[11] K. Cryns, S. Thys, L. van Laer, Y. Oka, M. Pfister, L. van Nassauw, R.J.H. Smith, J.P. Timmermans, G. Van Camp, The WFS1 gene, responsible for low frequent sensorineural hearing loss and Wolfram syndrome, is expressed in a variety of inner ear cells, Histochem. Cell Biol. 119 (2003) 247–256.
[12] H. Ishihara, T. Asano, K. Tsukuda, H. Katagiri, K. Inukai, M. Anai, M. Kikuchi, Y. Yazaki, J.I. Miyazaki, Y. Oka, Pancreatic beta cell line MIN6 exhibits characteristics of glucose metabolism and glucose-stimulated insulin secretion similar to those of normal islets, Diabetologia 36 (1993) 1139–1145.
[13] K.F. Ferri, G. Koemer, Organelle-specific initiation of cell death pathways, Nat. Cell Biol. 3 (2001) E255–E263.
[14] T. Michikawa, H. Hamanaka, H. Otsu, A. Yamamoto, A. Miyawaki, T. Furuichi, Y. Tashiro, K. Mikoshiba, Transmembrane topology and sites of N-glycosylation of inositol 1,4,5-trisphosphate receptor, J. Biol. Chem. 269 (1994) 9184–9189.
[15] S. Hofmann, C. Philbrook, K.D. Gerbitz, M.F. Bauer, Wolfram syndrome: structural and functional analyses of mutant and wild-type wolframin, the WFS1 gene product, Hum. Mol. Genet. 12 (2003) 2003–2012.
[16] K.P. Kearse, D.B. Williams, A. Singer, Persistence of glucose residues on core oligosaccharides prevents association of TCR alpha and TCR beta proteins with calnexin and results specifically in accelerated degradation of nascent TCR alpha proteins within the endoplasmic reticulum, EMBO J. 273 (1994) 17064–17072.
[17] S.H. Keller, J. Lindstrom, P. Taylor, Inhibition of glucose trimming with castanospermine reduces calnexin association and proteosome degradation of the alpha-subunit of the nicotinic acetylcholine receptor, J. Biol. Chem. 273 (1998) 17064–17072.
[18] Y. Chen, F. Caherec, S.L. Chuck, Calnexin and other factors that alter translocation affects the rapid binding of ubiquitin to apoB in the Sec61 complex, J. Biol. Chem. 273 (1998) 11887–11894.
[19] T.K. Lee, A.S. Koh, Z. Cui, R.H. Pierce, N. Ballatori, N-glycosylation controls functional activity of Oatp1, an organic anion transporter, Am. J. Physiol. Gastrointest. Liver Physiol. 285 (2003) G371–G381.
[20] L. Niu, M.L. Heaney, J.C. Vera, D.W. Golde, High-affinity binding to the GM-CSF receptor requires intact N-glycosylation sites in the extracellular domain of the β subunit, Blood 95 (2000) 3357–3362.
[21] Q. Gong, C.L. Anderson, C.T. January, Z. Zhou, Role of glycosylation in cell surface expression and stability of HERG potassium channels, Am. J. Physiol. Heart Circ. Physiol. 283 (2002) H77–H84.
[22] J. He, A.M. Castleberry, A.G. Lau, R.A. Hall, Glycosylation of β_1-adrenergic receptors regulates receptor surface expression and dimerization, Biochem. Biophys. Res. Commun. 297 (2002) 565–572.
[23] N. Buhlmamm, A. Aldecoa, K. Leuthauser, R. Gujer, R. Muff, J.A. Fischer, W. Born, Glycosylation of the calcitonin receptor-like receptor at Asn(60) or Asn(112) is important for cell surface expression, FEBS Lett. 486 (2000) 320–324.
[24] M. Ramanujam, J. Hofmann, H.L. Nakhasi, C.D. Atreya, Effect of site-directed asparagine to isoleucine substitutions at the N-linked E1 glycosylation sites on rubella virus viability, Virus Res. 81 (2001) 151–156.
[25] M. de Virgilio, C. Kitzmueller, E. Schwaiger, M. Klein, G. Kreibich, N.E. Ivessa, Degradation of a short-lived glycoprotein from the lumen of the endoplasmic reticulum: the role of N-inked glycans and the unfolded protein response, Mol. Biol. Cell 10 (1999) 4059–4073.
[26] T. Hirokawa, S. Boon-Chieng, S. Mitaku, SOSUI: classification and secondary structure prediction system for membrane proteins, Bioinformatics 14 (1998) 378–379.

AUDIOLOGICAL FEATURES AND MITOCHONDRIAL DNA SEQUENCE IN A LARGE FAMILY CARRYING MITOCHONDRIAL A1555G MUTATION WITHOUT USE OF AMINOGLYCOSIDE

TATSUO MATSUNAGA, MD, PHD
TOKYO, JAPAN

HIROSHI KUMANOMIDO, MD
TOCHIGI, JAPAN

MASAE SHIROMA, PHD
TOCHIGI, JAPAN

YU-ICHI GOTO, MD, PHD
TOKYO, JAPAN

SHIN-ICHI USAMI, MD, PHD
MATSUMOTO, JAPAN

To elucidate the pathophysiological and genetic mechanisms of hearing loss associated with the homoplasmic mitochondrial A1555G mutation in the absence of aminoglycoside exposure, we conducted audiological and genetic analyses on 67 maternally related members of a large Japanese family carrying this mutation. A consistent pattern was evident in the audiograms, with features of sensory presbycusis, cochlear origin at all levels of hearing loss, and a high degree of vulnerability of outer hair cells. That the degree of hearing loss was similar in affected subjects within the same sibling group but differed between sibling groups suggests the involvement of nuclear modifier genes. Total mitochondrial DNA sequences were completely identical among subjects with various levels of hearing loss, and lacked additional pathogenic mutations. For the diagnosis of sensorineural hearing loss, the mitochondrial A1555G mutation should be considered when these features are present even in the absence of aminoglycoside exposure.

KEY WORDS — cochlea, hereditary hearing loss, mitochondria, nonsyndromic hearing loss.

