Case Report

Congenital hypomyelinating neuropathy due to a novel *MPZ* mutation

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Running headline: A novel mutation in the *MPZ* gene

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Abstract Congenital hypomyelinating neuropathy (CHN) is a severe inherited neuropathy with neonatal or early infancy onset, reduced nerve conduction velocity, and pathological evidence of hypomyelination. We describe a case of CHN that presented with neonatal hypotonia and a progressive downhill clinical course, developing cranial nerve dysfunction and respiratory failure. The nerve conduction velocities were severely slowed and sural nerve biopsy revealed non-myelinated and poorly myelinated axons, with no typical onion bulbs. The mutational screening showed that our proband harbored a novel missense mutation, p.S121F, in the *MPZ* gene. *In silico* analyses and molecular modeling predicted that the replacement of a serine by a phenylalanine is a non tolerated change and may affect the folding and the stability of the protein. Sub-cellular location studies were performed and revealed that the mutant protein loses its correct location on the cell membrane surface and is mainly expressed in the cytosol, reducing its adhesive properties. This case illustrates the clinical heterogeneity that exists in neuropathies associated with *MPZ* mutations and highlights that in patients with mild hypotonia in the first months that develop a very severe demyelinating neuropathy, the *MPZ* gene must be taken into account.

Key words: Congenital hypomyelinating neuropathy (CHN); Déjérine-Sottas syndrome (DSS); Charcot-Marie-Tooth (CMT); *MPZ* gene; nerve biopsy.

Introduction

Congenital hypomyelinating neuropathy (CHN; MIM 605253) is a hereditary demyelinating neuropathy characterized by neonatal or early infancy onset, hypotonia, areflexia and severe slowing of nerve conduction velocity. There are two clinically distinct groups of patients with CHN (*Phillips et al., 1999*). Some patients present in the neonatal period with severe hypotonia, weakness, and frequently develop respiratory failure, while a second group present beyond the neonatal period with hypotonia, delayed motor development, and generally have a milder prognosis. In any case, the sural nerve pathology is quite consistent and shows an almost total lack of myelin sheaths with good preservation of axons. Mutations in several genes encoding for proteins involved in peripheral nerve myelination (*MPZ*, *PMP22*, *EGR2*, *MTMR2* and *SOX10*) have been described in patients who suffer from CHN, being *de novo* mutations in the *MPZ* gene the most frequent cause. The clinical phenotypes associated with *MPZ* mutations range from severe CHN and Déjérine-Sottas syndrome (DS), to demyelinating Charcot-Marie-Tooth (CMT1) or late onset axonal Charcot-Marie-Tooth (CMT2). Correlation between specific mutations and the phenotype has been studied (*Shy et al., 2004*), although further studies are necessary to fully characterize the molecular basis underlying the clinical heterogeneity of *MPZ*-associated neuropathies.

Case report

The patient is a 4-year-old boy who was caesarean born after an uneventful pregnancy. At birth Apgar scores were 10 after 1 and 5 min, and physical examination was normal. Hypotonia was first detected at 4 months of age, but was not studied until he was 6 months old and had not won weight in the last 3 months. On clinical examination the patient had good visual contact, weak crying and very prominent hypotonia, especially in the axial muscles. He was unable to turn in bed, raise his head or put his feet in the mouth, but could raise his hands in the air and touch one with the other. Deep tendon reflexes were absent. During his instay he developed a respiratory insufficiency that needed ventilatory support and intubation in the critical care unit. Cerebrospinal fluid analysis showed protein levels of 87 mg/dL (normal 15-45 mg/dL). MRI (Magnetic Resonance Imaging) of the brain was normal. Nerve conduction studies (Table 1) and sural nerve biopsy (Fig. 1A) at the age of 7 months revealed a severe hypomyelinating neuropathy. Electron microscopy discovered scattered atypical onion-bulbs formed by redundant and reduplicated basal lamina, but no typical onion bulbs (Fig. 1B). Muscle biopsy showed fiber size variation, but no indirect signs of denervation like group atrophy (Fig. 1C-D).

