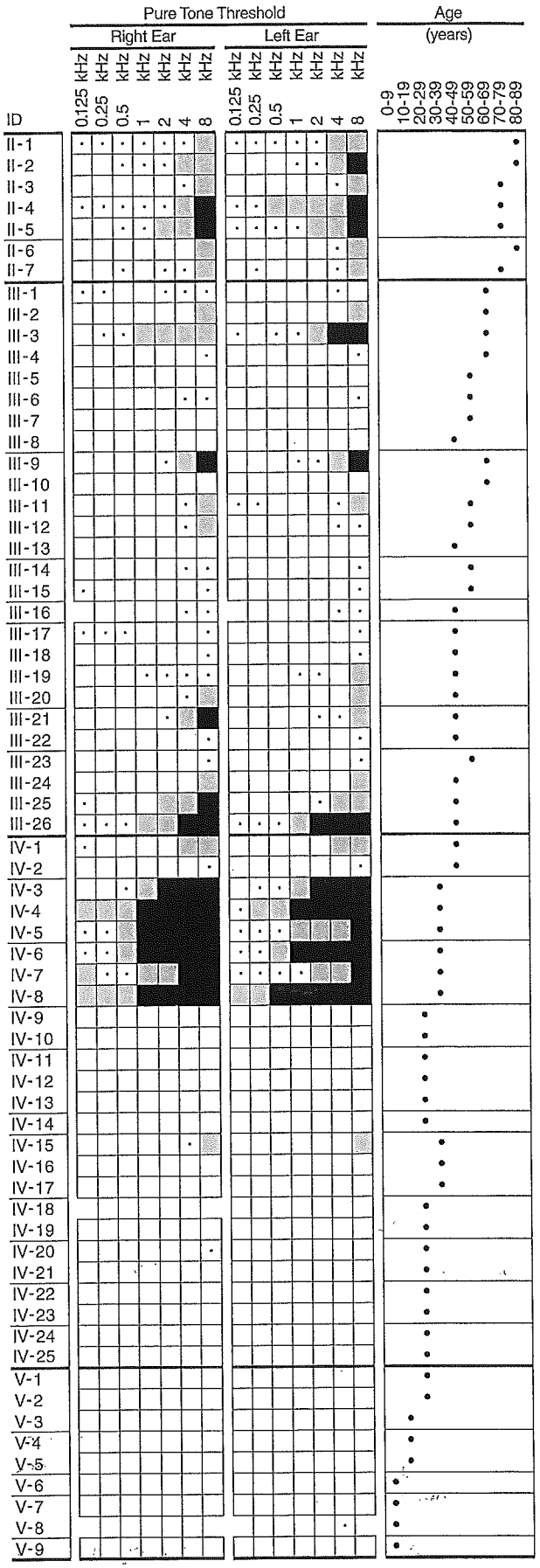

cal changes existed in their hearing, because the pure tone thresholds at 8 kHz were most prominently affected in the subjects with hearing loss. Thus, subjects whose ages ranged between 18 and 70 years and whose ears were free of otitis media were eligible for this analysis. Sixty-three ears of 32 subjects met this criteria, and 11 of the 63 ears (17.5%) exhibited significantly elevated pure tone thresholds at 8 kHz (>95th percentile) in comparison to the normal range for their respective ages and sexes.[14] A statistical analysis performed with the binomial test on which the threshold probability of the target population is .05 revealed the frequency of the elevated pure tone thresholds at 8 kHz (17.5%) to be significantly higher than the frequency expected in the ears of the otologically normal population ($p < .0001$).

Speech Recognition Test. The relationship between the maximum speech recognition score and PTA 0.5-2 kHz is shown for each ear (Fig 3). The score ranged from 100% in ears with normal hearing to 0% in ears with profound hearing loss. None of the subjects exhibited a disproportionately poor maximum speech recognition score in relation to the magnitude of pure tone thresholds. In 15 of 38 tested ears, the maximum speech recognition score was >50%, and the rollover index of the performance-intensity function could be reliably determined in these 15 ears. Significant amounts of rollover are pathological and are associated with retrocochlear hearing loss. That the rollover index was <40% in all of the 15 ears suggests that retrocochlear dysfunction did not contribute significantly to hearing loss.