INTRODUCTION

Various mitochondrial DNA mutations have been reported to cause hearing loss, either on their own or in association with other clinical symptoms such as neuromuscular disorders and diabetes.[1] The homoplasmic A1555G mutation in the mitochondrial 12S ribosomal RNA gene has been the first mitochondrial DNA mutation to be associated with nonsyndromic sensorineural hearing loss.[2] The A1555G mutation was initially identified primarily in subjects with hearing loss following aminoglycoside exposure. Indeed, it has been reported that the increased binding affinity of ribosomal RNA to aminoglycosides as a result of the mutation constitutes the pathogenetic mechanism underlying ototoxic susceptibility.[3] Subsequently, this mutation was also found in subjects who developed hearing loss in the absence of aminoglycoside exposure.[4-8] In these cases, the clinical phenotype ranged from profound congenital hearing loss to moderate progressive hearing loss of later onset to only slight hearing loss. Although these phenotypic differences may be the result of additional mutations in the mitochondrial or nuclear DNA, or of unknown environmental factors, the exact mechanism has not been determined. Furthermore, the pathophysiological mechanism of hearing loss due to the A1555G mutation in the absence of aminoglycoside exposure has not been defined, because there are no reports on temporal bone histopathology in patients with this mutation, and the audiological evaluation of patients has been limited to pure tone audiometry (PTA) in most previous studies. Only one study carried out detailed audiological evaluations, but most subjects exhibited profound hearing loss.[9] Thus, such detailed audiological evaluations of subjects with various levels of hearing loss, especially those with mild or moderate hearing loss, remain to be performed to uncover the pathophysiological mechanism underlying the development of hearing loss.

We previously identified a large Japanese family in which the A1555G mutation is prevalent. None of the family members were previously exposed to aminoglycosides, and the prevalence of hearing loss in maternally related members was much higher than that in the general population.[10] To further elucidate the pathophysiological and genetic mechanisms of

From the Department of Otolaryngology, National Tokyo Medical Center (Matsunaga), and the Department of Mental Retardation and Birth Defect Research, National Center of Neurology and Psychiatry (Goto), Tokyo, the Department of Speech-Language Pathology and Audiology, School of Health Science, International University of Health and Welfare, Tochigi (Matsunaga, Kumanomido, Shiroma), and the Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto (Usami), Japan. This work was supported by a Health Science Research Grant from the Ministry of Health, Labor and Welfare of Japan.

CORRESPONDENCE — Tatsuo Matsunaga, MD, PhD, Dept of Otolaryngology, National Tokyo Medical Center, Higashigaoka 2-5-1, Meguro-ku, Tokyo 152-8902, Japan.

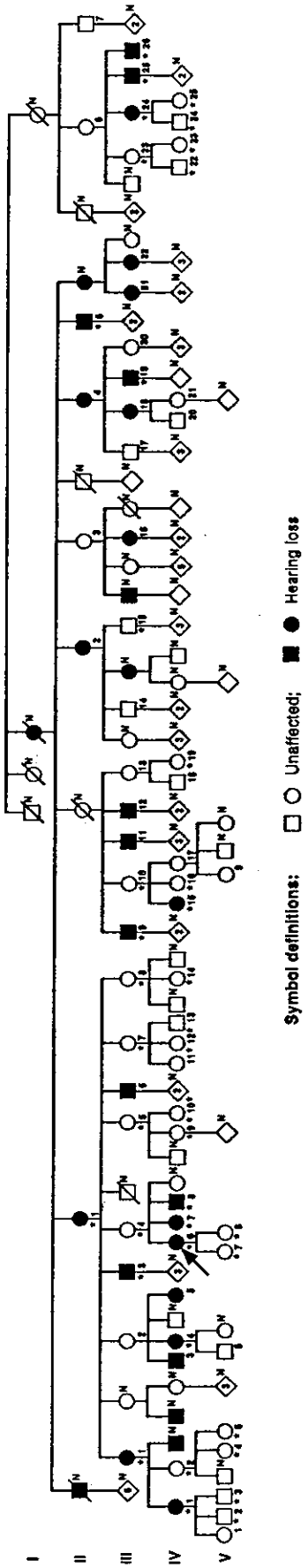


Fig 1. Part of pedigree shows intrafamilial relationship of subjects. Generations are indicated on left in roman numerals, and numbers under symbols represent identification numbers. Family members who were not subjects of this study are indicated by "N" to upper right of symbol. Subjects who reported hearing loss by interview are indicated by solid symbols. Asterisks indicate subjects who were previously tested for A1555G mutation. Arrow indicates proband of family.

the hearing loss due to this mutation, we conducted a battery of audiological tests and sequenced the entire mitochondrial DNA in maternally related members of this family.

MATERIALS AND METHODS

Subjects. The subjects were 67 maternally related members (23 male, 44 female) of a large Japanese family with the homoplasmic mitochondrial A1555G mutation (Fig 1). During interviews prior to PTA testing, 26 of the 67 subjects reported a hearing loss. The original family included 124 maternally related members in 6 generations. The medical histories, clinical phenotypes, and genetic features of these members have been reported previously.[10] In 123 maternally related members whose information about hearing was reliably obtained by interviews, 33 members (penetrance, 26.8%) were considered to have a hearing disability and handicap. The inheritance pattern was maternal and not paternal in this family. Apart from hearing loss, no other significant defects related to mitochondrial mutations were noted in this family. None of the family members had a history of aminoglycoside exposure. All 41 maternally related members who were tested for the A1555G mutation exhibited the mutation in a homoplasmic form. All 41 of these subjects participated in the present study.

Evaluation of Auditory Function. After otoscopic examination, PTA testing was conducted on all subjects. An AA75 audiometer (Rion, Tokyo, Japan) was used in a soundproof room for most subjects. For some subjects, PTA testing was conducted with an AA72B audiometer (Rion) and circumaural earphones in quiet rooms in which background noise was lower than 40 dB sound pressure level (SPL; as measured with an NA29 sound level meter; Rion) with A-weighting. Both air-conducted and bone-conducted thresholds were measured. Subjects who exhibited a pure tone threshold of 30 dB hearing level (HL) or worse at any frequency were given further detailed audiological tests when possible (Table 1). A speech recognition test was conducted with the 67-S monosyllable list (Japan Audiological Society, Tokyo) in 19 subjects. The performance-intensity function was made separately for right and left ears in each subject, and both the maximum speech recognition score and the rollover index were determined.[11] The short increment sensitivity index (SISI) test was performed to examine cochlear dysfunction at 1 or 2 frequencies in 14 subjects. The level of sound stimulation was set at 20 dB above the level of the pure tone threshold at the tested frequencies. Transient evoked otoacoustic emissions (TEOAEs) and distortion product otoacoustic emissions (DPOAEs) were examined to evaluate outer hair cell function with

TABLE 1. SUBJECTS OF DETAILED AUDIOLOGICAL TESTS

Test	*Subjects*
Speech recognition test	II-1, II-5, III-1, III-3, III-9, III-15, III-19, III-21, III-22, III-24, III-25, III-26, IV-1, IV-4, IV-5, IV-6, IV-7, IV-8, IV-15
Short increment sensitivity index test	III-1, III-3, III-9, III-15, III-19, III-22, III-24, III-25, III-26, IV-2, IV-5, IV-7, IV-8, IV-15
Transient evoked otoacoustic emissions and distortion product otoacoustic emissions	II-1, III-3, III-9, III-15, III-19, III-25, III-26, IV-15
Auditory brain stem response	II-1, III-3, III-9, III-26, IV-4, IV-6, IV-8

the ILO292 Otoacoustic Emission Systems (Otodynamics, Hatfield, England) in 8 subjects. For TEOAE analysis, a nonlinear click stimulus train was used at 80 dB SPL, and the number of responses to be averaged was set at 260. The DPOAE measurement was performed at 3 points per octave across the F2 stimulus frequency range of 1,000 Hz to 6,000 Hz with an F2-F1 ratio of 1.221 and at F1 and F2 levels of 70 dB SPL. Each DPOAE result was evaluated with a DP audiogram. The auditory brain stem response (ABR) was evaluated to locate the site of the lesion in the auditory pathway with the Neuropack Σ5504 (Nihon Kohden, Tokyo) in 7 subjects. Alternating click stimulation was presented monaurally at a rate of 10/s through an earphone while the contralateral ear was masked with white noise. The responses were recorded with vertex-earlobe electrodes. A total of 1,000 sweeps were added for each measurement. Thresholds of wave I and wave V were determined, and the latencies of wave I and wave V were measured with the click stimulation presented at 90 dB normal hearing level (nHL).

