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Pathology and physiology of auditory neuropathy with a novel mutation in the *MPZ* gene (Tyr145→Ser)

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Summary

We studied a family with hereditary sensory motor neuropathy and deafness accompanying a missense mutation in the *MPZ* gene. Pathological examination of the cochlea in one of the family members revealed marked loss of auditory ganglion cells and central and peripheral auditory nerve fibres within the cochlea. The inner hair cells were of normal number with preserved morphology. The outer hair cells were normal in number except for a 30% reduction in just the apical turn. Examination of the sural nerve and the auditory nerve adjacent to the brainstem showed marked loss of fibres with evidence of incomplete remyelination of some of the remaining fibres. Studies of auditory function in surviving family members using electrophysiological and psychoacoustic methods provided evidence that the hearing deficits in this form of auditory neuropathy were probably related to a decrease of auditory nerve input accompanying axonal disease. Altered synchrony of discharge of the remaining fibres was a possible additional contributing factor.

Keywords: Charcot–Marie–Tooth (CMT); deafness; N100; pathophysiology; psychoacoustics

Abbreviations: ABR = auditory brainstem response; AN = auditory neuropathy; CMs = cochlear microphonics; CMT = Charcot–Marie–Tooth; HSMN = hereditary sensory-motor neuropathy; MPZ = myelin protein zero; OAEs = otoacoustic emissions; PMP22 = peripheral myelin protein (22 kDa)

Introduction

Auditory neuropathy (AN) is a recently described hearing disorder characterized by abnormal auditory nerve function [absent auditory brainstem responses (ABRs)] in the presence of normal cochlear receptor hair cell activity reflected by preserved otoacoustic emissions (OAEs) and/or cochlear microphonics (CMs) (Starr *et al.*, 1991, 1996). Individuals with AN have impaired speech comprehension and sound localization, and an elevated detection for brief silent intervals in noise (gap detection), percepts that depend on temporal cues in the acoustic signal (Starr *et al.*, 1991; Zeng *et al.*, 1999). Amplification is typically ineffective in helping AN subjects to understand speech. Evidence that the auditory nerve is affected by a neuropathic disorder in AN subjects is indirect, based on observations that adults with AN often have a sporadic or inherited peripheral neuropathy (Starr *et al.*, 1996; Starr, 2001). A neuropathy of the auditory nerve with demyelination and axonal loss would affect both the synchrony of firing and the capacity of the nerve fibres to discharge at high rates (Starr *et al.*, 2001a). A disturbance of synchrony would interfere with the precise encoding of temporal cues (Zeng *et al.*, 1999; Kraus *et al.*, 2000) and impair speech comprehension and gap detection. This disturbance of temporal encoding would also affect the precise timing of neural discharges and the ability to obtain an ABR (Starr *et al.*, 1991). A loss of auditory nerve fibres and the inability of remaining fibres to achieve high discharge rates could account for both the absence of middle ear muscle reflexes to high intensity sounds and the failure of sound amplification to benefit hearing (Starr *et al.*, 1996).

Mutations in the myelin protein zero (*MPZ*) gene have been reported in patients with deafness and Charcot–Marie–Tooth (CMT) disease (Chapon *et al.*, 1999; De Jonghe *et al.*, 1999; Misu *et al.*, 2000). Deafness is also a feature in members of two CMT families with peripheral myelin protein (22 kDa) (PMP22) mutations (Boerkoel *et al.*, 2002; Kovach *et al.*, 2002), and in two CMT families with GJB1 mutations

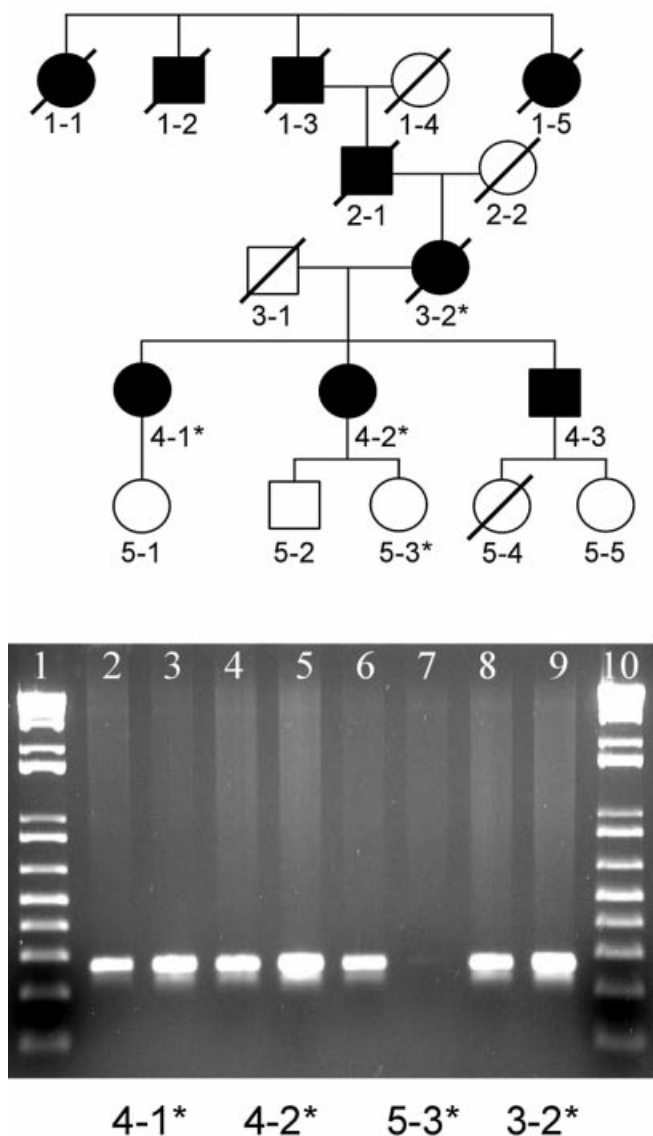## MPZ Family with Auditory Neuropathy

Fig. 1 (*Top*) The pedigree structure for the family in this study with HSMN and deafness accompanied by an *MPZ* mutation. The subjects examined for this report are indicated by an asterisk. (*Bottom*) Allele-specific PCR for the normal allele (lanes 2, 4, 6 and 8) and the mutant allele (lanes 3, 5, 7 and 9). Subjects 4-1* (lanes 2 and 3), 4-2* (lanes 4 and 5) and 3-2* (lanes 8 and 9) have both the normal and the mutant allele, while for 5-3* (lanes 6 and 7) the normal allele is present, but the mutant allele is absent.

(Stojkovic *et al.*, 1999; Boerkoel *et al.*, 2002). Additionally, CMT and deafness are also major findings in the autosomal recessive sensory-motor neuropathy known as HSMN-Lom (hereditary sensory-motor neuropathy–Lom), which has been described in gypsy populations (Kalaydjieva *et al.*, 1998; Butinar *et al.*, 1999). This disorder is associated with mutations in a gene called *NDRG1*, which encodes a ubiquitously expressed protein that is present at high levels

in Schwann cells (Kalaydjieva *et al.*, 2000). The deafness in members of the HSMN-Lom families as well as those with mutations in PMP22 have been studied using ABRs and OAEs, and show findings of AN (Berlin *et al.*, 1996; Butinar *et al.*, 1999; Kovach *et al.*, 2002). However, extensive audiological characterization of the deafness in patients with *MPZ* mutations has not been reported. Several other hereditary neurological disorders affecting both central pathways and peripheral nerves have deafness with some clinical features of AN including Refsum's disease (Oysu *et al.*, 2001), Friedreich's ataxia (Satya-Murti *et al.*, 1980) and several of the mitochondrial disorders (Zwirner *et al.*, 2001).

The pathology of AN has not yet been defined but is likely to be similar to temporal bone studies of individuals with a 'sensorineural deafness' and hereditary peripheral neuropathy published ~30 years ago (Spoendlin, 1974; Hallpike *et al.*, 1980). Those studies showed selective loss of auditory nerve and ganglion cells in the presence of preserved inner and outer hair cells. The authors noted their subjects had difficulties with speech comprehension out of proportion to the pure tone loss, a finding characteristic of most patients with AN (Starr *et al.*, 1996). Recently, Nadol (2001) reviewed temporal bone findings from adults with 'sensorineural deafness' and peripheral neuropathy and showed depletion of ganglion cells with relatively normal hair cell populations. The hearing disorder in these patients was not tested in sufficient detail to confirm a diagnosis of AN.

