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A novel stop codon mutation in the PMP22 gene associated with a variable phenotype

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Abstract

The most frequent inherited peripheral neuropathy is the peripheral myelin protein 22 (PMP22) gene related disease. Duplication, deletion, and point mutations in that gene are associated with phenotypic variability. Here we report a family carrying a novel mutation in the PMP22 gene (c. 327C > A), which results in a premature stop codon (Cys109stop). The family members who carry this mutation have a Charcot-Marie-Tooth type 1 variable phenotype, ranging from asymptomatic to severely affected. These findings suggest that the fourth transmembrane domain of the PMP22 gene may play an important role, although the intrafamilial clinical variability reinforces the observation that pathogenic mutations are not always phenotype determinant and that other factors (genetic or epigenetic) modulate the severity of the clinical course.

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1. Introduction

Charcot-Marie-Tooth disease (CMT) includes a group of peripheral nervous system disorders that are clinically and genetically heterogeneous [1]. Demyelinating CMT (CMT1) is the most frequent form. Clinical features include progressive distal motor deficit, distal muscle atrophy, often with absence of tendon reflexes and presence of pes cavus deformity and conduction velocities uniformly slowed [1,2]. On the other hand, hereditary neuropathy with liability to pressure palsy (HNPP) is characterized by recurrent nerve palsies precipitated or not by minor compression. On clinical and neurological examination, tendon reflexes may be diminished or abolished but bone abnormalities are rare [1]. In HNPP, the electrophysiological studies range from classic multiple focal slowing of nerve conduction at usual sites of entrapment to diffuse slowing abnormalities of nerve conduction [1,3].

Phenotypic heterogeneity, ranging from asymptomatic carriers to severe symptomatic patients, has been described in both disorders as well as in PMP22 non-related diseases [1,4–7]. Severe demyelinating clinical phenotypes, classified as Dejerine–Sottas syndrome (DSS) were found to be linked to chromosomes 1, 10, 17, 19, or X [8], which illustrates how difficult is the classification of hereditary motor and sensory neuropathies.

Molecular genetic studies have shown that the majority of patients with autosomal dominant CMT1 forms have mutations either in the peripheral myelin protein 22 gene (PMP22) at 17p11.2-p12 (CMT1A) [9,10], the most frequent form; in the myelin protein zero gene (MPZ) at 1q22-q23 (CMT1B) [11–13]; in the lipopolysaccharide-induced tumor necrosis factor- α factor gene (LITAF/SIMPLE) at 16p13.1-p12.3 (CMT1C) [14,15]; or in the early growth response 2 gene (EGR2) at 10q21.1-q22.1 (CMT1D) [16,17]. More than 90% of CMT1A patients have a 1.5 Mb tandem duplication of chromosome 17p11.2-p.12 region where lies the PMP22 gene [9,18]. A deletion in the same region that is duplicated in CMT1A is observed in most HNPP families [5–7,19]. The presence of duplications

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or deletions of the PMP22 gene points out for a gene dosage effect as the pathological mechanism of CMT1A [9,20–24]. In addition, the identification of point mutations in the PMP22 gene in non-duplicated CMT1A patients has provided additional evidence for its important role in the disease phenotype [5–7,25,26].

Here, we describe clinical, electrophysiological, histological and molecular findings in members of a family with a CMT1 phenotype carrying a novel stop codon mutation in the PMP22 gene.

2. Patients and methods

2.1. Family study

Patients were ascertained in the Department of Neurology, Hospital das Clínicas, and were referred for

molecular analysis at the Human Genome Research Center, Institute of Biosciences, at the University of São Paulo. All studies were done following informed consent. The index patient (II-5) reported onset at age 16, and was severely affected at age 61, when first examined because of weakness and difficulty to walk. Pedigree analysis revealed autosomal dominant inheritance. The proband's father, deceased at age 72, was clinically affected and reported he had one 59-year-old affected sister and four unaffected sibs (two brothers and two sisters). The proband also referred that he had two sons and one niece who were affected (Fig. 1A). Neurological examination was performed by standard technique and by the same examiner for all subjects. The score of strength was measured on ordinal scale recommended by the Medical Research Council [27]. Vibration sense was quantified with aid of a pallesthesiometer (Cibertron, PL11) as compared to normal control values [28].

