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Original Article

Charcot-Marie-Tooth disease: a novel Tyr145Ser mutation in the myelin protein zero (*MPZ, P0*) gene causes different phenotypes in homozygous and heterozygous carriers within one family

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Abstract. Charcot-Marie-Tooth disease type 1B (CMT 1B) is caused by mutations in the gene coding for peripheral myelin protein zero (MPZ, P0) that plays a fundamental role in adhesion and compaction of peripheral myelin. Here we report a Costa Rican family with a hereditary peripheral neuropathy due to a novel Tyr145Ser MPZ mutation. Four family members were

heterozygously affected; two siblings of two heterozygous carriers were homozygous for this mutation. On neurological examination the heterozygous parents and their homozygous children both showed distal sensory deficits. The mother and the siblings displayed impaired deep tendon reflexes and mild sensory ataxia. The homozygous individuals were more severely affected with an earlier age of onset, distal motor weakness, and pupillary abnormalities. Electrophysiological studies revealed both signs of demyelination and axonal nerve degeneration. The sural nerve biopsy of one sibling showed thinly myelinated nerve fibers, onion bulb formation, and clusters of regenerating fibers. On electron microscopy axonal degeneration and decompaction of inner myelin layers were found. This Costa Rican family shows phenotypic variability depending on the homozygous or heterozygous state of the Tyr145Ser mutation carriers.

Keywords MPZ, P0 - CMT1B - HMSN1B - Charcot-Marie-Tooth disease

A. Leal and C. Berghoff contributed equally to this work.

Introduction

Hereditary motor and sensory neuropathies (HMSN) are a clinically and genetically heterogeneous group of disorders of the peripheral nervous system. The most-common form is HMSN type 1, synonymous to Charcot-Marie-Tooth (CMT) disease type 1. Although the clinical course is often relatively mild, inter- and intrafamilial phenotypic variations are observed. Asymptomatic mutation carriers, as well as patients showing severe muscular weakness and atrophy, have been reported. One diagnostic hallmark of the demyelinating CMT type 1 is a reduced nerve conduction velocity (NCV); patients with the axonal or CMT type 2 show almost normal NCVs [*1*]. Mutations in at least four different genes can cause CMT type 1: the peripheral myelin protein 22 gene (*PMP22*), the myelin protein zero gene (*MPZ/P0*), the connexin 32 (*Cx32/GJB1*) gene, and the early growth response 2 gene (*EGR2*) [*2*]. The vast majority of patients carry a 1.4-Mb duplication in chromosome 17p11.2–12, including the *PMP22* gene [*3*, *4*]. Less frequently, mutations in *P0* are observed as a cause of CMT type 1B disease (CMT1B) [Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (MIM 118200)] [*5*].

P0 is the major structural protein of peripheral myelin, an integral membrane glycoprotein (28 kilodaltons) that is expressed exclusively in the Schwann cells. It is composed of an extracellular domain (immunoglobulin related), a transmembrane domain, and a basic intracellular domain. It contributes to the compaction of the intraperiod and major dense line [*6*, *7*]. The gene is located on chromosome 1q22-q23 and is composed of six coding exons. Both extracellular and intracellular domains are important for adhesion and myelination [*8*, *9*]. Mutations in P0 may affect the ability to form homotetramers or to mediate homophilic adhesion, processes that are fundamental for the compaction of myelin sheaths in peripheral nerves [*10*]. Morphological studies of patients with CMT 1B indicate signs of demyelination, such as thinly myelinated nerve fibers, impaired Schwann cell proliferation, tomacula, and uncompacted myelin layers. In addition to the pathological changes of the myelin sheath, axonal degeneration may be observed [*11*].

It is difficult to establish a correlation between genotype and phenotype of P0-related peripheral neuropathies [2]. Two severe, early onset forms of peripheral neuropathies, the Dejerine-Sottas syndrome and congenital hypomyelinating neuropathy, demonstrate the wide phenotypic variation of patients carrying *P0* mutations, depending on the codon position and the nature of the substitution [12]. Additionally the Roussy-Levy syndrome could be associated with a specific mutation in the extracellular domain of P0 [13].

