



Original research

Phenotypic spectrum of myelin protein zero-related neuropathies: a large cohort study from five mutation clusters across Italy

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ABSTRACT

Background We aimed to investigate the clinical features of a large cohort of patients with myelin protein zero (*MPZ*)-related neuropathy, focusing on the five main mutation clusters across Italy.

Methods We retrospectively gathered a minimal data set of clinical information in a series of patients with these frequent mutations recruited among Italian Charcot-Marie-Tooth (CMT) registry centres, including disease onset/severity (CMTES-CMT Examination Score), motor/sensory symptoms and use of orthotics/aids.

Results We collected data from 186 patients: 60 had the p.Ser78Leu variant ('classical' CMT1B; from Eastern Sicily), 42 the p.Pro70Ser (CMT2J; mainly from Lombardy), 38 the p.Thr124Met (CMT2J; from Veneto), 25 the p.Ser44Phe (CMT2I; from Sardinia) and 21 the p.Asp104ThrfsX13 (mild CMT1B; from Apulia) mutation. Disease severity (CMTES) was higher ($p < 0.001$) in late-onset axonal forms (p.Thr124Met = 9.2 ± 6.6 ; p.Ser44Phe = 7.8 ± 5.7 ; p.Pro70Ser = 7.6 ± 4.8) compared with p.Ser78Leu (6.1 ± 3.5) patients. Disease progression (Δ CMTES/year) was faster in the p.Pro70Ser cohort (0.8 ± 1.0), followed by p.Ser44Phe (0.7 ± 0.4), p.Thr124Met (0.4 ± 0.5) and p.Ser78Leu (0.2 ± 0.4) patients. Disease severity (CMTES = 1.2 ± 1.5), progression (Δ CMTES/year = 0.1 ± 0.4) and motor involvement were almost negligible in p.Asp104ThrfsX13 patients, who, however, frequently (78%, $p < 0.001$) complained of neuropathic pain. In the other four clusters, walking difficulties were reported by 69–85% of patients, while orthotic and walking aids use ranged between 40–62% and 16–28%, respectively.

Conclusions This is the largest *MPZ* (and late-onset CMT2) cohort ever collected, reporting clinical features and disease progression of 186 patients from five different clusters across Italy. Our findings corroborate the importance of differentiating between 'classical' childhood-onset demyelinating, late-onset axonal and mild *MPZ*-related neuropathy, characterised by different pathomechanisms, in view of different therapeutic targets.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Previous studies acknowledged the wide phenotypic variability, explained by different pathomechanisms, within myelin protein zero (*MPZ*)-related neuropathy spectrum, encompassing infancy-onset hypomyelinating/dysmyelinating, 'classical' childhood-onset demyelinating, mild demyelinating and late-onset axonal Charcot-Marie-Tooth (CMT) forms.

WHAT THIS STUDY ADDS

⇒ This is the largest study ever performed on *MPZ*-related neuropathy spectrum, and in particular on late-onset CMT2. We took advantage of a single-mutation-based approach to maximise sample homogeneity and gain information on disease features and progression. In our cohort, late-onset CMT (p.Pro70Ser, p.Ser44Phe and p.Thr124Met) was clearly associated with higher disease severity and progression compared with 'classical' CMT1B (p.Ser78Leu), while p.Asp104ThrfsX1-related CMT1B had negligible disease burden.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Differentiating the distinct forms within the *MPZ*-related neuropathy spectrum, considered the heterogeneity in their onset, severity and progression, is of paramount importance in view of natural history studies and future clinical trials.

INTRODUCTION

Charcot-Marie-Tooth (CMT) disease encompasses a heterogeneous group of inherited peripheral neuropathies with a pooled prevalence of 18:100 000 across different populations.¹ The myelin protein zero (*MPZ*) gene on chromosome 1q23.3 encodes the most abundant structural component (P0 protein) of the peripheral myelin sheath, and, when mutated, variably causes severe



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Table 1 Relative frequency of *MPZ* mutations in previous national and international Charcot-Marie-Tooth cohorts

	All patients, n	Patients with <i>MPZ</i> -related neuropathy, n (%)
USA, 2011 ⁵	787	45 (5.7)
UK, 2012 ⁶	425	13 (2.7)
Germany, 2013 ⁹	589	21 (3.6)
Spain, 2013 ¹¹	438	19 (4.3)
Inherited Neuropathy Consortium, 2014 ⁷	1427	70 (4.9)
Germany, 2015 ⁸	1206	29 (2.4)
Hungary, 2018 ¹⁰	531	24 (4.5)
Japan, 2019 ²⁶	1005	51 (5.1)
Turkey, 2022 ²⁷	302	6 (2)
Italy, 2023 ¹²	805	82 (10.2)
MPZ, myelin protein zero.		

infancy-onset hypomyelinating/dysmyelinating neuropathy (Dejerine-Sottas disease), 'classical' childhood-onset demyelinating CMT (CMT1B) and late-onset axonal forms (CMT2I/J).^{2,3} Still other mutations cause mild CMT1B with a slight decrease of nerve conduction velocities (NCVs) in the intermediate range.⁴ Previous works on large national and international cohorts showed that *MPZ* is the third (USA,⁵ UK,⁶ International Neuropathy Consortium,⁷ Germany,^{8,9} Hungary¹⁰), or, less commonly, the fourth (Spain¹¹), most frequently mutated gene in CMT, with a relative prevalence ranging between 2 and 5.7% (table 1).