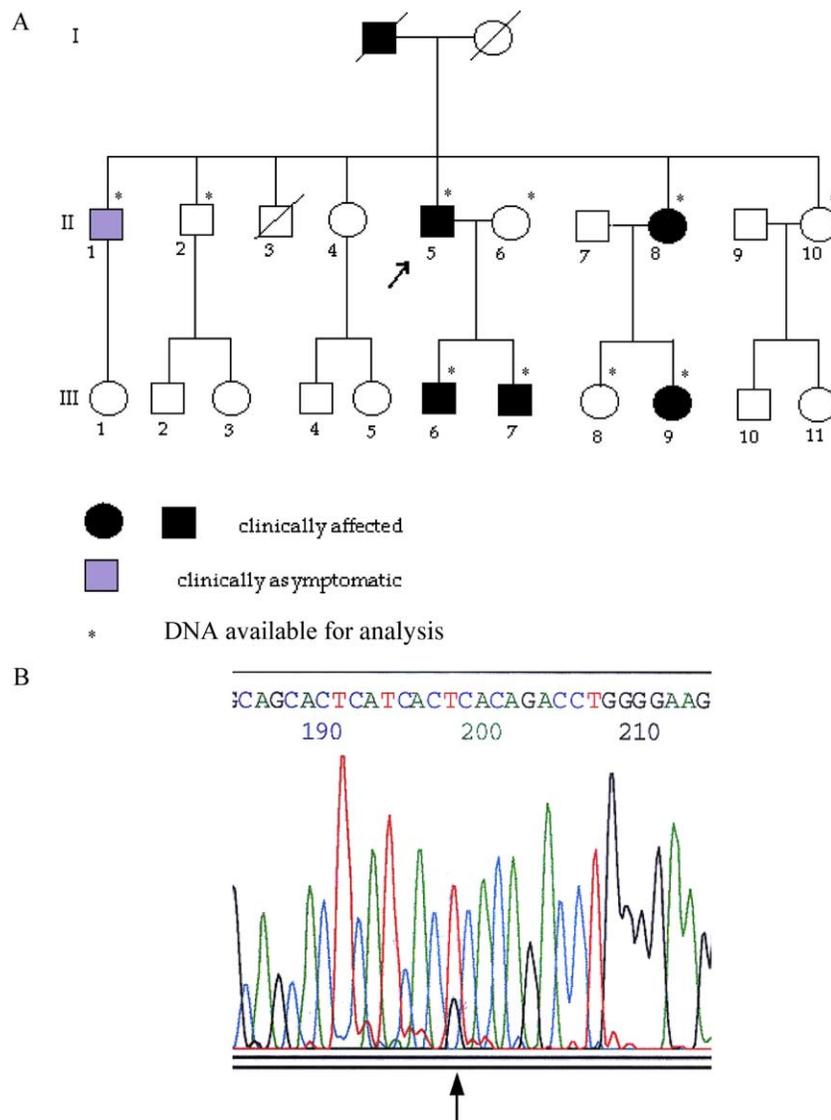


Fig. 1. (A) Pedigree of the family. (B) Identification of the C > A mutation in PMP22 exon 4 (reverse strand).

2.2. Electroneuromyography

Electrophysiological studies were performed on clinically affected patients using a Polimed model PL 1002 or a Nihon Kohden model neuropack 2. Electrodiagnostic studies consisted of motor nerve conduction studies in the arms (median and/or ulnar nerves) and legs (peroneal and/or tibial nerves), sensory nerve conduction studies (median, ulnar, and sural nerves), late responses from several nerves (H-reflex and F-wave) and a needle electromyography in proximal and distal muscles of upper and lower limbs.

2.3. Biopsy and histological techniques

The patient II-5 was submitted to a whole sural nerve biopsy, which was taken just above the lateral malleolus under local anesthesia, following informed consent. The nerve sample was fixed in 2.5% glutaraldehyde, at pH 7.4, in 0.1 M phosphate buffer and processed with Araldite. Transverse sections at 1 μm were stained with toluidine blue for light microscopy, and ultra thin sections with uranyl acetate and lead citrate for electron microscopy (JEOL 100CX) [1]. The density of myelinated fibers was determined in 1 μm section by counting under immersion objective (Kpl, X8, Zeiss). Unmyelinated axons were counted in electron micrographs 7000 \times amplification with the aid of a 144-point multipurpose grid. Morphometric analyses were carried out by stereology-based measurements [29] and the results were expressed as median per mm^2 of fascicular area. About 100 myelinated fibers were teased and analyzed for demyelination, remyelination, and myelin ovoids. They were categorized according to their morphological appearance [1].