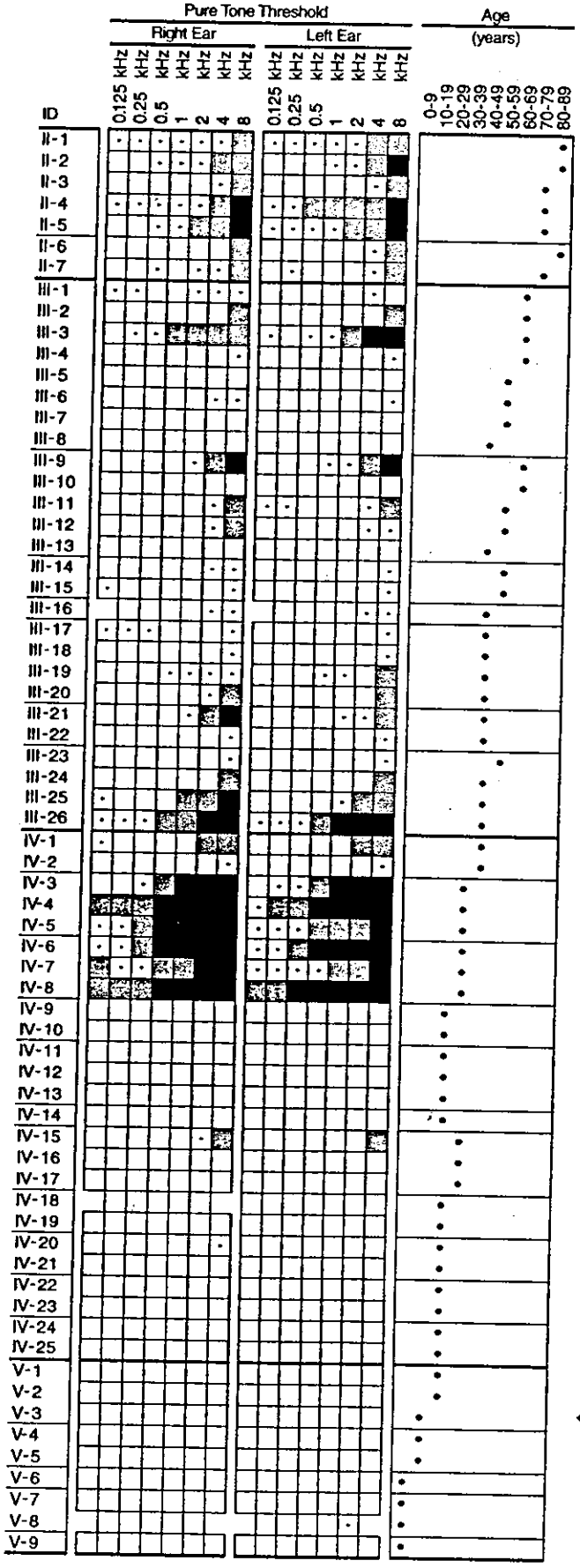
Total Mitochondrial DNA Sequencing. Total mitochondrial DNA was sequenced for 8 subjects with various degrees of hearing loss. The 8 subjects consisted of the proband (IV-6), her daughter (V-7), her mother (III-4), her grandmother (II-1), and 4 siblings (III-23, III-24, III-25, III-26). Genomic DNA was isolated from peripheral leukocytes of the subjects by conventional methods. As in a previous study,[12] to avoid nuclear pseudogene amplification, we applied the long polymerase chain reaction–based sequencing method. With 96 primer sets designed for sequencing, we sequenced the polymerase chain reaction products using the BigDye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, Foster City, California). Each reaction product was then analyzed with an ABI 3700 automated sequencer (PE Applied Biosystems) according to the manufacturer's protocol. The sequence data were compared with those in MITOMAP (http://www.mitomap.org),[13] as well as those from 200 unrelated Japanese without hearing loss.

The study protocol was approved by the Ethics Committee of the National Tokyo Medical Center, and the study was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all individuals who participated in the study.

RESULTS

Pure Tone Audiometry. The results of PTA testing in all of the subjects are summarized in Fig 2. Hearing loss was categorized with respect to the mean air-conducted pure tone thresholds at 0.5, 1, and 2 kHz ("PTA 0.5-2 kHz"),[11] by which 59% of the subjects were classified as having normal hearing (≤15 dB HL), 14% had slight hearing loss (16 to 25 dB HL), 9% had mild hearing loss (26 to 40 dB HL), 4% had moderate hearing loss (41 to 55 dB HL), 5% had moderately severe hearing loss (56 to 70 dB HL), 3% had severe hearing loss (71 to 90 dB HL), and 5% had profound hearing loss (>90 dB HL). The PTAs were symmetric in the right and left ears in the majority of the subjects, in that 56 subjects exhibited the same category of hearing loss on both sides. The remaining 11 subjects showed somewhat asymmetric hearing loss, but the categories differed by only 1 level. All subjects with hearing loss exhibited sloping or sharp sloping audiograms except for 1 subject (III-1) who had a history of noise exposure. This subject's audiogram was typical of noise-induced hearing loss (ie, increased bone-conducted thresholds at 4 kHz). The degree of hearing impairment was similar in affected subjects within the same sibling group, but differed between sibling groups.

In 41 subjects who did not report any hearing loss at the time of interview, normal hearing was detected in both ears by PTA over 0.5, 1, and 2 kHz in 32 subjects, slight or mild hearing loss in one or both ears in 8 subjects, and slight hearing loss due to otitis media in 1 subject. The age of the 8 subjects (II-3, II-6, II-7, III-2, III-15, III-17, III-20, III-23) with slight or mild hearing loss ranged from 42 to 80 years. Considering the ages and the degree of hearing loss in these 8 subjects, the lack of reported hearing loss was considered to be reasonable in these subjects. In these 41 subjects, the results of PTA at 8 kHz were analyzed in order to find out whether any subclini-



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.

