JKMS

Original Article
Human Genetics & Genomics

Check for updates

Address for Correspondence:
Jinsei Jung, MD, PhD
Department of Otorhinolaryngology, Yonsei
University College of Medicine, 50-1 Yonsei-ro,
Seodaemun-gu, Seoul 03722, Korea.
Email: jsjung@yuhs.ac

Heon Yung Gee, MD, PhD
Department of Pharmacology, Graduate
School of Medical Science, Brain Korea 21
Project, Yonsei University College of Medicine,
50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722,
Korea.
Email: hygee@yuhs.ac

*Sun Young Joo and Seung Hyun Jang
contributed equally to this work.

ORCID iDs
Sun Young Joo
https://orcid.org/0000-0001-8121-9714
Seung Hyun Jang
https://orcid.org/0009-0002-1147-1476
Jung Ah Kim
https://orcid.org/0000-0002-1264-8328

Prevalence and Clinical Characteristics of Mitochondrial DNA Mutations in Korean Patients With Sensorineural Hearing Loss

Sun Young Joo [iD],[1,2*] Seung Hyun Jang [iD],[1,2*] Jung Ah Kim [iD],[1,2] Se Jin Kim [iD],[1,2] Bonggi Kim [iD],[3] Hye-Youn Kim [iD],[1,2] Jae Young Choi [iD],[2,3] Heon Yung Gee [iD],[1,2] and Jinsei Jung [iD] [2,3]

[1]Department of Pharmacology, Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, Korea
[2]Won-Sang Lee Institute for Hearing Loss, Seoul, Korea
[3]Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea

ABSTRACT

Background: Mutations in mitochondrial DNA (mtDNA) are associated with several genetic disorders, including sensorineural hearing loss. However, the prevalence of mtDNA mutations in a large cohort of Korean patients with hearing loss has not yet been investigated. Thus, this study aimed to investigate the frequency of mtDNA mutations in a cohort of with pre- or post-lingual hearing loss of varying severity.

Methods: A total of 711 Korean families involving 1,099 individuals were evaluated. Six mitochondrial variants associated with deafness (*MTRNR1* m.1555A>G, *MTTL1* m.3243A>G, *MTCO1* m.7444G>A and m.7445A>G, and *MTTS1* m.7471dupC and m.7511T>C) were screened using restriction fragment length polymorphism. The prevalence of the six variants was also analyzed in a large control dataset using whole-genome sequencing data from 4,534 Korean individuals with unknown hearing phenotype.

Results: Overall, 12 of the 711 (1.7%) patients with hearing loss had mtDNA variants, with 10 patients from independent families positive for the *MTRNR1* m.1555A>G mutation and 2 patients positive for the *MTCO1* m.7444G>A mutation. The clinical characteristics of patients with the mtDNA variants were characterized by post-lingual progressive hearing loss due to the m.1555A>G variant (9 of 472; 1.9%). In addition, 18/4,534 (0.4%) of the Korean population have mitochondrial variants associated with hearing loss, predominantly the m.1555A>G variant.

Conclusion: A significant proportion of Korean patients with hearing loss is affected by the mtDNA variants, with the m.1555A>G variant being the most prevalent. These results clarify the genetic basis of hearing loss in the Korean population and emphasize the need for genetic testing for mtDNA variants.

Keywords: Mitochondrial DNA; Restriction Fragment Length Polymorphism; Sensorineural Hearing Loss

Se Jin Kim (iD)
https://orcid.org/0009-0006-5244-4887
Bonggi Kim (iD)
https://orcid.org/0009-0005-9708-2659
Hye-Youn Kim (iD)
https://orcid.org/0000-0003-2090-6427
Jae Young Choi (iD)
https://orcid.org/0000-0001-9493-3458
Heon Yung Gee (iD)
https://orcid.org/0000-0002-8741-6177
Jinsei Jung (iD)
https://orcid.org/0000-0003-1906-6969

Disclosure
The authors have no potential conflicts of interest to disclose.

Author Contributions
Conceptualization: Gee HY, Jung J. Data curation: Joo SY, Kim JA, Kim SJ. Formal analysis: Joo SY, Kim B, Kim HY. Funding acquisition: Gee HY, Choi JY, Jung J. Investigation: Joo SY, Jang SH. Methodology: Joo SY, Jang SH. Software: Joo SY, Jang SH. Validation: Gee HY, Jung J. Visualization: Joo SY, Jang SH. Writing - original draft: Joo SY, Jang SH. Writing - review & editing: Gee HY, Jung J.

INTRODUCTION

Hearing loss is the most prevalent sensory organ disorder, affecting approximately 1–2 per 1,000 newborns. More than half of congenital hearing loss cases are due to genetic causes,[1,2] with 80% of genetic hearing loss inherited in an autosomal recessive manner and 20% inherited in an autosomal dominant manner or through less prevalent inheritance patterns, such as X-linked or mitochondrial inheritance.[3] However, the proportion of post-lingual genetic hearing loss cases caused by mitochondrial DNA (mtDNA) variants increases with ethnic diversity. Causative mtDNA variants have been identified in 5% of Caucasians with post-lingual non-syndromic hearing loss[4] and in 2.5–4.4% of Asians.[5-7]

Although the frequency of mtDNA variants varies by ethnicity, the m.1555A>G variant of *MTRNR1* is the most common mitochondrial variant associated with deafness, especially aminoglycoside-induced or non-syndromic hearing loss.[5,6,8-10] Several other variants, including m.3243A>G in *MTTL1*,[11,12] m.7444G>A and m.7445A>G in *MTCO1*,[13-16] m.7471dupC and m.7511T>C in *MTTS1*,[17-20] have been causally linked to deafness in multiple studies.[21] In addition to non-syndromic hearing loss, mitochondrial variants cause syndromic hearing loss. The m.3243A>G variant in the *MTTL1* gene, which encodes leucine transfer RNA, has been linked to maternally inherited diabetes and deafness (MIDD) and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS).[11,12] In addition to post-lingual and progressive deafness, the *MTCO1* m.7445A>G variant is also associated with palmoplantar keratoderma.[13,14]

Despite increasing reports of new mtDNA mutations associated with hearing impairment, the proportion of congenital or post-lingual hearing loss attributable to mtDNA mutations in cohorts with varying degrees of hearing loss remains unknown. Thus, this study aimed to investigate the frequency of mtDNA mutations in a cohort of individuals with pre- or post-lingual hearing loss of varying severity. Towards this goal, we screened m.1555A>G, m.3243A>G, m.7444G>A, m.7445A>G, m.7471dupC, and m.7511T>C variants of the mitochondrial genome, variants highly associated with sensorineural hearing loss. In addition, the frequencies of these variants, as well as the clinical and audiological characteristics of patients identified as carrying these mitochondrial variants, were investigated.