We have had the opportunity to study a family with deafness and an inherited peripheral neuropathy associated with a novel *MPZ* mutation. Affected family members had a mild-to-moderate pure tone loss with audiological and neurophysiological findings typical of AN. One of the family members died and the temporal bones, central portions of auditory/vestibular nerves, and sural nerves were examined. We also made quantitative physiological and psychophysical studies of affected family members to provide objective measures of temporal synchrony and intensity processes in AN.

The family (see Fig. 1) originated from Costa Rica and medical information for the first and second generations was derived from family histories. Subject consents for the research were obtained according to the Declaration of Helsinki and followed the guidelines of the Institutional Review Board of the University of California, Irvine, for research involving human subjects. Three of the four members of the first generation (1-1, 1-2, 1-3) were affected by 'heavy legs'. There was no information about their hearing. Subject 1-3 had only one child (2-1) who immigrated to the US and developed 'heavy legs, foot drop, small hands' at 35 years of age. Again, no information on hearing function was available. An autopsy was said to show a 'small spinal cord'. Subject 2-1 had only one child, 3-2, who developed motor loss and deafness at age 50 years. We examined subject 3-2 and two of her affected children, 4-1 and 4-2. All showed hearing loss and sensory/motor disabilities. Subject 4-3 has not been examined but family members report the presence of

a hearing loss. We have also examined one subject in the fifth generation, 5-3, whose neurological and audiological functions were normal at age 32 years. No hearing or motor problems have been reported for members of the fifth or sixth generations.

Subject 3-2, was a surgical nurse, and was noted on a routine physical examination at age 49 years, to have hypertension and non-reactive pupils (Adie's). One year later, at age 50 years, she complained of numbness in her hands and feet. At age 53 years, she discontinued working in the operating room because of increasing numbness of hands and clumsiness affecting her ability to handle surgical instruments. At age 59 years, she experienced hearing problems affecting her ability to understand speech. At age 64 years, she developed urinary and fecal incontinence and enlargement of the tongue. She underwent retropubic urethropexy and posterior colporrhaphy for stress incontinence and rectocele. Following the surgical operation, she began self-catheterization for urinary retention. She became progressively weak and unsteady in her legs requiring a wheel chair for mobility when she was in her 70s.

At 74 years of age, the patient had transient ischaemic attacks and underwent right carotid endarterectomy. Two months later, she had symptoms consistent with a small brainstem infarction and also developed right temporal lobe haemorrhage and a right subdural haematoma. Several generalized seizures occurred with focal features. Intracerebral and subdural haematoma resolved without surgical intervention.

Neurological examination on June 25, 1997 at age 75 years, revealed bilateral Adie's pupils, deafness, unsustained nystagmus on lateral gazes, atrophy and weakness of the hands and feet with preserved proximal strength, and absent deep tendon reflexes in the lower limbs. Sensory functions (touch, vibration and proprioception) in the toes were markedly impaired, but only slightly affected in the fingers. The hearing loss had progressed so that she appeared totally deaf.

Two and a half years later, she was admitted to a hospital with increasing lethargy. She lapsed in coma and died. An autopsy was performed and the brain, proximal portions of the sural nerves, and both temporal bones were removed and processed for histological studies.

Her two daughters (subjects 4-1, 4-2) developed hearing loss during their fourth decades a few years after the appearance of symptoms of peripheral nerve involvement (numbness and weakness). Neurological examination in the siblings revealed Adie's pupils, absent ankle jerks, elevated threshold to vibration in the feet, slight weakness of distal muscles and no enlargement of peripheral nerves.

Patients and methods
Genetics
Blood samples were drawn from three affected family members (3-2, 4-1, 4-2) and from subject 5-3, who was unaffected. DNA was extracted from these samples, and each of the six exons of *MPZ* was amplified and sequenced in the samples from the affected individuals using the primers and conditions given in Nelis *et al.* (1994). Additionally, exon 3 was PCR amplified and sequenced in the unaffected family member and 50 unaffected control samples. In brief, for exon 3 the intronic primers, $P_o$ex3-P4 (5′-TCATTAGGGTCCT-CTCACATGC-3′) and $P_o$ex3-M21 (5′-GCCTGAATAAA-GGTCCTTAGGC-3′), were used for amplification resulting in a 368 base pair product. The temperatures and times for each of the 40 cycles of amplification were 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, with an initial denaturation at 95°C for 5 min and a final extension at 72°C for 10 min (Perkin Elmer Gene Amp PCR system 9700). The PCR products were loaded on a 1% agarose gel to verify size and quantity, then the DNA fragments were extracted from the gel and purified using the Qiaex II Gel Extraction Kit (Qiagen). Manual sequencing in both the forward and reverse directions was carried out using the ThermoSequenase Radiolabeled Terminator Cycle Sequencing Kit according to the manufacturer's protocol (USB).

Allele specific PCR was performed using the forward primer, $P_o$ex3-P4, with two different reverse primers each designed to amplify either the normal or the mutant allele. The reverse primer sequences used were $P_o$ex3-M21N (5′-CTCACACCTTTTTCAAAGACAT-3′) to amplify the normal allele and $P_o$ex3-M21A (5′-CTCACACCTTTTTCAAA-GACAG-3′) to amplify the mutant allele. The amplification conditions were an initial denaturation for 5 min at 95°C, followed by 35 cycles of 20 s at 95°C, 20 s at 62.5°C and 20 s at 72°C, with a final extension at 72°C for 10 min.

Clinical audiology
Family members (3-2, 4-1, 4-2, 5-3) were tested for pure tone thresholds, speech discrimination, acoustic middle ear muscle reflexes, and OAEs. Auditory brainstem potentials were tested in subjects 4-1 and 4-2 (for details of the test procedures see Starr *et al.*, 1996).

Vestibular function
Eye movements were measured in subjects 3-2 and 4-1 during caloric stimulation of the vestibular system (for details see Fujikawa and Starr, 2000).

Neurophysiology of peripheral nerve and muscles
Nerve conduction velocities were tested for subjects 3-2, 4-1 and 4-2 using surface recording and stimulating procedures. Electromyograms were tested in subjects 3-2 and 4-2.

Neuropathology

Sural nerve biopsies were performed on subject 3-2 at age 70 and 77 years, the latter as part of the *post mortem* studies. Sural nerve biopsy was performed on subject 4-2 at 46 years of age. The tissues were processed for routine neuropathology. Teased fibre studies and nerve fibre counts were performed in subject 4-2 by Dr Andrew Verity at the University of California, Los Angeles.

The brain, spinal cord, sural nerves and temporal bones were removed from subject 3-2 at time of death. The vestibular and cochlear nerves were sampled within 0.5 cm of the brainstem, immersed in 2% gluteraldehyde, and subsequently embedded in epon. Sections (1 μm) were prepared for examination. Vestibular and cochlear nerves were also taken from a decreased elderly subject without a history of hearing impairment to serve as a control.

The temporal bones were placed in 10% buffered formalin and shipped to the temporal bone laboratory of the House Ear Institute (Los Angeles, CA, USA). Upon arrival the left bone was perfused with 2% osmium tetraoxide to protect the myelin from the various solvents used in the routine staining processes. The stapes was removed using a tympanotomy approach and the osmium was introduced through the round window membrane by means of a No. 28 modified spinal needle using a gravity feed. Both bones were then put in fixative, which was changed weekly, for 3 weeks. Fixation was followed by decalcification in ethylenediaminetetraacetic acid until shown by X-ray, after 9 months, to be calcium free. The bones were then placed in graduated concentrations of celloidin. The resulting celloidin blocks containing the decalcified bones were cut in the horizontal plane into 20 μm sections using a sliding microtome. Every tenth section was stained with haematoxylin and eosin, mounted on a slide, and topped with a coverslip. Neurons were counted using the technique described by Schuknecht (1993). Hair cells were counted in the midmodiolar and two paramidmodiolar mounted sections and in the hook area for a total of 21 segments of the organ of Corti. The stria vascularis was also measured by the method described by Schuknecht (1993). Dendrites were so scant that they were not counted but described individually. The auditory nerve fibres were not counted as there was obvious severe degeneration that mirrored the loss of ganglion cells in the modiolus.

Pathophysiology

Psychoacoustics

Auditory temporal and frequency discrimination abilities were assessed in subjects 4-1 and 4-2, and a group of normal-hearing listeners. Temporal discrimination measures included gap detection and detection of amplitude modulations for noise bursts of 500 ms duration presented at a comfortable loudness level; frequency discrimination was measured at 250, 500, 1000, 2000, 4000 and 8000 Hz and was presented at the maximal comfortable loudness level. The pure tone for frequency discrimination was 200 ms in duration. The detection of signals in noise was also measured in subject 4-1 and an age-matched control with normal hearing. The signal was a 200 ms duration 1000 Hz tone and the noise was a white noise that had a 20–14 000 Hz bandwidth and a 500 ms duration. The 200 ms tone was temporally centered on the noise burst. All stimuli had 3 ms, cosine-squared ramps. The subject was seated in a double-walled, sound attenuating booth and listened to the monaurally presented stimuli through a Sennheiser HAD-200 headphone. An adaptive, three-interval, forced-choice, two-up and one-down procedure was employed to track the 70.7% correct response criterion (for detailed methods see Zeng *et al.*, 1999).

