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De novo Ser72Leu mutation in the peripheral myelin protein 22 in two Polish patients with a severe form of Charcot-Marie-Tooth disease✪

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To date, 12 cases of heterozygous Ser72Leu mutations in the peripheral myelin protein 22 have been reported in patients suffering from severe demyelinating form of Charcot-Marie-Tooth disease (CMT1) and congenital hypomyelinating neuropathy (CHN) [MIM# 605253]. In the present study we report two cases of *de novo* S72L mutations in the *PMP22* gene detected in patients of Polish origin suffering from CMT1 disease.

Charcot-Marie-Tooth disease (CMT) is the most common inherited neuromuscular disorder in man, with an overall prevalence of 1 in 2500 (Skre, 1974). CMT is an extremely heterogenous group of disorders. In general, it is characterized by progressive weakness and atrophy of distal muscles, absence of deep tendon reflexes, and sensory abnormalities (Harding & Thomas, 1980).

Between 1991 and 2004, thirty three genes have been shown to be mutated in different forms of CMT disease. The biological functions of the proteins encoded by the genes mutated in CMT cover a wide spectrum en-

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Abbreviations: CMT, Charcot-Marie-Tooth disease; HNPP, hereditary neuropathy with laiability of pressure palsies; SSCP, single-stranded conformation polymorphism.

compassing small ion channels (*Cx32)* and transcription factors (*EGR-2*) (Reilly, 2000).

The first gene mutation detected in CMT disease (Raeymaekers *et al.*, 1991) was duplication of the peripheral myelin protein gene (*PMP22*).

Peripheral myelin protein of 22 kDa (PMP22) [MIM # 601097] is a myelin molecule associated with Schwann cells of peripheral nerves. PMP22 is produced in a glycosylated form that appears to be generated from an 18 kDa precursor (Pareek *et al.,* 1993). The protein is associated structurally with Schwann cell membranes and similarly to other proteins of compact myelin it is produced when myelin is being formed. The role of the PMP22 protein in the peripheral nervous system is still unknown and two functions have been postulated so far. The *PMP22* gene was shown to be associated with the regulation of the cell cycle and myelin structure in the peripheral nervous system.

The *PMP22* mRNA is identical to growth arrest-specific-3 mRNA (Gas-3; Welcher *et al.,* 1991). The *Gas-3* gene has been shown to be associated with cellular growth arrest in the G0 phase of the cell cycle (Schneider *et al.*, 1988). On the other hand, the autosomal dominant point mutations in the *PMP22* gene were found in the trembler (Tr) and trembler-J (Tr^J) mice (Suter *et al.,* 1992a; 1992b). Those mice mutants show limb paralysis, tremor, seizures and severe hypomyelination of peripheral nerves.

In humans, *PMP22* gene duplication was shown to segregate with the most common inherited peripheral neuropathy, i.e. Charcot-Marie-Tooth type 1A disease (CMT1A) [MIM# 118220] (Timmerman *et al.*, 1992). Eleven mutations in the *PMP22* gene have been shown to segregate with hereditary neuropathy with liability to pressure palsies (HNPP). In patients suffering from Dejerine-Sottas disease, 19 mutations in the *PMP22* gene have been reported (Web site). Only one mutation, i.e. S72L, was reported in the congenital hypomyelinating neuropathy (CHN) (Roa *et al.,* 1993).

In this study we report on two unrelated patients of Polish origin harboring *de novo* S72L substitutions in the PMP22. Until now, the S72L substitution in the PMP22 has not been reported in Polish patients.

MATERIAL AND METHODS

Genomic DNA was extracted from white blood cells of two patients and their parents. In the two patients, a diagnosis of congenital hypomyelinating neuropathy was established (Dr. H. Drac). Four exons of the peripheral myelin protein 22 gene (*PMP22*) were amplified by polymerase chain reaction (PCR) using previously published primers (Roa *et al.,* 1993). For mutation screening, single stranded conformation polymorphism analysis (SSCP) was performed in the probands and their healthy parents as previously described. Amplification products of exon 3 of the *PMP22* gene were sequenced using Big Dye Terminator Sequencing Ready Reaction Kit (Applied Biosystems). Samples were run and analyzed on an ABI PRISM 373 fluorescent DNA sequencer (Applied Biosystems).

RESULTS AND DISCUSSION

An altered SSCP pattern of exon 3 of the *PMP22* gene was found in the two patients.