Fig. 1A-D

At 11 months the patient required gastrostomy because of malnutrition. From then on the course has been clearly progressive; the patient is now 4 years old, requires assisted ventilation and enteral feeding and is unable to perform any voluntary movement at all except with the eyes.

Methods and Results

All protocols performed in this study complied with the ethics guidelines of the institutions involved. The patient's parents were aware of the nature of the studies and gave their consent.

Mutations in the codified regions of the genes *PMP22* (NM_000304.2), included the CMT1A duplication by MLPA (Multiplex Ligation-dependent Probe Amplification, SALSA kit P033 CMT1, MRC-Holland), *EGR2* (NM_000399.3), and *SOX10* (NM_006941.3) were discarded. The analysis of the *MPZ* gene (NM_000530.4) revealed a novel mutation, c.362C>T (p.S121F; NP_000521.2), in heterozygosis (Fig. 1E). His healthy parents did not carry this change, which was neither identified in 318 chromosomes from healthy controls of Spanish ancestry, suggesting a pathogenic effect for the *MPZ* p.S121F.

Fig. 1E

We investigated *in silico* the biological relevance of the *MPZ* p.S121F mutation as previously described (*Espinós et al., 2009*). The residue S121 is an evolutionary conserved amino acid, invariant across more than one hundred different species (data not shown). Computational analyses performed with the SIFT and PolyPhen algorithms predicted that the *MPZ* p.S121F mutation was probably damaging. Visualization of the structure and of the consequences of the mutations on the 3D structure of the MPZ extracellular domain (Protein Data Bank, PDB; entry 1NEU) (*Shapiro et al., 1996*) was carried out using the program Coot (*Emsley and Cowtan 2004*). The structure of the extracellular domain of MPZ showed that the side chain hydroxyl group of S121 forms a hydrogen bond with T44 (Fig. 1F). The p.S121F mutation implies a replacement of a small polar serine with a large and aromatic highly hydrophobic residue of phenylalanine which would prevent the hydrogen bond and would elicit the

Fig. 1F

misfolding of the protein and so alter its stability.

The human full-length cDNA of *MPZ* was obtained using the human MGC Verified FL cDNA clone (ID: 3926008; Open Biosystems), which was subcloned in-frame into the mammalian expression vector pcDNA3-HA, to produce the MPZ-HA construct. The construct p.S121F-HA was generated with specific primers containing the nucleotide change using the QuickChange® Site-Directed Mutagenesis kit (Stratagene). HeLa cells were grown, transiently transfected with either MPZ-HA or p.S121F-HA construct, and further analysed as described elsewhere (*Lupo et al., 2009*). The subcellular localization studies showed that the MPZ-HA construct was correctly expressed on the cellular surface, and in contrast, the p.S121F-HA construct presented a cytoplasmatic expression (Fig. 1G). The mutant protein therefore lost its correct localization on the cell surface.

Fig. 1G

Discussion

The proband suffered from a CHN caused by a *de novo* *MPZ* p.S121F mutation, which has deleterious effects on the protein function according to the *in silico* and molecular modeling analyses performed. Moreover, the subcellular localization studies showed that the mutant protein is retained in the cytoplasm and potentially decreases the *MPZ*-mediated adhesion. Other *MPZ* mutants retained in the cytoplasm have been demonstrated to provoke unfolded protein response and apoptosis (*Khajavi et al., 2005; Wrabetz et al., 2006; Pennuto et al., 2008*), possibly related to the accumulation of excessive improperly folded proteins (*Harding et al., 2002*). Independently of the molecular pathomechanism of *MPZ* mutant proteins trapped within the cytosol, the reduction of adhesive functions has been demonstrated in some of them and usually leads to early-onset demyelinating neuropathies (*Grandis et al., 2008; Lee et al., 2008*).