SISI Test. The SISI score and the pure tone threshold at the respective frequencies in each ear are shown in Fig 4. We regarded SISI scores of 70% or higher as positive for cochlear dysfunction, while those between 30% and 70% were regarded as semipositive, and those of 30% or lower as negative.[11] The SISI scores were mostly negative at frequencies for which the pure tone threshold was lower than 30 dB HL. In contrast, the SISI scores were predominantly positive at frequencies for which the pure tone threshold was 30 dB HL or higher. A few subjects exhibited

Fig 2. Pattern of pure tone thresholds for all tested frequencies in each subject. Subjects are listed in order of generation and identification number (ID) as designated in Fig 1. Age of each subject is indicated by dot at corresponding division of age scale classified at top. Thin horizontal lines divide different sibling groups, and thick horizontal lines divide generations. Air conduction pure tone thresholds of right and left ears are indicated by following symbols: white square, ≤30 dB HL; dot in white square, 31 to 60 dB HL; gray square, 61 to 90 dB HL; black square, ≥91 dB HL; blank, not tested. Bone conduction pure tone thresholds are shown instead of air conduction thresholds in 2 subjects (IV-18 and V-8) who had otitis media at time of test.

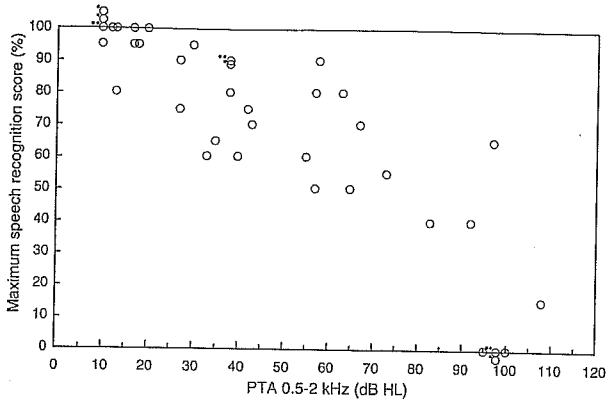

Fig 3. Maximum speech recognition score as function of mean of pure tone thresholds at 0.5, 1, and 2 kHz ("PTA 0.5-2 kHz") for each ear. For ease of visualization, overlapping symbols were moved from original position (indicated by double dots) to neighboring positions (indicated by dot).

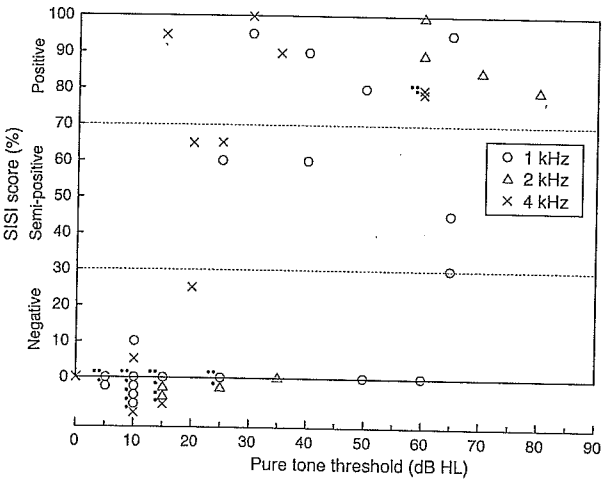

Fig 4. Short increment sensitivity index (SISI) score as function of pure tone threshold at SISI-tested frequency for each ear. Tests were conducted at 1, 2, and 4 kHz. Overlapping symbols were moved as indicated in Fig 3.

semipositive or negative SISI scores despite elevated pure tone thresholds (mostly at 1 kHz). Such occurrences have been noted in previous studies reporting that SISI scores are occasionally semipositive or negative at low frequencies (including 1 kHz) even in ears with cochlear dysfunction.[15]

TEOAE. The TEOAE results were evaluated by the response of the spectral amplitude against noise across a broad frequency range (Fig 5A), as well as by the reproducibility of the time waveform (Fig 5B). The data were plotted against the PTA 0.5-2 kHz in each ear. The response and reproducibility were lower in ears with a PTA 0.5-2 kHz higher than 20 dB HL than in ears with a PTA 0.5-2 kHz of 20 dB HL or lower. No TEOAEs were detected in any of the 6 ears with a PTA 0.5-2 kHz higher than 40 dB HL.