METHODS

Study design and patients
Patients with hearing loss were enrolled in the Yonsei University Hearing Loss (YUHL) cohort after providing informed consent for the study and publication of their clinical data. Molecular analysis was conducted on probands and additional family members from 711 Korean families, encompassing a total of 1,099 individuals. The inclusion criterion was hearing loss patients aged 0 to 75 years with multiple affected family members or suspected to harbor sporadic genetic mutations. Hearing loss caused by congenital cytomegalovirus infection or other medical conditions primarily affecting hearing function was excluded.

Clinical evaluation
All patients underwent a comprehensive physical examination and history interviews. Audiological evaluations using otoscopy, tympanometry, and pure-tone audiometry were conducted for all patients as well as for their affected and unaffected family members. Air and

bone conduction thresholds were measured in a double-walled audio booth at frequencies of 250–8,000 Hz and 250–4,000 Hz, respectively. The level of hearing loss was categorized as mild (26–40 dB), moderate (41–70 dB), severe (71–90 dB), or profound (> 90 dB) based on the average threshold of the four frequencies (500, 1,000, 2,000, and 4,000 Hz). The audiogram pattern was defined as ascending when the average thresholds for high frequencies (2,000 Hz and 4,000 Hz) were 25 dB less than those for low frequencies (250 and 500 Hz), ski-sloping when high frequencies were more than 25 dB than low frequencies, and flat when the difference between high and low frequencies was within 25 dB.

Control data collection

We used the whole-genome sequencing (WGS) data of 4,534 Korean individuals from the National Project of Bio Big Data. As part of this project, the gVCFs of 6,886 individuals from 4,534 unrelated families were generated using the GRCh38 assembly. We created a genomics database (genomicsDB) for the complete mitochondrial region (chrM:1-16569) of 6,886 individuals, called variants, using HaplotypeCaller in the Genome Analysis Toolkit (version 4.1.9.0, Broad Institute, Boston, USA).

Molecular analyses

Genomic DNA was extracted from peripheral blood lymphocytes using red blood cell lysis, cell lysis, and protein precipitation solutions (iNtRon Biotechnology, Inc., Seongnam, Korea). After extraction, polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were performed to detect mtDNA variants. For the six target mtDNA variants, PCR was performed in 1,099 DNA templates from 711 families using the primers listed in **Supplementary Table 1**. After identifying the target band size in 1% agarose gels under ultraviolet light, RFLP was performed using the enzymes listed in **Supplementary Table 1**. Finally, 2% agarose gel electrophoresis was performed to determine whether the tested samples were positive for the variants.[22] Mutation analyses of *GJB2*, *TRMU* p.A10S, *MTND1* m.3308T>C, and *MTND6* m.14484T>C were performed using the primers listed in **Supplementary Table 2**. The PCR products were analyzed using Sanger sequencing. The sequences were assembled and compared to those of *GJB2* (NC_000013.11), *TRMU* (NC_000022.11), *MTND1* (NC_012920.1), and *MTND6* (NC_012920.1).

Haplogroup analysis of the mtDNA genome

To amplify the hypervariable region (HVR) of the mtDNA genome, PCR amplification of the template DNA was performed using the F15971 and R638 primers.[23] After visualization in 1% agarose gel, the PCR product was analyzed using Sanger sequencing with primers F16328, F015, F314, R16509, R240, and R569, in addition to the amplification primers (**Supplementary Table 3**). Pairwise comparison and convergence of sequences were performed using the CLC Workbench version 20.0.1 (Qiagen, Hilden, Germany) to create a FASTA format file of the individual's HVR sequence. Haplogroup analysis was performed using the 'Classify' function of HaploGrep2.[24]

Ethics statement

This study was approved by the Institutional Review Board (IRB) of Severance Hospital, Yonsei University Health System (IRB #4-2015-0659) and was conducted according to the tenets of the Declaration of Helsinki. We obtained written informed consent from individuals with hearing loss for their participation in this study and the publication of their clinical data.

RESULTS

Mutational screening of mtDNA variants associated with hearing loss

Of the 12 families that were identified with mtDNA variants, 10 out of 711 (1.4%) carried the m.1555A>G mtDNA variant whereas 2/711 (0.28%) were with the m.7444G>A variant (**Table 1**). None of the 759 individuals from the 373 unrelated families had the m.3243A>G, m.7445A>G, m.7471dupC, or m.7511T>C variants, indicating that these variants are extremely rare causes of hearing loss associated with mtDNA in Korea (**Table 1**). Overall, 1.7% of the YUHL cohort carried mitochondrial variants causing deafness (**Fig. 1A**). The detection rate in the phenotypic subgroup of individuals with post-lingual hearing loss with moderate-to-profound severity was 2.1%, suggesting that carriers of mtDNA variants, particularly those carrying the m.1555A>G variant, share clinical characteristics (**Fig. 1B**).

Twelve YUHL families with mtDNA variants did not have a pathogenic variant in *GJB2*, one of the most common genes responsible for non-syndromic hearing loss.[25-27] In addition, since whole exome or genome sequencing data were available for seven individuals with mtDNA variants, we analyzed genes associated with hearing loss and found no likely pathogenic or pathogenic variants that could explain their hearing loss in five patients (YUHL58-21, 82-21, 206-21, 379-21, and 401-21). The c.671G>T variants in *EYA1* and c.491C>G in *POU4F3* were found in YUHL379-21 and 849-21, respectively (**Supplementary Table 4**). Despite of the fact that both variants are reported as pathogenic in the Deafness Variation Database (http://deafnessvariationdatabase.org/), their allele frequencies (AFs) in East Asians and functional evidence indicate that they are insufficient to explain their hearing loss.

Given that hearing loss caused by the m.1555A>G or m.7444G>A variant is predominantly post-lingual and progressive, we hypothesized that these mitochondrial variants may be overlooked in people with adult-onset hearing loss. In the Genome Aggregation Database (gnomAD), the AFs of the homoplasmic m.1555A>G and m.7444G>A variants were 0.001 and 0.005, respectively (**Table 1**). Although gnomAD provides reliable AF for various populations, Koreans are underrepresented in the database. Therefore, we investigated mitochondrial variants in the Korean population using 4,534 WGS data from the National Project of Bio Big Data. In the WGS data, we detected four mitochondrial variants corresponding to four of the six mtDNA variants screened for YUHL: m.1555A>G, m.3243A>G, m.7444G>A, and m.7445A>G (**Table 1**). Regarding the four variants identified in the Korean control data, the m.1555A>G and m.7444G>A variants were identified in multiple individuals, with the m.1555A>G variant present in six individuals and m.7444G>A in ten individuals, whereas the m.3243A>G and m.7445A>G variants were only identified in one individual each in the heteroplasmic state. Four of the six m.1555A>G variant carriers and all m.7444A>G variant

Table 1. Results of screening of deafness-causing mutations in mtDNA in Korean patients