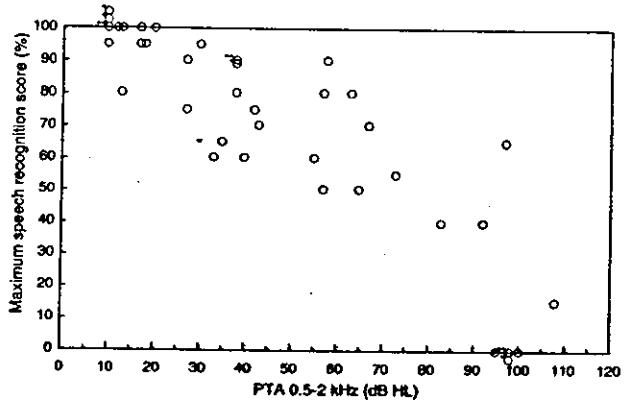


Fig 3. Maximum speech recognition score as function of mean of pure tone thresholds at 0.5, 1, and 2 kHz ("PTA 0.5-2 kHz") for each ear. For ease of visualization, overlapping symbols were moved from original position (indicated by double dots) to neighboring positions (indicated by dot).

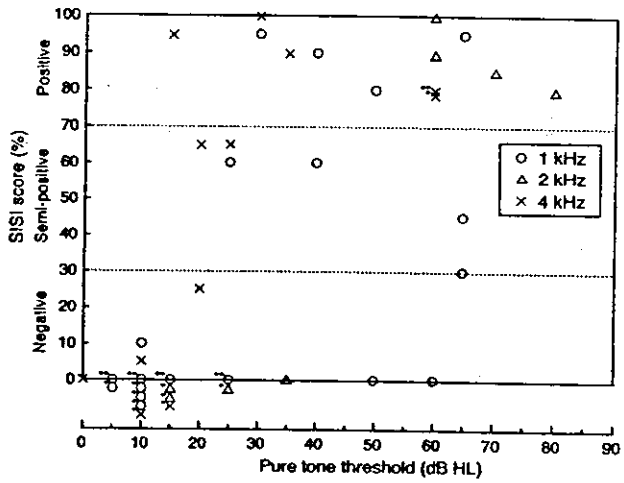


Fig 4. Short increment sensitivity index (SISI) score as function of pure tone threshold at SISI-tested frequency for each ear. Tests were conducted at 1, 2, and 4 kHz. Overlapping symbols were moved as indicated in Fig 3.

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-

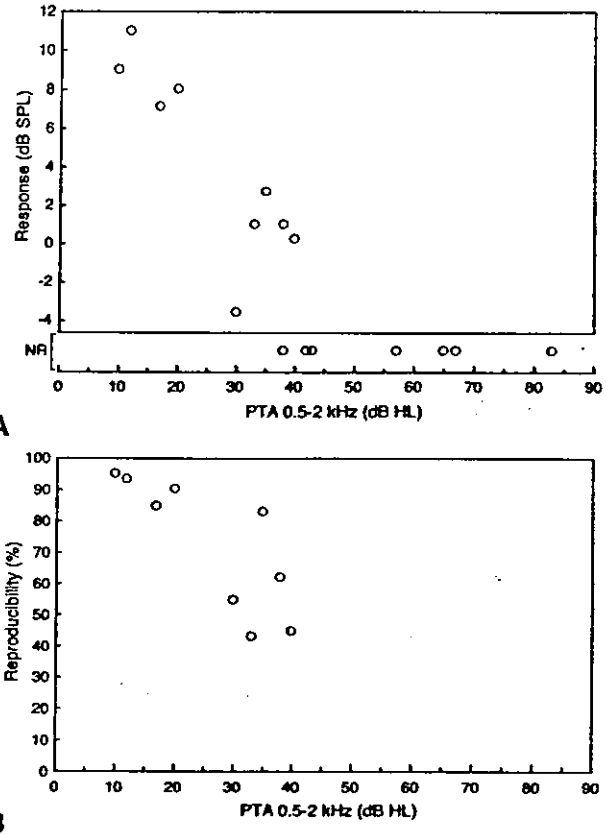


Fig 5. Response (A) and reproducibility (B) of transient evoked otoacoustic emissions as function of mean of pure tone thresholds at 0.5, 1, and 2 kHz ("PTA 0.5-2 kHz") for each ear.

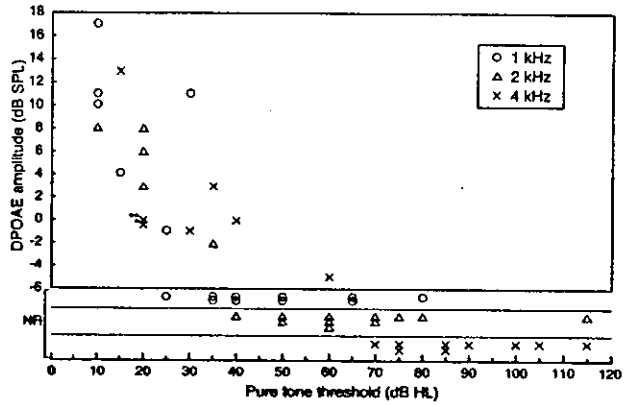


Fig 6. Distortion product otoacoustic emission (DPOAE) amplitude as function of pure tone threshold at DPOAE-tested frequency for each ear. Tests were conducted at 1, 2, and 4 kHz. Symbols between horizontal lines in lower part of Figure (indicated by NR) represent no DPOAE response. Overlapping symbols 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)* *I*	*V*	*Latency† (ms)* *I*	*V*	*PTA 2-4 kHz (dB HL)*	*Threshold* (dB nHL)* *I*	*V*	*Latency† (ms)* *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.

REFERENCES

1. Fischel-Ghodsian N. Mitochondrial deafness mutations reviewed. Hum Mutat 1999;13:261-70.

2. Prezant TR, Agapian JV, Bohlman MC, et al. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nat Genet 1993;4:289-94.

3. Hamasaki K, Rando RR. Specific binding of aminoglycosides to a human rRNA construct based on a DNA polymorph-

ism which causes aminoglycoside-induced deafness. Biochemistry 1997;36:12323-8.

4. Matthijs G, Claes S, Longo-Mbenza B, Cassiman J-J. Non-syndromic deafness associated with a mutation and a polymorphism in the mitochondrial 12S ribosomal RNA gene in a large Zairean pedigree. Eur J Hum Genet 1996;4:46-51.

5. Usami S, Abe S, Kasai M, et al. Genetic and clinical features of sensorineural hearing loss associated with the 1555 mitochondrial mutation. Laryngoscope 1997;107:483-90.

6. el-Schahawi M, Lopez de Munain A, Sarrazin AM, et al. Two large Spanish pedigrees with nonsyndromic sensorineural deafness and the mtDNA mutation at nt 1555 in the 12S rRNA gene: evidence of heteroplasmy. Neurology 1997;48:453-6.