DPOAE. DPOAEs with amplitudes higher than 2 standard deviations above the noise level were considered as positive responses, and DPOAE amplitudes tested at 1, 2, and 4 kHz were compared with the pure tone thresholds measured at the corresponding frequency in each ear (Fig 6). The DPOAE amplitudes were reduced in ears with pure tone thresholds of 20 dB HL or higher at the corresponding DPOAE-tested frequency, and the DPOAE was mostly absent in ears with the pure tone thresholds of 40 dB HL or higher.

ABR. The thresholds of wave I and wave V were determined with the click stimulation, and the latencies of these two waves at 90 dB nHL were measured. The thresholds were then compared with the mean of the air-conducted pure tone thresholds at 2 and 4 kHz ("PTA 2-4 kHz"; Table 2). This frequency range is known to produce the largest ABR components in the cochlea.[16] The relationships of wave I and wave V thresholds and PTA 2-4 kHz were consistent with cochlear dysfunction; ie, the wave V thresholds were almost equal to the PTA 2-4 kHz, and wave I thresholds were higher than wave V thresholds.[17] The wave V latency was within the range predicted by the PTA 2-4 kHz based on the relationship in ears with the corresponding degree of cochlear hearing loss[18] in all but 3 ears (left ear of III-2 and both ears of IV-4) that exhibited relatively long wave V latencies, indicating mild retrocochlear involvement. These 2 subjects were 87 and 62 years old, respectively, and both presented with mild cerebrovascular disease.

Total Mitochondrial DNA Sequence. The mitochondrial DNA sequences were identical in all 8 subjects examined. These subjects exhibited 40 base substitutions relative to the human mitochondrial DNA sequence in MITOMAP, including the A1555G mutation (Table 3). The 39 base substitutions excluding the A1555G mutation were previously reported as polymorphisms in MITOMAP or found in normal Japanese controls — a finding indicating that these substitutions were not related to the observed hearing loss.

DISCUSSION

In our previous study,[10] the proband of the present family exhibited the mitochondrial A1555G mutation in a homoplasmic pattern; ie, all of the mitochondrial genomes in different cells and tissues of the proband harbor the mutation. Because mitochondrial DNA exhibits exclusively maternal inheritance,[19] all of the maternally related members of this family were assumed to carry the A1555G mutation in a homoplasmic form, and this presumption was substantiated by genetic tests that revealed the mutation in a homoplasmic pattern in all 41 maternally related fam-