Auditory physiology

Subject 4-1 and an age- and gender-matched control with normal hearing were studied using long-latency, auditory cortical potentials evoked in response to a tone burst at several intensity levels. Brain potentials were recorded from midline frontal (Fz), central (Cz) and parietal (Pz) scalp sites referenced to linked mastoids. Eye movements were monitored from electrodes located above and below the right eye; a ground electrode was on the forehead. Amplifier bandpass was set between DC and 100 Hz. Continuous EEG records were digitized and stored to disk. The acoustic stimuli were presented from an electrically shielded transducer coupled to a 20 cm length of plastic tubing to a foam insert placed within the ear canal. The acoustic signals were 1000 Hz tone bursts (100 ms duration, 2 ms rise/fall times) presented at an interstimulus interval of 980 ms. A total of 300 tones were presented monaurally at five intensity levels above threshold.

Continuous records were processed off-line to adjust for DC drift and to correct for ocular artefacts. Single trial epochs were derived from the continuous records for a period of 100 ms before to 500 ms after the stimulus. Accepted single trials were then averaged and the latency and amplitude of the N100 cortical component in the average were computed.

The latency variability of the N100 component of the single trials comprising the averaged response was determined using a template matching procedure. The method involved calculating the correlation (Pearson product-moment) between the points in the template, the averaged N100 for that subject, with corresponding points in a segment of the raw EEG (for details see Michalewski *et al.*, 1986). A computer program positioned the template 50 ms before N100 and moved 100 ms while computing the correlations at each data point. N100 was identified in the EEG as the largest positive correlation between the template and corresponding single trial data points. Only trials with correlation coefficients >0.5 were used to define N100. N100 latency for each trial was estimated by computing how far (number of dwell points) the template was moved relative to the starting point. The SD of the estimated N100 latencies served as the measure of N100 variability. In control procedures we embedded the same N100 signal in EEG recorded in the absence of acoustic
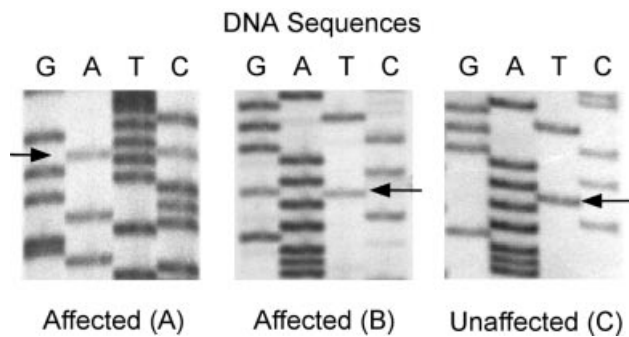
DNA Sequences

Fig. 2 (A) Forward and (B) reverse DNA sequences for a portion of exon 3 of *MPZ* in one of the affected individuals (4-1), and (C) the reverse sequence for the unaffected subject (5-3). The arrows point to sequence position 434. In A and B two bands are present at this position, showing that 4-1 is heterozygous (AC in the forward and TG in the reverse sequence). In contrast, only one band (T) is present for the (homozygous) unaffected individual (C). The C band in A and the G band in B indicate the presence of a mutation in individual 4-1.

stimulation and established that the N100/EEG amplitude ratio needed to be >0.5 to detect at least 80% of the negative potentials rather than the background EEG. Only the results from the Cz electrode site are considered here.

Results
Genetics
Figure 1 contains the pedigree (top panel) and the results of allele-specific PCR (bottom panel) for subjects 3-2, 4-1, 4-2 and 5-3. Both the normal and the mutant alleles are detected in the affected individuals (3-2, 4-1, 4-2), while only the normal allele is present in 5-3, demonstrating that the mutant allele segregates with the disease phenotype.

Sequencing revealed the presence of a missense mutation in exon 3 of the *MPZ* gene in each of the affected members. No sequence variants were detected in any of the other exons. Forward and reverse sequencing for a portion of exon 3 in individual 4-1 are shown in Fig. 2A and B, respectively. Both of these figures show clearly the presence of two bands at position 434 (marked by an arrow), indicating that 4-1 is heterozygous at this position. This A to C mutation at position 434 results in an amino acid change from tyrosine to serine at codon 145 (Tyr145→Ser), which is located in the hydrophobic extracellular domain of *MPZ*. Figure 2C shows that the unaffected family member (subject 5-3) has only one band at position 434, demonstrating that this individual is homozygous and does not have the A to C mutation. In addition, the PCR product for 4-1 was cloned and four independent clones were sequenced. Three of these clones had the A nucleotide at position 434 and one of them had the C nucleotide, again demonstrating that 4-1 is a heterozygote. Also, this mutation was not present in 100 control chromosomes (50 unrelated, unaffected individuals). Although the possibility that this is a rare variant is not excluded, our
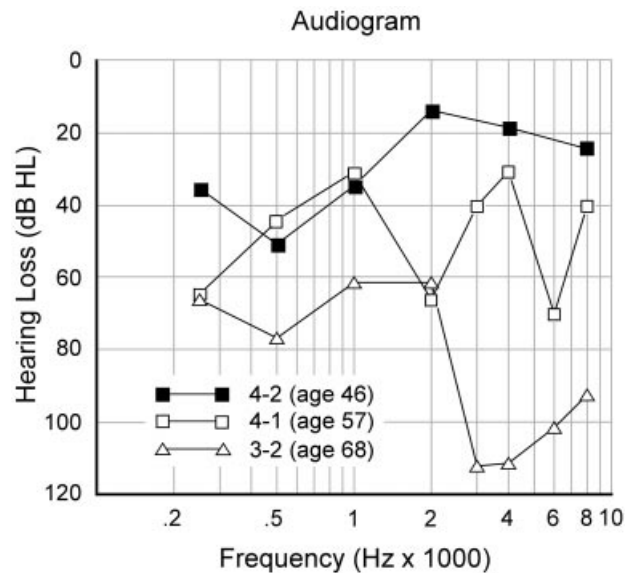
Audiogram

Fig. 3 Audiograms from the affected family members; an increased loss, particularly at frequencies >2000 Hz, is shown with age.

findings are consistent with the mutation being the cause of the phenotype. This conclusion is strengthened by the fact that this region is highly conserved, with both the nucleotide and the amino acid being identical in human, rat and mouse.

Clinical auditory functions (Table 1)
Audiogram, speech discrimination, reflexes
The pure tone audiograms for the three affected subjects are shown in Fig. 3 when they were ~10 years apart in age (46, 57 and 68 years). The extent of low frequency loss (<2000 Hz) differed by ~30 dB across the three subjects. In contrast, high tone frequency thresholds (>4000 Hz) differed by ~90 dB, being profound in subject 3-2 at age 68 years, moderate in subject 4-1 at age 57 years and mild in subject 4-2 at age 46 years. Speech discrimination was impaired in all even when the audiogram was only mildly affected [Table 1, subjects 3-2 and 4-1, compare pure tone audiogram and speech discrimination]. Middle ear muscle reflexes were absent in all subjects on all test sessions.

OAEs
Both transient and distortion product OAEs were present in the daughters (4-1 and 4-2) but absent in their mother (3-2).

