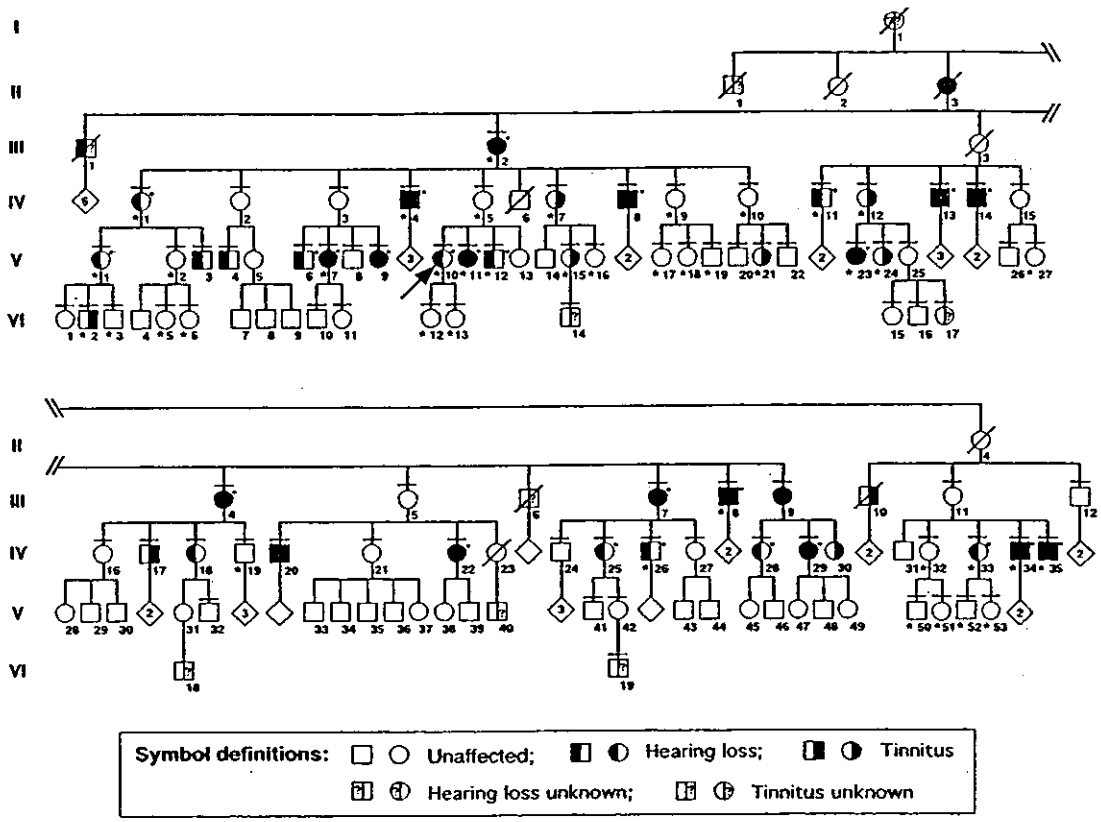

Fig. 1. Pedigree of the family. Generations are indicated by Roman numerals on the left side, and the numbers under the symbols identify the maternally related family members in each generation. Horizontal lines above the symbols indicate individuals who were personally interviewed, dots indicate individuals in whom pure-tone audiometry was conducted, and asterisks indicate individuals from whom DNA samples were obtained. The arrow indicates the proband of the family.

six generations in this family. None of the family members had a history of aminoglycoside exposure. The medical histories of the family members indicated no other significant defects related to mitochondrial mutations apart from hearing loss.

Genetic Analysis

Genetic analysis was performed in 41 maternally related family members (Fig. 1). DNA was extracted from blood samples and screened for the A1555G mutation by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) using endonuclease *Bsm*AI and was confirmed by sequencing as previously described.[8] By the PCR-RFLP analysis, only a single slower-migrating band of a DNA fragment can be detected in the case of the homoplasmic A1555G mutation, whereas two faster-moving fragments can be seen in the case of a normal control without the mutation. In addition, connexin 26 gene mutations were screened by sequencing a PCR-amplified DNA fragment containing the entire coding region as previously described.[13]

