Hearing loss as the first feature of late-onset axonal CMT disease due to a novel *P0* mutation

P. Seeman, MD; R. Mazanec, MD; K. Huehne, PhD; P. Šušlíková, MD; O. Keller, MD; and B. Rautenstrauss, PhD

Abstract—A Czech family with three individuals carrying a novel mutation, 290 A→T (Glu97Val), in the myelin protein zero gene (P0) is reported. The two eldest carriers developed progressive sensorineural hearing loss and abnormal pupillary reaction at age 18. These preceded the onset of the classic signs of Charcot–Marie–Tooth disease (CMT) by more than a decade. Sural nerve biopsy and nerve conduction studies were compatible with the axonal type of CMT. The authors show that progressive hearing loss can be the first symptom in *P0* mutation carriers.

NEUROLOGY 2004;63:733–735

Hereditary motor and sensory neuropathies (HMSNs) and Charcot–Marie–Tooth disease (CMT) have been traditionally classified into more frequent demyelinating (CMT1 or HMSN I) and rarer axonal (CMT2 or HMSN II) subtypes.[1,2] Mutations in the myelin protein zero gene (*P0*) are found in about 5% of CMT patients.[3] *P0* mutations show a phenotypic clustering.[4] A few *P0* mutations such as Ser44Phe in a Sardinian family[5] and Thr124Met in Belgian families are associated with a distinct CMT2 phenotype or with abnormal pupillary reaction[6,7] and hearing loss. However, hearing loss was not reported as the presenting feature of CMT.

We report an atypical CMT phenotype with progressive sensorineural hearing loss preceding the motor disability associated with a novel mutation in the *P0* gene by a number of years.

Case reports. A three-generation Czech family (figure 1) with two affected patients (nos. 645 and 622) and one clinically oligosymptomatic member (no. 623) developed progressive sensorineural hearing loss as a first feature of an atypical CMT2 polyneuropathy.

Patient 622. Patient 622 is a 41-year-old woman. At age 18 years she developed a progressive hearing impairment. At age 30 years she noticed progressive distal muscular weakness in the legs.

Neurologic examination revealed an absent or extremely slow pupillary reaction with slow light convergence reflex, moderate sensorineural hearing loss, symmetric distal muscle atrophies in her legs as well as in the hands and a profound foot drop, absent deep tendon reflexes, and distally markedly reduced vibration sense.

At age 40 years, brain MRI as well as CSF protein and cell counts were normal. Motor nerve conduction study in the median nerve showed severely decreased compound muscle action potential (CMAP) amplitudes (0.4 mV) and a moderately decreased motor nerve conduction velocity (MNCV) (30 m/s) (table). Visual evoked potentials (VEPs) had normal wave latencies. Brainstem auditory evoked potentials (BAEPs) showed evidence of peripheral lesion. Audiometry showed symmetric moderate sensorineural hearing loss of 30 to 40 dB hearing level (HL) in the middle frequencies and a severe hearing loss in low and high frequencies (see figure E- 1A in the supplementary material on the *Neurology* Web site; go to www.neurology.org).

Sequential nerve conduction studies in this patient in 1993, 1998, and 2001 showed that the decrease of the CMAP amplitudes was more pronounced and faster than either prolongation of the distal motor latencies or the slowing of MNCV, suggesting a more pronounced, progressive axonal loss of motor fibers of the median and ulnar nerves compared with the demyelination process, which is probably secondary (see figure E-2 in the supplementary material on the *Neurology* Web site).

A sural nerve biopsy, performed in Patient 622 at age 32 (1994), showed profound loss of large myelinated fibers, multiple clusters of regenerated axonal sprouts, no onion bulb formations, and no inflammatory infiltration (figure 2). All of these findings suggest the primary axonal lesion as the most probable. A biopsy from the tibialis anterior muscle showed small, angulated fibers, small grouped atrophy, and hypertrophic fibers, representing chronic neurogenic changes.

Patient 645. Patient 645 is a 69-year-old man who, at the end of high school, noticed a progressive hearing impairment. He became completely deaf at age 45 years. Distal weakness and atrophy started at age 40 years. Proximal muscle strength remains normal to the present.

Neurologic examination revealed absent pupillary reaction and profound deafness. Distal muscular weakness was mainly in the legs; active dorsiflexion was limited; distal muscle atrophies were mainly in the lower extremities. He has no foot deformity or scoliosis. Deep tendon reflexes were absent in all four extremities.

