Atypical hereditary neuropathy with liability to pressure palsies (HNPP): the value of direct DNA diagnosis

Maria Sessa, Raffaello Nemni, Angelo Quattrini, Ubaldo Del Carro, Lawrence Wrabetz, Nicola Canal

Abstract

We report two patients with suspected hereditary liability to pressure palsies. Neurophysiological studies showed a mixed axonal-demyelinating sensory-motor polyneuropathy with focal slowing of conduction velocities at the common sites of entrapment. Morphological studies on sural nerve biopsy from the proband showed active axonal regeneration without typical tomacula. Molecular analysis confirmed the presence of a deletion of chromosome 17p11.2 in both patients. Our observation confirms the heterogeneity of hereditary liability to pressure palsies and the relevance of DNA testing for the diagnosis of this hereditary neuropathy.

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Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal dominant disorder characterised by recurrent palsies or sensory disturbances after minor trauma to peripheral nerves. Pathological changes observed in peripheral nerves of patients with clinical and neurophysiological features of HNPP usually include sausage-like thickening of myelin sheaths, called tomacula.¹ The majority of HNPP cases are characterised by a 1.5 Mb deletion in chromosome 17p11.2.² After the introduction of DNA based tests for the diagnosis of HNPP, tomacula were described in the sural nerves from almost all biopsied patients with genetically defined HNPP.³⁻¹⁶

We report a family with clinical, neurophysiological, and genetic characteristics typical of HNPP in which morphological study performed in the proband did not show typical tomacula in the sural nerve biopsy.

Materials and methods

For morphological studies, a portion of sural nerve was fixed in 2% buffered glutaraldehyde and postfixed in 1% osmium tetroxide. After alcohol dehydration, nerve fragments were embedded in Epon 812. Transverse sections (0.5-1 µm thick) were stained with toluidine blue and examined by light microscopy. Morphometrical studies were performed as previously described.¹⁷

For molecular analysis, DNA was extracted from leucocytes from peripheral venous blood. Deletion of chromosome 17p11.2 was detected with quantitative or deletion breakpoint analysis by Southern blotting and by microsatellite polymorphisms by PCR. For Southern blotting, a single copy fragment of pVAW409R3a and pVAW412R3a, contained within the deletion, and a reference autosomal probe localised outside the deletion, detecting three *Eco*RI alleles of 4.2, 2.2, and 3.5 kb respectively, were used. The ratios pVAW409R3a/reference probe and pVAW412R3a/reference probe were expressed as a percentage of values obtained from control DNA.¹⁸ The breakpoints were detected on Southern blots of *Eco*RI/*Sac*I digested DNA and hybridised with probe pJ7.8P.¹⁹ PCR was performed using the GT strand (CAGAACCACAAAATGTCTTGCATTC) and the CA strand (GGCCAGACACCAGGCTCTGC) oligonucleotide primers flanking the (GT)_n repeat. The GT strand was labelled with γ-dATP using T4 polynucleotide kinase, according to the manufacturer's instructions. PCR was performed in a 100 µl final volume containing 300 ng genomic DNA, 50 mmol/l KCl, 200 mmol/l Tris HCl, pH 8.4, 1.5 mmol/l MgCl₂, 100 µg/µl acetylated BSA, 50 pmol of cold primer, 4 pmol of labelled primer, 200 µmol/l each dNTPs, and 1.25 U *Taq* polymerase (Perkin Elmer, Norwalk, CT).

Results

CASE REPORTS

The father, a 45 year old man, reported the acute onset, three months earlier, of weakness and paraesthesia in the right hand after sustained pressure. On admission, the neurological examination showed normal cranial nerve function. Motor deficit was present in the distribution of the right ulnar and median as well as the right peroneal nerves where muscle strength was reduced to 4/5. Deep tendon reflexes were present with the exception of the right achilles tendon, which was elicitable with reinforcement manoeuvres. Plantar responses were flexor. Pes cavus and hammer toes were present. Vibration sensation was reduced distally in the legs; the other sensory modalities were preserved. Routine blood screening was negative; anti-GM1 antibodies were absent.

The son, an 18 year old parachutist, reported acute onset of weakness in his left shoulder after parachuting. Neurological examination

Department of
Neurology, IRCCS San
Raffaele Scientific
Institute, University of
Milan, Via Olgettina
60, 20132 Milano, Italy
M Sessa
R Nemni
A Quattrini
U Del Carro
L Wrabetz
N Canal

Correspondence to:
Dr Sessa.

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Figure 1 Transverse section of the sural nerve of the proband. There is a preferential loss of large myelinated fibres and numerous clusters of small myelinated fibres. No tomacula were detected. (Toluidine blue.)

Figure 2 Southern blot hybridisation pattern with probes pVAW412R3a, pVAW409R3a, and reference probe. The ratios pVAW409R3a/reference probe and pVAW412R3a/reference probe are approximately 50% of the ratios obtained from control patients. c: control patient. d: duplicated patient.

showed hyposthenia in his left supraspinatus, infraspinatus, and serratus anterior muscles where strength was reduced to 3/5. The remaining examination was normal, with the exception of pes cavus. The motor deficits recovered completely after several weeks. He denied a history of similar episodes in the past.