Gene	Mutation	Tested[a]	Result[b]	Homoplasmic AF in gnomAD	Freqeucny in Korean[c]
MT-RNR1	m.1555A>G	711 (families)	10/711	0.001117	G = 0.00132[d] (6/4,534)
MT-TL1	m.3243A>G	759 (individuals)	0/759	0	G = 0.00022 (1/4,534)
MT-CO1	m.7444G>A	711	2/711	0.005353	A = 0.0022[d] (10/4,534)
MT-TS1	m.7445A>G	711	0/711	0	G = 0.00022 (1/4,534)
	m.7471dupC	759	0/759	0	-
	m.7511T>C	759	0/759	0	-

mtDNA = mitochondrial DNA, AF = allele frequency, gnomAD = Genome Aggregation Database.
[a]The number of unrelated families or individuals whose mtDNA is analyzed for variants.
[b]The number of individuals detected with mitochondrial variants.
[c]Frequency of mtDNA variants among the 4,534 Koreans from the National Project of Bio Big Data.
[d]Allele frequency of individuals carrying mtDNA mutations in both homoplasmic and heteroplasmic states.
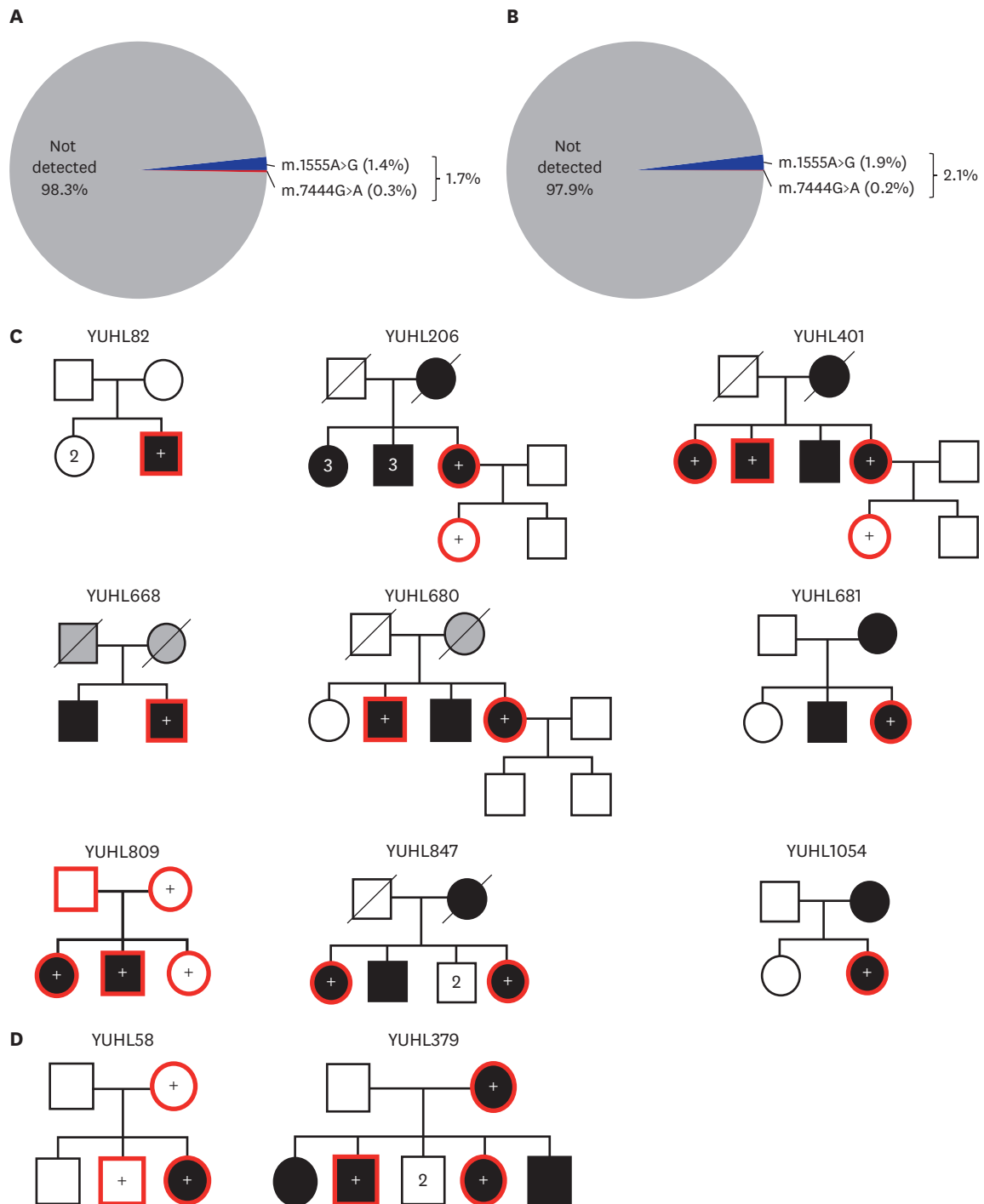
Fig. 1. Detection rate of pathogenic mtDNA variants in the YUHL cohort and pedigree information summarizing segregation analysis. (**A**) Detection rate of mtDNA mutations that cause deafness in the YUHL cohort. Among the 711 families screened for mtDNA mutations, 10 unrelated hearing loss patients with the m.1555A>G variant and 2 patients with the m.7444G>A variant are identified. (**B**) Detection rate of deafness-causing mtDNA mutations in a group of YUHL patients with moderate-to-profound post-lingual hearing loss. (**C**) Pedigree and segregation results of families carrying m.1555A>G variant. (**D**) Pedigree and segregation results of families with m.7444G>A variant. Individuals highlighted in red were those available for RFLP and Sanger sequencing. '+' indicated individuals with the mtDNA variants.
mtDNA = mitochondrial DNA, YUHL = Yonsei University Hearing Loss.

carriers identified in the Korean control data were in a homoplasmic state. Overall, a considerable proportion (18/4,534; 0.4%) of the Korean population is at risk of developing hearing loss, primarily due to the m.1555A>G variant.

Clinical and molecular characteristics of the 12 mitochondrial variant-bearing families

To evaluate the clinical characteristics of patients carrying mtDNA variants, the patients were classified according to age at diagnosis, sex, age at disease onset, degree of hearing loss, audiogram pattern, and mode of inheritance (**Table 2**). Hearing loss was predominantly familial with post-lingual onset and showed progressive features (**Table 2**). The mitochondrial variant m.1555A>G is frequently associated with variable onset and severity of hearing loss.[7,28] Patients with the m.1555A>G variant in Korea exhibited a phenotype similar to that described in previous studies, in that the severity of hearing loss ranged from mild to profound. Consistent with previous reports,[7,28-30] eight m.1555A>G carriers exhibited bilateral and symmetrical hearing loss. However, hearing loss in the other two carriers (YUHL847-21 and 1054-21) was asymmetric, and the unaffected sides had normal thresholds (**Table 3**). In addition, the average age at the onset of hearing loss for all m.1555A>G carriers was 48.5 years, but there was a wide range, with some carriers experiencing hearing loss from infancy and others not until their early 50s.

