of congenital deafness gene in Vietnam.

MATERIALS AND METHODS

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Written informed consent was obtained from the parents or guardians of underage participants in 100 families. 100 diagnosed hearing-impaired children and their families from Hanoi Medical University Hospital took part in the research, from 3/2017 till 10/2019. NGS was applied to detect 100 mutations on 18 genes which are the most common in hearing-impaired patients worldwide.

Congenitally deaf subjects, regardless of gender, meet the following criteria: Children who are deaf before language and do not suffer from diseases that cause hearing loss (meningitis, mumps, rubella, measles, chicken pox, head injury). They have been diagnosed with congenital deafness and already have cochlear implant indications.

The study used cross-sectional descriptive method combined with research results through genetic test by NGS, investigating the relationship between each gene mutation and congenital deafness.

Assay protocol of NGS

Extraction of DNA from 100 diagnosed hearing-impaired children's peripheral blood using DNeasy Blood & Tissue Kit (Qiagen).

To further search for the causative genes in these families, we performed targeted genomic capture and MPS. For cases in which novel variants were detected, segregation analysis was performed to assess 100 deafness children. This study was approved by the Ethical Committee of Hanoi Medical University for Human Studies. Targeted genomic capture and next-generation sequencing whole-exon regions of 18 deafness genes, namely GJB2, GJB3, SLC26A4, MT-RNR1, MT-CO1, MT-TL1, MT-TS1, MT-TH, DSPP, GPR98, DFNA5, TMC1, MYO7A, TECTA, DIABLO, COCH, MYO15A and PRPS1 (Table 1) were target-enriched using a Target Enrichment Kit (My Genostics Inc., Beijing, China) as previously described. A minimum of 3μg of DNA was used to generate indexed Illumina libraries according to the manufacturer's protocol. The final library size was 300 to 400 bp, including the adapter sequences.

Table 1. The result of gene mutation causing congenital deafness by NGS technique

Gene mutation	Type of mutation	Number of cases		The proportion of mutations detected (n=30), %	The proportion of patients studied (n=100), %	Number of parents with mutations
		Homozygous	Heterozygous			
GJB2	235delC	8	5*	53.34	16.00	24
	299_300delAT	0	3*			
SLC26A4	c.439A>G	0	2	10.00	3.00	4
	c.754T>C	0	1**			
	c.1229C>T	0	0**			
TMC1	c.1334G>A	0	1	23.33	7.00	12
	c.150delT	3	3			
12S-rARN (MT RNR1)	1555A>G	0	1	6.67	2.00	1
	1494C>T	0	1			
MT-TL1	m.3243A>G	0	1	3.33	1.00	1
MT-TH	m.1220T>C	0	1	3.33	1.00	1
Total		30		100.00	30.00	43

*In our study, 100 children all responded well with cochlear implantation.

*These gene mutations are all being studied for gene therapy.

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The enrichment libraries were sequenced on an Illumina HiSeq 2000 sequencer to generate 100 bp paired-endreads. High-quality reads were identified by filtering out low-quality reads and adaptor sequences using Solexa QA package and Cutadapt program, respectively. Variants were first selected if they appeared in the 1000 Genomes Project database with an MAF of >0.05, and then they were selected if they appeared in the 300 local Asian Genome database. The remaining variants were further processed according to the db SNP database. SNPs and indels were identified using SOAPsnp and GATK programs. Subsequently, the reads were realigned to the reference genome (NCBI37/hg19) using BWA software. Non-synonymous variants were evaluated by four algorithms, including PolyPhen, and Pmut, to determine pathogenicity.
The panel of 100 mutations on 18 genes is attached in the table in the Appendix A.

RESULTS AND DISCUSSION

In particular, we found two cases with double heterozygous: 1 case has 2 mutations 235delC and 299-300delAT on GJB2 gene; 1 case has 2 mutations c.754T> C and c.1229C> T on SLC26A4 gene (Table 2).