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From the Departments of Child Neurology (Drs. Seeman and Šušlíková) and Neurology (Dr. Mazanec), Second School of Medicine, Charles University Prague, and Department of Neurology (Dr. Keller), Thomayer Memorial and University Hospital, Prague Krč, Czech Republic; and Institute of Human Genetics (Drs. Kuehne and Rautenstrauss), University Erlangen, Germany.

Supported by grant from IGA MZ no. NF 6504-3 and by VZ 111300003. P.S. was supported by ENS. B.R. is supported by DFG and DGM.

Received December 4, 2003. Accepted in final form April 16, 2004.

Address correspondence and reprint requests to Dr. P. Seeman, DNA Laboratory, Department of Child Neurology, Second School of Medicine, Charles University Prague, V úvalu 84, 150 06 Praha 5, Czech Republic; e-mail: pavel.seeman@lfmotol.cuni.cz

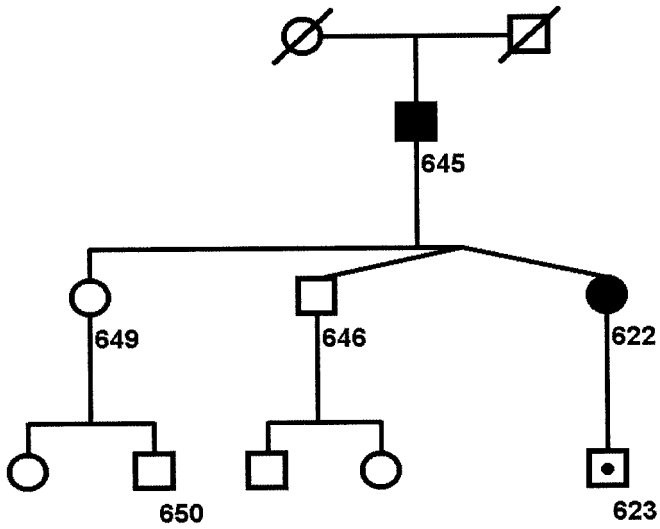

Figure 1. Family tree of the reported family. Detailed DNA testing was done in individuals marked with numbers 622 to 650. Individuals 645, 622, and 623 are described in this report. Circles = females; squares = males; filled symbols = persons affected by Charcot–Marie–Tooth disease.

Vibration sense was profoundly reduced distally in all four extremities.

Electrophysiology showed extremely low CMAP amplitudes (0.1 mV) recordable only from the median nerve and a comparably less pronounced decrease of MNCV (29 m/s) (see the table).

VEP showed normal wave latencies. Audiometry confirmed symmetric profound sensorineural hearing loss over 80 dB HL to practical full deafness. BAEP showed absent responses at age 69.

Patient 623. Patient 623 is a 12.5-year-old boy, subjectively without physical problems, who noticed a mild hearing impairment in the last year.

Neurologic examination revealed very slow pupillary reaction in the right eye, no muscle atrophies, and no foot deformities. His muscle strength was normal. Deep tendon reflexes were present, and vibration sense was preserved.

Median and ulnar nerve MNCV were normal; tibial MNCV was borderline, but amplitudes of action potentials were normal (see the table). BAEP revealed borderline wave I latencies, and audiometry showed mild, primarily sensorineural hearing loss of 25 to 30 dB HL at age 13 years (see figure E-1B on the *Neurology* Web site).

Comparison of the motor conduction studies from the median and ulnar nerves in the three mutation carriers showed normal values in the teenager (Patient 623), but age-dependent changes with signs of severe axonal damage accompanied by moderate, slowly progressive demyelination in the fourth and sixth decades (Patients 622 and 645) (see the table; also see figure E-3 on the *Neurology* Web site).

Molecular genetic studies. Direct sequencing analysis of the entire coding region of the *P0* gene using previously reported intronic PCR primers[8] revealed a novel, not yet reported, heterozygous missense mutation in exon 3: c.290 A→T, resulting in a Glu97Val amino acid exchange. This mutation segregates with the phenotype; it was detected in all three affected members (622, 645, and 623) in three generations of this family but in none of the unaffected relatives with normal hearing (see figure 1). The amino acid numbering includes the 29-amino acid leader sequence corresponding to the CMT mutation database.[9]

The mutation was confirmed independently by *Fsp*I restriction enzyme digestion of exon 3 of the *P0* gene, and it was not found on 100 chromosomes from healthy control individuals without any signs of polyneuropathy or hearing loss. We have not found any other report about a mutation affecting this codon.

Discussion. We describe an atypical CMT2 phenotype with progressive sensorineural hearing loss as a presenting feature with onset many years before the onset of neuropathic weakness associated with a novel Glu97Val mutation in the *P0* gene.