Direct DNA sequencing of exon 3 performed in the two patients revealed a heterozygous missense mutation at codon 72 (TCG to TTG) resulting in the serine to leucine amino acid change (Fig. 1). SSCP analysis of the *PMP22* gene in the patients healthy parents did not show any abnormality, suggesting that the *PMP22* gene was most likely not mutated.

In addition, DNA sequencing of exon 3 of the *PMP22* gene performed on the DNA sam-

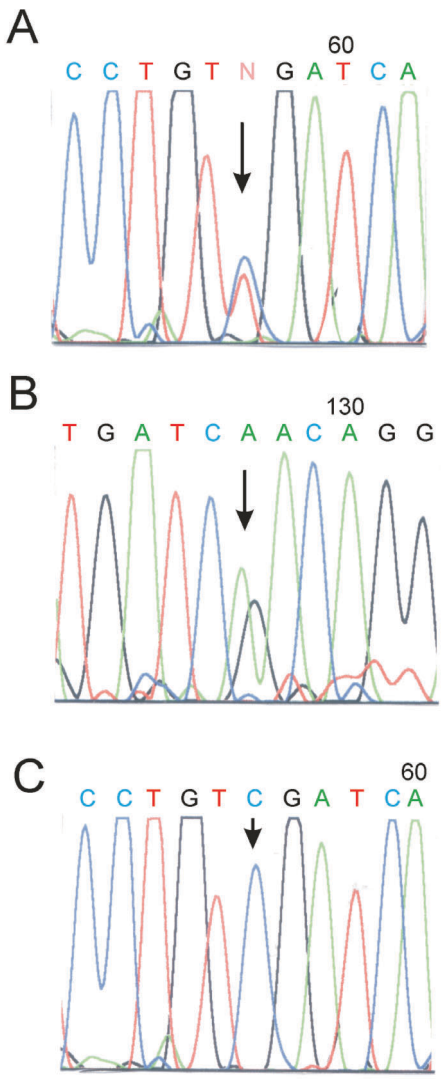

Figure 1. Fluorograms from direct sequencing of the *PMP22* gene exon 3.

A. Arrow indicates the C/T (sense strand) heterozygosity at the nucleotide position 264 (codon 72). **B**. Arrow indicates the G/A (antisense strand) heterozygosity at the nucleotide position 264 (codon 72). **C**. Wild type sequence in a healthy individual.

ples from the parents revealed a normal, wild-type sequence.

Our study is the first ones to document the presence of a heterozygous S72L mutation in patients originating from Poland.

Since its first description in 1993, the S72L mutation has been reported in 12 patients originating from America, Europe and Asia (Ionasescu *et al.*, 1996; Marques *et al.*, 1998; Numakura *et al.*, 2002). Only in one case was the S72L mutation reported to be transmitted from a mother to the child (Roa *et al.*, 1993).

The S72L mutation has been reported only in the heterozygous state. It is possible that homozygous S72L mutation may be lethal.

Whether the Ser72Leu substitution alters the structural or regulatory function of the PMP22 protein remains unknown.

Loss-of-function mutations in the *PMP22* gene are associated with the phenotype of HNPP [MIM #162500]. HNPP was previously reported to result from a deletion of one *PMP22* gene allele (Chance *et al.*, 1993). Although heterozygous Val30Met and Ala67Thr mutations do not result in deletion of the *PMP22* gene, they are associated with the HNPP phenotype (Sahenk *et al.*, 1998; Nodera *et al.*, 2003). Thus, the Val30Met and Ala67Thr mutations may be classified as loss-of-function substitutions. Recently, a Cys109stop mutation was reported in the *PMP22* gene in CMT1 affected patients. The heterozygous Cys109stop mutation, although classified as a loss-of-function, did not result in the HNPP phenotype. Thus, the identification of the Cys109stop mutation in the *PMP22* gene segregating with CMT1 phenotype suggests that the relationship between the type of mutation and the phenotype is not as straightforward as previously reported (Abe *et al.*, 2004).

In contrast to the loss-of-function mutations, the heterozygous S72L substitution in the PMP22 protein results in the severe phenotype of the Charcot-Marie-Tooth disease.

On the basis of the relatively high rate of S72L mutations in the *PMP22* gene, the Ser72Leu was designed as a possible "hot spot" in the PMP22 gene. The S72L mutation is located in a CpG rich region of the *PMP22* gene which is a hypermutable region (Marques *et al.*, 1998).

The molecular basis of the dominant negative effect of this mutation remains unknown. Further *in vitro* studies using cells transfected with the S72L mutated gene or transgenic animals are required to shed light on

the nature of the dominant negative effect of the S72L mutation in humans.

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