Among the 10 cases with the m.1555A>G variant, only 1 case (YUHL82-21) had sporadic inheritance, and the remaining 9 cases had familial inheritance (**Table 3**). Five of the nine

Table 2. Clinical characteristics of deafness-causing mtDNA variant carriers from the YUHL cohort

Category	Total[a]	mtDNA variants carrier[b]	m.1555A>G carrier	m.7444G>A carrier
Age[c]	39.8	47.1	48.5	40
Sex				
Male	363 (51)	4	4	0
Female	337 (47)	8	6	2
Unknown	11 (2)	-	-	-
Onset				
Pre-lingual	127 (17)	2 (16.7)	1 (10)	1 (50)
Post-lingual				
Total	571 (80)	10 (83.3)	9 (90)	1 (50)
> 10s	148 (21)	4 (33.3)	4 (40)	-
20–30s	167 (23)	3 (25)	2 (20)	1 (50)
Over 40s	256 (36)	3 (25)	3 (30)	-
Unknown	13 (3)	-	-	-
Severity				
Mild	82 (12)	1 (8.3)	1 (10)	-
Moderate	363 (51)	3 (25)	2 (20)	1 (50)
Severe	99 (14)	2 (16.7)	2 (20)	-
Profound	140 (20)	6 (50)	5 (50)	1(50)
Unknown	27 (4)	-	-	-
Progressiveness				
Progressive	517 (73)	10 (83.3)	8 (80)	2 (100)
Non-progressive	179 (25)	2 (16.7)	2 (20)	-
Unknown	15 (2)	-	-	-
Inheritance				
Familial	324 (46)	10 (83.3)	8 (80)	2 (100)
Sporadic or autosomal recessive	381 (53)	2 (16.7)	2 (20)	-
Unknown	6 (1)	-	-	-

Values are presented as number (%).
mtDNA = mitochondrial DNA, YUHL = Yonsei University Hearing Loss.
[a]Phenotype data of 711 individuals from the YUHL cohort.
[b]Phenotype data of 10 individuals with the m.1555A>G variant and 2 individuals with the m.7444G>A variant.
[c]Average age per group.

Table 3. Clinical and molecular characteristics of mtDNA m.1555A>G or m.7444G>A variant carriers in the Yonsei University Hearing Loss cohort

| Variant	Sample ID	Inheritance[a]	Onset	Sideness	Severity	Progressiveness	PTA (dbHL) Right	PTA (dbHL) Left	Pattern	mtDNA haplogroup	Exposure to aminoglycosides	Additional medical history
m.1555A>G	16-21	Familial	Late 40s	Both	Profound	Progressive	99	98	Ski-sloping	M51	-	-
	82-21	Sporadic	Early 10s	Both	Severe	Stable	88	90	Ski-sloping	D5a1	No	-
	206-21	Familial	Early 50s	Both	Moderate	Progressive	60	65	Flat	D5a2a1a	No	-
	401-21	Familial	Congenital	Both	Profound	Progressive	94	96	Ski-sloping	D5a1	No	Hypothyroidism Type 2 diabetes mellitus
	668-21	Familial	0s	Both	Profound	Progressive	95	99	Ski-sloping	D4e1a1	-	Visual impairment
	680-21	Familial	0s	Both	Profound	Progressive	94	101	Ski-sloping	D4e1a1	-	Dyslipidemia
	681-21	Familial	Mid 20s	Both	Moderate	Progressive	46	46	Ski-sloping	M7c1a2a1	No	-
	809-21	Familial	Late 10s	Both	Severe	Progressive	75	83	Ski-sloping	M10a1a1b	-	-
	847-21	Familial	Late 40s	Right	Profound	Progressive	119	25	Flat	D5a3a1	No	-
	1054-21	Familial	Early 20s	Right	Mild	Sudden	33	4	Flat	U6b3a	-	-
m.7444G>A	58-21	Sporadic	0s	Both	Moderate	Progressive	48	49	Flat	M9a1a1	No	-
	379-21	Familial	Early 20s	Both	Profound	Progressive	93	103	Flat	B4c1b2a	No	Exposure to noise in 20s

mtDNA = mitochondrial DNA, PTA = pure tone audiometry.
[a]Familial inheritance is a pedigree with more than two affected individuals. Sporadic inheritance is considered if only a single proband is present in the family.

familial cases (YUHL206-21, 401-21, 681-21, 847-21, and 1054-21) were suspected to be maternally transmitted despite incomplete penetrance (**Fig. 1C**). One of the m.1555A>G carriers (YUHL401-21) was also diagnosed with hypothyroidism and type 2 diabetes, and another m.1555A>G carrier (YUHL668-21) had severe visual impairment (**Table 3**). However, the exact association between these diseases and the m.1555A>G variant is unknown because of the lack of published reports. The individual and mean audiograms of the 10 individuals carrying the m.1555A>G variant are presented based on pure tone audiometry results in **Fig. 2**. These audiograms suggested that the audiometric patterns of the m.1555A>G variant carriers in our cohort were predominantly ski-sloping, although some patients (YUHL206-21, 847-21, and 1054-21) exhibited a flat configuration (**Fig. 2**).

In general, the m.7444G>A variant is associated with hearing loss of varying severity, frequently in conjunction with the m.1555A>G variant or aminoglycoside exposure.[15,16,31] We identified two patients in our cohort with the m.7444G>A variant but none with the m.1555A>G variant. Additionally, none of the m.7444G>A carriers in our study were exposed to aminoglycosides. These results suggested that the m.7444G>A variant alone may occasionally result in hearing loss, even in the absence of the m.1555A>G variant or aminoglycoside exposure. One of the carriers of the m.7444G>A variant had a sporadic inheritance, whereas the other had a suspected maternally inherited familial inheritance (**Table 3**, **Fig. 1D**). None of the carriers of the m.7444G>A variant in our cohort presented with any additional syndromic symptoms (**Table 3**).

Several factors have been linked to the variable expressivity and incomplete penetrance of hearing loss in individuals affected by mitochondrial variants. These include heteroplasmy levels,[30] environmental factors such as noise or aminoglycoside exposure,[6,15] modifier genes such as *TRMU*,[32,33] and other accompanying mtDNA variations,[15,29,34-36] which can influence the onset, severity, and penetrance of hearing loss. To assess the role of the p. A10S variant in the *TRMU* gene as a modifier of m.1555A>G, we conducted direct sequencing of
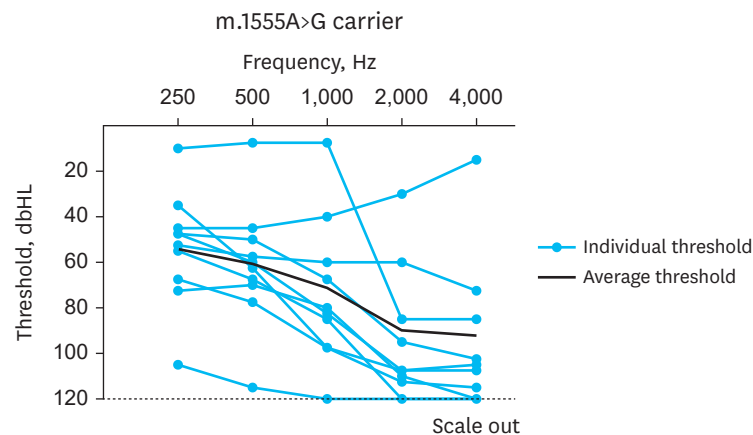
Fig. 2. Audiograms of m.1555A>G variant carriers. The transparent blue line depicts the individual thresholds, the average of the right and left ear (except for YUHL847-21 and 1054-21). The thresholds of the affected ear are indicated for YUHL847-21 and 1054-21. The black line represents the average of ten audiograms.
YUHL = Yonsei University Hearing Loss.