7. Casano RAMS, Bykhovskaya Y, Johnson DF, et al. Hearing loss due to the mitochondrial A1555G mutation in Italian families. Am J Med Genet 1998;79:388-91.

8. Estivill X, Govea N, Barcelo A, 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;62:27-35.

9. Braverman I, Jaber L, Levi H, et al. Audiovestibular findings in patients with deafness caused by a mitochondrial susceptibility mutation and precipitated by an inherited nuclear mutation or aminoglycosides. Arch Otolaryngol Head Neck Surg 1996;122:1001-4.

10. Matsunaga T, Kumanomido H, Shiroma M, et al. Delineation of the phenotypic expression in a large Japanese family with A1555G mitochondrial mutation. In: Abstracts of the Twenty-Sixth Midwinter Research Meeting of the Association for Research in Otolaryngology, Daytona Beach, Fla, 2003:487.

11. Gelfand SA. Essentials of audiology. 2nd ed. New York, NY: Thieme, 2001:139-71, 257-90.

12. Akanuma J, Muraki K, Komaki H, Nonaka I, Goto Y. Two pathogenic point mutations exist in the authentic mitochondrial genome, not in the nuclear pseudogene. J Hum Genet 2000; 45:337-41.

13. MITOMAP. A human mitochondrial genome database. (http://www.mitomap.org/)

14. ISO. Acoustics: Statistical Distribution of Hearing Thresholds as a Function of Age. ISO 7029. Geneva, Switzerland: International Organization for Standardization, 2000:1-9.

15. Jerger J. Diagnostic audiometry. In: Jerger J, ed. Modern developments in audiology. 2nd ed. New York, NY: Academic Press, 1973:75-115.

16. Sininger YS, Abdala C. Physiologic assessment of hearing. In: Lalwani AK, Grundfast KM, eds. Pediatric otology and neurotology. Philadelphia, Pa: Lippincott-Raven, 1998:127-54.

17. Coats AC, Martin JL. Human auditory nerve action potentials and brain stem evoked responses: effects of audiogram shape and lesion location. Arch Otolaryngol 1977;103:605-22.

18. Selters WA, Brackmann DE. Acoustic tumor detection with brain stem electric response audiometry. Arch Otolaryngol 1977;103:181-7.

19. Nussbaum RL, McInnes RR, Willard HF. Thompson and Thompson genetics in medicine. 6th ed. Philadelphia, Pa: WB Saunders, 2001:244-9.

20. Sajjadi H, Paparella MM, Canalis RF. Presbycusis. In: Canalis RF, Lambert PR, eds. The ear: comprehensive otology. Philadelphia Pa: Lippincott Williams & Wilkins, 2000:545-57.

21. Brierley EJ, Johnson MA, Lightowlers RN, James OF, Turnbull DM. Role of mitochondrial DNA mutations in human aging: implications for the central nervous system and muscle. Ann Neurol 1998;43:217-23.

22. Tono T, Ushisako Y, Kiyomizu K, et al. Cochlear implantation in a patient with profound hearing loss with the A1555G mitochondrial mutation. Am J Otol 1998;19:754-7.

23. Norton SJ. Cochlear function and otoacoustic emissions. Semin Hear 1992;13:1-14.

24. Pandya A, Xia X-J, Erdenetungalag R, et al. Heterogeneous point mutations in the mitochondrial tRNA Ser(UCN) precursor coexisting with the A1555G mutation in deaf students from Mongolia. Am J Hum Genet 1999;65:1803-6.

25. Guan M-X, Fischel-Ghodsian N, Attardi G. Biochemical evidence for nuclear gene involvement in phenotype of nonsyndromic deafness associated with mitochondrial 12S rRNA mutation. Hum Mol Genet 1996;5:963-71.

26. Bykhovskaya Y, Shohat M, Ehrenman K, et al. Evidence for complex nuclear inheritance in a pedigree with nonsyndromic deafness due to a homoplasmic mitochondrial mutation. Am J Med Genet 1998;77:421-6.

日本臨牀　62巻 増刊号4（2004年4月28日発行）別刷

痴呆症学(3)

—高齢社会と脳科学の進歩—

特　　論

ミトコンドリア機能異常と変性性痴呆との関連

後藤雄一

特　論

ミトコンドリア機能異常と変性性痴呆との関連

Association between mitochondrial dysfunction and degenerative dementia

後藤雄一

Key words: ミトコンドリア，ミトコンドリアDNA，アポトーシス

1. 概念，定義

ミトコンドリア内には，エネルギー代謝に関する多くの酵素が局在している[1]．ミトコンドリア病とは，ミトコンドリア自体およびミトコンドリア内に存在するDNAや蛋白に異常が存在し，ミトコンドリアにおけるエネルギー産生に障害を来した疾患群を総称している．当然のことながらミトコンドリア機能障害が神経細胞に起きると痴呆症状を来す場合があるが，多くは単なる痴呆だけではなく，精神症状・てんかんなどの他の中枢神経症状を同時にもっている．

ミトコンドリア内のエネルギー代謝異常のうち最も頻度の高い電子伝達系酵素の障害は，酵素異常と臨床症状とが必ずしも1対1に対応せず，しかも個々の症例で，極めて多彩な臨床症状が障害度を違えて認められる．また，電子伝達系酵素の一部はミトコンドリアDNA(mtDNA)にコードされており，ミトコンドリア(およびmtDNA)のもつ独自の細胞生物学的特徴を色濃く反映させている[2]．

本稿では，ミトコンドリア機能異常と痴呆との関係を解説する．

2. 痴呆を来すミトコンドリア病の臨床病型

ミトコンドリア病の臨床症状は多彩である．それは，ミトコンドリアが個体の(一部の例外を除き)あらゆる細胞に存在しているために，そのミトコンドリアの障害は種々の異常を引き起こすからである．このような臨床症状の多様性や症例ごとの違いという特徴がある中で，ミトコンドリア病では比較的エネルギー依存度の高い組織や細胞が障害されやすいことは容易に理解できる．実際，エネルギー依存度の高いと考えられる中枢神経，骨格筋，心筋などはミトコンドリア病の主な罹患臓器である．

ミトコンドリア病の代表的な疾患として，慢性進行性外眼筋麻痺症候群(chronic progressive external ophthalmoplegia: CPEO)，赤色ぼろ線維・ミオクローヌスてんかん症候群(myoclonus epilepsy associated with ragged-red fibers: MERRF)，ミトコンドリア脳筋症・乳酸アシドーシス・脳卒中様発作症候群(mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes: MELAS)がある．これら3病型は主症状である中枢神経症状によって分類されているものの，実際には臨床症状を重複してもつ症例や各病型の特徴的症状に乏しい症例などが多数存在している．