ABRs
A wave V of prolonged latency was identified without other neural components in subject 4-1 (when tested at age 52 years) and in subject 4-2 (the latter's responses are shown in the bottom traces of Fig. 4). Wave V was no longer present when subject 4-1 was retested at age 54, 57 and 59 years (top

Table 1 *Hearing and audiological functions in affected family members*

Subject	Age at onset (years)	Age tested (years)	PTA (dB)		Speech discr. (%)		Reflexes		TEOAEs (dB)		ABR V (ms)	
			AD	AS	AD	AS	AD	AS	AD	AS	AD	AS
3-2	59	68	65	58	4	8	–	–	NR	NR	–	–
4-1	40	49	20	20	84	76	–	–	–	–	–	–
		52	20	43	80	8	–	–	–	–	6.1	NR
		54	25	37	–	–	–	–	8	8	–	–
		57	48	50	10	10	NR	NR	–	–	NR	NR
		59	50	45	–	–	–	–	–	–	NR	NR
4-2	40	46	32	28	88	92	NR	NR	–	–	–	–
		52	40	48	60	76	NR	NR	11	11	6.2	6.8

AD and AS = right and left ear, respectively; TEOAEs = transient evoked otoacoustic emissions; PTA = average of pure tone threshold to tones of 500, 1000 and 2000 Hz; ABR = auditory brainstem potential, wave V; Speech discr. = speech discrimination; NR = no response obtained; – = not tested.
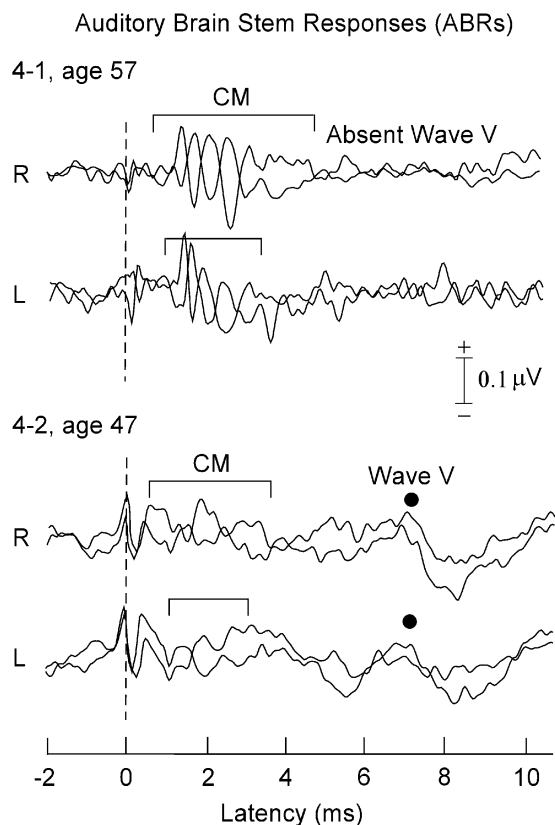

Fig. 4 ABRs from subjects 4-1 and 4-2 showing preserved receptor potentials (CMs in 4-1) and both CMs and a wave V (marked by a filled circle) that is delayed in latency in subject 4-2. L = left stimulation; R = right stimulation; dashed vertical lines indicate stimulus onset.

traces of Fig. 4 are responses at age 57 years). CMs, represented by the out-of-phase components labelled CM in Fig. 4, were present in both subjects. ABRs were not tested in subject 3-2.

Vestibular function
Subjects 3-2 and 4-1 had absence of eye movements (nystagmus) and symptoms of vertigo or dizziness when tested by caloric stimulation of the labyrinths.

Neurophysiology and pathology of peripheral nerve and muscles
Nerve conduction velocities in affected family members were slightly slow for both motor and sensory nerves (Table 2). The velocities ranged from 25 to 38 m/s depending on the nerve tested and the age of the subject. The amplitudes of the muscle potentials and compound nerve potentials were reduced in the upper extremities and absent in lower extremities (Table 2).

Electromyography in two affected subjects (3-2 and 4-2) showed acute and chronic denervation. Calf muscle biopsy showed denervation atrophy with reinnervation with prominent target fibre changes.

Sural nerve biopsy in two affected subjects (3-2 and 4-2) showed decrease in the number of large myelinated fibres, variation of internodal length and segmental hypomyelination (Table 3). The findings are consistent with an axonal neuropathy with features of incomplete remyelination.

Pathology
Cochlea
Some *post mortem* degeneration occurred due to the time interval between death and the removal of the bones. The findings were essentially the same in the two ears. The organ of Corti appeared normal throughout the cochlea except for the apical turn, where a 30% loss of outer hair cells was found (Fig. 5). The inner hair cells appeared normal (Fig. 6). There was a profound loss of ganglion cells (>95%). The surviving neuronal cells numbered 1161 and 1548 in the right and left ears, respectively; a normal count for the patient's age is

Table 2 *Peripheral nerve conduction tests*

Subject	Age at onset (years)	Age tested (years)	Nerve		Velocity (m/s) or distal latency (ms)	Amplitude
3-2	50	70	Motor	Median	**32**	**0.48 mV**
				Ulnar	43	7.4 mV
				Peroneal	**34**	**0.41 mV**
				Tibial	NR	NR
			Sensory	Median	<u>**4.5**</u>	**9.7 μV**
				Ulnar	<u>**4.8**</u>	31 μV
4-1	46	57	Motor	Median	**34**	3 mV
				Peroneal	NR	NR
			Sensory	Median	38	**3.8 μV**
				Sural	NR	NR
4-2	40	46	Motor	Ulnar	52	10 mV
				Peroneal	**34**	**1.2 mV**
				Tibial	38	9 mV
			Sensory	Ulnar	**38**	17 μV
				Radial	**23**	14 μV
				Sural	**34**	**2 μV**

Abnormal values are in bold type; distal latencies are underlined. NR = no response.

Table 3 *Sural nerve fibre count in subjects 3-2 and 4-2*

Parameter	Subject 3-2	Subject 4-2	Normal values
Myelinated fibres/mm	**5040**	8610	6000–10 000
Large myelinated fibres/mm	**1260**	**1050**	2800–4000
Large/small	**0.33**	**0.14**	0.4–0.6
Schwann cells/mm	**5460**	1260	<2500

Abnormal values are in bold type.

23 000 (Schuknecht, 1993). An example of one of the surviving neurons is shown in Fig. 5B. Most of the peripheral and central neurites that remained had a beaded appearance (Fig. 5C and D). Only an occasional peripheral process was found in the osseous spiral lamina. The stria vascularis exhibited a patchy atrophy, especially in the anterior middle turn; however, the total loss was ~35%, insufficient to account for a hearing loss.

The sensory epithelium of the vestibular organs appeared to be normal. A section of the vestibular nerve in AN is shown in Fig. 7. The number of nerve fibres between the vestibular receptors and the vestibular ganglion was reduced. However, the number of cells in the ganglia appeared normal. Approximately one-third of the laterally projecting vestibular fibres had the same irregular beaded appearance as the auditory nerve dendritic processes in the cochlea, in contrast to the medial vestibular fibres, which appeared normal. For some of the peripheral fibres, the myelin appeared to be separated into fragments about twice the diameter of the fibre. In others there were areas that resembled a number of doughnuts stacked together. These vestibular nerve changes were not found on reviewing temporal bones from normal subjects contained in the House Ear Institute Bone Bank. The vestibular nerve changes are consistent with a neuropathy of

vestibular nerve and would account for the abnormal vestibular function found in two of the affected family members (Fujikawa and Starr, 2000).

Microscopic examination of the central auditory nerves adjacent to the cochlear nucleus showed a marked depletion of the number of auditory nerve fibres in the AN subject (Fig. 8A) compared with the age-matched control (Fig. 8B). The myelin sheath on several of the remaining axons in the AN subject was thin consistent with incomplete remyelination. Comparison of the auditory and sural nerves in the AN subject at high gain (×1500; Fig. 8C and D) showed axonal loss with several of the remaining axons being incompletely myelinated (arrows in Fig. 8C and D).

Pathophysiology
Psychoacoustics
We obtained behavioral measures for temporal, frequency and signal-in-noise processes in the AN subjects of this report, an age-matched normal hearing control, and normal hearing young adults (Table 4). Thresholds for gap detection were slightly increased to 10 ms in subject 4-1 and 5 ms in subject 4-2 (normal subjects <3 ms). The detection of amplitude modulation as a function of modulation rate was

modeled as a first-order low-pass filter (Zeng *et al.*, 1999), in which the peak sensitivity and the cut-off frequency were derived from the measured data. Although subjects 4-1 and 4-2 had relatively normal peak sensitivity at low modulation rates, they could not follow temporal fluctuations faster than 40–50 Hz, whereas normal controls can follow modulation rates >200 Hz.

Frequency discrimination in subject 4-1 (Fig. 9) showed marked elevation for low frequency tones (125–2000 Hz), slight elevation for a mid-frequency (4000 Hz) and normal discriminations for a high frequency (8000 Hz).

Signal detection (1000 Hz tone) in noise for subject 4-1 and a normal age-matched control are shown in Fig. 10. Over a 40

dB noise range, the normal control showed a linear growth of masking, in which a 1 dB increase in noise level required a 1 dB increase in signal level (1 : 1) to maintain detection (filled circles). The neuropathy subject (open squares) had the same S/N (Signal/Noise) slope (1 : 1) when the noise masker was of low level (<10 dB sound pressure level, SPL) but to a much steeper slope (3 : 1) when the noise level was increased to moderate levels.