Analysis of Hearing Disability and Handicap, Tinnitus, and Medical Histories

Information about hearing disability and handicap, tinnitus, and medical histories were available in total of 123 maternally related family members, (i.e., all but I-1). Using a formatted questionnaire, 81 family members were directly questioned about hearing disability and handicap, tinnitus, aminoglycoside usage, general medical history, and other hearing-related incidents. The information of three other infant members was obtained from their mothers, and these infants were also examined for hearing-related behavior and reflexes. One (VI-14) of the three infants exhibited normal response in screening test for hearing loss using an ALGO Newborn Hearing Screener (Natus Medical, Foster City, CA). The information for the other 39 family members was obtained from family members who were in close contact with the subjects (usually living with the subjects) by using the same formatted questionnaire. First-degree relatives always confirmed the information provided by affected members, whereas that of unaffected members was confirmed either by first-degree relatives or, in a few cases, by second-degree relatives. Most subjects had been previously screened for hearing loss during their school years or for employment purposes. Five subjects (III-8, IV-1, IV-4, IV-25, and IV-26) had a previous history of noise exposure of at least 8 years' duration.

The degree of hearing disability and handicap was classified into the following categories: 1) no problem; 2) mild problem (difficulty in hearing during specific situations such as conversation over background noise, generally not requiring hearing aids); 3) moderate problem (need for hearing aids for daily conversation, and able to communicate by telephone with hearing aids); 4) severe problem (may carry on a conversation only with hearing aids under quiet, face-to-face situations, and inability to communicate by telephone even with hearing aids); and 5) profound problem (need for visual information or signing in addition to hearing aids for conversation in any situation). Tinnitus was considered pathologically significant if it continued for longer than 5 minutes and occurred more than once a week with a duration of 1 month or longer for intermittent tinnitus or 3 days or longer for continuous tinnitus.

Analysis of Hearing Impairment

The degree of hearing impairment was analyzed by pure-tone audiometry following otoscopic examination in 26 subjects with hearing disability and handicap (Fig. 1). The majority of the subjects were tested for pure-tone audiometry using an AA75 audiometer (Rion, Tokyo, Japan) in a soundproof room. For a minority of subjects, the test was conducted with an AA72B audiometer (Rion) using circumaural earphones in quiet rooms where background noise was less than 40 dB sound pressure level (SPL) (measured using a RION NA29 sound level meter with A-weighting). Both air-conducted and bone-conducted thresholds were examined.

The study protocol was approved by the Ethical Committee of the National Tokyo Medical Center (Tokyo, Japan) and was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained for all subjects participating in the study.

RESULTS

Genetic Analysis

The A1555G mutation was detected in a homoplasmic form (i.e., only a single slower-migrating band of a PCR-amplified DNA fragment could be detected) in all 41 maternally related family members who were screened. Forty of these 41 subjects were also screened for mutations in the nuclear connexin 26 gene (a technical problem prevented the analysis of one subject) that are related to hearing loss, and one individual (IV-19) exhibited such a mutation. This subject had a heterozygous 299–300delAT frameshift mutation (i.e., deletions of A and T at position 299 and 300) in the connexin 26 gene that altered the amino acid sequence from codon 100 and generated a stop at codon 113. The subject exhibited neither hearing loss nor tinnitus.

Hearing Disability and Handicap

In 123 subjects evaluated for hearing disability and handicap, 90 subjects exhibited no problem, and those with any problems were distributed as follows: 20 with mild problem, 8 with moderate problem, 3 with severe problem, and 2 with profound problem (Fig. 2). Overall, hearing disability and handicap were identified in 26.8% (33 of 123) of the subjects and were more frequent in older members than in younger members. There was no significant difference in occurrence between male (26.3%) and female (27.3%) subjects. It is important that affected subjects within the same sibling group tended to exhibit a similar degree of hearing disability and handicap, whereas the severity between sibling groups varied. For example, severe or profound hearing disability and handicap were aggregated in two sibling groups (V-6 to V-9 and V-10 to V-13). The age at onset varied, and neither congenital nor prelingual onset was identified. As was seen in the degree of hearing disability and handicap, the age at onset was similar in affected subjects within the same sibling group, whereas this parameter varied between distinct sibling groups.

Tinnitus

One hundred fifteen subjects could be evaluated for tinnitus. Tinnitus that met our criteria was noted in 24.3% (28 of 115) of the family members (Fig. 2). Tinnitus was continuous (24 h a day) in 15 subjects, intermittent in 11 subjects, and present but with unknown persistence in 2 subjects. The age at onset varied. The disappearance of tinnitus or a significant improvement was noted within 1 month after onset in three subjects, between 1 month and 1 year in one subject, between 1 year and 10 years in three subjects, and after 10 years in one subject. As was noted for hearing loss, both the age at onset and duration of tinnitus were similar among affected subjects within the same sibling group but varied among different sibling groups.