the *TRMU* gene in all patients with m.1555A>G variant. However, the p.A10S variant in *TRMU* was not detected in any of the ten individuals with the m.1555A>G variant. Intriguingly, although accurate records of aminoglycoside medication were impossible because of the retrospective nature of the history interviews, 7 of the 12 probands (58.3%) were not exposed to aminoglycosides, and none of the probands reported that their hearing loss was initiated by particular medications (**Table 3**). Heteroplasmy levels also influence the expressivity of the phenotype, particularly in the m.1555A>G variant.[30] After detecting the m.1555A>G variant by PCR-RFLP in eight patients (YUHL16-21, 82-21, 206-21, 401-21, 680-21, 681-21, 668-21, and 1054-21), Sanger sequencing of the *MTRNR1* gene was performed. All m.1555A>G variants in the eight patients appeared to be in a homoplasmic state (**Supplementary Fig. 1**), although low-level heteroplasmy could not be completely ruled out owing to amplification bias during PCR. In addition, the m.7444G>A variant in two patients (YUHL58-21 and 379-21) was confirmed by direct sequencing of the *MTCO1* gene, and the m.7444G>A variant in both patients was in a homoplasmic state (**Supplementary Fig. 1**).

Penetrance was determined by dividing the number of affected matrilineal relatives by the total number of matrilineal relatives in the family and then averaging the results according to the variant type.[7] In total, 14 individuals from 10 families affected by the m.1555A>G variant and 4 individuals from 2 families affected by the m.7444G>A variant were included in the segregation analysis and penetrance calculations (**Fig.1C and D**). The penetrance of hearing loss for the m.1555A>G variant ranged from 33.3% to 100%, with an average of 70.6%. Penetrance ranged from 33% to 67% in the two m.7444G>A variant-carrying individuals, with an average rate of 50%.

Haplogroup analysis of the mtDNA of the twelve affected patients

To determine whether mtDNA haplogroups influenced the variable expressivity of the mtDNA m.1555A>G and m.7444G>A variants, we amplified the HVR of the mtDNA genome and directly sequenced the PCR amplicons to identify the mtDNA haplogroups in 10 m.1555A>G and 2 m.7444G>A variant carriers. Four major haplogroups, namely, D, M, U, and B, were detected in the 12 pedigree patients (**Table 3**). In accordance with a previous report that analyzed the mtDNA haplogroups in Korean patients with hearing loss,[7] haplogroup D was the most prevalent (6/12, 50%), followed by haplogroup M (4/12, 33.3%). Eight

individuals were identified with haplogroups D5a2a1a, D5a3a1, M51, M7c1a2a1, M10a1a1b, M9a1a1, U6b3a, and B4c1b2a, whereas haplogroups D5a1 and D4e1a1 were identified in two individuals each.

In addition, we analyzed the distribution of haplogroups among 4,534 Korean control individuals. Macrohaplogroup D was the most prevalent lineage among these individuals, identified in 1,477 (32.57%) of them. The second most prevalent haplogroup was B (681 individuals; 15.01%), followed by M (14.18%) and G (405 individuals; 8.93%) (**Supplementary Table 5**). These results are consistent with previous findings regarding the prevalent mtDNA haplogroups observed in East Asians.[23,37]

DISCUSSION

To our best knowledge, this study represents the most extensive analysis of the major mtDNA mutations associated with hearing loss. Li et al.[6] screened 128 Chinese pediatric patients with hearing loss for mutations in the mitochondrial 12S rRNA gene and reported that approximately 2.9% of patients with nonsyndromic hearing loss carried the m.1555A>G variant. Yelverton et al.[38] examined 2,434 probands with hearing loss in the United States and found that 3.5% had either *MTRNR1* or *MTTS1* mutation. Although the detection rate of deafness-causing mtDNA variants in our cohort (approximately 1.7%) was slightly lower than that reported previously, it is important to consider the impact of ethnic background, demographic composition of the cohort, and environmental factors on the differences in prevalence. For instance, our cohort consists predominantly of adults with an average age of 39.8 years, in contrast to the study by Li et al.[6] that focused on pediatric patients. In addition, the hearing loss of our cohort ranged from mild to profound, in contrast to the cohort of Yelverton et al.[38] in whom the majority had profound hearing loss.

Maternally transmitted mtDNA is frequently used to determine maternal ancestry and evolutionary distance. Haplogroups D, G, M, and B are the most common haplogroups in East Asia.[23,37] Previous studies by Lu et al.[39] and Bae et al.[7] revealed that haplogroup D is the most common haplogroup among m.1555A>G variant carriers. These findings are supported by the fact that haplogroup D was the most prevalent in our cohort, followed by haplogroup M. In our cohort, we identified numerous specific haplogroups such as D5a2a1a, D5a3a1, D5a1, D4e1a1M51, M7c1a2a1, M10a1a1b, M9a1a1, and B4c1b2a, as well as rare East Asian haplogroups such as U6b3a. However, no definite correlations among expressivity, penetrance, and mtDNA haplogroups were observed. This indicates that the mtDNA haplogroup does not influence the penetrance and expressivity of mtDNA mutations that cause deafness. Additional research employing a larger pedigree is required to determine the relationship between mtDNA haplogroups and the expressivity of deafness-causing mtDNA variants.

Patients with mtDNA m.1555A>G variant show variable expressivity, ranging from profound congenital hearing loss to adulthood-onset moderate hearing loss.[7,28] In accordance with previous reports, the m.1555A>G carriers in this study exhibited varying onset (birth to age 50s) and severity (mild to profound). In contrast to earlier findings indicating that the m.1555A>G variant typically causes bilateral and symmetric hearing loss,[7,28,30] some m.1555A>G carriers (YUHL847-21 and 1054-21) in our study exhibited unilateral and asymmetric hearing loss, with the normal unaffected side. Additionally, a flat audiometric pattern was observed in some m.1555A>G carriers (YUHL206-21, 847-21, and 1054-21),

whereas the stereotypical m.1555A>G audiogram exhibited a ski-sloping pattern. These results expand the phenotypic variety of the m.1555A>G variant. In addition, our data revealed that the average penetrance of the m.1555A>G variant was 70.6%, higher than the 54.1–65.4% reported in other studies.[7,9,39,40] Notably, none of the m.1555A>G carriers in our study reported exposure to aminoglycosides, suggesting that penetrance may be influenced by factors other than aminoglycosides.