Auditory cortical evoked potentials

Long-latency auditory evoked potentials consisting of P50, N100 and P200 components are shown for subject 4-1 and a
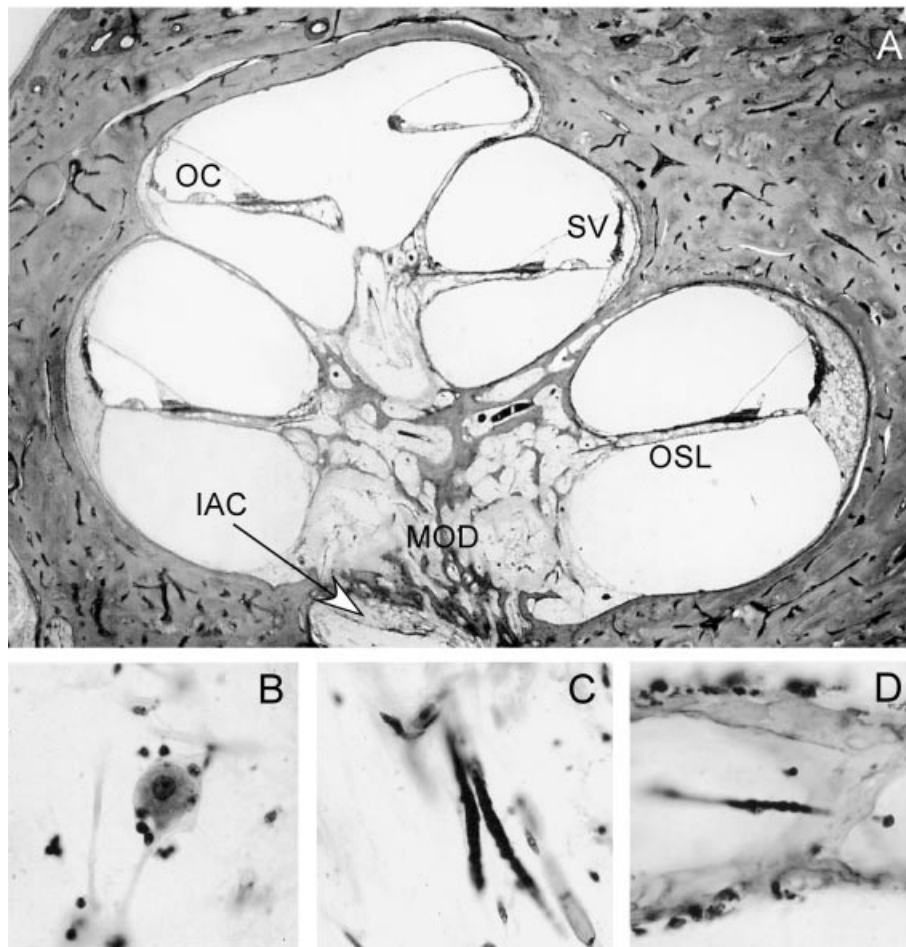

Cochlea in Auditory Neuropathy

Fig. 5 (**A**) Midmodiolar section of the left cochlea showing a normal organ of Corti (OC), except for a loss of a few outer hair cells in the apex. A few (5%) neurons, represented by small black dots, are present in the modiolus (MOD). There is a corresponding loss of peripheral neurites in the osseous spiral lamina (OSL) and in the internal auditory canal (IAC, indicated by arrow). The stria vascularis (SV) is partly atrophic in the anterior middle turn [osmium tetraoxide, haematoxylin and eosin (O, H & E), ×25]. (**B**) Remaining spiral ganglion neuron with attached central process (O, H & E, ×650). (**C**) Central axons in the atrophic auditory nerve illustrating a beaded appearance (O, H & E, ×650). (**D**) Beaded peripheral process between the lamella of the osseous spiral lamina (O, H & E, ×650).
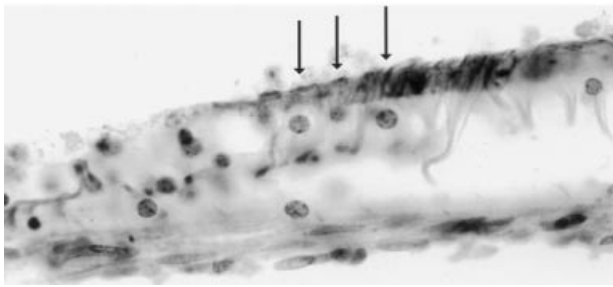
Inner Hair Cells in Auditory Neuropathy

Fig. 6 Inner hair cells (arrows) as seen between the inner sulcus and inner pillar cells in a section cut on the bias (osmium tetraoxide, haematoxylin and eosin, ×650).
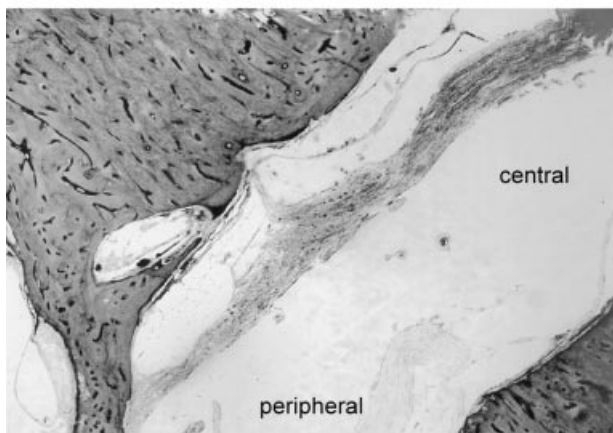
Vestibular Nerve in Auditory Neuropathy

Fig. 7 Inferior vestibular nerve and ganglion (small dots) from right ear with degenerated peripheral processes but more normal central axons (haematoxylin and eosin, ×25).

Auditory Nerve (400x)

Fig. 8 Auditory nerve adjacent to brainstem from AN subject (**A**) and aged-matched control (**B**) (osmium tetroxide, ×400). High power (×1500) of auditory nerve (**C**) and sural nerve (**D**) from the AN subject. The arrows are directed to thinly myelinated fibres in both nerves reflecting incomplete remyelination.

normal hearing subject of comparable age as a function of signal intensity (Fig. 11, lower traces). The relationships between N100 amplitude and latency as a function of signal intensity in dB SL are plotted in the upper panels. The peak latency of N100 to high intensity signals (75–80 dB SL) was similar in AN and control (106 versus 92 ms, respectively). However, a latency disparity between AN and control developed as signal intensity was reduced amounting to almost 70 ms at 35–40 dB SL. In the control, N100 was identified close to threshold (5 dB sensation level, SL) and amplitude increased in a linear fashion over a 55 dB range. In AN, N100 was identified when the signal was 30 dB SL and increased in amplitude only when stimuli were 70–80 dB SL.

Measures of N100 latency variability in the single trials were compared with stimulus intensities of 80 dB SL in AN and 35 dB SL in the control, to provide equivalent amplitudes of the averaged N100 relative to background EEG amplitude (ratio of 0.5). The distribution of the peak latencies of the individual trials in both the AN subject and the control at
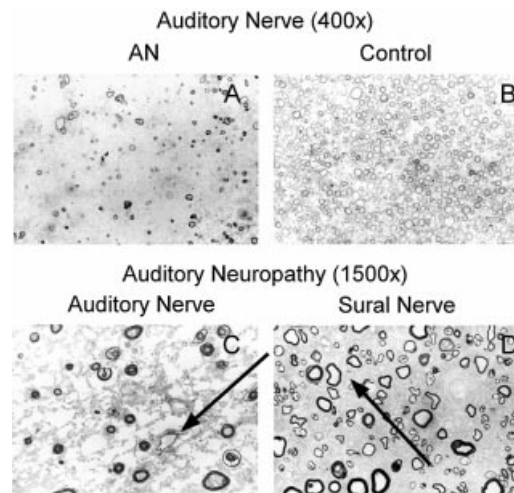
these intensities clustered around the averaged peak latency of N100 component (Fig. 12). The SD of N100 latency was 30 ms in AN and 32 ms in control, and the difference was not statistically significant. Increasing signal intensity in the control (Fig. 12B) resulted in a shortening of the peak latency without a significant change in the latency variability (SD = 31 ms).

Discussion

Results from this family with HSMN and deafness accompanying an *MPZ* mutation provide data relevant for defining both the site of pathology and underlying physiology of the hearing loss.