Hearing loss was recently reported in some patients with late-onset CMT2 disease,[6,7] but it was not described as a feature present more than a decade before classic signs of CMT such as distal weakness and atrophy developed. Furthermore, a follow-up electromyographic study over a longer period in Patient 622

Table *Results from motor and sensory nerve conduction studies of all three mutation carriers in the family*

Patient no.	Motor nerves				Sensory nerves		
	Nerve	DML, ms	CMAP, mV	MNCV, m/s	Nerve	SNAP, μV	SNCV, m/s
645	Tibial	None	None	None	Sural	None	None
	Median	9.4	0.1	29	Median	None	None
	Ulnar	3.6	3.4	35	Ulnar	None	None
622	Tibial	None	None	None	Sural	None	None
	Median	4.8	0.4	30	Median	7.0	44
	Ulnar	2.5	5.0	47	Ulnar	4.0	44
623	Tibial	6.2	10.9	40	Sural	16.0	47
	Median	3.3	13.3	47	Median	26.0	47
	Ulnar	2.3	10.5	53	Ulnar	Not done	

Nos. 645, 622, and 623 correspond with the individual numbers from the family tree. Note very low amplitudes in the eldest person (no. 645), less so, but also a severely decreased amplitude in his daughter (no. 622) and normal amplitudes in her 12-year-old son (no. 623), who is still a physically unaffected mutation carrier. Decrease of the amplitudes of nerve action potentials and the slowing of nerve conduction velocity in the three mutation carriers correlate directly with the age of the individual and with the duration of the disease.

DML = distal motor latency; CMAP = compound muscle action potential; MNCV = motor nerve conduction velocity; SNAP = sensory nerve action potential; SNCV = sensory nerve conduction velocity.

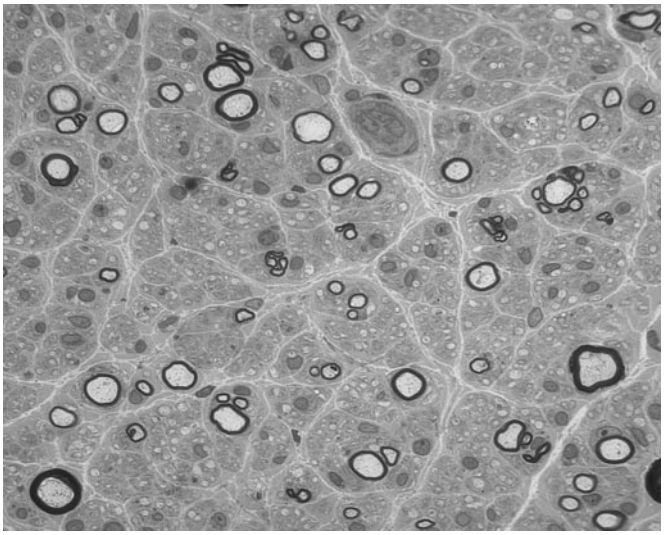

Figure 2. Sural nerve biopsy from Patient 622 at age 32 (1994). Semithin section shows profound loss of large myelinated fibers, multiple clusters of regenerated axonal sprouts, no onion bulb formations, and no inflammatory infiltration. Toluidine blue; ×1,000.

revealed valuable data about age-dependent progression of axonal damage in motor nerves accompanied by a less pronounced, slowly progressive demyelination associated with this *P0* mutation.

The manifestation of progressive sensorineural hearing loss and abnormal pupillary reaction long before any signs of polyneuropathy may indicate that the Glu97Val mutation results in an earlier abnormality of cranial nerves rather than in an abnormality in other peripheral nerves.

Why some *P0* mutations result in a severe, infantile-onset demyelinating CMT and others in a late-onset axonal CMT remains unclear. The substantial phenotypic differences resulting from the mutations of the neighbor amino acids, such as Lys96Glu (CMT1), Glu97Val (CMT2), Arg98Cys (Dejerine–Sottas), Arg98His (CMT1), and Ile99Thr (steroid-responsive polyneuropathy),[9] make additional pathophysiologic mechanisms besides the location of the mutation and the *P0* adhesion capability more probable.

Another conclusion of our study is that normal clinical findings and normal median and ulnar nerve conduction studies in childhood and adolescence may not be sufficient to exclude a *P0* mutation carrier status with a high probability of a CMT disease later on in life.

Patients with a clear family history of autosomal dominant, adult-onset, inherited, sensorineural hearing loss should be further examined for peripheral neuropathies and subsequently for underlying *P0* mutations.

Acknowledgement

The authors thank Vladimir Beneš for technical assistance.

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