Moreover, the phenotypic variability of *P0* mutation carriers comprises axonally pronounced peripheral neuropathies (CMT2), which has been shown for a Thr124Met mutation [14, 15]. Carriers of this Thr124Met mutation also show Argyll Robertson-like pupils, and deafness and dysphagia frequently accompany the disease. Here we present CMT patients of a Costa Rican family who carry a previously undescribed Tyr145Ser mutation of the *P0* gene in the heterozygous and homozygous state. The severity of the peripheral neuropathy corresponds to the genetic state of this particular *P0* mutation.

Materials and methods

A Costa Rican family of four generations with six living CMT patients was investigated. DNA from the family members was obtained from peripheral blood, following standard methods.

All six coding *P0* exons were amplified for the following individuals: III:1, III:2, III:3, III:4, III:5, III:6, III:7, IV:1, and IV:2, using primer sequences already published [16]. Sequencing was performed in both directions, using the same primers and BigDye Terminator version 3.0 (Applied Biosystems) with an ABI 3100 DNA Sequencer (Applied Biosystems), and analyzed with the Sequence Analyzer (Applied Biosystems) and Seqman II (DNAstar) software. For all these procedures, informed consent from the family members was obtained.

Family history was collected, in order to find a genealogical relationship between individuals III:4 and III:5, and to document the phenotype of the family ancestors. To evaluate this relationship experimentally, microsatellite markers close to *P0* were investigated in the family members. Specific primers for repeats in loci D1S2705 and D1S2771 were used for polymerase chain reaction (PCR), and the products were analyzed with an ABI 3100 Sequencer and Genescan and Genotyper software (Applied Biosystems).

A physical examination was performed in subjects III:4, III:5, IV:1, and IV:2. Motor weakness and muscle wasting, sensory perception for light touch, pain, vibration, and joint position, deep tendon reflexes, and skeletal deformities were assessed. The cranial nerve status was also examined. Standard electrophysiological tests [17] were performed in the median, ulnar, peroneal, tibial, and sural nerves in all subjects. A sural nerve biopsy was taken from individual IV:1 and processed for light and electron microscopy according to standard techniques. The sural nerve was fixed with glutaraldehyde and embedded in epoxy resin. For light microscopy 1-μm sections were stained with methylene blue. For electron microscopy, 80-nm ultrathin sections were stained with lead citrate and uranyl acetate. Morphometric parameters were analyzed by light (×200 enlargement) and electron microscopy (between ×1,600 and ×16,700 enlargement).

Results

The hereditary peripheral neuropathy in family CR-CMT2 follows an autosomal dominant pattern of inheritance (Fig. 1). The family history of subjects III:4 and III:5, indicated that individuals I:1, II:1, II:4, and II:5 possibly also presented the disease.