Interestingly, one m.1555A>G carrier (YUHL401-21) was diagnosed with type 2 diabetes and hypothyroidism, whereas another carrier (YUHL668-21) displayed visual impairment. In a Chinese family with Leber's hereditary optic neuropathy (LHON) and hearing loss, Wei et al.[41] reported co-segregation of the *MTND6* m.14484T>C and *MTRNR1* m.1555A>G mutations in a Chinese family with LHON and hearing loss. In addition, Mezghani et al.[42] identified the coexistence of *MTND1* m.3308T>C and *MTRNR1* m.1555A>G mutations, as well as multiple mitochondrial deletions, in two related Tunisian patients with MIDD. However, no study has linked the m.1555A>G variant as the sole cause of LHON or MIDD. None of the maternal relatives of the two patients (YUHL401-21 and 668-21) was diagnosed with type 2 diabetes or visual impairment, indicating that these conditions occurred independently. In our analysis, neither of the two patients (YUHL401-21 and 668-21) possessed any additional mtDNA variants such as m.14484T>C or m.3308T>C. Determining the functional contribution of the m.1555A>G variant to LHON and MIDD requires further research.

The pathogenicity of the m.7444G>A variant in hearing loss remains unknown because this variant alone is insufficient to cause a phenotype and requires additional modifiers such as the m.1555A>G variant or aminoglycoside exposure.[15,16,31] Notably, the m.7444G>A variant carriers in our cohort lacked the m.1555A>G mutation and were not exposed to aminoglycosides. The m.7444G>A variant modifies the stop codon of the *CO1* gene and adds three additional amino acids (Lys-Gln-Lys) to the C terminus of the protein, potentially resulting in aberrant protein function.[15,31,39] Moreover, the m.7444G>A variant is located close to the 3' end endo-nucleolytic processing site of L-strand polycistronic RNA precursors spanning tRNA$^{Ser(UCN)}$ and ND6 mRNA, which may result in splicing defects in RNA processing and a decrease in steady-state tRNA$^{Ser(UCN)}$ precursor level, similar to the m.7445A>G variant.[43,44] Our results indicated that the m.7444G>A variant may be the sole cause of hearing loss in certain ethnic and nuclear genetic backgrounds. Therefore, additional comprehensive functional investigations are required to establish the pathogenicity of the m.7444G>A variant in hearing impairments.

Aminoglycosides are commonly used to treat gram-negative sepsis,[45,46] infective endocarditis,[47] and multidrug-resistant *Mycobacterium tuberculosis* infections.[48] However, this medication may have adverse effects, including ototoxicity and nephrotoxicity.[49,50] In addition, patients with susceptible mtDNA mutations may experience hearing loss, even with a single therapeutic dose of aminoglycosides. The mtDNA variants m.1555A>G and m.7444G>A were detected in 16 individuals with unknown hearing phenotypes: 6 carried m.1555A>G (0.13%) and 10 individuals the m.7444G>A variant (0.22%) in the Korean control data. Therefore, 0.35% of the Korean population is at a risk of aminoglycoside-induced hearing loss based on this result. Before administering aminoglycosides, physicians should inquire about the history of hearing loss in the relatives of patients and screen for susceptible mtDNA variants if a suspicious family history is present.

In conclusion, this study presents a comprehensive examination of the frequencies of the main mtDNA mutations that cause deafness in East Asian populations. The detection rates of deafness-causing mtDNA variants such as m.1555A>G and m.7444G>A are consistent with those of previous studies, albeit with differences presumably caused by ethnic background, demographic composition, and environmental factors. This study also highlights the diversity of phenotypic expression associated with the m.1555A>G variant, such as variations in hearing loss onset, degree, and audiometric patterns. Additional medical conditions are observed in some m.1555A>G carriers, suggesting the involvement of multiple systems. The m.7444G>A variant is also analyzed, emphasizing the need for additional functional research to determine its pathogenicity in hearing loss. These findings improve the understanding of the genetic and clinical characteristics of hearing loss caused by mtDNA mutations.

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIALS

Supplementary Table 1
List of primers and enzymes utilized in the polymerase chain reaction–restriction fragment length polymorphism analysis of six mitochondrial DNA mutations associated with sensorineural hearing loss

Click here to view

Supplementary Table 2
List of primers used for mutational analysis of variable expressivity in carriers of the m.1555A>G or m.7444G>A variant

Click here to view

Supplementary Table 3
List of primers for polymerase chain reaction and sequencing of hypervariable regions of mitochondrial DNA

Click here to view

Supplementary Table 4
Information on pathogenic variants in hearing loss-associated nuclear genes that were found in YUHL379-21 and 847-21

Click here to view

Supplementary Table 5
Macrohaplogroups derived from mitochondrial genomes of 4,534 Korean participants from the National Project of Bio Big Data

Click here to view

Supplementary Fig. 1
RFLP result and sequence chromatograms from segregation analysis of families identified with mtDNA variants. (**A**) RFLP result of an individual with or without mtDNA mutation. (−) indicated RFLP result of individuals with no mtDNA mutations; (+) indicated carriers of m.1555A>G or m.7444G>A mutations. Band specific to mutation carriers indicated by asterisk marks. (**B**) Sequence chromatograms showing the m.1555A>G mutation in the *MTRNR1* gene and the m.7444G>A mutation in the *MTCO1* gene of the affected patients.

Click here to view

REFERENCES

1. Morton NE, Shields DC, Collins A. Genetic epidemiology of complex phenotypes. *Ann Hum Genet* 1991;55(4):301-14.
   PUBMED | **CROSSREF**

2. Yuan Y, You Y, Huang D, Cui J, Wang Y, Wang Q, et al. Comprehensive molecular etiology analysis of nonsyndromic hearing impairment from typical areas in China. *J Transl Med* 2009;7(1):79.
   PUBMED | **CROSSREF**

3. Morton NE. Genetic epidemiology of hearing impairment. *Ann N Y Acad Sci* 1991;630(1):16-31.
   PUBMED | **CROSSREF**

4. Jacobs HT, Hutchin TP, Käppi T, Gillies G, Minkkinen K, Walker J, et al. Mitochondrial DNA mutations in patients with postlingual, nonsyndromic hearing impairment. *Eur J Hum Genet* 2005;13(1):26-33.
   PUBMED | **CROSSREF**

5. Usami S, Abe S, Akita J, Namba A, Shinkawa H, Ishii M, et al. Prevalence of mitochondrial gene mutations among hearing impaired patients. *J Med Genet* 2000;37(1):38-40.
   PUBMED | **CROSSREF**

6. Li Z, Li R, Chen J, Liao Z, Zhu Y, Qian Y, et al. Mutational analysis of the mitochondrial 12S rRNA gene in Chinese pediatric subjects with aminoglycoside-induced and non-syndromic hearing loss. *Hum Genet* 2005;117(1):9-15.
   PUBMED | **CROSSREF**

7. Bae JW, Kim DB, Choi JY, Park HJ, Lee JD, Hur DG, et al. Molecular and clinical characterization of the variable phenotype in Korean families with hearing loss associated with the mitochondrial A1555G mutation. *PLoS One* 2012;7(8):e42463.
   PUBMED | **CROSSREF**