Mutation proportion and the role of genes in congenital hearing loss

The proportion of mutation in hearing impairment in the study group is 30%. The genes in the nucleus with the highest mutation were GJB2 (16%), TMC1 (7%), SLC26A4 (3%), respectively. In addition, we identified three gene mutations in mitochondria that are associated with reduced hearing ability: MT-RNR1 (2%), MT-TL1 (1%), MT-TH (1%).
Bai Lin Wu et al. (2002) studied 324 non-syndrome hearing loss (NSHL) children in the US (in which 70% of congenital deaf children were familial) and found that 127 children with mutations related to congenital hearing loss accounted for a proportion of 39.2% [8]. Another study by Jing Pan et al. (2017) on 380 NSHL patients in Northern China found that the proportion of gene mutation in patients was 19.2% [9].
The big difference here is due to the following 3 factors:
- These gene mutations are specific to each race. These studies were conducted on groups of patients of different races.
- Our sample size is not large enough.
- The study subjects were randomized patients while our study subjects were specific congenital deaf patients who had cochlear implant indications, not focusing on children with high genetic factors (children with father, mother, siblings born deaf).
The GJB2 gene is on chromosome 13, encoding connexin 26 (Cx26) [7]. This is the first gene that has been proved to be associated with sensorineural hearing loss (SNHR). According to numerous studies, GJB2 is the most common cause of genetic hearing loss worldwide [10]. The rate of GJB2 is different from studies around the world. In this study, it is 16%, about 2 times more than the study of Jing Pang (2017) 8.9% [9], but fit with the rate of author Du et al. (2014) 17.27% [11] and Mariana Dória et al. (2015) 19.77% [12].
The TMC1 gene on chromosome 9, which regulates protein channels, helps conduct electrical potential across the membrane of the hair cell in the inner ear [13]. This gene mutation has both dominant and recessive forms, of which the dominant form often causes progressive deafness, and the recessive form often causes severe congenital deafness. The result 7% is significantly higher than that of author Hong Hanh et al. (2017) 1.67% [1], Santos et al. (2005) 4.4% [14]. The ratio could be explained with the difference in sample size.
The SLC26A4 gene encodes the protein pendrin that transports the anion Cl- and HCO3- on chromosome 7 [15]. A mutation of the SLC26A4 gene leads to altered ion transport across the cell membrane in the cochlea, reducing or having congenital hearing loss in a variety of degrees. The rate of 3% is mildly higher than other studies from Vietnam, Hong Hanh et al. (2017) 1.67% [1], Hoa et al. (2016) 1.1% [16].

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Besides the genes in the nucleus, we found three genes in the mitochondria also affect hearing MT-RNR1 (2%), MT-TL1 (1%), MT-TH (1%) which fit with the result of Hong Hanh et al. (2017) MT-RNR1 (3.33%), MT-TL1 (1.67%), MT-TH (1.67%) [1]. Notably, the gene MT–RNR1 encodes for rARN 12S. The early detection of this gene in children is very important because children younger than 10 years of age carrying the gene using aminoglycosides have a high risk of hearing impairment, especially in our country when the rate of antibiotic use indiscriminately still very high.

Table 2. The results of congenital deafness treatment on the basis of gene mutations in some studies

Gene name	Main cellular expression sites in the cochlea	Level of hearing-impaired	Effective intervention	Reference
GJB2	Conexin 26 supporting cells	Severe deafness with syndrome or non-syndrome	High efficiency cochlear implant Low efficiency hearing aids	Sloan-Heggen et al. [3].
TMC1	Protein channels transport ions at inner and outer hair cells	Severe deafness with non-syndrome	High efficiency cochlear implant Low efficiency hearing aids	Eppsteiner et al. [4].
SLC26A4	Cl- transport channel in epithelial cell at the spiral limbus	Severe deafness with syndrome or non-syndromic	High efficiency cochlear implant Low efficiency hearing aids	Eppsteiner et al. [4], Rose et al. [5].
MT-RNR1	Mitochondrial12S ribosome is encoded	Using aminoglycosides caused a wide range of hearing loss	High efficiency cochlear implant Aminoglycosides are limited in use	Li et al. [6].
MT-TH	Unknown	MELAS syndrome	High efficiency cochlear implant	Eppsteiner et al. [4].
MT-TL1	ATPs were reduced at the K+ channels of outer hair cells in the Corti organ	MELAS syndrome	High efficiency cochlear implant	Eppsteiner et al. [4].
STRC	Extracellular protein at outer hair cells	Mild	Low efficiency hearing aids	Sloan-Heggen et al. [3].
TMPRSS3	Cochlear ganglion cells	Severe	Low efficiency cochlear implant	Eppsteiner et al. [4].

Orienting the value of identifying congenital deafness genetic mutation by next generation sequencing (NGS)

Identifying mutations is very important in treatment. Based on the fact that each gene mutation specifies a different type and extent of the hearing condition, we can choose the most appropriate option. According to a study by Christina M. Salan-Heggen et al. 2016, it is shown that the majority of mutations in STRC cause only mild-to-moderate hearing loss and respond well to hearing aids, while mutations in GJB2 cause severe hearing loss or deafness and require cochlear implant system (CI) [3]. Robert Eppsteiner et al. showed that CI is very effective in mutations that occur in GJB2, TMC1, mitochondrial genes, but has little to no effect on mutations in cochlear nerve cells such as TMPRSS3. [4]. It is consistent with our study, the selected children have mutations that were claimed to have good results with cochlear implantation, were well responsive.