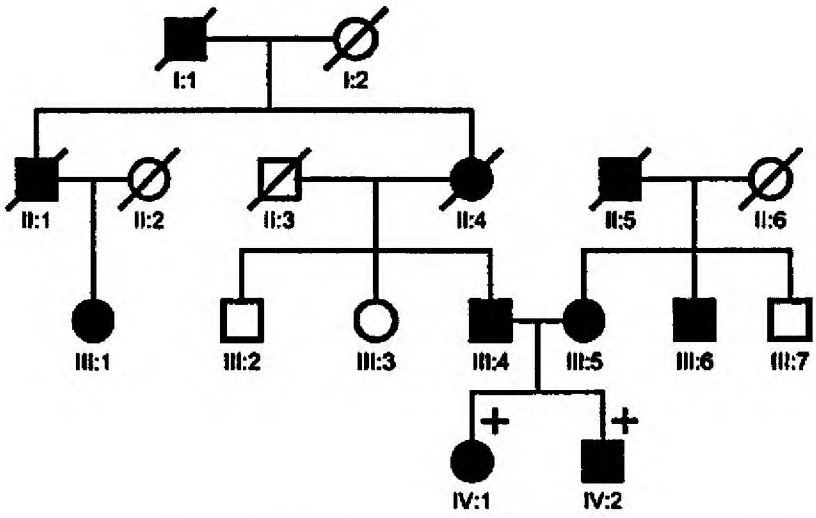

Fig. 1. Pedigree of the Costa Rican family CR-CMT2 with autosomal dominant Charcot-Marie-Tooth disease. Phenotypes of members of the first and second generation are assumed according to testimonies of descendents. Individuals IV:1 (+) and IV:2 (+) are homozygous for a mutation in *MPZ* and presented a more-severe phenotype than the heterozygous members

No mutations were found in the *P0* exons 1, 2 and 4–6 in any family member. In contrast, in the *P0* exon 3 an A→C transversion (c.434A>C) was found, resulting in a novel missense mutation Tyr145Ser. Individuals III:1, III:4, III:5, and III:6 are heterozygous for this mutation, whereas individuals IV:1 and IV:2 (children of two heterozygous patients) are homozygous carriers (Fig. 2). Healthy individuals of the family do not carry the mutation. This transversion has not been reported previously, either as a polymorphism or as a pathogenic mutation (Inherited Peripheral Neuropathies Mutation Database, http://molgen-www.uia.ac.be/cmtMutations/). In addition, 50 healthy non-related controls were screened who did not carry this Tyr145Ser mutation.