2.4. Genetic analysis

DNA was obtained from 10 individuals (including one unrelated spouse) and extracted from whole blood using standard methods. A duplication or deletion on chromosome 17p11.2-p12, was excluded through polymerase chain reaction (PCR) and Southern blot analysis according to standard procedures [9,30]. In order to identify the pathogenic mutation, single-strand conformation polymorphism (SSCP) analysis of the four coding exons of PMP22 was performed using exon-specific primer pairs [31]. Sequencing of the abnormal fragment was done in ABI Prism 377 automatic sequencer.

3. Results

3.1. Mutation analysis

SSCP analysis of PMP22 gene exons showed an abnormal migration pattern for exon four and sequencing

analysis in affected subjects showed a cytosine to adenine substitution at nucleotide position 327 (c. 327C > A) (Fig. 1B). This mutation, which results in a premature stop codon at position 109 (Cys109stop) was found in six family members (including individual II-1, who was asymptomatic) and was segregating with the disease. This change was not found in 150 ethnic matched controls (300 normal chromosomes).

3.2. Neurological and complementary exams

In five of the six subjects carrying the Cys109stop mutation who were personally examined, the onset of symptoms ranged from 2 to 16 years and there were no history of single or recurrent episodes of nerve palsy, alcoholism or toxic exposition. All affected patients had a progressive difficulty for walking but were able to walk without help when last examined. Muscle strength was normal in upper limbs. In lower limbs, altered tendon reflexes, motor and vibration deficits with symmetric and distal predominance were observed (Table 1). Peripheral nerves were not palpably enlarged. Temperature, touch, pricking pain, and joint position (thumb and great toe) were preserved in all affected subjects. Except the patient III-6 who had hyperhidrosis in hands and feet, there were no changes in color or texture of the skin, sweating or bladder function. None of the other patients had a more severe recent deterioration in clinical symptoms. All laboratorial investigations, including glucose, vitamin B12, thyroid function and cerebrospinal fluid (CSF) were normal and dismissed another cause of neuropathy. Following DNA analysis, subject II-1, who was apparently asymptomatic at age 72, was submitted to a careful clinical neurological examination as well as nerve conduction studies. The results are detailed in Table 1. Electrophysiological study confirmed a demyelinating neuropathy in all family members carrying the Cys109stop mutation including him. Slowing motor and sensory nerves conductions with temporal dispersion of proximal motor potentials were observed in all patients but the velocities were not uniformly slowed in all tested nerves of patients II-5 and II-8 (Table 2).

3.3. Histology

The sural nerve biopsy showed that the median density of myelinated and unmyelinated axons were 3752 and 12 776 fibers/ mm^2 , respectively (our normal age-matched values for the sural nerve were 10 479 [4166–13 940] myelinated fibers per mm^2 and 32 560 [17 320–47 450] unmyelinated fibers per mm^2) [32]. Teased fiber examination showed many fibers with myelin wrinkling, variation in internodal length, and demyelinated paranodal areas. Linear myelin ovoids were seen in several fibers. Many fibers were found to be thinly myelinated with infolded loop of myelin. In these fibers, axonal damage was characterized by vacuolization of axoplasm and