Site of pathology

The histology of the cochlea from an affected family member who died at age 77 years showed a profound loss of spiral ganglion cells, from 23 000 found in normals compared with 1161 and 1548 cells in the two cochleae of this subject. The inner hair cells were normal in number and appearance. The outer hair cells in the apical turns showed some depletion, compatible with the subject's age. There was also some modest changes in the stria vascularis but insufficient to account for the hearing disorder. Thus, the basis for the hearing impairment defined in this family is from a neuropathy of the auditory nerve as part of a generalized neuropathy due to a missense mutation of the *MPZ* gene. The type of cochlear pathology we found is quite similar to what was described previously in instances of 'sensorineural deafness' associated with a hereditary peripheral neuropathy (Spoendlin, 1974; Hallpike *et al.*, 1980; Nadol, 2001). The procedures (ABRs, OAEs, CMs) now used to distinguish

Table 4 *Auditory psychophysical measures*

Test	Subject 4-1	Subject 4-2	Normal
Gap detection (at 40 dB SL) (ms)	**11**	**5**	1–3
Peak sensitivity* of AM detection (dB)	**–14**	–20	–18 to –23
Cutoff frequency of AM (Hz)	**51**	**42**	200–238

Values in bold type are abnormal. *Peak sensitivity = 20 log (m) where m is modulation depth. For example, –20 dB peak sensitivity indicates that the subject can detect 10% amplitude modulation. Peak sensitivity in dB. AM = amplitude modulation.
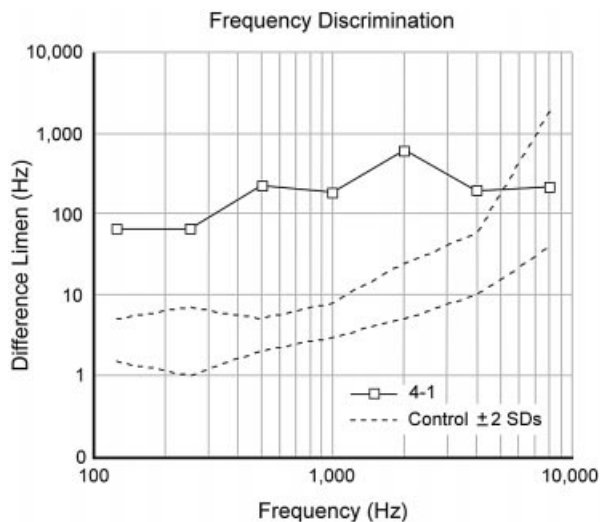

Fig. 9 Frequency discrimination in subject 4-1 compared with normal hearing adults, ages 25–38 years. The dashed lines signify the boundaries for ±2 SD limits in the controls. Note that discrimination is markedly abnormal in the AN subject from 125 to 2000 Hz, and normal at 8000 Hz. The values at 2000 Hz are slightly beyond the normal limits.
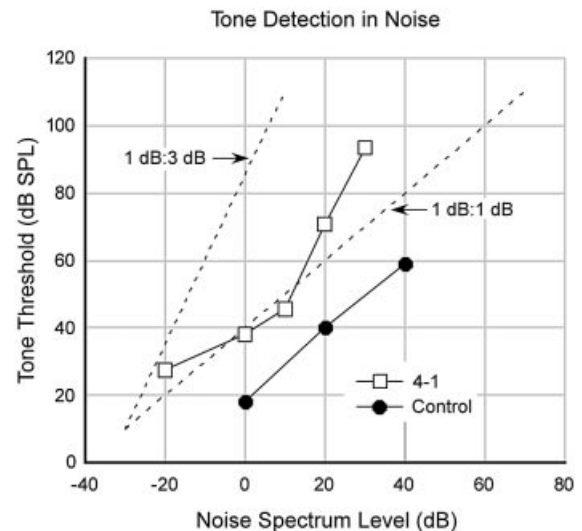

Fig. 10 Detection of 1000 Hz tone in noise in AN subject and age-matched control as a function of signal and noise intensity. Note that in the normal subject, an increase of noise level by 20 dB requires an equivalent 20 dB increase of signal for detection. In AN this 1 : 1 slope is maintained for low intensities (30–45 dB SPL) but at 45–90 dB SPL signal intensity needed to be increased 3 dB for every 1 dB increase of noise.

between hearing losses accompanying cochlear receptor damage from those accompanying auditory nerve involvement were not available until recently. However, the similarities between the cochlear pathology in these former cases to that found in the present family make it likely that AN would have been a diagnosis considered for the hearing loss in the aforementioned studies.

The microscopic changes found in auditory nerve fibres had features similar to those found in sural nerves from the same individual (3-2) and from another affected family member (4-2). Both the sural and proximal auditory nerves showed a loss of large myelinated fibres, consistent with a primary axonal disorder, and thinning of the myelin sheath of many of the remaining axons, consistent with incomplete remyelination. The few distal auditory nerve fibres found in the cochlea also had a beaded appearance, consistent with incomplete remyelination. In addition, beading occurred in many of the fibres comprising the distal but not proximal portions of the vestibular nerve, suggesting that distal axons of the auditory/vestibular nerve may be disproportionately affected in this disorder.

The hearing disorder in this family can be attributed to a neuropathy of the auditory nerve without changes in the number and appearance of inner hairs. This constellation of findings is appropriate for the clinical designation of the hearing disorder as AN. The modest change of outer hair cell number and the loss of OAEs are attributed to cochlear changes with aging rather than remote effects from the neuropathy. We would expect that the pattern of audiological findings (absent ABRs, normal OAEs and/or CMs) could also occur with lesions of inner hair cells and/or nerve terminals. For example, animal studies have shown that application of neurotoxins to the cochlea (e.g. kainic acid, Zheng *et al.*, 1996; carboplatinum, Ding *et al.*, 1999; or a period of generalized anoxia, Harrison *et al.*, 1998) is accompanied by swelling and degeneration of both terminal nerve dendrites and inner hair cells. The nerve damage may be a consequence of excess glutamate transmitter release by inner hair cells, leading to excitotoxic damage of terminal dendrites and subsequent retrograde degeneration of spiral ganglion cells
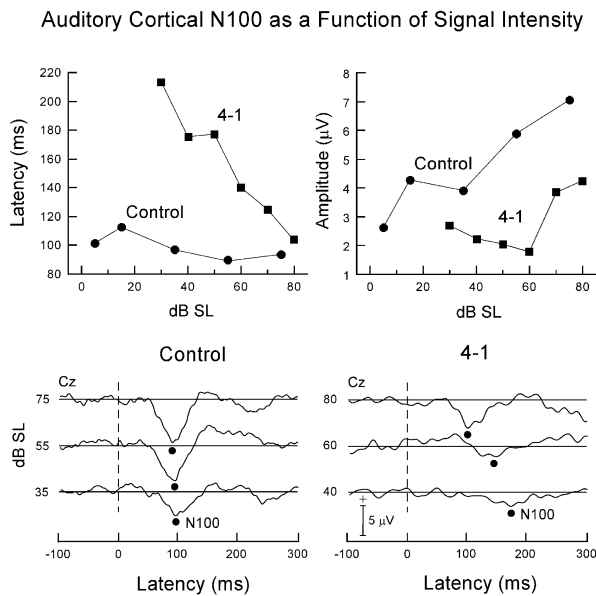
Auditory Cortical N100 as a Function of Signal Intensity

Fig. 11 Measures of peak N100 latency and amplitude as a function of signal intensity for subject 4-1 and age-matched control are shown in the *top panels*. Averaged auditory cortical potentials are shown in the *bottom panels* for the AN subject (*right*) and the control (*left*) as a function of signal intensity. The N100 component is indicated by a filled circle. Note the marked disparity of N100 latency between AN and control when signal intensity was reduced.

N100 Single Trial Latency

Fig. 12 Percentage histogram of N100 peak latency of the individual trials occurring between 60 and 160 ms contributing to the averaged cortical N100 potential that is plotted above each histogram; (**A**) AN subject 4-1; (**B**, **C**) age-matched control. The auditory signal was a 100 ms duration, 1000 Hz tone, presented at 80 dB SL (**A**), 75 dB SL (**B**) and 35 dB SL (**C**). The amplitude calibration on the right is for the average potential in each panel. Note that the peak latency of the averaged potentials corresponds to the modal value of the histogram and that the distribution of latencies of the single trials contributing to the averaged N100 component was similar in AN and the control.

(Puel *et al.*, 2002). Animals with such experimental lesions have abnormal or absent auditory nerve responses, while outer hair cell functional measures are preserved, a constellation of findings similar to AN in humans. Recently, Amatuzzi *et al.* (2001) reported that some premature infants who died following anoxia and who had failed a hearing ABR screening test had a selective loss of inner hair cells with normal numbers of ganglion cells. We suggest that the inner hair cell nerve terminals were likely to have been affected by the anoxia, but that the time period intervening before death was insufficient for retrograde ganglion cell degeneration to have appeared.