3. MELASの病態と痴呆

MELASは，脳卒中様症状を主徴とするミトコンドリア病であり比較的若年で発症する(80%が20歳以前)．臨床症状は，極めて多彩である．卒中様症状を示した急性期や亜急性期に脳

Yu-ichi Goto: National Institute of Neuroscience, NCNP　国立精神・神経センター神経研究所

0047-1852/04/¥50/頁/JCLS

CTやMRIを行うと病変を認め，また症状の回復とともに画像所見も正常化するのが普通である．しかし不可逆性の変化を起こす場合もある．画像で確認できる病変部位は血管の支配領域とはかならずしも一致せず，急性経過中に病変部が増大したり，周辺部に移動したり，更には半球を越えて進展したりすることが示されている[3]．

このような病変の増大や移動，可逆性および不可逆性変化の原因として，小動脈の血管機能の障害が主体か，神経細胞自体の代謝性の変化が主体であるかの議論がある．大浜らは，MELAS患者の脳の小動脈，特に脈絡層の血管平滑筋のミトコンドリアに著明な変化のあることを報告し，ミトコンドリアアンギオパチーという呼称を提唱した[4]．その後同様な病変は，骨格筋の小動脈にも存在することが判明した．この病変は生検筋のコハク酸脱水素酵素(succinate dehydrogenase: SDH)染色で容易に検出できることから，SSV(strongly SDH-reactive blood vessel)と呼ばれ，ミトコンドリア形態異常の代表である赤色ぼろ線維(ragged-red fiber: RRF)とともに重要な病理所見と考えられる[5]．更にこの発見から同様な血管病変は全身性に存在しているであろうと考えられる．一方，脳卒中様発作の原因が神経細胞自体の変性を主体とするという考え方があるが，その場合でも細胞自体の脆弱性とともに血管透過性の亢進や浮腫性変化の関与が想定されており[3]，血管機能とミトコンドリア異常との関係は今後更に検討を有する課題である．

また，大きな脳卒中様発作を経験しないために明らかな梗塞像に似た画像所見はないが，年齢とともに大脳や小脳の萎縮が進行する症例が数多く報告されている．実際は，このような大脳や小脳の全般性の萎縮が痴呆症状と密接な関係があると想定されている．

MELAS以外の臨床病型や分類不能の症例などに痴呆症状が認められる．またAlzheimer病などの代表的な痴呆疾患においても，ミトコンドリア機能の低下がかかわるという報告もある．このような痴呆にかかわるミトコンドリア機能異常の議論は，神経細胞をはじめとする脳組織を構成する種々の細胞の病態を理解することが鍵になる．次に，ミトコンドリア機能異常から起きる細胞病態を考える．

4. ミトコンドリア異常の細胞病態(図1)

最近の細胞生物学的な研究により，ミトコンドリアは多面的な機能を有することが判明し，したがってミトコンドリア機能異常を理解するためにはこれら多面的な病態解析が必要になっている．紙面の都合でその詳細は論じられないが，主に総説を参考文献としてあげておくので，興味のある方はご参照願いたい．

a. エネルギー代謝

ミトコンドリアはそもそもエネルギーを産生する細胞小器官であるので，ミトコンドリア機能低下はATP産生低下を招く．実際に，ミトコンドリア内に存在するTCA回路，電子伝達系酵素，脂肪酸代謝酵素などの異常によりATP産生が低下する．

その中で電子伝達系酵素異常症は特異な位置を占めている．その理由は，構成する酵素サブユニットの一部がmtDNAにコードされており，mtDNAの特殊性が色濃く反映しているからである．mtDNAは，約16kbの環状2本鎖DNAであり，ミトコンドリア内で蛋白を合成するための2個のリボソームRNA，22個の転移RNAと，電子伝達系酵素群のサブユニットの一部を構成する蛋白を計13個コードしている．重要なことは，1つの細胞内に数十-数百個存在する個々のミトコンドリア内に，mtDNAは5-10個ずつ存在しているため，1細胞では数百-数千個存在することになる(マルチコピー性)．また，核DNAに比べ，変異の起こりやすさが5-10倍程度とされている(易変異性)．そして，受精の際にミトコンドリアはすべて卵に由来することから，mtDNAも母からしか子に伝わらない(母系遺伝形式)．

実は，このような性質をもつmtDNAは，体細胞で種々の変異(欠失，点変異)を起こし，それがミトコンドリア機能に少なからず影響を与える可能性が示されている．言うなれば，mtDNAは個体内で一律同じ塩基配列ではなく，

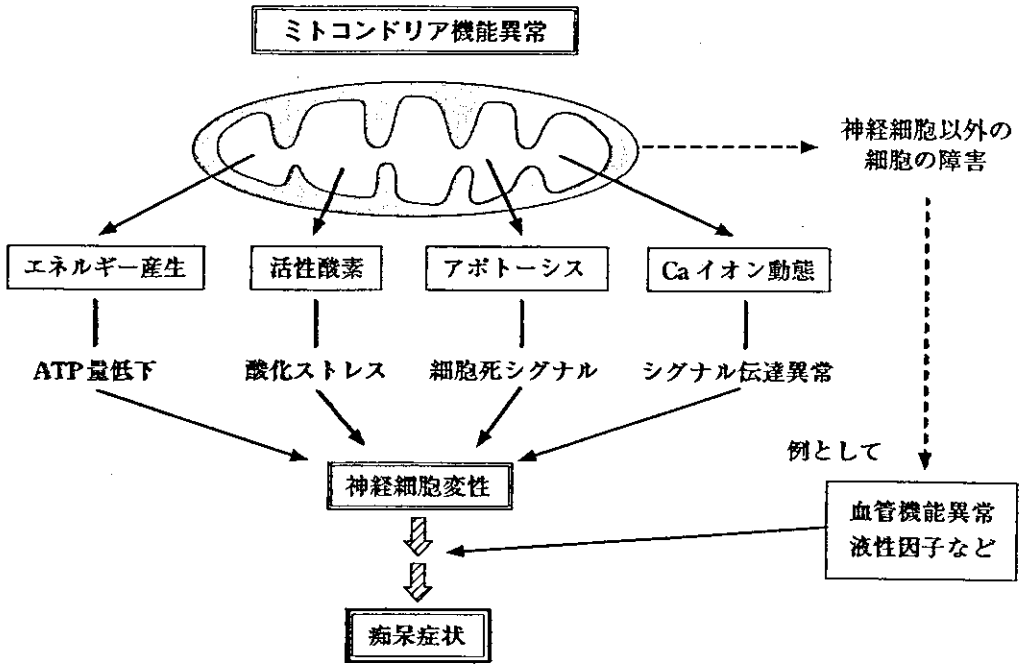


図1 ミトコンドリア機能異常の細胞病態

種々の割合で変異や多型をもったものとして存在しており，細胞内のミトコンドリア機能やひいては細胞機能に影響を与えていると考えるべきである．Alzheimer病患者と年齢対照者の脳を用いた研究で，患者群では標準塩基配列と比較すると明らかに変異や多型が多いと報告されている[6]．