Table 1
Neurological examination of family members

Clinical characteristic	Patients								
	II-1	II-2	II-5	II-8	II-10	III-6*	III-7	III-8	III-9
Sex	M	M	M	F	F	M	M	F	F
Ae (years)	72	69	61	59	55	17	14	35	30
Ao (years)	–	–	16	2	–	13	3	–	12
Strength									
Quadriceps	5	5	4	5	5	4	5	5	5
Hamstrings	5	5	4	4	5	4	4	5	4
Dorsiflexors	4	5	3	3	5	3	3	5	3
Plantar flexors	5	5	4	3	5	3	3	5	4
Reflexes									
Biceps brachii	nl	nl	hypo	hypo	nl	hypo	hypo	nl	nl
Triceps brachii	nl	nl	hypo	hypo	nl	hypo	hypo	nl	nl
Brachioradialis	nl	nl	hypo	hypo	nl	hypo	hypo	nl	nl
Quadriceps femoris	nl	nl	A	hypo	nl	hypo	hypo	nl	hypo
Triceps surae	hypo	nl	A	hypo	nl	A	A	nl	A
Vibration									
Elbow	13.5	15.0	15.0	12.0	10.0	8.5	12.5	10.0	12.5
Ring finger	8.5	6.0	12.5	8.5	6.0	17.5	11.0	5.5	10.0
Tibial tuberosity	27.5	15.0	27.0	25.0	10.0	27.5	15.0	10.0	27.5
Great toe	>50.0	6.0	>50.0	45.0	5.0	>50.0	22.5	5.0	>50.0
Clinical features									
Pes cavus	P	A	P	P	A	A	P	A	P
Scoliosis	A	A	P	P	A	A	A	A	A
Peroneal atrophy	A	A	P	A	A	A	P	A	P

M, male; F, female; Ae, age of first neurological examination; Ao, age of onset of clinical symptoms; degree of strength, 3, normal movement against gravity, but not against additional resistance; 4, normal movement is overcome by resistance; 5, normal power; nl, normal; hypo, hyporeflexia; A, absent; P, present; our normal range of vibration sense, 10–15 for elbow and tibial tuberosity, 5–8 for proximal phalanx of ring finger and great toe; *, hyperhidrosis in hands and feet.

disorganization of cytoskeletal elements (Fig. 2A). Occasional basal lamina onion bulb formations were visible, and clusters of axonal regeneration were rare. Small number of lamellae and normal myelin periodicity were observed (Fig. 2B). There were no inflammatory infiltrates, and we did not observe tomacula in nerve.

4. Discussion

Here, we report a Caucasian Brazilian family where five of six members carrying a novel stop codon mutation in the *PMP22* gene have a CMT1 clinical phenotype.

The diagnosis of CMT1 was based on clinical weakness and atrophy of distal limb muscle, diminished or absent tendon reflexes, diminished vibratory sensation, pes cavus deformity and no history of episodic nerve palsy. These clinical features are similar to those from the classic descriptions of CMT [1,2,33]. Enlargement of peripheral nerves may be found in one fourth of affected persons but it did not occur in our patients [1]. Phenotypic variability, observed in our family, has been reported in axonal and demyelinating inherited neuropathies as well as in non-related PMP22 disorders [3–7,31,34–36].

In the patients here reported we observed the presence of abnormal temporal dispersion of the proximal motor

potentials in all patients. Non-uniform slowing velocity was found in all tested nerves, motor and sensory of two patients only but conduction block was not found. Familial history, clinical course, and systematic laboratorial search excluded an acquired demyelinating neuropathy, particularly chronic inflammatory demyelinating polyneuropathy (CIDP) in our family. A uniform slowing motor conduction velocity characterizes the nerve conduction abnormalities in CMT1 [1,2]. Multifocal slowing, conduction block, and abnormal temporal dispersion are prominent features in chronic acquired demyelinating polyneuropathies and may be used to distinguish CMT1 from CIDP, particularly if a clear family history of CMT1 is absent or if the clinical course is unusual. Although uncommon, conduction block was described in 5.3–64% while temporal dispersion was reported in 36% of patients with CMT1 [37–39]. Here, the non-uniform conduction velocity is a consequence of abnormal temporal dispersion. This may be due to an excessive desynchronization of impulses in nerve fibers resulting in a phase cancellation between fast and slow conducting motor or sensory fibers [1] and suggesting differences in the expressivity of the mutation in Schwann cells.

In some aspects, sural nerve biopsy (patient II-5) showed an overlap of pathological features. Segmental demyelination, myelin wrinkling, and onion bulbs

Table 2
Electrophysiological study of motor nerve in carriers of the Cys109stop mutation