The distinction between sensory hearing loss and AN can be lost as patients are followed over time. Almost one-third of the children with AN lose their OAEs, consistent with the development of an outer hair cell disorder (Starr, 2001). Temporal bone studies in deaf subjects often reveal loss of both sensory receptors (inner and outer hair cells) and a reduction in the number of ganglion cells (Nadol *et al.*, 2001). Additional clinical methods are needed to refine the designation of deafness as being sensory, neural, or a combination of sensory and neural disorders.

AN accompanying hereditary neuropathies

The peripheral nerve alterations in CMT are classified, in part, by nerve conduction studies as: (i) primary demyelinat-
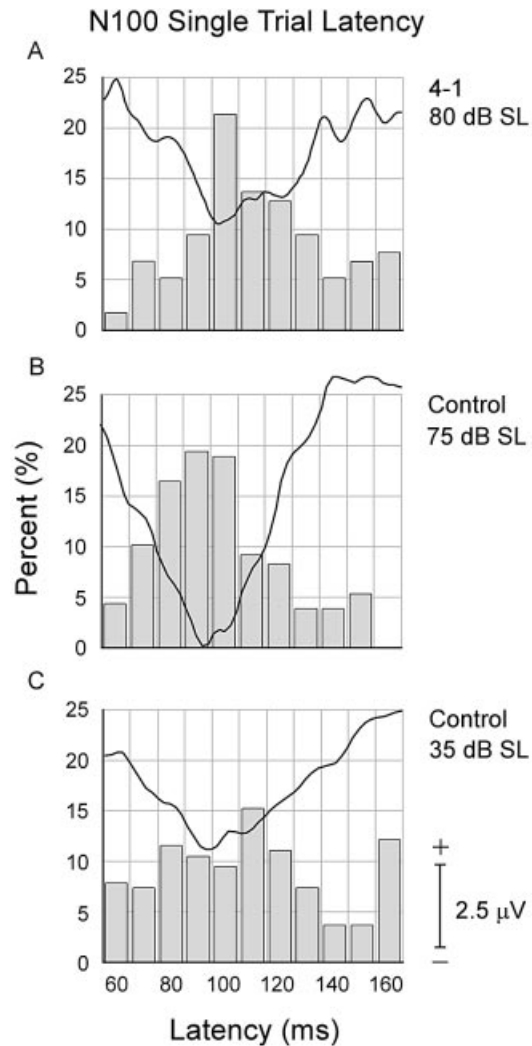
ing (CMT1) with slow conduction velocities and normal amplitudes of the compound action potential; and (ii) primary axonal loss (CMT2) with minimal slowing of nerve conduction velocity (>38 m/s) and reduced amplitudes of the compound action potential. CMT patients with hearing loss, in whom *MPZ* mutations have been reported, have a CMT2 phenotype. The *MPZ* mutations are Thr124→Met (Chapon *et al.*, 1999; De Jonghe *et al.*, 1999) and Asp75→Val (Misu *et al.*, 2000). The family in the present paper with the *MPZ* missense mutation (Tyr145→Ser), CMT and deafness also had nerve conduction findings consistent with CMT2. However, why patients with these three mutations have

hearing loss, while those with other nearby mutations do not, is unclear. Boerkoel *et al.* (2002) suggest that the phenotype
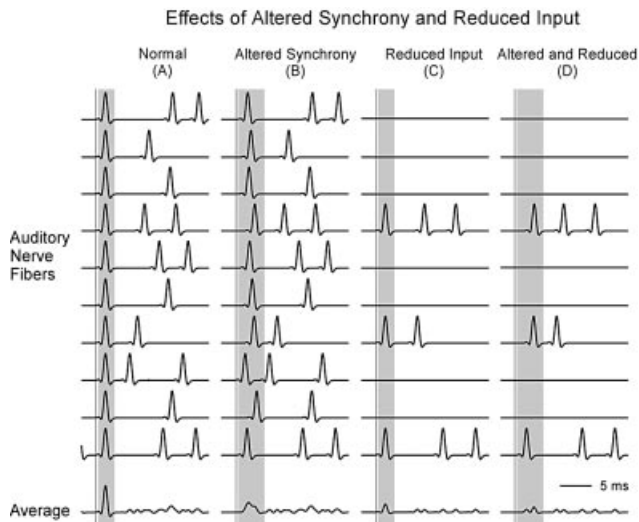

Fig. 13 A computer model of the discharges of 10 auditory nerve fibres synaptically related to a single inner hair cell. The fibres are spontaneously active and also discharge at short latency to a transient sound stimulus presented at the vertical line. The average potential of these fibres is shown below each column: (**A**) normal, all fibres discharge synchronously to the acoustic stimulus; (**B**) altered synchrony, all fibres discharge but their latency is delayed up to 1 ms from **A**; (**C**) reduced input, only three fibres are active without a change in synchrony; (**D**) combination of altered (synchrony) and reduced input. Note that the averaged nerve activity is both reduced in amplitude and prolonged in duration in **B**; reduced in amplitude in **C** and reduced in amplitude and prolonged in duration in **D**, making it indistinguishable from spontaneous activity levels.

may be determined by the specific amino acid substitution in the P0 protein.

The clinical course and clinical findings in *MPZ* families with deafness are similar. The onset of symptoms occurs when subjects are adults; the disorder is slowly progressive; there are associated findings of deafness, Argyll–Robertson pupils, and often other autonomic dysfunction. The peripheral nerve pathology in affected family members with this constellation of findings consists of axonal loss with incomplete remyelination (Chapon *et al.*, 1999; De Jonghe *et al.*, 1999; Misu *et al.*, 2000; and the subjects of this report). Deafness has not been reported accompanying *MPZ* mutations expressing a CMT1 phenotype. However, the presence or absence of deafness with *MPZ* disorders is probably unrelated to demyelination versus axonal loss, since other demyelinating forms of CMT, particularly those due to PMP22 mutations or duplication of 17p12, may have deafness as an associated finding. The family described by Kovach *et al.* (2002), with a PMP22 mutation, had audiological findings consistent with both AN (absent or abnormal ABRs) and cochlea sensory deafness (absent OAEs). The audiological findings for the two sisters in family Hou 216 (Boerkoel *et al.*, 2002) are provided in Berlin *et al.* (1996), where they are designated as patients 2 and 3. They both have a diagnosis of AN (preserved OAEs, no ABRs). Deafness due to AN is found in HSMN-Lom (Butinar *et al.*, 1999) with peripheral nerve changes of both primary demyelination and axonal loss. The affected protein in this disorder may play a role in regulating the Schwann cell signalling necessary for axonal survival (Kalaydjieva *et al.*, 2000), meaning that both myelin and axons may be affected early in the disease. Thus CMT disorders with axonal loss and demyelination, and a combination of the two processes have

Table 5 *Neural mechanisms contributing to AN*

Test	Result	Mechanism	
		Reduced input	Desynchrony
Electrophysiology			
ABR	Wave V delayed	+	–
ABR	Absent	+	+
Acoustic middle ear reflexes	Absent	+	–
ABR with body temperature	Lost when febrile	+	–
Long-latency auditory EPs	N100 delayed	+	–
N100 latency variability	Normal	–	–
EABR	Present	+	+
Psychoacoustics			
Signal/noise	Non-linear	+	–
Gap detection	Prolonged	+	+
TMTF detection	Abnormal at fast rates	+	+
Speech discrimination	Impaired	+	+
Low frequency DL	Abnormal	–	+
High frequency DL	Normal	–	–

ABR = auditory brainstem response; EABR = electrically evoked auditory brainstem response; EPs = evoked potentials. TMTF = temporal modulation transfer function; DLs = difference limens.

an associated auditory nerve involvement. Additional studies of hearing function in CMT and other neuropathic disorders may reveal features specific to their pathology.

Pathophysiology of the auditory dysfunction

Two mechanisms have been proposed to account for abnormalities of auditory function found in AN: (i) impaired synchrony among nerve fibres (Starr *et al*., 1991; Kraus *et al*., 2000); and/or (ii) reduced neural input (Starr *et al*., 2001*a*). Neural synchrony in the auditory pathway may enhance encoding of temporal features of acoustic signals used for speech perception and sound localization. The neural basis for synchrony is organized structurally in the auditory periphery, where ~25 afferent auditory nerve fibres make synaptic contact with each inner hair cell. Spontaneous and click-evoked discharges of 10 fibres (computer model) are represented in Fig. 13 to show the effects on the averaged compound action potential accompanying demyelination, axonal loss and their combinations. When the hair cell is activated, there is normally synchronous excitation of discharges in nerve fibres in contact with the inner hair cell (Fig. 13A), leading to a clear averaged compound action potential that is distinguished from spontaneous discharges. Alterations of the synchrony of discharge with demyelination of auditory nerve fibres or impaired synaptic coupling between inner hair cells and auditory nerve dendrite result in changes of both amplitude and duration of the averaged nerve responses (Fig. 13B). When the numbers of fibres are reduced due to axonal loss, the compound action potential is unchanged in form but markedly reduced in amplitude (Fig. 13C). In addition, many of the nerve fibres that were partially damaged would be unable to discharge at high rates due to the development of conduction block affecting auditory perceptual abilities involving suprathreshold discriminations (Turner and Nelson, 1982; Zeng and Turner, 1992). A combination of reduced neural input and desynchrony (Fig. 13D) would have profound effects on the formation of a detectable compound action potential.