b. 活性酸素の産生

ミトコンドリアは細胞内で活性酸素を産生する主要な部位である．特に，電子伝達系酵素機能が低下すると，活性酸素の産生が増加することが知られている．その結果，酸化蛋白質，8-OhdGなどの酸化DNA，ヒドロキシノネナールなどの過酸化脂肪が生成され，細胞障害が生じると考えられている[7]．

c. アポトーシス

アポトーシスの場としてのミトコンドリアの重要性は，既に確固たるものになった[8]．チトクロームcの放出をはじめとして，アポトーシスのキープレーヤーがミトコンドリアに局在する蛋白であったり，ミトコンドリアに移動してくる蛋白であったりしている．また，ミトコンドリア膜電位の崩壊が細胞死の直接の引き金になっていることも明らかにされている．このアポトーシスに関連する現象，関係する物質の研究は，痴呆を理解するために必要な病態の理解とともに，治療法開発の重要なターゲットである．

d. カルシウムイオン動態

細胞内のカルシウムイオン濃度の変化は，種々のカルシウムイオン濃度依存性蛋白の機能変化を介して細胞に影響を与える．ミトコンドリアは急激な濃度変化に対応して，カルシウムイオンを貯蔵する働きももつ．最近はアポトーシスとの関連でも，カルシウムイオン濃度の浮動がアポトーシスを増強することなどが報告され，その重要性が示されている[9]．

■文　献

1) Alberts B, et al: Energy conversion: mitochondria and chloroplasts. In: Molecular Biology of the Cell, 4th ed, p767-830, Garland Publishing, New York, 2002.
2) Larsson N-G, Clayton DA: Molecular genetic aspects of human mitochondrial disorders. Annu Rev

Genet 29: 151–178, 1995.
3) Iizuka T, et al: Slowly progressive spread of the stroke-like lesions in MELAS. Neurology 61: 1238–1244, 2003.
4) Ohama E, et al: Mitochondrial angiopathy in cerebral blood vessels of mitochondrial encephalomyopathy. Acta Neuropathol 74: 226–233, 1987.
5) Hasegawa H, et al: Strongly SDH-reactive blood vessels in muscle from patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. Ann Neurol 29: 601–605, 1991.
6) Lin MT, et al: High aggregate burden of somatic mtDNA point mutations in aging and Alzheimer's disease brain. Hum Mol Genet 11: 133–145, 2002.
7) 康 東天：ミトコンドリアにおける酸化ストレスの発生と防御機構．日本臨牀 60(増刊：ミトコンドリアとミトコンドリア病)：181–186, 2002.
8) Zamzami N, Kroemer G: The mitochondrion in apoptosis: how Pandra's box opens. Nat Rev Mol Cell Biol 2: 67–71, 2001.
9) Pacher P, Hajnóczky G: Propagation of the apoptotic signal by mitochondrial waves. EMBO J 15: 4107–4121, 2001.

Overview●

特集●ミトコンドリア病

ミトコンドリア病の分子メカニズム

後藤雄一

Molecular Medicine Vol.41 No.3 2004（p.266～268の別刷）　中山書店

OVERVIEW

ミトコンドリア病の分子メカニズム

後藤雄一
国立精神・神経センター神経研究所疾病研究第二部

●ミトコンドリア機能の多様性

ミトコンドリアは細胞内小器官（オルガネラ）の一つであり，細胞に必要なエネルギーを産生する働きをもつ．しかしながら最近の研究によって，ミトコンドリアは単にエネルギー産生の場であるだけでなく，カルシウムイオンを取り込んで細胞内イオン濃度を調節し，さらにアポトーシスにかかわる因子の局在する場としても重要であることがわかってきている．

ミトコンドリアは，三大栄養素である糖，脂肪，タンパク質がエネルギーとして代謝される場としてよく知られている．クエン酸回路，β酸化系などを経て最終的に電子伝達系を経て効率的にエネルギー産生が行われる．そこにかかわる酵素タンパク質は優に百を越える数になる．しかも，細胞内のエネルギー必要量をモニターしながら，ミトコンドリア内のエネルギー産生を増加させたり減少させたりするシステムが存在するはずであるが，その詳細もまだわかっていない．

このミトコンドリア内のエネルギー産生には，活性酸素が不可避的に生じることをあげなくてはならない．細胞の維持や増殖に必要なエネルギーを産生する働きがありながら，一方で細胞に傷害を引き起こす活性酸素を生むミトコンドリアは，細胞の生理学的状態とも病理学的状態とも深くかかわっていることは想像に難くない．

さらに最近になって，アポトーシスとミトコンドリアとの密接な関係が明らかにされてきた．アポトーシスのプロセスを進めるシグナルがミトコンドリアに達し，シトクロムcがミトコンドリアから放出されて細胞死の過程が一気に進行する．

このようにエネルギー代謝，シグナル伝達，細胞死などと深くかかわっているミトコンド

Molecular pathomechanisms of mitochondrial diseases

Yu-ichi Goto
ごとう・ゆういち　1980年北海道大学医学部卒業．小児科医として研修を受けた後，88年国立精神・神経センター神経研究所での研究を始める．スタンフォード大学医学部への留学の後，94年に国立精神・神経センター神経研究所微細構造研究部室長，99年同研究所疾病研究第二部部長（現在に至る）．現在の研究テーマ：ミトコンドリア病の病因・病態・治療研究，精神遅滞の分子遺伝学的研究．

リアは，細胞を維持させる方向へと働くのか，細胞を消滅させる方向に進ませるのかを，いったいどのように決めているのであろう．この重大な選択に関する詳細な機構は明らかになっていない．小説「パラサイト・イヴ」のように，ミトコンドリアに意思があると考えたくなるのもうなずけるほどである．

●ミトコンドリア機能異常の分子メカニズム

ミトコンドリア病とは，ミトコンドリア機能異常を本態とする病気の総称である．ミトコンドリア機能の多様性から考えると，その病的状態である機能異常もエネルギー産生低下，活性酸素による細胞傷害，シグナル伝達異常，アポトーシスの変調など，質的に多様になるのは当然である．しかも，ミトコンドリア機能異常がどのように細胞に傷害を与え，その影響が組織や臓器レベルでどうなるかを知ることが病態の理解につながり，新しい治療法や予防法へとつながる研究となる．

実は，これまでのミトコンドリア病研究はエネルギー産生低下にかかわる研究が主体であった．近年，ミトコンドリアにかかわるタンパク質をコードする核遺伝子やミトコンドリア内に存在するミトコンドリアDNAの変異がミトコンドリア病患者で見出され，その分子レベル，細胞レベルの効果が詳細に検討されている．本特集ではその点を解説していただくことにした．そして，病気のメカニズムを知るには，まず分子，細胞レベルの機能異常を知ることが前提であり，これらの知識をもってより大きいレベルである組織，臓器での機能異常を理解することが重要である．