Nerve		Patients						
		Normal values	II-1	II-5	II-8	III-6	III-7	III-9
Motor	Median							
	d-Amp	≥ 5000	4000	500	8000	7600	10 000	1000
	p-Amp*	≥ 5000	3500	300	500	2600	2400	200
	DML	≤ 3.8	9.1	11.1	8.0	10.9	10.5	10.3
	MCV	≥ 47.0	33.8	16.1	18.3	13.5	11.7	16.2
	F-wave	≤ 30.0	55.4	Absent	54.1	132.1	117.2	42.7
	Ulnar							
	d-Amp	≥ 5000	5200	ND	10 000	5500	700	6000
	p-Amp*	≥ 5000	2800	ND	8000	1800	900	6000
	DML	≤ 3.0	6.9	ND	4.2	16.9	7.3	4.9
	MCV	≥ 47.0	35.9	ND	34.8	11.9	15.5	13.9
	F-wave	≤ 30.0	52.2	ND	40.5	135.1	Absent	34.2
	Tibial							
	d-Amp	≥ 5000	4500	300	5200	600	200	ND
	p-Amp*	≥ 5000	120	600	1000	U	100	ND
	DML	≤ 5.0	8.5	8.8	5.2	11.1	11.9	ND
	MCV	≥ 47.0	23.2	1.7	19.6	U	11.2	ND
	F-wave	≤ 50.0	110.0	Absent	75.6	Absent	Absent	ND
	Fibular							
	d-Amp	≥ 2500	2200	2300	200	200	U	400
	p-Amp*	≥ 2500	2100	1800	500	180	U	400
	DML	≤ 5.0	11.2	4.2	9.9	14.1	U	12.5
	MCV	≥ 45.0	20.7	31.8	34.5	16.4	U	11.8
	F-wave	≤ 50.0	120.7	Absent	Absent	Absent	Absent	Absent
Sensory	Median							
	Amp	≥ 15	4	U	6	U	U	U
	L	≤ 3.0	4.2	U	5.0	U	U	U
	SCV	≥ 47.0	34.5	U	24.0	U	U	U
	Ulnar							
	Amp	≥ 15	U	U	5	U	U	8
	L	≤ 3.0	U	U	4.8	U	U	7.3
	SCV	≥ 47.0	U	U	20.8	U	U	14.3
	Sural							
	Amp	≥ 5.0	5	U	8	U	U	U
	L	≤ 3.0	4.2	U	3.2	U	U	U
	SCV	≥ 45.0	32.1	U	32.8	U	U	U
	H-response	≤ 32.0	65.8	Absent	62.4	Absent	Absent	Absent

d-Amp, amplitude of distal compound muscle action potential (CMAP) (μV); p-Amp, amplitude of proximal CMAP (μV); DML, distal motor latency (ms); MCV, motor conduction velocity (m/s); SCV, sensory conduction velocity (m/s); Amp, amplitude of evoked potential, peak to peak (μV); L, latency of sensory action potential (ms); ND, not done; U, unexcitable nerve; *, CMAP with low amplitude and temporal dispersion.

characterized the pathologic alterations as a demyelinating process [1,5,40–42]. A decrease in the number of myelinated fibers was observed in accordance to other reports [1,41]. Basal lamina onion bulbs, which are described with a variable frequency in humans and experimental models of inherited peripheral neuropathies, were a rare feature in our case. They represent a non-specific consequence of chronic demyelination and remyelination as the classical onion bulbs, both described in CMT1 [3,20,24,40,43]. Focal thickenings of myelin sheath, tomacula, were not seen in our patient's nerve, however, there were many fibers with infolded loops of myelin. Tomacula occur as non-specific features in several clinical syndromes, which are associated with genetic or immunological defects of myelin [44]. Some authors accept the formation of

redundant myelin loops as a sign of hypermyelination, most probably reflecting tomacula as found in HNPP nerves [22,44]. Despite of reduced unmyelinated fibers density (probably including nerve sprouts), our patient had no symptoms or signs of autonomic dysfunction although subject III-6 had hyperhidrosis. Several authors have reported that unmyelinated fibers are not affected in CMT1A [1,40,41]. However, an alteration of unmyelinated fibers is assumed from the observed abnormalities of autonomic tests in many cases of CMT1 [1]. Features of axonal damage were seen in our case as well as many fibers with infolded loops of myelin associated with disorganization of cytoskeletal elements, suggesting that this disorganization may contribute for a physical interruption of axonal flux and consequent distal degeneration. In short,