We will examine the role that desynchrony and reduced neural input play in the hearing and clinical test abnormalities found in subjects with AN (see Table 5, containing electrophysiological and psychoacoustic test abnormalities arranged according to their probable mechanisms).

Test results related to reduced neural input in AN

(i) ABR tests of auditory function earlier in the hearing disorder (age 49 years in subject 4-1, age 46 years in subject 4-2) showed wave V, the component generated in the region of the lateral lemniscus (Achor and Starr, 1980), to be present to high signal intensities (75 dB normal hearing level, nHL). The latency of wave V was abnormally delayed to 6.1 and 6.8 ms in subjects 4-1 and 4-2, respectively (upper limit of

normal at 75 dB nHL is 5.8 ms). Three years later, wave V in subject 4-1 was unobtainable bilaterally. We have not retested subject 4-2. The presence of a wave V is evidence of preserved neural synchrony sufficient to evoke time-locked brainstem electrical activity in the ABR. The subsequent loss of the ABR with progression of the neuropathic disorder is consistent with continued loss of auditory nerve fibres and/or the development of altered neural synchrony among remaining fibres.

The prolonged latency of wave V in the AN subjects of this report is probably related to slowed conduction velocity of the auditory nerve. The human auditory nerve is ~2.5 cm in length and has an average conduction velocity of 20 m/s (Moller *et al*., 1994). The delay in wave V in our AN patients was ~1 ms, a value that would occur if the averaged conduction velocity were reduced to ~11 m/s. A prolonged ABR wave V has been found in close to 50% of AN patients (Starr *et al*., 2001*b*) suggesting that there is slow nerve conduction in a significant number of AN subjects.

(ii) Acoustic middle ear muscle reflexes are absent in AN, whereas their activation by nonacoustic stimuli is preserved (Starr *et al*., 1998). Intense sounds (~100 dB nHL) normally activate a large proportion of auditory nerve fibres that discharge at high rates for several seconds, leading to reflex excitation of stapedial motor neurons. The absence of acoustic middle ear muscle reflexes in AN subjects is consistent with reduced numbers of auditory nerve fibres being activated and/or their ability to discharge at high rates (Wang *et al*., 1997).

(iii) AN is sensitive to hyperthermia. In some AN subjects, profound deafness and loss of ABRs develop when body temperature becomes elevated 1–2°C (Starr *et al*., 1998). Hearing and ABRs are restored promptly when body temperature returns to normal. These findings are consistent with a reduction of neural input accompanying the development of conduction block in demyelinated fibres accompanying slight rise (1–2°C) of core temperature.

(iv) N100 latency of auditory cortical potentials to moderate intensity tones (75–80 dB SL) was quite similar in AN (106 ms) and controls (92 ms). However, when intensity was reduced to 20–40 dB SL, the latency of N100 in AN lengthened disproportionately, reaching 171 ms, whereas in the control the latency shifted only to 102 ms. The extent of the latency difference between AN and control (69 ms) is far in excess of the 25–30 ms latency shift encountered in normal subjects when signal intensity and the resulting auditory nerve input is reduced (Picton *et al*., 1977). The steep slope for changes of latency with signal intensity in AN may be a form of 'central recruitment' accompanying the hearing impairments. Cody *et al*. (1968) described an abnormal growth of N100 amplitude as a function of signal intensity in individuals with 'sensorineural' hearing loss, and speculated as to its relationship to abnormal growth of loudness often encountered in such patients. For AN subjects, psychoacoustic measures of intensity processes are normal in contrast to their marked abnormality of temporal processes (Zeng *et al*.,

2000). The mechanisms underlying altered cortical excitability in AN may reside within the cortex. An animal model of AN showing increased excitability of auditory cortex did not have a corresponding excitability change of inferior colliculus (Salvi *et al.*, 1999). The abnormal excitability of auditory cortex in AN may be likened to the central excitability changes encountered in disorders of other sensory systems following deafferentation.

(v) The detection of a 1000 Hz signal in background noise is impaired in AN patients (Fig. 10). In normal subjects, a 1 dB increase in noise requires an equal 1 dB increase of the signal to maintain detection independent of signal intensity. In subject 4-1, a 1 : 1 ratio was found at low noise intensities, but with moderate intensity noise tone intensity had to be raised by 3 dB for each dB of noise to restore detection. Auditory nerve fibres discharge in phase (synchrony) to low frequency tones relatively independent of intensity levels (Johnson, 1980; Paolini *et al.*, 2001) making it likely that the impaired detection of a 1000 Hz signal in noise in our AN subject is best accounted for by reduction of available neural elements or their discharge rate as intensity is raised.

Test results related to neural desynchrony in AN

(i) The measures of latency variability of the N100 cortical potential in the individual trials contributing to the averaged potential for the AN and the normal subject were both ~30 ms. Desynchrony of auditory nerve discharges in AN may amount to only a few milliseconds to result in impaired auditory function but of insufficient magnitude to affect the latency variability of auditory cortical potentials.

(ii) The finding that ABRs in AN subjects can be evoked by electrical stimulation of auditory nerve through a cochlear implant in subjects who did not have an ABR to acoustic stimulation (Shallop *et al.*, 2001; Buss *et al.*, 2002; Madden *et al.*, 2002) is evidence that the auditory nerve is capable of synchronous activation in AN. Electrical stimulation by a cochlear implant is thought to be effective by activating ganglion cells and not peripheral dendrites or inner hair cells (van den Honert and Stypulkowski, 1987). Thus, the finding of an electronically evoked ABR is consistent with a distal site of lesion of auditory nerve in AN without distinguishing whether the distal lesion affected a reduction of neural input and/or desynchrony of discharge.

(iii) Frequency difference limens are abnormal in AN for low but not high frequency tones (Fig. 9). Because the auditory nerve is capable of phase locking to frequencies up to 5000 Hz (Johnson, 1980), pitch may be coded by temporal cues at low frequencies and by place cues at high frequencies. The results in the AN subject of this and another report (Starr *et al.*, 1991) appear to support a temporal desynchrony theory in AN, since frequency discrimination at low frequencies (<4000 Hz) was impaired whereas discrimination at high frequencies (8000 Hz) was normal.

Test results compatible with both reduced neural input and desynchrony

Most of the psychoacoustic abnormalities (gap detection, temporal modulation transfer function, speech discrimination) found in AN are compatible with reduced neural input and/or altered neural synchrony. A desynchronous response of the auditory nerve would affect temporal and speech performance directly. A phenomenological model and its partial validation has been provided by Zeng *et al.* (1999) showing that temporal smearing alone can account for decreased speech performance in AN subjects. On the other hand, gap detection threshold can be elevated tens of milliseconds in normal listeners when signals are at low sensation levels (Zeng *et al.*, 1999). This intensity effect in normals indicates that reduced neural input may account for impaired temporal processing because, at low sensation levels, the number of activated neurons is quite limited whereas synchrony of discharge is preserved (Johnson, 1980; Paolini *et al.*, 2001).

Conclusions

This study of a family with deafness of the AN type and peripheral neuropathy has identified pathological and physiological processes underlying the auditory functional impairment. The pathological findings suggest that the disorder is related to damage of auditory nerve fibres beginning distally in the nerve while the inner hair cells are spared. The nerve fibre disorder results in a reduction of neural input due to (i) loss of auditory ganglion cells and nerve fibres and (ii) reduction in discharge rates of remaining fibres. Desynchrony of discharge in the remaining nerve fibres was considered a possible additional factor contributing to the hearing disorder. There are sites in the auditory periphery other than the ganglion cells that, if affected, could also lead to a reduction and desynchrony of auditory nerve input and the clinical picture of AN. These sites include inner hair cells, synapses between inner hair cells and dendrites of auditory nerve, terminal dendrites of auditory nerve, or dendrites and axons of auditory nerve. Physiological and pathological studies of the auditory periphery in AN will probably help to define the varieties of AN.

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