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.1-4 In addition, the cochlea is highly dependent on respiratory chain metabolism. The estimated incidence of SNHL in these multisystem disorders is approximately 70%.3

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 heteroplasy. 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.6,7

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.8 Tiranti et al9 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, dystonia, 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-

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PATIENTS AND METHODS

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

Pure-tone audiograms were obtained in a sound-treated room with an interacoustic AC40 audiometer calibrated to International Standards Organization 389 according to the International Standards Organization 8253-1 standard. Air and bone conduction thresholds were measured in decibels of hearing level. The method of International Standards Organization 7029 was followed to calculate the 95th percentile (P95) threshold values for presbyacusis for each patient at each frequency. Two individuals (III1 and III9) were investigated at their home with a portable audiometer (Madsen DSA 84, Assens, Copenhagen, Denmark). We measured selected partners to exclude the possibility of genetic transmission of deafness from a different family.

Vestibular examinations were performed for individuals with a high Fletcher index (ie, average threshold in decibels at 1000-2000-4000 Hz) of more than 25 dB. All of them were questioned specifically about any symptoms of dizziness or motion sickness. Vestibular tests were performed in the dark while the patient’s eyes were open. Eye movements 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 evaluated for 20° calibration saccades. Smooth pursuit was tested with a sinusoidal stimulus (20°/s peak velocity). Optokinetic nystagmus was elicited with shadow bands (width and separation, 7.5°) projected onto a hemicylindrical screen (90° x 110°) at constant velocities of 40° and 60°/s. The vestibulo-ocular reflex was tested with velocity steps at a frequency 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°. Methods, results, and computer analysis have been previously described.

Blood samples were taken from the patients for DNA extraction. Large rearrangements in mtDNA were studied with Southern blot analysis. Because hearing loss was the main clinical symptom in all family members, the T7445C, A1555G, and Cins7472 mutations were investigated. The T7445C mutation was analyzed as described by Vernham et al. The A1555G mutation was analyzed by testing for the loss of a BamHI restriction site in a polymerase chain reaction (PCR) product encompassing the 1555 nucleotide region. Modified PCR amplification followed by digestion with XcmI was used to analyze the Cins7472 mutation in the tRNA(ser)UCN gene. An insertion at position 7472 can be easily detected since this modified PCR primer creates an artificial site in the PCR product. The results of this genetic study will be presented elsewhere in detail.

A specimen of the proband’s (III10) quadriceps muscle obtained at the age of 68 years was processed for histochimical and biochemical findings. The oxidation rates for pyruvate and malate and the production of adenosine triphosphate and creatinine phosphate of the intact mitochondria were determined in a 600 g supernatant of the fresh muscle. The activities of the enzyme complexes of the respiratory chain and pyruvate dehydrogenase complex were measured in the supernatant from fresh muscle according to the methods of Fischer et al.

CLINICAL STUDY OF THE FAMILY

All individuals born to the hearing-impaired great grandmother (II1) were audiometrically assessed. The pedigree of 105 living individuals demonstrates that the maternal trait of deafness is present in 9 different sibships (Figure 1). Two children (III1 and III6) experienced a decrease in hearing ability reported by amaness in the first week of treatment with dihydrostreptomycin for tuberculosis spondylitis. A similar decrease of 50 dB, which could be evaluated audiometrically, occurred in a 4-year-old cousin (IV25).

INDEX CASE

The proband (III10) was diagnosed as having progressive bilateral hearing impairment with tinnitus and dizziness 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 frequencies. 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]; normal 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, dysdiadochokinesis, and motor and sensory polyneuropathy in both arms and legs. Exercise tolerance had decreased severely from the age of 40 years, when neurologic symptoms had developed. Recent magnetic resonance imag-
ing of the brain showed advanced atrophy of the superior cerebellar vermis and multiple subcortical white matter lesions in the semioval center. Serum myoglobin concentrations were slightly elevated (108/g [normal range, 100/g]) 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 (IV41) who was not a maternal relative and another (IV44) who was both experienced noise 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 (III 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.

Figure 1. Pedigree of 5 generations with maternally transmitted deafness.
The average annual threshold increase was in the range of 0.8 to 1.5 dB/y at all frequencies (Figure 3). An analysis of longitudinal data from 11 individuals confirmed that at a given age, high-frequency impairment was more severe than low-frequency impairment. However, instead of confirming congenital impairment, this analysis indicated that onset of hearing impairment was in the first or second decade of life (Figure 4). Progression seemed to be nonlinear, i.e., with more rapid progression in the initial stage of the disease.

Vestibulo-ocular functions were evaluated in 20 of 32 persons with hearing impairment. Optokinetic nystagmus responses were normal in all except for the proband (III10), who showed response deterioration on stimulation at a frequency of 60/s. Five patients, all without vertigo or dizziness, had normal vestibular responses. One (IV25) showed vestibular areflexia with an enhanced cervico-ocular reflex, which was attributed to previous treatment with streptomycin. Thirteen patients exhibited vestibular hyperactivity, 3 (III10, IV19, and IV23) of whom had dizziness and 2 (IV9 and IV11) of whom were particularly susceptible to motion sickness.

Three of the patients with hyperactive vestibulo-ocular reflex (III10, IV19, and IV23) were extremely nervous during their examinations, and 2 (IV19 and IV23) had hyperventilation syndrome. Another patient (V24) susceptible to motion sickness, whose vestibulo-ocular reflex was normal except for notable asymmetry, showed marked unilateral caloric hyporeflexia.

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, heteroplasmy, 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 7472insC 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.

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, heteroplasmy 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 heteroplasmy. The percentage of heteroplasmy varied among family members from 20% (V24) to 100% (III12, III19, IV19, IV23, IV25, and IV31). In most individuals with hearing impairment (>90%), heteroplasmy varied from 80% to 100%. It seemed that the widest range of heteroplasmy was in the youngest individuals with the lowest level of hearing impairment.
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 Torbergsen 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.

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.
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 (III3) 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.9 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.9 It may be that the insertion of an extra C predisposes 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 heteroplasmacy can make proper counseling difficult.

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