8. Fischel-Ghodsian N, Prezant TR, Chaltraw WE, Wendt KA, Nelson RA, Arnos KS, et al. Mitochondrial gene mutation is a significant predisposing factor in aminoglycoside ototoxicity. *Am J Otolaryngol* 1997;18(3):173-8.
   PUBMED | **CROSSREF**

9. Estivill X, Govea N, Barceló E, Badenas C, Romero E, Moral L, et al. Familial progressive sensorineural deafness is mainly due to the mtDNA A1555G mutation and is enhanced by treatment of aminoglycosides. *Am J Hum Genet* 1998;62(1):27-35.
   PUBMED | **CROSSREF**

10. Casano RA, Bykhovskaya Y, Johnson DF, Hamon M, Torricelli F, Bigozzi M, et al. Hearing loss due to the mitochondrial A1555G mutation in Italian families. *Am J Med Genet* 1998;79(5):388-91.
    PUBMED | **CROSSREF**

11. Manouvrier S, Rötig A, Hannebique G, Gheerbrandt JD, Royer-Legrain G, Munnich A, et al. Point mutation of the mitochondrial tRNA(Leu) gene (A 3243 G) in maternally inherited hypertrophic cardiomyopathy, diabetes mellitus, renal failure, and sensorineural deafness. *J Med Genet* 1995;32(8):654-6.
    PUBMED | **CROSSREF**

JKMS

12. Majamaa K, Moilanen JS, Uimonen S, Remes AM, Salmela PI, Kärppä M, et al. Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: prevalence of the mutation in an adult population. *Am J Hum Genet* 1998;63(2):447-54.
    PUBMED | **CROSSREF**

13. Martin L, Toutain A, Guillen C, Haftek M, Machet MC, Toledano C, et al. Inherited palmoplantar keratoderma and sensorineural deafness associated with A7445G point mutation in the mitochondrial genome. *Br J Dermatol* 2000;143(4):876-83.
    PUBMED | **CROSSREF**

14. Reid FM, Vernham GA, Jacobs HT. A novel mitochondrial point mutation in a maternal pedigree with sensorineural deafness. *Hum Mutat* 1994;3(3):243-7.
    PUBMED | **CROSSREF**

15. Yuan H, Qian Y, Xu Y, Cao J, Bai L, Shen W, et al. Cosegregation of the G7444A mutation in the mitochondrial COI/tRNA$^{Ser(UCN)}$ genes with the 12S rRNA A1555G mutation in a Chinese family with aminoglycoside-induced and nonsyndromic hearing loss. *Am J Med Genet A* 2005;138A(2):133-40.
    PUBMED | **CROSSREF**

16. Pandya A, Xia XJ, Erdenetungalag R, Amendola M, Landa B, Radnaabazar J, et al. Heterogenous point mutations in the mitochondrial tRNA Ser(UCN) precursor coexisting with the A1555G mutation in deaf students from Mongolia. *Am J Hum Genet* 1999;65(6):1803-6.
    PUBMED | **CROSSREF**

17. Friedman RA, Bykhovskaya Y, Sue CM, DiMauro S, Bradley R, Fallis-Cunningham R, et al. Maternally inherited nonsyndromic hearing loss. *Am J Med Genet* 1999;84(4):369-72.
    PUBMED | **CROSSREF**

18. Wong LC, Chen T, Wang J, Tang S, Schmitt ES, Landsverk M, et al. Interpretation of mitochondrial tRNA variants. *Genet Med* 2020;22(5):917-26.
    PUBMED | **CROSSREF**

19. Hutchin TP, Navarro-Coy NC, Van Camp G, Tiranti V, Zeviani M, Schuelke M, et al. Multiple origins of the mtDNA 7472insC mutation associated with hearing loss and neurological dysfunction. *Eur J Hum Genet* 2001;9(5):385-7.
    PUBMED | **CROSSREF**

20. Subathra M, Ramesh A, Selvakumari M, Karthikeyen NP, Srisailapathy CR. Genetic epidemiology of mitochondrial pathogenic variants causing nonsyndromic hearing loss in a large cohort of South Indian hearing impaired individuals. *Ann Hum Genet* 2016;80(5):257-73.
    PUBMED | **CROSSREF**

21. Brandon MC, Lott MT, Nguyen KC, Spolim S, Navathe SB, Baldi P, et al. MITOMAP: a human mitochondrial genome database--2004 update. *Nucleic Acids Res* 2005;33(Database issue):D611-3.
    PUBMED | **CROSSREF**

22. Kokotas H, Grigoriadou M, Korres GS, Ferekidou E, Kandiloros D, Korres S, et al. Detection of deafness-causing mutations in the Greek mitochondrial genome. *Dis Markers* 2011;30(6):283-9.
    PUBMED | **CROSSREF**

23. Lee HY, Yoo JE, Park MJ, Chung U, Shin KJ. Mitochondrial DNA control region sequences in Koreans: identification of useful variable sites and phylogenetic analysis for mtDNA data quality control. *Int J Legal Med* 2006;120(1):5-14.
    PUBMED | **CROSSREF**

24. Weissensteiner H, Pacher D, Kloss-Brandstätter A, Forer L, Specht G, Bandelt HJ, et al. HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Res* 2016;44(W1):W58-63.
    PUBMED | **CROSSREF**

25. Van Camp G, Willems PJ, Smith RJ. Nonsyndromic hearing impairment: unparalleled heterogeneity. *Am J Hum Genet* 1997;60(4):758-64.
    PUBMED

26. Estivill X, Fortina P, Surrey S, Rabionet R, Melchionda S, D'Agruma L, et al. Connexin-26 mutations in sporadic and inherited sensorineural deafness. *Lancet* 1998;351(9100):394-8.
    PUBMED | **CROSSREF**

27. Shin JW, Lee SC, Lee HK, Park HJ. Genetic screening of GJB2 and SLC26A4 in Korean cochlear implantees: experience of soree ear clinic. *Clin Exp Otorhinolaryngol* 2012;5 Suppl 1(Suppl 1):S10-3.
    PUBMED | **CROSSREF**

28. Subathra M, Selvakumari M, Ramesh A, Ramakrishnan R, Karan KR, Kaur M, et al. Complete mitochondrial genome analysis and clinical documentation of a five-generational Indian family with mitochondrial 1555A>G mutation and postlingual hearing loss. *Ann Hum Genet* 2014;78(3):217-34.
    PUBMED | **CROSSREF**

29. Ding Y, Li Y, You J, Yang L, Chen B, Lu J, et al. Mitochondrial tRNA^Glu A14693G variant may modulate the phenotypic manifestation of deafness-associated 12S rRNA A1555G mutation in a Han Chinese family. *J Genet Genomics* 2009;36(4):241-50.
   PUBMED | **CROSSREF**

30. del Castillo FJ, Rodríguez-Ballesteros M, Martín Y, Arellano B, Gallo-Terán J, Morales-Angulo C, et al. Heteroplasmy for the 1555A>G mutation in the mitochondrial 12S rRNA gene in six Spanish families with non-syndromic hearing loss. *J Med Genet* 2003;40(8):632-6.
   PUBMED | **CROSSREF**