Gene therapies have the potential to conserve or restore auditory retrieval with more natural sound perception because stooling their frequency range is much higher than that of cochlear implants. These

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bio-based interventions are also able to reproduce hearing without any implants; the convenience and lower financial burden for such biologically based interventions will be of greater benefit to the patients. A study of Nist-Lund et al. (02/2019) has tested gene therapy on mice and has shown some positive results. They designed gene replacement therapies using adeno vectors, transferring the gene (which codes for the Transmembrane Channel-Like 1) into the hair cells of mice that lack the ability to hear and balance due to mutation in TMC1. They found the rehabilitation of the hair cells, enhanced life of them, restored cochlear and vestibular function, restored nerve responses in the auditory cortex and restored behavioral responses to hearing and vestibular stimulation [17].

In conclusion, it is necessary to find the rate of congenital deafness mutation specific to Vietnamese community for gene therapy applications in Vietnam.

CONCLUSIONS

Through this study we find that:

- The proportion of hearing-impaired mutation in the study group is 30%, in which the rate of GJB2 gene mutation accounts for the highest rate of 16%, TMC1 7%, SLC26A4 3%, MT-RNR1 2%, MT-TL1 1%, MT-TH 1%.
- The gene mutations detected in this study all have good results with cochlear implants, consistent with studies around the world.
- Different gene mutations cause different levels of hearing loss and can be applied to help guide diagnosis, treatment, and prevention early.

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APPENDIX A. 100 mutations of 18 genes in the topic

N	Gen	Variable type
1	**GJB2**	c.235delC
2		c.299-300delAT
3		c.35delG
4		c.176-191del16
5		c.167delT
6		c.512insAACG
7		c.456C>A
8		c.456C>G
9		c.427C>T
10		c.416G>A
11		c.257C>G
12		c.253T>C
13		c.109G>A
14		c.99delT
15		c.94C>T
16		IVS7-2A>G
17		c.2168A>G
18		c.1229C>T
19		c.1147A>T
20		c.1975G>C
21		c.2027T>A
22		c.2162C>T
23	**SCL**	c.589G>A
24	**26A4**	c.1226G>A
25		c.281C>T
26		IVS15+5G>A
27		c.2086C>T
28		c.745T>C
29		c.1079C>T
30		c.259G>T
31		c.1343C>T
32		c.1540C>T
33		c.1919G>T
34		c.2000T>C
35		c.679G>C
36		IVS14-2A>G
37		c.919-18T>G
38		c.920C>T
39		c.109G>T
40		c.1160C>T
41		ct.1181_1183delTCT
42		c.1318A>T
43		c.1336C>T
44		c.1555_1556delAA
45		c.1586T>G
46		c.1594A>C
47		c.1634T>C
48		c.1673A>T
49		c.1717G>T
50		c.1746delG
51		c.2054G>G
52	**SCL**	c.2082delA
53	**26A4**	c.2107C>G
54		c.227C>T
55		c.230A>T
56		c.269C>T
57		c.589G>A
58		c.349delC
59		c.387delC
60		c.404A>G
61		c.439A>G
62		c.697G>C
63		c.812A>G
64	**GJB3**	IVS10-12T>A
65		IVS13+9C>G
66		IVS14+1G>A
67		IVS14-1G>A
68		IVS16-6G>A
69	**GJB3**	c.538C>T
70		c.574G>A
71		c.423delATT
72		c.497A>G
73		c.421A>G
74	**MT-**	m.1555A>G
75	**RNR1**	m.1494C>T
76		m.827A>G
77		m.961delTinsC
78	**MT-CO1**	m.7444G>A
79	**MT-TL1**	m.3243A>G
80	**MT-TS1**	m.7445A>G
81		m.7505T>C
82		m.7511T>C
83	**MT-TH**	m.12201T>C
84	**DSPP**	c.52G>T
85	**GPR98**	c.10088_10091delTAAG
86	**DFNA5**	IVS8+4A>G
87	**TMC1**	c.150delT
88		c.1334G>A
89	**MYO7A**	c.625G>A
90		c.731G>C
91	**TECTA**	c.4525T>G
92	**DIABLO**	c.377C>T
93	**COCH**	c.1535T>C
94		c.1625G>A
95	**MYO15A**	c.8183G>A
96		c.8767C>T
97		c.193G>A
98	**PRPS1**	c.259A>G
99		c.869T>C
100		c.916G>A