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Molecular genetic diagnosis of the 1.5 Mb deletion causing hereditary neuropathy with liability to pressure palsies (HNPP) in a Polish family

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Abstract. A molecular genetic analysis was performed in one Polish family with hereditary neuropathy with liability to pressure palsies (HNPP), using two distinct molecular genetic methods, i.e. RFLP and STR analysis. This permitted to reveal the presence of a 17p11.2 HNPP deletion both in the proband and in her mother. The molecular analysis in the proband was supplemented with nerve conduction rate tests and sural nerve biopsy. We conclude that the relatively low prevalence of HNPP in Poland is caused most probably by lack of access to molecular genetic testing. In the future HNPP molecular testing should be offered to all patients in Poland.

Key words: HNPP neuropathy, RFLP analysis, STR analysis.

Introduction

Hereditary neuropathy with liability to pressure palsies (HNPP) is a rare autosomal dominant disorder of the peripheral nervous system characterised by recurrent palsies of the peripheral nerves with recovery within weeks or months. Usually weakness develops after trauma, but in some cases traumatic aetiology cannot be found. The clinical features of the disease were first described by

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DE JONG in 1947. In the next years several cases of HNPP were recorded. The diagnosis of HNPP is based on the clinical features and morphology of sural nerve biopsy in which numerous focal thickenings of myelin (so-called tomaculae) characteristic for the disease can be seen. The clinical picture of HNPP is characterised by a wide polymorphism of symptoms. In some cases HNPP is manifested as a slowly progressive disorder of the peripheral nervous system; in other cases no clinical symptoms are present. Only electrophysiological and histopathological investigations can help to determine the HNPP diagnosis. In contrast to the uninformative clinical picture in the majority of HNPP cases, nerve conduction rate tests reveal that the HNPP process is more generalised, not limited to certain nerves. In the majority of HNPP cases a 17p11.2-p12 submicroscopical deletion was detected. In Charcot-Marie-Tooth type 1A (CMT1A) patients, a reciprocal 1.5 Mb tandem duplication was identified previously (LUPSKI et al. 1991, RAEYMAEKERS et al. 1991). The peripheral myelin protein (PMP22) has been assigned to the middle of the 1.5 Mb region, deleted in HNPP and duplicated in CMT1A (TIMMMERMAN et al. 1992, VALENTIJN et al. 1992, PATEL et al. 1992, MATSUNAMI et al. 1992). As a consequence, HNPP deletion patients carry only one copy of the *PMP22* gene, i.e. the gene located in the normal homologous chromosomal 17p11.2 region (CHANCE et al. 1993).

Recently several molecular genetic techniques have been developed to detect the HNPP deletion in the 17p11.2 region. Here we present results of a molecular genetic analysis of a Polish HNPP family using restriction fragment length polymorphism (RFLP) and short tandem repeat (STR) analysis.

Material and methods

The proband (III.2) (Figure 1), aged 15 years, during the last 7 years had a transitional paresis of the right peroneal muscles twice. There were no precipitating factors. A neurological examination performed after these episodes did not detect any abnormality. Conduction rate tests of peripheral nerves revealed a generalised abnormality in motor and sensory fibres associated with axonal and demylinating changes, more advanced in the lower limbs. In sural nerve biopsy nearly all teased fibres showed de- and remyelination and the presence of numerous focal thickenings (Figures 2, 3).

For DNA analysis, informed consent from all the family members was obtained. High molecular weight DNA was isolated from total blood samples according to standard isolation procedures. DNA samples were stored at –20°C.