Neurophysiological study was performed in both patients. Motor and sensory conduction velocities of, respectively, the peroneal, median, and ulnar nerves and of the sural, median, and ulnar nerves were examined bilaterally. Distal latencies of MAP of the median nerves were markedly increased. Motor and sensory conduction velocities were slightly reduced in all nerves tested. A marked conduction slowing of motor velocity of the ulnar nerves at the elbow groove and of sensory velocity of the median nerve at the wrist was detected bilaterally. Sensory and motor action potential amplitude was at the lower normal limit. Needle examination showed, in both patients, chronic

Figure 3 RM11-GT microsatellite polymorphism analysis by PCR showing that both father and son have a single RM11-gt allele each of different size. c: control patient.

potentials especially in the distal muscles. In the son, fibrillation and fasciculation potentials were detected in supraspinatus, infraspinatus, and serratus anterior muscles.

A sural nerve biopsy was performed in the father. Histopathological studies showed no inflammatory cell infiltration and no vascular abnormalities. A preferential loss (fig 1) of large myelinated fibres and numerous clusters of small myelinated fibres, indicating active axonal regeneration after axonal injury, were observed. Almost every fibre had a thin myelin sheath when compared with axon diameter. Only a few myelin fibres had a normal axon/myelin ratio. No axonal degeneration was observed. No tomacula were detected in any of the fascicles examined. Morphometrical analysis confirmed a preferential loss of the large myelinated fibres, 12% being larger than 8 μ m (NV 22.8-33.6%).

SOUTHERN BLOT ANALYSIS

Quantitative analysis by Southern blot showed that the ratios pVAW409R3a/reference probe and pVAW412R3a/reference probe were approximately 50% of the ratios obtained from healthy subjects (fig 2), suggesting that both patients have a single copy of pVAW409R3a and pVAW412R3a.

PCR ANALYSIS

Microsatellite analysis by PCR showed that both father and son have a single RM11-GT allele, each of different size (fig 3). The size of the son's allele was the same as that of the mother. These data show that he inherited a deleted allele from his father and a normal allele from his mother.

BREAKPOINT ANALYSIS

In the mother four *EcoRI/SacI* restriction fragments of 6.0, 5.0, 2.8, and 1.8 kb respectively, corresponding to the CMT1A-REP restriction map, were detected. The additional 7.8 kb junction fragment generated by crossing over between the differential *SacI* and *EcoRI* sites in the CMT1A-REPs was detected both in the father and in the son, confirming the presence of the deletion in both patients (fig 4).

Discussion

HNPP is a distinct genetic entity whose clinical presentation may be extremely variable and should be differentiated from other hereditary or acquired neuropathies.

Figure 4 Southern blot hybridisation pattern of *EcoRI/SacI* digested DNA with probe pJ7.8P showing the junction fragment (arrow) in the father and in the son.

We report a patient with a clinical presentation suggestive of HNPP in whom pathological features were atypical and no tomacula were found in the sural nerve. The diagnosis, in the father and subsequently in his son, was confirmed by DNA testing.

Since the introduction of molecular studies, there has been only one report of three related HNPP patients in which tomacula were not found in the peripheral nerve.¹¹ As pointed out by those authors, genetic analysis of that family was difficult because no probes from the deleted region were fully informative. They then performed quantitative analysis by Southern blotting using a reference probe located outside the deletion, which is a less sensitive technique. Moreover, one of the three patients with a normal biopsy was found not to be deleted.

Pathological studies are not sufficient for the diagnosis of HNPP. In fact, in our patient, sural nerve biopsy suggested axonal damage based on the presence of numerous clusters of small myelinated fibres that indicated axonal regeneration. Furthermore, many thinly myelinated fibres were present and no tomacula were detected. In the past, HNPP has been defined as tomaculous neuropathy because of the presence, in the peripheral nerves, of characteristic sausage-like formations of the myelin sheath.²⁰⁻²² Even if present, tomacula are not pathognomonic of HNPP, having been de-

scribed also in other hereditary, acquired, and experimental neuropathies.²³⁻²⁹ Rather than on their presence, diagnosis of HNPP should rely on the number of tomacula.³⁰ Generally, cases in which tomacula are not found have a more advanced neuropathy.³¹⁻³² This observation has been confirmed by studies in transgenic PMP deficient mice, suggesting that tomacula are unstable formations predisposing to subsequent myelin degeneration.³³

Recently, HNPP has been shown to be characterised by a 1.5 Mb interstitial deletion in chromosome 17p11.2.² The introduction of DNA based tests for the diagnosis of HNPP has shown that the majority of clinically defined HNPP are deleted, although point mutations in the PMP-22 gene have been described³⁴⁻³⁵ and other genetic defects at other loci may be responsible for non-deleted patients.⁷ It has been reported that patients with PMP-22 point mutation showed a more severe phenotype, which is similar to the CMT1A phenotype.³⁶

It is now clear that HNPP is much more heterogeneous than previously thought from a clinical, morphological, and genetic point of view. Differential diagnosis is important for a correct prognostic evaluation and genetic counselling as well as for therapy.

Our observation, together with the previous report of Gonnaud *et al*,¹¹ supports the relevance of DNA analysis for the diagnosis of HNPP, especially when a family history is lacking. If the unequal crossing over hypothesis is correct,³⁷ the prevalence of HNPP should be roughly similar to that of CMT1A. Up to now, the prevalence of HNPP seems to be underestimated both because of misdiagnosis and paucity of symptoms. Molecular analysis, which is less invasive than nerve biopsy, represents an important and specific tool for diagnosis of HNPP and should be used first. The routine use of molecular analysis would permit an estimate of the prevalence of the deletion and the selection of non-deleted patients to be analysed for new mutations.

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