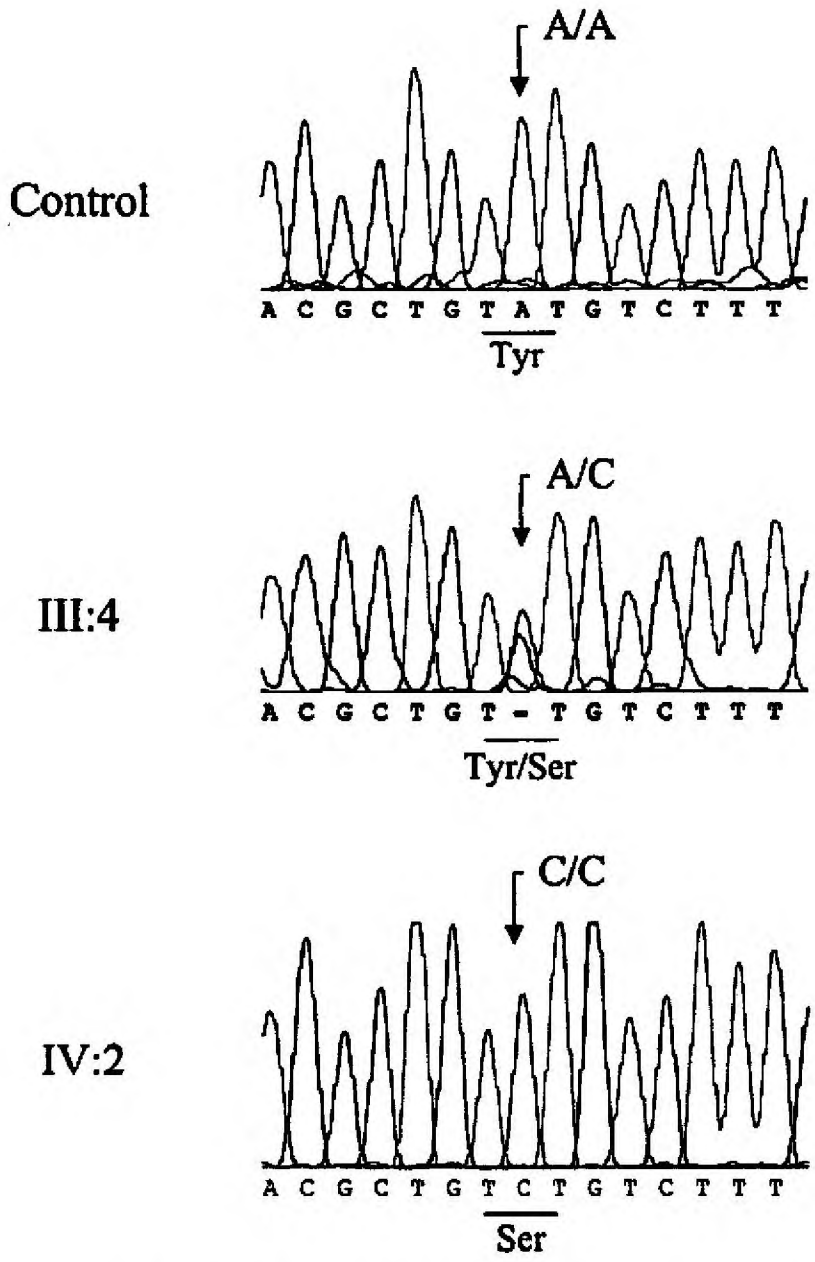

Fig. 2. DNA sequence of the exon 3 of *MPZ* in a healthy control, a heterozygous (III:4) and a homozygous (IV:2) carrier of the Tyr145Ser mutation. The nucleotide exchange (A→C) results in an amino acid change in codon 145 (Tyr→Ser)

Although it was not possible to establish a genealogical relationship between the paternal and the maternal branches, analysis with polymorphic markers showed that in both branches the mutated allele is related to a 148-bp PCR product of marker D1S2705 and a 250-bp PCR product of marker D1S2771.

Upon clinical examination, individuals III:1, III:4, III:5, and III:6 had a mild peripheral

neuropathy characterized by a late age of onset (after 51 years), whereas individuals IV:1 and IV:2 had an earlier age of onset (38 and 39 years, respectively) and a more-severe neuropathy. The parents III:4 and III:5, heterozygous for the Tyr145Ser mutation, as well as their children IV:1 and IV:2, homozygous for the Tyr145Ser mutation, were studied in more detail. The father (patient III:4) did not report a serious impairment of his daily activities at 65 years of age. Disease duration ranged from 2 to 4 years in the siblings, and was 11 years in the mother (III:5); the father's (III:4) disease duration could not be defined exactly. All mutation carriers showed distal sensory deficits in a stocking-glove pattern for light touch and pin perception, which were more pronounced in the lower limbs. Vibration perception in the feet was severely impaired in all patients. Individuals III:5, IV:1, and IV:2 showed mild sensory ataxia. Motor weakness and wasting of distal muscles were present only in the children IV:1 and IV:2. Deep tendon reflexes of the lower limbs (ankle jerk, patellar and adductor deep tendon reflexes) were reduced or absent in the mother and children, whereas the father's patellar deep tendon reflex was still preserved. Pupillary abnormalities with mydriasis and no reaction to light were also only present in the siblings IV:1 and IV:2. The daughter IV:1 reported progressive deafness; she presented mild hypacusis of both ears. The electrophysiological studies revealed reduced or absent compound muscle action potentials and normal or reduced motor NCVs in the lower limbs. Sensory nerve action potentials of the sural nerve could be detected only in the daughter, with a reduced amplitude and a prolonged sensory NCV. Electromyographic studies of the anterior tibial muscle were compatible with either active or chronic denervation as a sign of axonal lesion. An increased number of polyphasic motor unit potentials (MUPs) and increased amplitudes of the MUPs were found. A pattern of reduced recruitment activity to full effort was also observed. The diagnosis was further confirmed by a sural nerve biopsy of the daughter.

Analysis of the semithin sections revealed a moderate decrease in myelinated nerve fiber density. Clusters of mainly four to six thinly myelinated nerve fibers were observed, showing regenerating axonal sprouts. Additionally, several thinly myelinated axons could be found, often surrounded by proliferating Schwann cells forming onion bulbs. The ultrastructural examination showed peri- and intra-axonal vacuoles as a sign of axonal degeneration. Clusters of unmyelinated axons in bands of Büngner indicated wallerian degeneration. Furthermore, signs of myelin degeneration, with decompacted lamellae of the myelin layers and abnormal myelin thickening forming loops and folds, were detected. These findings confirmed the electrophysiological findings of both axo