Mutations in *MPZ* result in a wide spectrum of clinical phenotypes, which is probably determined by the location and type of the pathogenic mutation. Several arguments support this hypothesis. First, the same mutation has been reported in different subjects with identical clinical phenotype, as the sporadic p.Q215X (*Warner et al., 1996; Mandich et al., 1999*) or familial p.L184AfsX51 (*Smit et al., 2008*). Second, several mutations, like the p.F64del (*Ikegami et al., 1996*) and p.V102del (*Pareyson et al., 1999*) deletions have been described in homozygous and heterozygous states with different severity. The heterozygous individuals present with a mild CMT1 phenotype while the homozygous carriers have a severe DS phenotype. Third, certain missense mutations in the *MPZ* gene affect the same codon but with a different amino acid change resulting in completely different phenotypes. The p.T124K mutation causes CHN

(Nowakowski and Kochanski 2004) while the p.T124M mutation causes a milder CMT2 phenotype (De Jonghe et al., 1999). In fact, the p.S121C mutation which affects the same residue as in our patient has been reported associated with a CMT1 phenotype (Mandich et al., 2009).

For a concise diagnosis of CHN, compatible nerve pathology is mandatory, because the differentiation from DS on clinical grounds alone can be quite difficult. This has resulted in some inconsistency in the nosology of the literature, and after a comprehensive review of the available pathological phenotypes, nine *MPZ* mutations actually seem to be associated with CHN (Table 2). The concept of hypomyelination implies a congenital onset and non-progressive or slowly progressive course unless axon degeneration intervenes. Clinically, most cases show a slow sustained improvement over time instead of progressive decline, but some have a very severe phenotype, with neonatal hypotonia, progressive weakness, ventilatory support, and sometimes resulting in death.

The muscle biopsy in our case revealed no classic features of denervation, and good fiber type differentiation. In a patient reported with the *MPZ* c.550_552delinsG mutation, there was no differentiation of the fiber type (Szigeti et al., 2003). This could be related to the different ages at time of biopsy or to the severity of clinical findings at birth. In our patient there was probably an acceptable postnatal innervation, in fact clinical features went unnoticed during the first three months, while the patient without fiber type differentiation was born with arthrogriposis and severe respiratory failure.

The propositus presented has a phenotypic peculiarity because he had normal development until the age of three months, followed by 3-4 months of

stabilization and from then on a progressive decline until he developed complete paralysis of all voluntary muscles except for the eyes, thus resembling a locked-in syndrome. A similar clinical course was described in a patient with the *MPZ* p.R69C mutation reported as a DS phenotype, but the clinical course and pathological findings seem to be consistent with CHN (*Meijerink et al. 1996*).

In summary, the patient reported suffered a severe rapidly progressive CHN neuropathy caused by a previously unreported *MPZ* p.S121F mutation. The spectrum of phenotypes and *MPZ* mutations is broad and must be taken into account in patients with mild hypotonia in the first months that develop a very severe demyelinating neuropathy with complete paralysis.

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Table 1. Electrophysiological findings.

Nerve	Distal latency	CMAP	MNCV	SNEP
Median	13.7 ms	559 μ V	2.9 m/s	NR
Ulnar	12.3 ms	99 μ V	3.3 m/s	--
Posterior tibial	21.7 ms	143 μ V	3.6 m/s	--
Sural				NR

CMAP= Compound Muscle Action Potential; MNCV= Motor Nerve Conduction Velocity; SNEP= Sensory Nerve Evoked Potential; NR= No response.