31. Rydzanicz M, Cywińska K, Wróbel M, Pollak A, Gawęcki W, Wojsyk-Banaszak I, et al. The contribution of the mitochondrial *COI/tRNA*^Ser(UCN) gene mutations to non-syndromic and aminoglycoside-induced hearing loss in Polish patients. *Mol Genet Metab* 2011;104(1-2):153-9.
   PUBMED | **CROSSREF**

32. Meng F, Cang X, Peng Y, Li R, Zhang Z, Li F, et al. Biochemical evidence for a nuclear modifier allele (A10S) in TRMU (methylaminomethyl-2-thiouridylate-methyltransferase) related to mitochondrial tRNA modification in the phenotypic manifestation of deafness-associated 12S rRNA mutation. *J Biol Chem* 2017;292(7):2881-92.
   PUBMED | **CROSSREF**

33. Guan MX, Yan Q, Li X, Bykhovskaya Y, Gallo-Teran J, Hajek P, et al. Mutation in TRMU related to transfer RNA modification modulates the phenotypic expression of the deafness-associated mitochondrial 12S ribosomal RNA mutations. *Am J Hum Genet* 2006;79(2):291-302.
   PUBMED | **CROSSREF**

34. Chen B, Sun D, Yang L, Zhang C, Yang A, Zhu Y, et al. Mitochondrial ND5 T12338C, tRNA^Cys T5802C, and tRNA^Thr G15927A variants may have a modifying role in the phenotypic manifestation of deafness-associated 12S rRNA A1555G mutation in three Han Chinese pedigrees. *Am J Med Genet A* 2008;146A(10):1248-58.
   PUBMED | **CROSSREF**

35. Zhao L, Wang Q, Qian Y, Li R, Cao J, Hart LC, et al. Clinical evaluation and mitochondrial DNA sequence analysis in two Chinese families with aminoglycoside-induced and non-syndromic hearing loss. *Biochem Biophys Res Commun* 2005;336(3):967-73.
   PUBMED | **CROSSREF**

36. Young WY, Zhao L, Qian Y, Li R, Chen J, Yuan H, et al. Variants in mitochondrial tRNAGlu, tRNAArg, and tRNAThr may influence the phenotypic manifestation of deafness-associated 12S rRNA A1555G mutation in three Han Chinese families with hearing loss. *Am J Med Genet A* 2006;140(20):2188-97.
   PUBMED | **CROSSREF**

37. Rishishwar L, Jordan IK. Implications of human evolution and admixture for mitochondrial replacement therapy. *BMC Genomics* 2017;18(1):140.
   PUBMED | **CROSSREF**

38. Yelverton JC, Arnos K, Xia XJ, Nance WE, Pandya A, Dodson KM. The clinical and audiologic features of hearing loss due to mitochondrial mutations. *Otolaryngol Head Neck Surg* 2013;148(6):1017-22.
   PUBMED | **CROSSREF**

39. Lu J, Qian Y, Li Z, Yang A, Zhu Y, Li R, et al. Mitochondrial haplotypes may modulate the phenotypic manifestation of the deafness-associated 12S rRNA 1555A>G mutation. *Mitochondrion* 2010;10(1):69-81.
   PUBMED | **CROSSREF**

40. Bykhovskaya Y, Shohat M, Ehrenman K, Johnson D, Hamon M, Cantor RM, et al. Evidence for complex nuclear inheritance in a pedigree with nonsyndromic deafness due to a homoplasmic mitochondrial mutation. *Am J Med Genet* 1998;77(5):421-6.
   PUBMED | **CROSSREF**

41. Wei QP, Zhou X, Yang L, Sun YH, Zhou J, Li G, et al. The coexistence of mitochondrial ND6 T14484C and 12S rRNA A1555G mutations in a Chinese family with Leber's hereditary optic neuropathy and hearing loss. *Biochem Biophys Res Commun* 2007;357(4):910-6.
   PUBMED | **CROSSREF**

42. Mezghani N, Mnif M, Mkaouar-Rebai E, Kallel N, Charfi N, Abid M, et al. A maternally inherited diabetes and deafness patient with the 12S rRNA m.1555A>G and the ND1 m.3308T>C mutations associated with multiple mitochondrial deletions. *Biochem Biophys Res Commun* 2013;431(4):670-4.
   PUBMED | **CROSSREF**

43. Guan MX, Enriquez JA, Fischel-Ghodsian N, Puranam RS, Lin CP, Maw MA, et al. The deafness-associated mitochondrial DNA mutation at position 7445, which affects tRNA^Ser(UCN) precursor processing, has long-range effects on NADH dehydrogenase subunit ND6 gene expression. *Mol Cell Biol* 1998;18(10):5868-79.
   PUBMED | **CROSSREF**

44. Li X, Zhang LS, Fischel-Ghodsian N, Guan MX. Biochemical characterization of the deafness-associated mitochondrial tRNA$^{Ser(UCN)}$ A7445G mutation in osteosarcoma cell cybrids. *Biochem Biophys Res Commun* 2005;328(2):491-8.
   PUBMED | **CROSSREF**

45. Kumana CR, Yuen KY. Parenteral aminoglycoside therapy. Selection, administration and monitoring. *Drugs* 1994;47(6):902-13.
   PUBMED | **CROSSREF**

46. Driessen RG, Groven RV, van Koll J, Oudhuis GJ, Posthouwer D, van der Horst IC, et al. Appropriateness of empirical antibiotic therapy and added value of adjunctive gentamicin in patients with septic shock: a prospective cohort study in the ICU. *Infect Dis (Lond)* 2021;53(11):830-8.
   PUBMED | **CROSSREF**

47. Habib G, Lancellotti P, Antunes MJ, Bongiorni MG, Casalta JP, Del Zotti F, et al. 2015 ESC guidelines for the management of infective endocarditis: the task force for the management of infective endocarditis of the European Society of Cardiology (ESC). Endorsed by: European Association for Cardio-Thoracic Surgery (EACTS), the European Association of Nuclear Medicine (EANM). *Eur Heart J* 2015;36(44):3075-128.
   PUBMED | **CROSSREF**

48. World Health Organization. *WHO Consolidated Guidelines on Tuberculosis: Module 4: Treatment - Drug-Resistant Tuberculosis Treatment, 2022 Update.* Geneva, Switzerland: World Health Organization; 2022.

49. Wargo KA, Edwards JD. Aminoglycoside-induced nephrotoxicity. *J Pharm Pract* 2014;27(6):573-7.
   PUBMED | **CROSSREF**

50. Le TA, Hiba T, Chaudhari D, Preston AN, Palowsky ZR, Ahmadzadeh S, et al. Aminoglycoside-related nephrotoxicity and ototoxicity in clinical practice: a review of pathophysiological mechanism and treatment options. *Adv Ther* 2023;40(4):1357-65.
   PUBMED | **CROSSREF**