For RFLP analysis, genomic DNA was digested with the *MspI* restriction enzyme, and digested DNA fragments were separated on 0.8% agarose gels and transferred to nylon membranes (Hybond N+, Amersham, England). Hybridisation was performed with two radiolabelled single copy probes pVAW409R3a (D17S122) and pEW401HE (D17S61). Additionally, probe SF85(D21S48) was

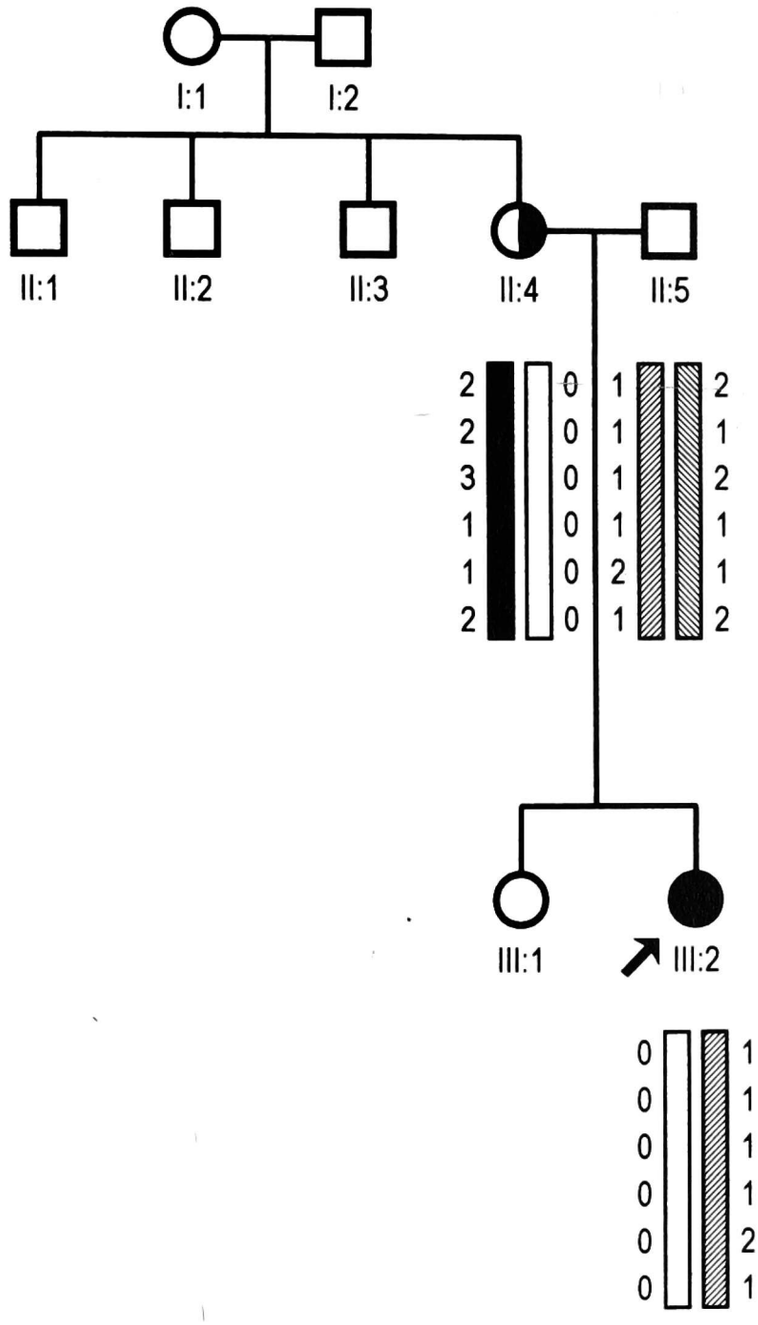


Figure 1: Pedigree chart of a Polish HNPP family

Explanations: circles – females, squares – males, filled symbol – HNPP, half filled symbol – asymptomatic HNPP individual, blank symbol – unaffected individual. The hemizygosity was detected both in the proband (III.2) and proband's mother (II.4). The haplotypes, indicated below symbols, are derived from the segregation data obtained with markers in order from centromere to telomere: RM11-GT and pVAW409R3a(D17S122)-103B11ac1(D17S1357)-pEW401HE(D17S61)-133C4ac1(D17S1358) -AFM191xh12(D17S921).

used as a control to estimate visually the density of *MspI* alleles of pVAW409R3a and pEW401HE in the dosage analysis approach (RAEYMAEKERS et al. 1992). The probes were labelled with [α-^{32}P] dCTP by random priming method. The hybridisation patterns were obtained by autoradiography using Kodak T-mat films and developed according to standard procedures.