With designation of hearing loss based on reported hearing disability and handicap, both hearing loss and tinnitus were noted in 19 subjects. Fourteen subjects had hearing loss only, and nine subjects had tinnitus only. The onset and duration of hearing loss and tinnitus for all subjects are presented in Figure 3. Of 19 subjects who initially noted tinnitus not associated with hearing loss, 10 subjects later developed hearing loss.

Hearing Impairment

Pure-tone audiometry in 26 subjects with hearing disability and handicap revealed sloping or sharp-sloping audiograms for all but one subject (subject IV-1) who had a previous history of noise exposure and exhibited an audiogram typical for noise-induced hearing loss (i.e., increased bone-conducted thresholds at 4 kHz). In 18 of the 26 subjects, the pure-tone average (PTA) over the frequencies of 0.5, 1, 2, and 4 kHz in the better ear was 25 dB HL or worse, which meets the common lower-threshold definition of hearing impairment.[14] The remaining eight subjects also exhibited significantly elevated pure-tone thresholds at least at 8 kHz (>95th percentile or >50 dB HL), compared with the normal range for their respective ages and sexes.[15] Hearing impairment was sensorineural in type and mostly symmetrical when detected.

The relationship between the degree of hearing impairment and the age at onset of hearing loss in the 26 subjects is shown in Figure 4. The association of severe to profound hearing loss with an onset of hearing loss before age 10 years was evident. In six subjects (three men and three women) with a mean age of 36 years (age range, 31–40 y), who developed hearing loss before 10 years of age, the binaural PTA at the three middle frequencies (binaural PTA at 0.5–2 kHz) ranged from 75 to 103 dB HL (mean value, 89.2 dB HL [SD = 11.7 dB HL]) and the binaural PTA at the three higher frequencies (binaural PTA at 2–8 kHz) ranged from 103 to 112 dB HL (mean value, 108.3 dB HL [SD = 3.7 dB HL]). In 20 subjects (8 men and 12 women) with a mean age of 55 years (age range, 33–87 y), who developed hearing loss at age 10 years or later, the binaural PTA at 0.5 to 2 kHz ranged from 1.5 to 61.5 dB HL (mean value, 29.5 dB HL [SD = 19.6 dB HL]) and binaural PTA at 2 to 8 kHz ranged from 27 to 91.5 dB HL (mean value, 56.6 dB HL [SD = 22.7 dB HL]). Statistical analysis performed using Mann-Whitney *U* test on the Statview statistical software program (SAS Institute Inc., Cary, NC) exhibited a significant difference between these two groups in both the binaural PTA at 0.5

