Early-Onset Sensorineural Hearing Loss and Late-Onset Neurologic Complaints Caused by a Mitochondrial Mutation at Position 7472

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Objectives: To detect a mitochondrial mutation responsible for maternally transmitted hearing loss with late-onset neurologic features in a 3-generation Dutch family, and to describe the hearing loss, associated symptoms, and vestibular dysfunction.

Patients and Methods: All maternally related family members (n = 69) were investigated using standard audiometry. In a selected group, vestibulo-ocular examinations and additional neurologic and ophthalmologic examinations were performed. Twenty milliliters of venous blood was taken from all participants for genetic studies.

Setting: University medical center.

Results: All maternally related individuals carried an extra C at position 7472 of the mitochondrial genome. Hearing loss was the only symptom or presenting symptom in most family members and most pronounced at higher frequencies. Hearing loss at lower frequencies was demonstrated in individuals 10 years and older. Most patients had vestibular hyperreactivity and were susceptible to motion sickness, suggesting vestibulocerebellar dysfunction. Neurologic complaints were infrequent and presented by older individuals; however, numerous enlarged mitochondria were found in a muscle biopsy specimen of an individual with hearing impairment but without neurologic symptoms.

Conclusions: Respiratory chain dysfunction should be considered as a possible cause of progressive sensorineural hearing loss. More research into the causes of high-frequency impairment should be considered, especially when sensorineural hearing loss, syndromal or nonsyndromal, is exclusively maternally transmitted. Maternal transmission of hearing impairment can also be valuable in the diagnosis of unclear neurologic syndromes.


In recent years, sensorineural hearing loss (SNHL) has been recognized as one of the symptoms or even the only abnormal feature in maternally inherited disorders. Most inherited mitochondrial disorders come to expression in tissues with high energy requirements such as nerves and muscles. Neurologic and ophthalmologic manifestations are the prominent features. In addition, the cochlea is highly dependent on respiratory chain metabolism. The estimated incidence of SNHL in these multisystem disorders is approximately 70%.

Mitochondrial DNA (mtDNA) differs in many aspects from nuclear DNA. The mtDNA is small; it contains 16 569 base pairs in a closed circular molecule and is inherited exclusively via the ovum. In most inherited mtDNA diseases, individuals carry 2 distinct types of mtDNA, a condition that is known as heteroplasmy. Phenotypic expression depends on the proportion of transmitted mutant mtDNA and the energy requirement of the tissue. The nuclear DNA has a major role in mitochondrial metabolism because it codes for most of the more than 70 distinct polypeptides involved in the respiratory chain. The involvement of nuclear genes in mitochondrial diseases has already been demonstrated.

High-frequency hearing loss in combination with type 2 diabetes mellitus associated with the mitochondrial A to G substitution at nucleotide 3243 has been described. Tiranti et al report a maternally inherited disorder with SNHL as the first and most consistent symptom; in some family members, SNHL was the only manifestation of the disease. Ataxia, dysarthria, and focal myoclonus were present in a more advanced stage of the disease.

Sensorineural hearing loss with variable age of onset was recently reported in 2 different families as the only manifesta-
PATIENTS AND METHODS

The medical histories of the patients were documented with special attention to hearing loss, neurologic symptoms, visual impairment, diabetes mellitus, and ototoxicity. A general oto-
logic examination, pure-tone audiometry, and analysis of pre-
vious audiograms were performed. In selected cases, neuro-
logic and ophthalmologic examinations were performed.

Pure-tone audiograms were obtained in a sound-
treated room with an interacoustic AC40 audiometer cali-
brated to International Standards Organization 389 accord-
ing to the International Standards Organization 8253-1
standard.15 Air and bone conduction thresholds were
measured in decibels of hearing level. The method of In-
ternational Standards Organization 7029 was followed to
calculate the 95th percentile (P95) threshold values for pres-
byacusis for each patient at each frequency.16 Two individ-
uals (III1 and III9) were investigated at their home with a
portable audiometer (Madsen DSA 84, Assens, Copen-
hagen, Denmark). We measured selected partners to ex-
clude the possibility of genetic transmission of deafness from
a different family.