STR markers 103B11ac1 (D17S1357), 133C4ac1 (D17S1358), RM11 (D17S122) and AFM191xh12(D17S921) were fluorescently labelled and PCR

amplified according to methods previously described (NAVON et al. 1995). An aliquot of the PCR products was mixed with formamide and a fluorescently labelled size standard marker, and denatured by heating. PCR products were loaded on polyacrylamide sequencing gel and electrophoresed in the automated DNA sequencer 377 (Applied Biosystems Inc. Foster City, USA). Finally, the data were collected and analysed using the GENESCAN-ABI software which estimated the size of the STR alleles and computed a calibration curve permitting quantification of the signals based on peak heights and peak areas.

Results

In the present study we performed a DNA analysis of the HNPP patient (III.2) and her parents (II.4) and (II.5) using two distinct molecular genetic methods, i.e. RFLP and STR analysis. All DNA markers used in this study are located within the 1.5 Mb HNPP deletion region in 17p11.2 (LUPSKI et al. 1991, RAEYMAEKERS et al. 1992, BLAIR et al. 1995). In the proband (III.2) as well as in her mother (II.4), hemizygosity of the *MspI* alleles was detected for the RFLP markers pVAW409R3a and pEW401HE. In contrast, the father II.5 was homozygous for the *MspI* alleles (Figure 4). The hemizygosity in the proband (III.2) and the homozygosity in proband's father (II.5) were determined by visual control of the density of *MspI* alleles of pVAW409R3a (data not shown) and pEW401HE probes.

STR analysis of markers AFM191xh12(D17S921) and 103B11ac1(D17S1357) clearly demonstrated hemizygosity in the mother (II.4) and the proband (III.2). In the unaffected father (II.5), two different STR alleles were detected (Figure 5).

Discussion

The present study is one of the first molecular genetic diagnoses concerned inherited peripheral neuropathies performed in Poland (KOCHAŃSKI et al. 1998, 1999). The molecular methods used in this study revealed the presence of a 17p11.2 interstitial deletion in two HNPP individuals belonging to one family. In 1993, an interstitial chromosome 17p11.2 submicroscopical deletion of 1.5 Mb was detected in nearly all HNPP patients (CHANCE et al. 1993). In HNPP families, the 1.5 Mb deletion is inherited as an autosomal dominant trait. *De novo* HNPP deletion is caused by an unequal crossing-over event between repeat elements flanking the 1.5 Mb region (REITER et al. 1996, TIMMERMAN et al. 1996). In Europe, the HNPP deletion frequency was estimated to be 84% (NELIS et al. 1996a). Interestingly, a few HNPP patients without the 1.5 Mb interstitial deletion have been reported. These patients have point mutations in the *PMP22* gene (NICHOLSON et al. 1994, reviewed in NELIS et al. 1999). The presence of point