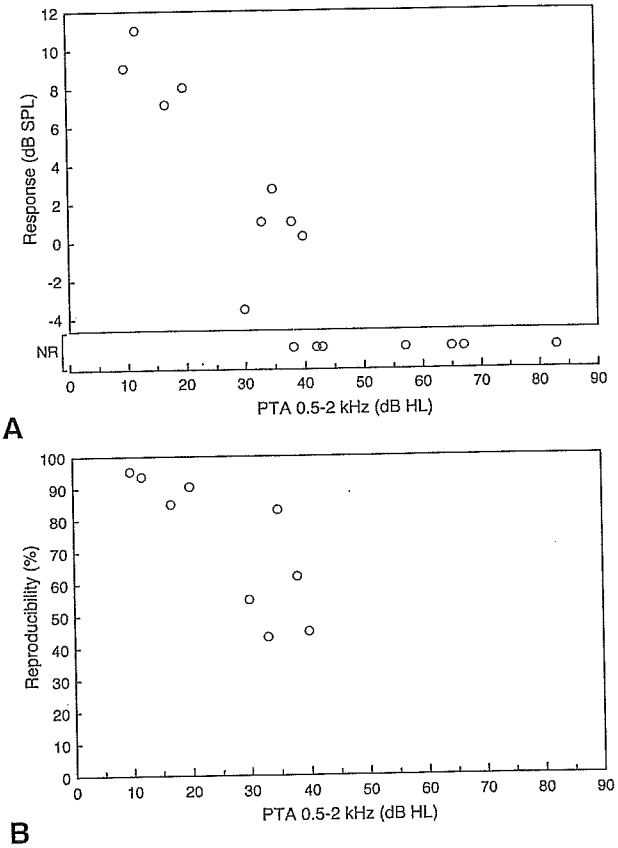

Fig 5. Response (**A**) and reproducibility (**B**) of transient evoked otoacoustic emissions as function of mean of pure tone thresholds at 0.5, 1, and 2 kHz ("PTA 0.5-2 kHz") for each ear.

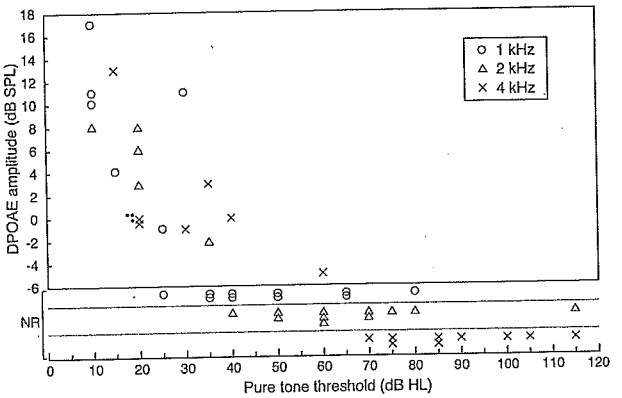

Fig 6. Distortion product otoacoustic emission (DPOAE) amplitude as function of pure tone threshold at DPOAE-tested frequency for each ear. Tests were conducted at 1, 2, and 4 kHz. Symbols between horizontal lines in lower part of Figure (indicated by NR) represent no DPOAE response. Overlapping symbols were moved as indicated in Fig 3.

ily members who were tested.[10] Thus, all of the present subjects who were maternally related members of this family can be considered to carry the A1555G mutation, and all of the present audiological findings can be considered to represent the effects of the A1555G mutation.

A battery of audiological tests conducted in the present study showed a consistent pattern of audiological characteristics, indicating a common pathophysiological mechanism in the development of hearing loss due to the A1555G mutation. Exclusively sloping or sharp sloping audiograms were noted in all subjects with hearing loss except for 1 individual whose hearing loss resulted from long-term noise exposure. In subjects with slight or mild hearing loss according to the PTA 0.5-2 kHz, the pure tone thresholds at 8 kHz were always the most elevated. Even in the subjects who did not report any hearing loss at the time of interview, 11 of the 63 ears (17.5%) exhibited significantly elevated pure tone thresholds at 8 kHz. This frequency was significantly higher than the frequency expected in ears of an otologically normal population. As a result, the relatively frequent occurrence of elevated pure tone thresholds at 8 kHz was considered to be a subclinical audiological feature associated with the mitochondrial A1555G mutation.

These audiogram characteristics have been known in sensory presbycusis, a type of age-related audi-

TABLE 2. CHARACTERISTICS OF AUDITORY BRAIN STEM RESPONSES

	Right Ear					*Left Ear*				
Subject	*PTA 2-4 kHz (dB HL)*	*Threshold* (dB nHL)*		*Latency† (ms)*		*PTA 2-4 kHz (dB HL)*	*Threshold* (dB nHL)*		*Latency† (ms)*	
		I	*V*	*I*	*V*		*I*	*V*	*I*	*V*
III-2	55	90	70	1.9	5.9	60		90		7.1
IV-4	80		70		6.4	87.5		70		6.8
IV-11	72.5		80		5.9	67.5	80	70	2.2	6.0
IV-35	92.5	105	100			115		105		
V-7	115					110		105		
V-10	115					115				
V-12	115					115				

PTA 2-4 kHz — average of pure tone thresholds at 2 kHz and 4 kHz.

*Threshold of wave I and wave V.

†Latency of wave I and wave V with click stimulation at 90 dB nHL.