Vestibular examinations were performed for individu-
als with a high Fletcher index (ie, average threshold in deci-
bels at 1000-2000-4000 Hz) of more than 25 dB. All of them
were questioned specifically about any symptoms of dizzi-
ness or motion sickness. Vestibular tests were performed in
the dark while the patient’s eyes were open. Eye move-
ments were recorded by means of direct-current electro-
oculography. Gaze positions were tested to see if there was
any gaze-evoked nystagmus. Saccade metrics were evalu-
ated for 20° calibration saccades. Smooth pursuit was tested
with a sinusoidal stimulus (20º/peak velocity). Optoki-
netic nystagmus was elicited with shadow bands (width and
separation, 7.5°) projected onto a hemicylindrical screen
(90°×110°) at constant velocities of 40° and 60°/s. The ves-
tibulo-ocular reflex was tested with velocity steps at a fre-
quency of 90°/s using a rotary chair and caloric stimulation.
The cervico-ocular reflex was tested in 1 case of vestibular
areflexia by sinusoidal body rotation under the head, which
was fixed in space (the test was performed in the dark while
the patient’s eyes were open), at a frequency of 0.1 Hz and
an amplitude of 30°. The results of this genetic study will be presented elsewhere in detail.

A specimen of the proband’s (III9) quadriceps muscle
obtained at the age of 68 years was processed for histo-
chemical and biochemical findings. The oxidation rates for
pyruvate and malate and the production of adenosine tri-
phosphate and creatinine phosphate of the intact mito-
chondria were determined in a 600g supernatant of the fresh
muscle. The activities of the enzyme complexes of the res-
piratory chain and pyruvate dehydrogenase complex were
measured in the supernatant from fresh muscle according
to the methods of Fischer et al.18

INDEX CASE

The proband (III10) was diagnosed as having progres-
Siv e bilateral hearing impairment with tinnitus and diz-
ness at the age of 18 years. His latest examination, at
the age of 68 years, revealed a hearing loss of 84 dB high
Fletcher index that was most pronounced at higher fre-
quencies. Other symptoms included angina pectoris and
impaired renal function, which was probably due to late-
onset diabetes mellitus (serum creatinine, 203 μmol/L [2.7
mg/dL]; normal range, <60-110 μmol/L [<0.8-1.4 mg/
dl]); serum urea nitrogen, 16.7 mmol/L [46.8 mg/dL]; nor-
mal range, <3-7 mmol/L. [<8.4-19.6 mg/dL].

NEUROLOGIC EXAMINATION

An examination of the proband revealed an ataxic gait,
severe dysarthria, truncal and limb ataxia, dysdiado-
chokinesis, and motor and sensory polyneuropathy in both
arms and legs. Exercise tolerance had decreased se-
verely from the age of 40 years, when neurologic symp-
toms had developed. Recent magnetic resonance imag-
ination of disease caused by the mitochondrial T7445C mu-
tation.10,11

The mutation responsible for susceptibility to
aminoglycosides (12S ribosomal RNA-A1555G) was later
detected in an isolated family that showed maternal trans-
mision of deafness.12-14

We describe herein a large 3-generation Dutch fam-
ily with progressive SNHL. All maternally related mem-
bers carried the C inserted at base 7472 mutation. For
many family members, hearing loss was assessed with ser-
ial audiometry. The proband, who was the oldest fam-
ily member, developed neurologic signs and symptoms
that were similar to those reported in mitochondrial en-
cephalomyopathies.5

RESULTS

CLINICAL STUDY OF THE FAMILY

All individuals born to the hearing-impaired great grand-
mother (II1) were audiometrically assessed. The pedi-
gree of 105 living individuals demonstrates that the ma-
ternal trait of deafness is present in 9 different sibships
(Figure 1). Two children (III1 and III9) experienced a
decrease in hearing ability reported by anamnesis in the
first week of treatment with dihydrostreptomycin for tu-
berculous spondylitis. A similar decrease of 50 dB, which
could be evaluated audiometrically, occurred in a 4-year-
cold cousin (IV25).
ing of the brain showed advanced atrophy of the superior cerebellar vermis and multiple subcortical white matter lesions in the semiomial center. Serum myoglobin concentrations were slightly elevated (108 g [normal range, <100 g/l]) and creatine kinase levels were normal. Findings from a neurologic examination of 1 younger brother (III12) were normal. An older brother (III5) had died within 1 year of an unspecified rapidly progressive neurologic disease that had been diagnosed as possible amyotrophic lateral sclerosis. Other family members had no history of neurologic signs or symptoms. Various psychiatric disorders, such as paranoid schizophrenia, psychosis, and depression, had occurred over a long period in the great grandmother and some of her children.

OPHTHALMOLOGIC EXAMINATION

No tapetoretinal degenerations were found on examination of 4 individuals (III10, IV26, IV31, and IV49); however, all of their lenses were not as clear as was expected for their age. Noninsulin-dependent hyperglycemia had caused the cotton-wool lesions of diabetic retinopathy in the index case (III10). His 2 sisters (III13 and III19) are being treated for insulin-dependent hyperglycemia that started at the age of approximately 45 years.