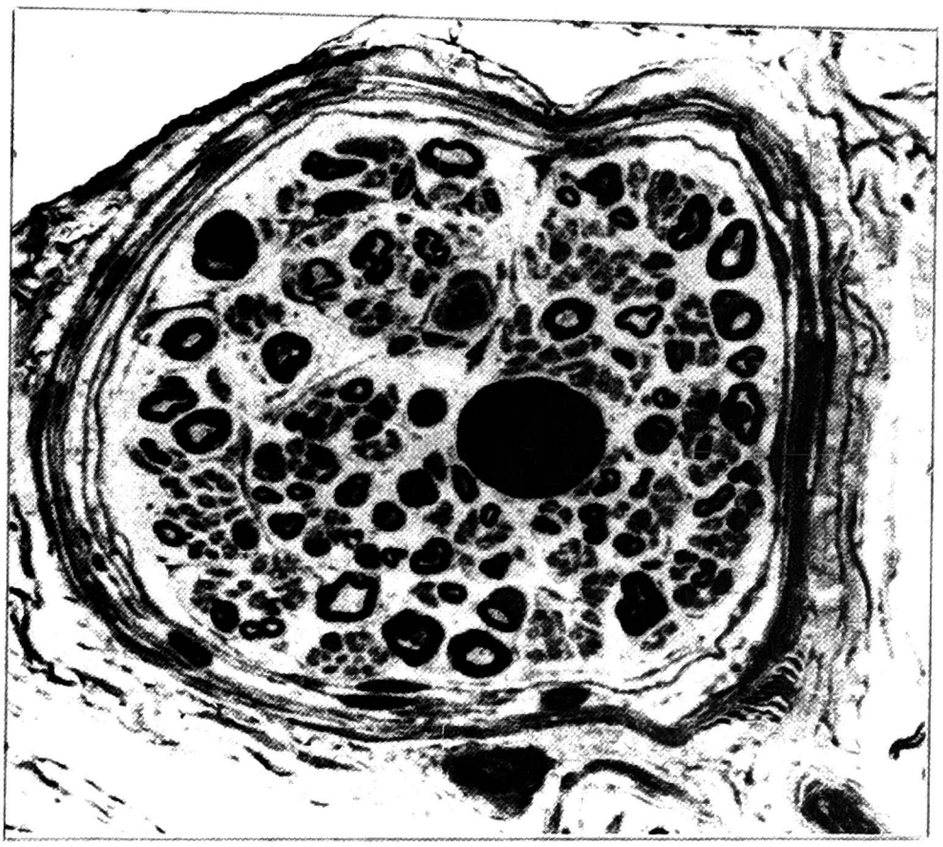


Figure 2. Cross-section of a fascicle from the sural nerve
A semi-thin Epon section stained by the Pal-Kultschizky method: a slight decrease in the density of myelinated fibres, one fibre with a thick myelin sheath (× 430)