TABLE 3. MITOCHONDRIAL DNA SEQUENCE VARIANTS IN SUBJECTS

Gene Product	*Nucleotide Change*
D-loop	A73G, T152C, A263G, 311insC, T489C
12S rRNA	A750G, A1438G, A1555G
16S rRNA	A2706G, A3145G
NADH dehydrogenase 2	A4715G, A4769G
Cytochrome c oxidase 1	T6632C, A6752G, C7028T, C7196A
Cytochrome c oxidase 2	A8188G
ATP synthase 6	G8584A, A8701G, A8860G, T9090C
Cytochrome c oxidase 3	T9540C
NADH dehydrogenase 3	A10398G, C10400T
NADH dehydrogenase 4	T10873C, G11719A
NADH dehydrogenase 5	C12705T
NADH dehydrogenase 6	C14668T
Cytochrome b	C14766T, T14783C, G15043A, G15301A, A15326G, A15487T, T15784C
D-loop	C16185T, C16186T, C16223T, C16260T, T16298C

tory impairment resulting from the degeneration of sensory hair cells and supporting cells primarily at the basal turn of the cochlea.[20] Several other mitochondrial DNA mutations have been proposed to play roles in age-related dysfunction in organs such as the central nervous system and muscle,[21] and therefore, the A1555G mutation may act analogously to promote auditory dysfunction by a mechanism similar to that of sensory presbycusis.

The speech audiometry results in the present subjects indicated cochlear dysfunction in subjects with slight to severe hearing loss, and these subjects did not exhibit features of retrocochlear dysfunction. The SISI and OAE tests also detected cochlear dysfunction almost simultaneously with or even earlier than the deterioration of pure tone thresholds, indicating that cochlear dysfunction, especially outer hair cell dysfunction, occurred at quite an early stage of hearing loss in the affected subjects. The observed ABR thresholds and latencies also indicated cochlear damage. In agreement with these results, excellent auditory performance with a cochlear implant has been reported in a patient with profound hearing loss due to the A1555G mutation.[22] Given that selective damage to the outer hair cells induces only mild to moderate hearing loss,[23] it would be expected that other cochlear components would thus be damaged in cases of more advanced hearing loss.

The PTA testing confirmed various levels of hearing loss in the present subjects, none of whom had a history of aminoglycoside exposure. To explore possible genetic factors that may have contributed to such phenotypic differences, we sequenced the entire mitochondrial DNA for 8 subjects who presented with various levels of hearing loss. Previously, the coexistence of two mitochondrial mutations, A1555G and G7444A, was identified in Mongolian subjects with hearing loss, and these subjects appeared to present earlier onset and increased severity of hearing loss as compared to patients with the A1555G mutation alone.[24] This finding suggests that an additional new mitochondrial DNA mutation may be responsible for the intrafamilial phenotypic differences in this family. However, our analysis revealed that all 8 subjects had identical mitochondrial DNA sequences, thus indicating that the observed phenotypic differences were not related to any variations in the mitochondrial DNA. In addition, except for the A1555G mutation, no known pathogenic mutations were found in the total mitochondrial DNA sequences; thus, the A1555G mutation is probably the only mitochondrial mutation involved in hearing loss in this family. The degree of hearing loss was similar in the affected subjects within the same sibling group, but varied between the sibling groups. These results suggest that nuclear modifier genes may also be involved in phenotypic differences in the present family, as previously reported in an Arab-Israeli family.[25,26]

In conclusion, our study revealed that various degrees of hearing loss could be caused by an A1555G mutation in the mitochondrial DNA with identical sequences, without any additional pathogenic mutations, even in the absence of aminoglycoside exposure. The affected subjects exhibited audiograms that are characteristic of sensory presbycusis, and also shared common audiological features such as a cochlear origin for all levels of hearing loss and a high degree of vulnerability of outer hair cells. These results further our understanding of the genetic and pathophysiological mechanisms of hearing loss associated with the A1555G mutation, and may aid in the diagnosis and development of new therapies for the treatment of this genetic hearing loss.

ACKNOWLEDGMENTS — We thank Dr Minako Sato, Dr Hidenobu Taiji, and Prof Seiji Niimi for their support during the data collection. We also thank Dr Seiji Bito for his help with the statistical analysis.

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