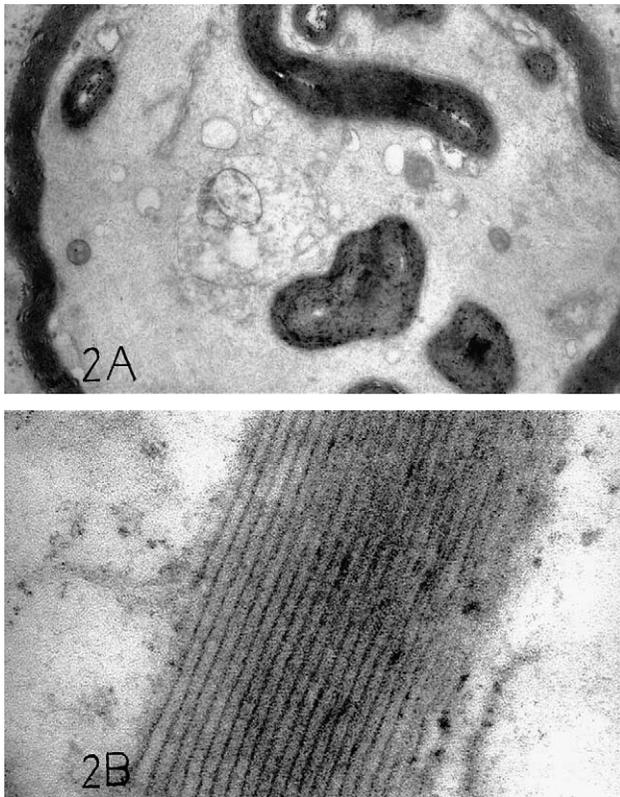


Fig. 2. Transverse electron micrographs of sural nerve (patient II-5, 61 years old). (A) Axonal vacuolization in proximity of infolded loop of myelin sheath (12000 \times). (B) Normal periodicity of lamellae of myelin sheath (140000 \times).

probably, many factors may act together for axonal degeneration in CMT1A such as defects in Schwann cell–axon interactions, altered expression of trophic factors and adhesion molecules [20,45–47].

Deletion, duplication, and point mutations, as previously identified in the *PMP22* gene, are considered as dominant mutations when they lead to a mutant phenotype in the presence of a normal copy of the gene. The phenotypes associated with dominant mutations may represent either a loss or a gain of function [48]. The CMT1 phenotype is most frequently caused by a 1.5 Mb tandem duplication in the region encompassing the *PMP22* gene while deletions of the same region are commonly associated with HNPP phenotype. More severe clinical features in homozygous patients, elevated levels of *PMP22* mRNA in nerve biopsies as well as animal models carrying duplications and deletions support the dosage hypothesis as a disease mechanism [9,20–23]. Usually, missense mutations in the *PMP22* gene are associated with CMT1 or DSS phenotypes through a gain of function mechanism while frameshift or non-sense mutations in that gene exhibit an HNPP phenotype generated by a mechanism which mimic haploinsufficiency [5,6,25].

The family here reported carries a stop codon mutation in *PMP22* gene associated with a CMT1 phenotype. It is difficult to predict the intracellular effect of a mutation on

protein function but we assume that this Cys109stop mutation leads to premature termination of translation resulting in a polypeptide that is shorter than the wild type *PMP22*. Therefore, we would expect a milder HNPP phenotype and not a more severe course as observed in five of our patients. In addition, the finding of one asymptomatic family member is still more intriguing. However, the unexpected phenotype and clinical variability reported in association with deletion, frameshift and missense mutations in *PMP22* gene [5,22,49,50] is still not understood. The myelination requires coordinated synthesis, trafficking, and assembly of myelin constituents. Mutated *PMP22* protein might impair wild type protein transport through the endoplasmic reticulum to the cell surface or could modify the interactions with normal *PMP22* [51,52] or protein zero [53] limiting the number of functional *PMP22* molecules available for myelination [22,54,55].

Understanding the intrafamilial clinical variability in patients carrying the same mutation remains a great challenge. Phenotypic variability has been reported in demyelinating and axonal inherited neuropathies as well as in several other neuromuscular disorders such as limb-girdle and facioscapulohumeral muscular dystrophies [36,56–59]. The multiplicity of symptoms and signs cannot be explained on the basis of the specific mutation alone and depends on interactions with other genetic and/or epigenetic factors.

It is important to point out that the minor signs found in individual II-1 at neurological examination would probably remain unnoticed if he would be an isolated case. As observed by other authors, it is possible that the relative proportion of individuals who have mutations in this gene may be underestimated.

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