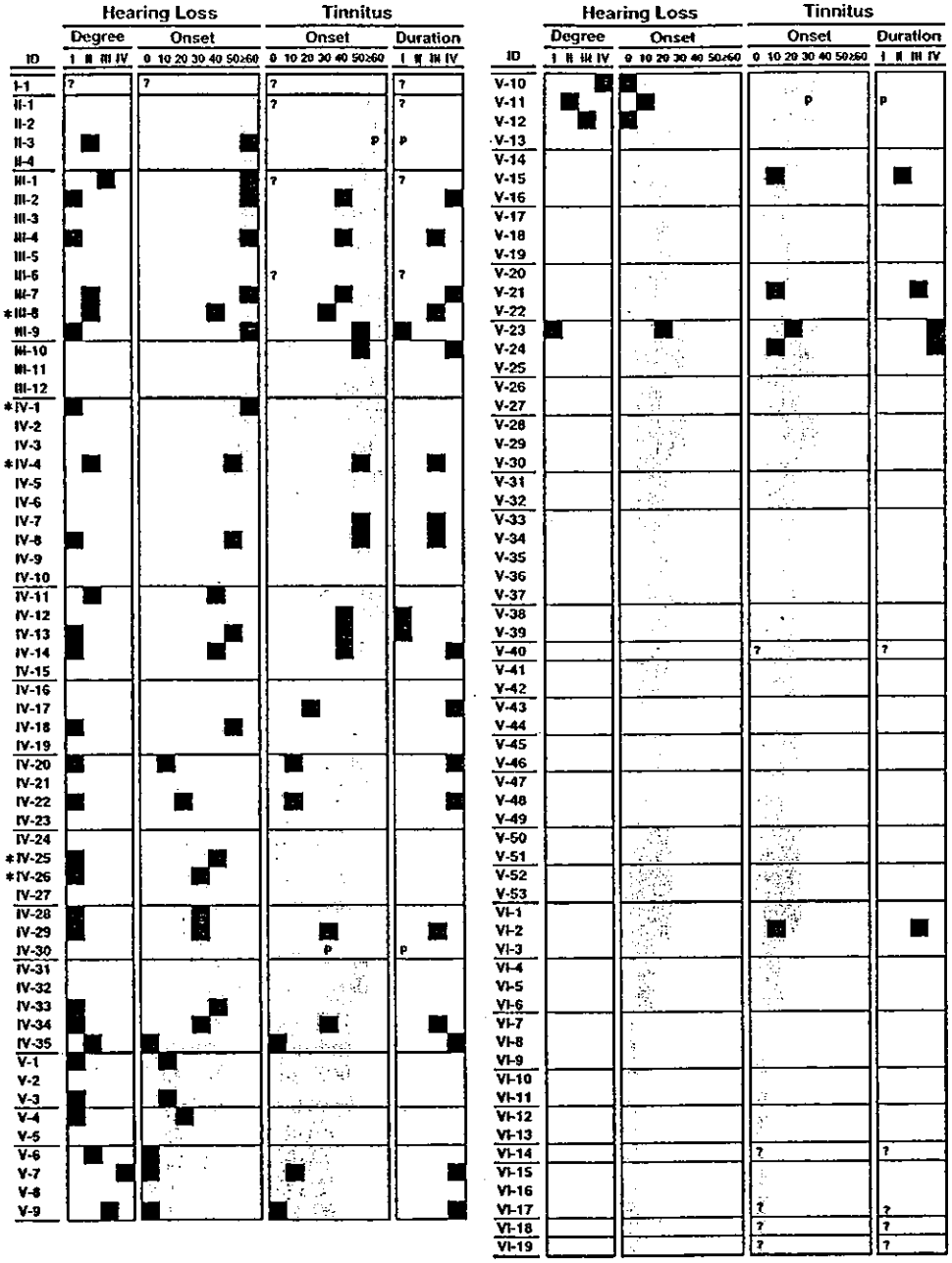

Fig. 2. Summary of phenotypic expression in all maternally related family members ordered by generation and identification number (ID) as designated in the pedigree. Horizontal lines divide different sibling groups. Asterisks indicate individuals with a long history of noise exposure. Each subject is categorized by age in 10-year increments as indicated by horizontal gray bars. Black squares represent degree and onset of hearing loss based on interviews about hearing disability and handicap and onset and duration of tinnitus as classified at the top of the columns. The degree of hearing loss is classified as follows: I, mild; II, moderate; III, severe; and IV, profound (for details, see "Materials and Methods" section). Ages are indicated in 10-year increments as follows: 0, 0–9 years; 10, 10–19 years; 20, 20–29 years; 30, 30–39 years; 40, 40–49 years; 50, 50–59 years; ≥60, 60 years or older. Duration of tinnitus is classified as follows: I, less than 1 month; II, 1–11 months; III, 1–9 years; IV, 10 years or more. The symbol 'p' indicates that the individual presented with tinnitus but the age at onset and the duration of the tinnitus was undefined. The absence of a black square indicates that the individual did not exhibit the corresponding phenotype. Question marks denote undefined phenotypes.

to 2 kHz (P = .0003) and the binaural PTA at 2 to 8 kHz (P = .0003).

DISCUSSION

Homoplasmic A1555G Mitochondrial Mutation in Maternally Related Family Members

In the proband of the family in the present study, the A1555G mutation was detected in a homoplasmic pattern by the PCR-RFLP analysis. This result indicates that all the mitochondrial genomes in different cells and tissues of the proband harbor the mutation. Besides, all the maternally related family members of the present family should carry the A1555G mutation also in a homoplasmic form because the mitochondrial DNA exhibits exclusively maternal inheritance.[16] This was substantiated by the detection of this homoplasmic mutation in all of the 41 subjects who were screened.