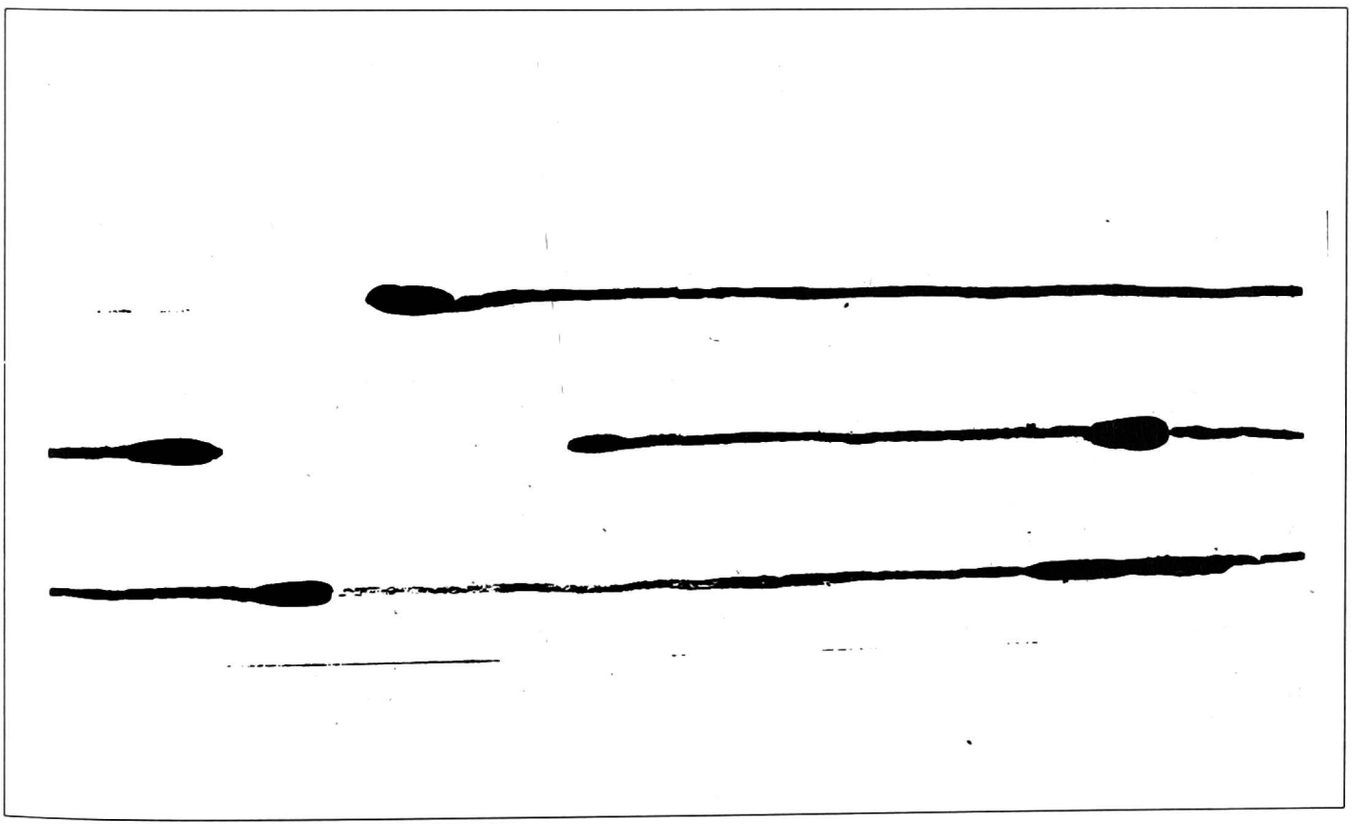


Figure 3. Consecutive portions of teased myelinated fibres showing several myelin thickenings, a remyelinating internode (upper part) and two demyelinated segments (middle and lower part) (× 120)

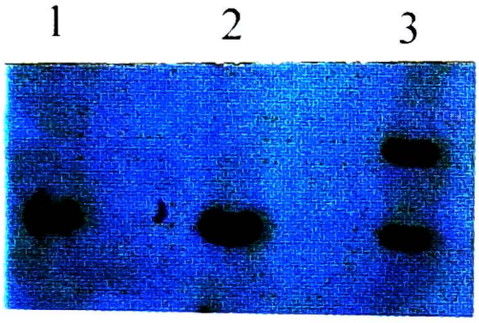
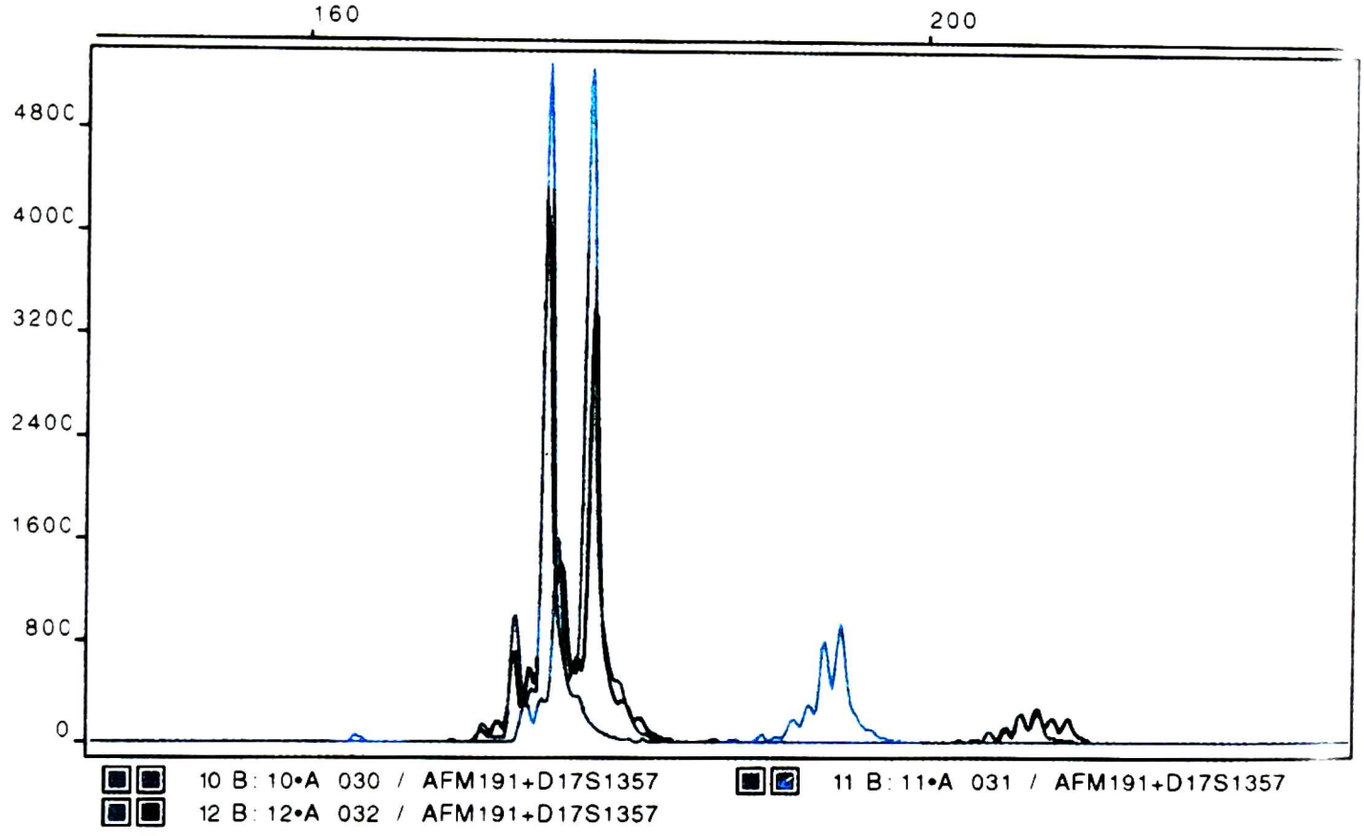