Table 2 Mutations in *MPZ* with phenotype of Congenital Hypomyelinating Neuropathy

Phenotype	SR & MNCV	Biopsy	Mutation	Reference
Hypotonia at 10 months walks a few steps unassisted at 3½y. Scoliosis at age 3 y. Last time visited 8 y.	SR: absent MNCV: 6 m/s (10 m)	Thin myelin sheaths, rare rudimentary OB with EM. CSF: 75 mg%	p.Q215X	(Warner et al. 1996)
Hypotonia at 6 months, then stabilization. Downhill clinical course from 10 m on. Death 22m.	SR: NA MNCV: 8.5 m/s	Thin myelin sheaths, uncompacted myelin	p.R69C	(Meijerink et al. 1996)
5 months: Hypotonia, walked with braces at 2½ y. At 5 y Gowers manoeuvre. Ataxic gait.	SR: absent (2 y) MNCV: 9 m/s	Severe hypomyelination with basal lamina OB	p.R69C	(Phillips et al. 1999)
At 12m: delayed motor milestones, hypotonia & scoliosis. At 7 y distal muscle wasting and sensory ataxia.	SR: absent MNCV: 4.2 m/s	Loss of myelin fibres, thin myelin sheaths. Atypical OB	p.Q215X	(Mandich et al. 1999)
Hypotonia & arthrogyrosis at birth with respiratory failure. NSV until 31 m. At this time she was able to crawl and kneel.	SR: absent MNCV: 11 m/s	Axons with little to no compact myelin and few basal lamina OB.	c.550_552delinsG	(Szigeti et al. 2003)
Floppy infant & delayed motor milestones. At 7 y able to walk with support. Scoliosis & chest deformation. At 12 y unable to walk.	SR: absent (7 y) MNCV: 3 m/s	Lack of normally myelinated fibres. Basal lamina OB.	p.T124K	(Nowakowski and Kochanski 2004)
Hypotonia, arthrogyrosis, difficulty in swallowing & pneumonia. Respiratory support until 3 m.	SR: NP (10 d) MNCV: 4 m/s		p.L184AfsX51	(Smit et al. 2008)
Father: 27 y. Infancy: Hypotonia, swallowing & respiratory difficulties. Scoliosis. Walks with aid at 4 y, not progressive. Partly wheelchair bound.	MNCV: NR.	No myelinated fibres. No OB.	p.L184AfsX51	(Smit et al. 2008)
5 m. Hypotonia & failure to thrive	No SR or MNCV	Nearly complete absence of myelination	c.368_382del	(McMillan et al. 2010)
3y. Hypotonia delayed motor acquisitions. Walk 2½years	No SR or MNCV	Demyelination. Occasional OB-like formations	p.N131K	(McMillan et al. 2010)

SR= Sensory Response; MNCV= Motor Nerve Conduction Velocity; OB= Onion Bulb; CSF= Cerebrospinal Fluid; NA= Not available; NR= No response; NP= No performed.

Table Legends

Table 1. Electrophysiological findings.

CMAP= Compound Muscle Action Potential; MNCV= Motor Nerve Conduction Velocity; SNEP= Sensory Nerve Evoked Potential; NR= No response.

Table 2. Mutations in *MPZ* with phenotype of Congenital Hypomyelinating Neuropathy.

SR= Sensory Response; MNCV= Motor Nerve Conduction Velocity; OB= Onion Bulb; CSF= Cerebrospinal Fluid; NA= Not available; NR= No response; NP= No performed.

Figure Legend

Figure 1. Sural and muscle nerve biopsy, electrophoregram, cellular and structural findings related to the *MPZ* p.S121F mutation. Sural nerve biopsy shows (A) severe reduction of myelinated fibres in semi-thin sections with few scattered thinly myelinated axons (arrows), (epoxy section, toluidine blue stain, x100); and (B) reduplicated basal lamina around thin myelinated fiber (arrows), (electron microscopy). Muscle biopsy (C) shows fiber size variation (HE, x40) and (D) type I fibre hypotrophy and predominance; no signs of neurogenic atrophy were observed (ATPase pH9.4, x40). (E) Electrophoregram of the c.362C>T mutation identified in the *MPZ* gene in heterozygosis (arrow). (F) Detail of the MPZ structure to show the interactions mediated by S121 (left panel) and the effect of p.S121F mutation (right panel). (G) Subcellular localization of the wild MPZ protein on the cellular surface (left panel) and of the p.S121F protein in the cytoplasm (right panel).