Prevalence of Hearing Loss

Based on the interviews about hearing disability and handicap in the present study, 26.8% of the maternally related members had hearing loss. The prevalence of hearing loss according to age was as follows: 0%, less than 18 years of age; 21.7%, 18 to 44 years of age; 44.4%, 45 to 64 years of age; and 47.1%, 65 years of age or older. None of the present subjects had congenital or prelingual hearing loss; however, 10% of adult subjects exhibited postlingual hearing loss before the age of 20 years. Because the prev-

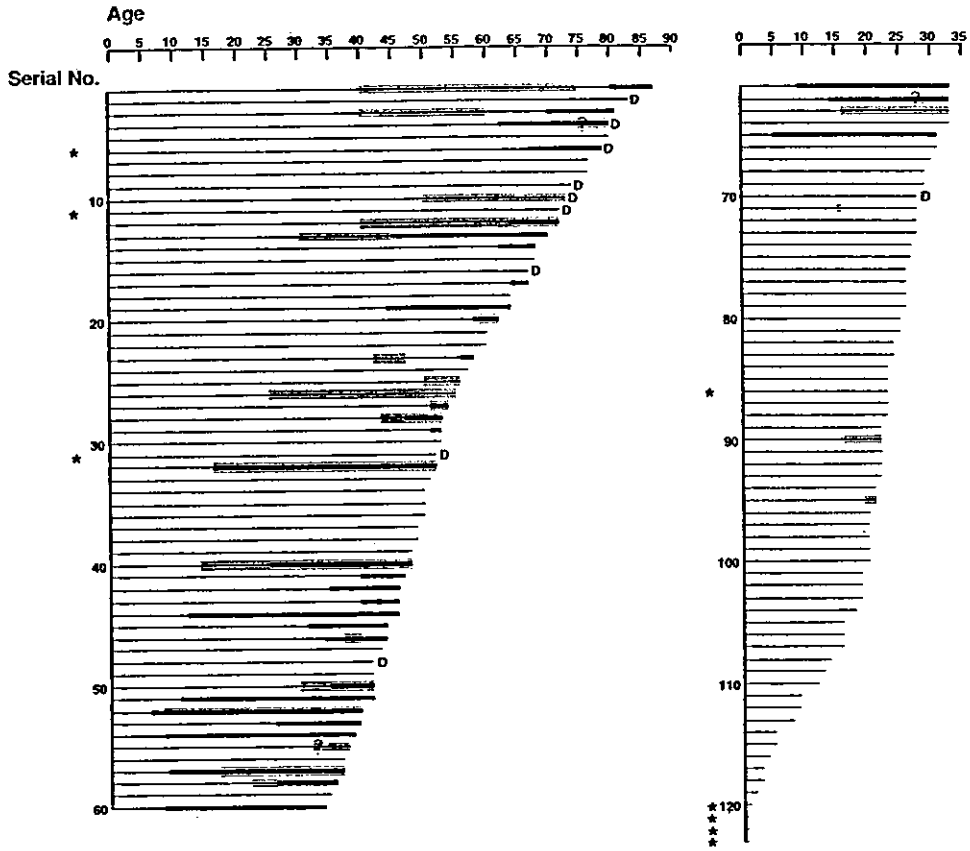

Fig. 3. Age at onset and duration of hearing loss and tinnitus based on interviews in all maternally related family members except I-1, ordered by age. The thin horizontal lines represent the age of each subject. Thick black and gray horizontal lines delineate the presence of hearing loss and tinnitus, respectively. Question marks to the left of the thick gray lines indicate cases in which the age at onset of tinnitus was unknown. Asterisks to the left of the thin lines denote subjects for whom tinnitus information was not obtained. Individuals who were deceased (D) at the time of the present study are also indicated. Age = years.

alence of hearing loss has not been studied by the method similar to the present study in Japan, comparison of the prevalence of hearing loss in the present subjects and that in general Japanese population could not be conducted. In a similar, previous study employing interviews about hearing handicaps in the United States, the prevalence of hearing loss in the general population according to age was as follows: 1.6%, less than 18 years of age; 4.8%, 18–44 years of age; 12.8%, 45–64 years of age; and 30.0%, 65 years of age or older.[17] Although there is a difference in the population examined, comparison of the report just cited with the present study suggests that maternally related members of the family with the A1555G mitochondrial mutation may have much higher risk for developing postlingual hearing loss than the general population even in the absence of aminoglycoside exposure.