MUSCLE BIOPSIES

Findings from light microscopic examinations of biopsy specimens were normal. An ultrastructural examination revealed numerous enlarged mitochondria with paracrystalline inclusions in the proband (III10) and 1 of his brothers (III12) (Figure 2). The muscle respiratory chain enzyme complexes in the proband showed a marked reduction in activities specific to complex I (7.2 µmol/min per milligram of mitochondrial protein; normal range, 24-87 µmol/min per milligram) and complex IV (19 µmol/min per milligram; normal range, 68-347 µmol/min per milligram).

HEARING

Audiometry in 32 of 69 individuals demonstrated a high hearing threshold relative to the P95 presbyacusis values. One individual (IV40) who was not a maternal relative and another (IV41) who was both experienced trauma. Five family members whose previous audiograms had been obtained elsewhere showed progressive high-frequency hearing impairment. All of those individuals had a negative history for acquired causes of hearing impairment (noise exposure, trauma, infectious ear disease, or ototoxic drugs).

Two subjects (III3 and III8) were excluded from analysis because they only showed presbyacusis. Regression analysis of serial audiograms seemed to suggest that high-frequency impairment (4-8 kHz) may already have been present at birth; however, hearing impairment at the lower frequencies (0.25-2 kHz) occurred in some individuals younger than 10 years and was almost always present in those younger than 20 years.
HYPERVENTILATION SYNDROME. Another patient (V 24) showed vestibular areflexia with an enhanced vertigo or dizziness, had normal vestibular responses. One noted unilateral caloric hyporeflexia. The progression of hearing impairment varied from 26% (V24) to 100% (III12, III19, IV7, IV25, and IV31). In most individuals with hearing impairment (>90%), heteroplasmia varied from 80% to 100%. It seemed that the widest range of heteroplasmia was in the youngest individuals with the lowest level of hearing impairment.

Disorders with mitochondrial inheritance are characterized by a progressive course and a broad spectrum of abnormalities. Most mutations occur in highly conserved tRNA genes. Homoplasmic mutations in infancy are generally manifested as fatal pediatric diseases. Only 2 mutations in the mitochondrial genome, the A1555G and T7445C mutations, have been reported in association with nonsyndromic hereditary deafness. In 2 unrelated families with the T7445C mutation, the incidence of deafness varied considerably. Environmental factors, heteroplasmia, and the presence of an extra C nucleotide at position 7472 have been suggested to explain this phenomenon. The C insertion in asymptomatic carriers raises the possibility of an additional cause of hearing loss. The T7445C mutation is situated in the same tRNA gene as the Cins7472 mutation; however, it causes a different disorder. The susceptibility to aminoglycosides related to a remarkable decrease in hearing ability in individuals initially raised our suspicions of the A1555G mutation.

The analysis of hearing loss in this family shows a progression at all frequencies similar to that previously described in another family with maternally inherited hearing impairment. However, in the family we studied, hearing impairment developed earlier and was more pronounced in younger individuals at higher frequencies. Early predominance of involvement of the high frequencies was reported previously, but no report suggested a congenital component, which we were inclined to suggest only on the basis of an analysis of the most recent audiograms. However, longitudinal analysis of hearing impairment data disclosed nonlinear progression with onset of hearing impairment in the first or second decade, and presumably more rapid progression in the initial stage of the disease.

Methodological considerations and implications for genetic counseling are discussed. A Southern blot analysis of the Dutch family (peripheral blood leukocytes; muscle biopsy index case, individual III10) revealed no large rearrangements. Polymerase chain reaction amplification and restriction enzyme analysis excluded the presence of the T7445C and A1555G mutations. However, in all maternally related relatives, we found the insertion of an extra C at nucleotide position 7472 in the tRNA(Ser)UCN gene. This mutation was not present in 100 healthy controls. Because the normal mtDNA PCR product remained visible after restriction enzyme analysis in almost all family members, heteroplasmacy was confirmed. Sequence analysis of the PCR fragment containing the Cins7472 mutation confirmed the presence of a seventh C at nucleotide position 7472.

No correlation was found between the high Fletcher index, age, and degree of heteroplasmacy. The percentage of heteroplasmacy varied among family members from 26% (V24) to 100% (III12, III19, IV7, IV25, and IV31). In most individuals with hearing impairment (>90%), heteroplasmacy varied from 80% to 100%. It seemed that the widest range of heteroplasmacy was in the youngest individuals with the lowest level of hearing impairment.