ミトコンドリアDNAは，核DNAと違って1細胞内に存在するコピー数が多く（核DNAは1対，ミトコンドリアDNAは数千コピー），その複製の仕方も核DNAと大きく異なるとされてきた．しかしながら，最近の研究で，これまで信じられてきたミトコンドリアDNAの特異な複製の仕方に疑義が出された．ミトコンドリア異常によって起こるミトコンドリア病を理解するためにもこの問題は重要である（安川論文）．

ミトコンドリアDNA変異で最も頻度の高いのは3243位の点変異である．この変異はミトコンドリアDNAのロイシンtRNA上に存在し，若年者に発症する脳卒中様症状を特徴とするMELAS（mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes）と関係が深い．また，糖尿病患者の約1％がこの3243変異によるものであることが日本を含め各国の研究で明らかにされている．その3243変異がもたらす効果として，ロイシンtRNAのアンチコドン修飾異常が発見された．この発見は，治療の可能性を含めて，さらなる研究の進展が期待できる（鈴木論文）．

また，ミトコンドリアDNAは顕著な病的効果を表す変異以外に，正常者でも見出される多型がよく知られている．この多型は，分子進化学，法医学などにも応用されているが，実は老化や神経変性疾患，糖尿病，心筋症など，ミトコンドリア病の一症状と考えられる病気の疾患感受性を上げる効果があるのではないかと考えられている．このような観点からのミトコンドリアDNA研究について解説していただいた（田中，武安，福ならびに藤田論文）．

また，ミトコンドリアDNA変異の効果を細胞レベルばかりでなく，組織・臓器レベルでの効果を研究するには，モデル動物が必要である．ミトコンドリアDNA変異をもつ動物を世界に先駆けて作製した筑波大学の林純一教授のグループに解説をいただく（中田，林論文）．

最後に，ミトコンドリアの機能異常はミトコンドリアDNA異常ばかりでなく，核DNA上に存在する数多くの遺伝子の変異でも惹起されることが明らかにされている．特に，ミトコンドリアの生合成，維持にかかわる因子はすべて核DNA上に存在しており，これらの遺伝子変異がミトコンドリアDNAの異常をきたすことで二次的にミトコンドリア機能異常をきたす病態メカニズムが徐々に明らかにされてきている．この核-ミトコンドリア相互作用とその破綻についても，解説をお願いした（小牧，後藤論文）．

●ミトコンドリア病研究のもたらすもの

ミトコンドリアは，エネルギー産生，シグナル伝達，細胞死などの生物現象に深くかかわることで，細胞の機能や運命を決めている．これら複雑で多様な機能をもつミトコンドリアの異常によって起こるミトコンドリア病を研究することは，細胞レベルの基本的生物現象を理解する絶好の機会を得ることになる．

一方，ミトコンドリア機能異常は全身のいろいろな臓器の症状をきたすことが知られており，その病態への関与の軽重もさまざまである．ミトコンドリア機能異常が病因の主体である，いわゆるミトコンドリア病と称されている疾患群ばかりでなく，老化や多因子性遺伝病（アルツハイマー病，糖尿病など）においても，ミトコンドリア機能異常が重要な因子としてかかわっている可能性が高い．

以上のように，ミトコンドリアおよびミトコンドリア病の研究は，細胞生物学などの生物学研究と広範囲の疾患を対象とする医学研究の両者に大きなインパクトを与えるものである．日本ミトコンドリア研究会などの場を最大限利用して，基礎研究者と臨床研究者が共同して研究を推進させていくことが望まれる．

ミトコンドリア脳筋症の病態と治療への展望

後藤　雄一

神経治療学　第21巻　第5号　別刷
平成16年9月25日発行
Reprinted from Neurological Therapeutics, Vol. 21, No. 5, p. 521-528, September 2004

ミトコンドリア脳筋症の病態と治療への展望*

後藤　雄一**

Key Words: mitochondrial encephalomyopathy, complex I, anticodon modification, Mito-Mice, L-arginine

はじめに

ミトコンドリア脳筋症（MEM）は，1962年のLuftらによる疾患概念の提唱にはじまる．生化学的，形態学的手法に加えて，1988年のHoltらによるミトコンドリアDNA（mtDNA）欠失の発見から分子遺伝学的手法が加わり，病因・病態に関する研究が大きく進展した．最近は，核DNA上に存在するミトコンドリア病にかかわる遺伝子変異が次々と同定され，その知見に基づいた詳細な病態・治療研究が進められている．さらに，ミトコンドリアが細胞内エネルギー産生以外に，酸化ストレス・アポトーシス・細胞内カルシウム動態に重要な役割を持つことが明らかにされ，MEMばかりでなく，種々の神経変性疾患（Alzheimer病，Parkinson病，Huntington病など）の病態における修飾因子としてのミトコンドリア機能異常が注目されてきている．

本稿では，MEM研究に関する最近の進歩を解説する．

I．病因に関する新しい知見

1．核DNA上の遺伝子変異

a．電子伝達系酵素を構成するサブユニットの遺伝子

MEMの原因は，ミトコンドリアDNA変異と核DNA上の遺伝子変異である（**Fig. 1**）．最近の病因研究での進歩は，核DNA上に存在する遺伝子変異の発見があげられる（**Table 1**）．まず，電子伝達系酵素複合体の核DNA由来のサブユニット遺伝子変異として，複合体Iの*NDUFV1*，*NDUFS4*，*NDUFS7*，*NDUFS8*，*NDUFS2*，*NDUFS1*の6個に発見された[1~6]．複合体Iの核由来サブユニットは30数個知られており，これ以外のサブユニット遺伝子の変異が存在することは否定できない．しかし，すべての複合体I欠損症に対して，30個以上の遺伝子を網羅的に検索することは困難であり，今のところ遺伝子診断に臨床応用することはできていない．これらの多くは，生化学的には複合体I欠損を示し，臨床的にはLeigh脳症，心筋症があるのが特徴である．また，あるLeigh脳症患者では複合体IIのフラボプロテインサブユニットをコードしている*SDHA*遺伝子の変異が報告された[7~9]．

b．酵素複合体の集合にかかわる因子の遺伝子

電子伝達系酵素複合体サブユニットそのものではなく，集合にかかわる因子の遺伝子異常も報告されている．その代表は，複合体IV（チトクロームc酸化酵素：COX）欠損を伴うLeigh脳症で認める*SURF-1*変異である．この遺伝子産物の機能はまだ明らかにされていないが，欠損すると

* The Prospects for Pathogenesis and New Therapy of Mitochondrial Encephalomyopathy.

** 国立精神神経センター　Yu-ichi Goto: National Institute of Neuroscience, National Center of Neurology and Psychiatry