Figure 4: Detection of the 1.5 Mb HNPP deletion by Southern blot analysis
RFLP results with probe pVAW401HE, showing hemizygosity for *MspI*, 4.4 kb allele in the proband (III:6) – lane1, and homozygosity for *MspI*-4.4 kb allele in proband's father (II.5) – lane 2. In lane 3 the heterozygosity with 5.5 kb and 4.4 kb *MspI* alleles in the control sample from an unaffected person can be seen.



Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
10B, 43	66.35	175.31	5359	47962	2488
10B, 47	75.20	206.11	227	2290	2820
11B, 37	64.11	178.34	5341	55103	2404
11B, 43	68.67	194.11	940	11778	2575
12B, 47	66.45	175.37	4203	29500	2492
12B, 49	67.28	178.24	3411	29574	2523
12B, 53	75.31	206.01	265	2124	2824
12B, 55	75.87	207.95	192	1933	2845

Figure 5: Detection of the 1.5 Mb HNPP deletion by STR-PCR analysis
Results of detection of D17S1357 with the ABI 373A automated DNA sequencer. The pattern for the proband (III.2- 10 B: 030) illustrates the hemizygosity for D17S1357 (206 bp allele). The hemizygosity for proband's mother (II.4-11B: 031) is shown by D17S1357 (194 bp allele). In contrast to the proband and proband's mother, the results of proband's father (II.5-12B: 032) show the heterozygosity for D17S1357 (206bp and 208bp alleles).

mutations in the *PMP22* gene in HNPP patients supports the hypothesis that the *PMP22* gene plays a major role in the molecular pathology of HNPP.

In the light of the above, the accuracy of the HNPP diagnosis is determined by the molecular genetic analysis and S if needed S neuropathological analysis.

The relatively low incidence of HNPP in Poland is most probably due to the unrecognised genetic background of the disease, misdiagnosis and lack of access to molecular genetic testing. To conclude, DNA diagnosis for HNPP and other inherited peripheral neuropathies such as CMT type 1, can now be offered to all patients in Poland.

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