**COMMENT**

Disorders with mitochondrial inheritance are characterized by a progressive course and a broad spectrum of abnormalities. Most mutations occur in highly conserved tRNA genes. Homoplasmic mutations in infancy are generally manifested as fatal pediatric diseases. Only 2 mutations in the mitochondrial genome, the A1555G and T7445C mutations, have been reported in association with nonsyndromic hereditary deafness. In 2 unrelated families with the T7445C mutation, the incidence of deafness varied considerably. Environmental factors, heteroplasmacy, and the presence of a second nuclear locus have been suggested to explain this phenomenon. The C insertion in asymptomatic carriers raises the possibility of an additional cause of hearing loss. The T7445C mutation is situated in the same tRNA gene as the Cins7472 mutation; however, it causes a different disorder. The susceptibility to aminoglycosides related to a remarkable decrease in hearing ability in 3 individuals initially raised our suspicions of the A1555G mutation.

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Analyses of hearing impairment in the youngest individuals at risk are obviously needed to evaluate the early condition and course of their hearing loss. Observations of the higher frequencies (8-18 kHz) by Elverland and Torbergsen²⁰ suggest detection of high-frequency impairment in young children at risk.

The proband’s optokinetic response deterioration with sustained stimulation at 60°/s can be interpreted as another type of exercise fatigue. The major vestibular finding in this study was the high prevalence of vestibular hyperactivity in 13 of 20 individuals with hearing impairment. Dizziness and susceptibility to motion sickness were found in 3 of the 13 patients with vestibular hyperactivity, although susceptibility to motion sickness was also found in 1 individual without vestibular hyperactivity but with unilateral caloric hyporeflexia and asymmetry of the vestibulo-ocular reflex.

Vestibular hyperactivity has been described in association with multiple sclerosis, vestibulocerebellar dysfunction, hyperventilation syndrome, and vestibulocerebellar dysfunction in children with congenital hyperbilirubinemia. The latter finding illustrates the vulnerability of the vestibulocerebellum to metabolic disturbances, presumably owing to the high energy requirement. In the Gunn rat, Purkinje cells that are inhibitory

![Figure 3: Regression analysis of the most recent audiogram in 28 family members with maternally inherited sensorineural hearing loss. The intercept of approximately 15 dB at 4 to 8 kHz seems to suggest the possibility of congenital hearing impairment. The intercept of approximately 8 years at 0.25 to 2 kHz suggests postnatal onset of hearing impairment.](image)

![Figure 4: Trend analysis of serial audiograms in 11 individuals with sufficient audiological data. In particular, observations at the lower frequencies (left) suggest the possibility of postnatal onset and initial nonlinear progression of hearing impairment. SNHL indicates sensorineural hearing loss.](image)
to vestibular nuclear neurons are damaged by congenital hyperbilirubinemia.23

The prevalence of neurologic abnormalities seems to be low among the individuals with hearing impairment in this family. However, the index case exhibited characteristics of a mitochondrial encephalopathy, as his deceased brother (III5) probably did. Another younger brother of the proband with hearing impairment displayed enlarged mitochondria and paracrystalline inclusions on muscle biopsy. Some depressive disorders have been found among members of a family with multiple deletions of mtDNA.24

The Cins7472 mutation was first detected in a small Sicilian family with hearing impairment, in which members demonstrated local myoclonus and neurologic symptoms at an early age.25 Both index cases had striking atrophy of the superior cerebellar vermis and showed similar light microscopic and ultrastructural findings on muscle biopsy. Subcortical white matter lesions were not described before.

Mitochondrial inheritance might explain the higher prevalence of hearing impairment in mothers with type 2 diabetes mellitus. The A3243G mutation was detected in association with SNHL as well as type 2 diabetes mellitus. Type 2 diabetes mellitus usually appears before SNHL, but may also present in the third or fourth decade of life. It may be that the insertion of an extra C predisposes individuals to late-onset type 1 diabetes mellitus, as was observed in this family. However, this possibility needs further investigation.

Individuals with a respiratory chain disease usually present with such a wide variety of signs and symptoms that it may seem almost impossible to establish a syndromal diagnosis. It appears that hearing impairment, ocular myopathy, and maternal inheritance are most helpful in establishing the diagnosis.23

Identification of the mitochondrial nature of the neurologic disorder in the proband occurred only after the evaluation of the maternal inheritance pattern of hearing impairment. These identifications are particularly relevant to genetic counseling, although heteroplasmy can make proper counseling difficult.

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