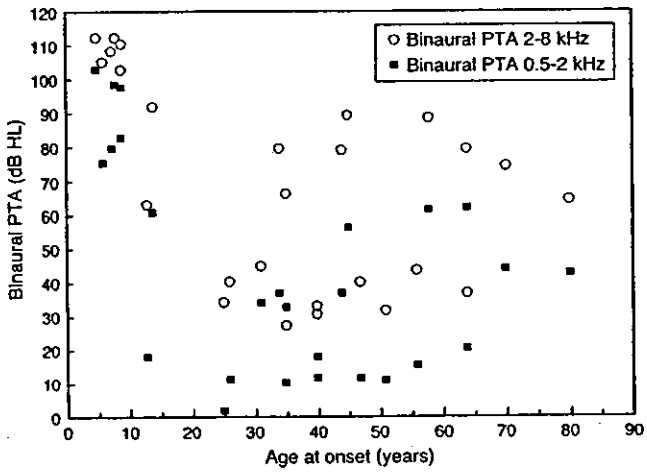

Fig. 4. Binaural pure-tone average (PTA) as a function of age at onset of hearing loss in 26 subjects with reported hearing disability and handicap. Binaural PTA of 2 to 8 kHz represents an average of pure-tone thresholds at 2, 4, and 8 kHz in both ears, and binaural PTA of 0.5 to 2 kHz represents an average of pure-tone thresholds at 0.5, 1, and 2 kHz in both ears.

Similar Phenotypic Expression Patterns Within Same Sibling Group

The degree and onset of hearing loss based on interviews about hearing disability and handicap were similar in affected subjects within the same sibling group but were distinct in different sibling groups. In addition, both affected and nonaffected subjects were found within the same sibling group. These phenotypic expression patterns were also observed in the onset and duration of tinnitus. The subjects in the present study were maternally related, therefore sharing the same mitochondrial DNA including the A1555G mutation, and had no history of aminoglycoside exposure. Thus, these phenotypic expression patterns suggest that nuclear background may be involved in modulating the phenotypic expression of the mutation, as was previously demonstrated in an Arab-Israeli kindred.[18–20]

The connexin 26 gene was recently reported as a candidate aggravating factor in the phenotypic expression

of the A1555G mutation.[21] Mutations in the connexin 26 gene are the most prevalent genetic cause of recessive sensorineural hearing loss in many populations,[22] including the Japanese.[13] One subject in the present study was heterozygous for a 299–300delAT mutation in the connexin 26 gene. However, this subject did not present with significant hearing loss. This observation is inconsistent with a previous study that reported synergy between a 235delC mutation (i.e., a deletion of C at position 235) in the connexin 26 gene and the A1555G mutation with regard to hearing loss.[21] This discrepancy may be due to the different functional properties of the two connexin 26 mutations or differences in the genetic backgrounds of the two families. Linkage analysis in this family, such as that conducted in the aforementioned Arab-Israeli kindred,[23] will be required to ascertain the unknown modifier genes.

Clinical Significance of Tinnitus

Tinnitus is a disorder that manifests as an abnormal sensation perceived in the head or ear without any external sound stimulus.[24] In the present study, 24.3% of the subjects had tinnitus that met our criteria at some time in their lives. It is important that approximately 50% of the family members who initially experienced tinnitus alone developed hearing loss at a later time, indicating that tinnitus is a clinically significant indication of future hearing loss associated with the A1555G mutation. Thus, it is important that individuals who carry the A1555G mutation and experience tinnitus take proper precautions against environmentally induced hearing loss (i.e., by avoiding loud noises and undergoing periodic hearing tests).

Association of Severe to Profound Hearing Loss With Early Onset

The association of severe to profound hearing loss with an onset of hearing loss before age 10 years was clearly shown by the pure-tone audiometry in subjects with hearing disability and handicap. This association suggests that the defensive or reparative system of the cochlea may be immature during early childhood. It has been reported that developing mammals, including humans, are more sensitive to noise, as well as chemical and drug-induced ototoxicity, than adults, although the molecular mechanisms of this hypersensitivity have not been delineated.[25,26] This association also illustrates the importance of careful audiological evaluation and follow-up in maternally related family members who carry the A1555G mutation and who are suspected of having hearing loss during early childhood.

CONCLUSION

The present study elucidated the characteristics, prevalence, and intrafamilial patterns of the auditory dysfunction in maternally related members of a family with the A1555G mitochondrial mutation in the absence of aminoglycoside exposure. The risk for developing postlingual hearing loss was likely to be much higher in the subjects than in the general population. The phenotypic expression patterns in the subjects suggested the involvement of nuclear backgrounds, but the connexin 26 gene was not the nuclear modifier gene in the present family. Tinnitus was identified as a clinically significant warning sign for future hearing loss. Association of severe to profound hearing loss with early onset highlighted the importance of careful audiological evaluation in individuals with the A1555G mutation in case hearing loss is suspected during early childhood.

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