However, in 2023, Pesciotta and colleagues¹² found a double-to-triple frequency (10.2%) of *MPZ* mutations in Italy, due to the presence of five large mutation clusters. These clusters were the following: (1) the p.Thr124Met missense variant (associated with CMT2J) in Northern Italy (mainly in Veneto), (2) the p.Pro70Ser missense variant (associated with CMT2I) in Southern Lombardy and Emilia, (3) the p.Ser44Phe missense variant (CMT2I) in Sardinia, (4) the p.Asp104ThrfsX13, previously labelled as p.Val102fs, a frameshift variant associated with mild CMT1B¹³ in Apulia and (5) the p.Ser78Leu missense variant (CMT1B) in Sicily. While the first variant has been reported worldwide, the others have not been reported outside Italy. For this reason, in the Italian cohort *MPZ*, along with gap junction protein beta 1 (10.3%, associated with CMTX1), is the second most frequently mutated gene. We investigated the disease features, severity and progression of these five *MPZ* variant clusters across Italy.

METHODS

Data were collected for patients carrying these five mutations across the centres belonging to the Italian CMT registry.¹² Both patients from the registry and additional affected subjects identified among the historical series of the centres were included. We retrospectively gathered a minimal data set of genetic, electrophysiological and clinical information, including type of mutation, family history, disease onset, disease severity (CMTES-CMT Examination Score¹⁴), ability to walk, need for shoe inserts, ankle foot orthoses (AFOs), walking aids or wheelchair, upper limb involvement, neuropathic pain, pupillary abnormalities and hearing loss (see Pesciotta *et al*¹²). Age of onset was considered the age of onset of walking difficulties. As we could not retrieve the age of hearing dysfunction occurrence, patients with hearing loss older than 70 years were excluded from this item. Clinical assessments were performed by neurologists with long-lasting experience in CMT. Data were pseudo-anonymised.

Statistical analysis

Participants' characteristics at baseline were described in terms of absolute numbers and percentages for categorical data and means with SDs and ranges for continuous data. Comparisons of clinical characteristics among CMT subtypes were performed using the age-adjusted analysis of variance or χ^2 test, as appropriate. The strength and direction of association between ranked variables were investigated through the Spearman's rank correlation coefficient (rs) with the corresponding p value. A p value <0.05 was considered significant.

RESULTS

Overall, we collected data from 186 patients (93 women; mean age 50.8±16.6 years) carrying one of the five selected variants in heterozygosity: 60 patients had the p.Ser78Leu amino acid change associated with a 'classical' demyelinating CMT1B phenotype, all from Eastern Sicily; 42 subjects carried the p.Pro70Ser variant associated with late-onset axonal CMT2I, 34 of whom were from Southern Lombardy and 8 from Emilia region; 38 patients harboured the p.Thr124Met mutation causing the late-onset axonal CMT2J, 36 of whom were from Veneto (Northeastern Italy) and 2 from Piedmont (Northwestern Italy); 25 other subjects had the p.Ser44Phe variant associated with CMT2I and all originated from Sardinia; eventually, 21 harboured the p.Asp104ThrfsX13 change associated with mild CMT1B, all from Apulia (figure 1).

76% of patients (141/186) had a positive family history (table 2).

Nine subjects (one p.Ser78Leu, four p.Pro70Ser, one p.Thr124Met and three p.Ser44Phe) were asymptomatic carriers diagnosed during the family assessment, as they showed neither motor nor sensory signs/symptoms and had unremarkable electrophysiological study and therefore were excluded from further analyses.

Data on clinical features and disease severity of the five clusters are reported in table 2. In detail, disease onset, as assessed by the onset of walking difficulties, occurred earlier in the p.Ser78Leu group (34.7±20.3 years), followed by p.Ser44Phe (41.4±10.9), p.Thr124Met (45.2±9.4) and p.Pro70Ser (56.4±5.8) patients (p<0.001). Accordingly, age at first evaluation was earliest for p.Ser78Leu cohort (39.6±19.6 years) and latest for p.Pro70Ser (62.0±10.3) patients (p<0.001). None of the patients with the p.Asp104ThrfsX13 mutation showed walking difficulties, thus we were not able to precisely determine disease onset for them. However, interestingly, the p.Asp104ThrfsX13 cohort presented to medical attention earlier (46.6±12.5) as compared with axonal forms, suggesting a non-motor symptom at the onset.

Disease severity (CMTES) was highest in patients with the p.Thr124Met (9.2±6.6) mutation, with p.Ser44Phe (7.8±5.7), p.Pro70Ser (7.6±4.8), p.Ser78Leu (6.1±3.5) and p.Asp104ThrfsX13 (1.2±1.5) patients showing progressively lower burden of disease (p<0.001). Through cross-sectional analysis we assessed that the correlation between age and CMTES was strongest in p.Pro70Ser patients (rs=0.81, p<0.001) followed by those with the p.Ser44Phe (rs=0.72, p=0.003), p.Ser78Leu (rs=0.56, p<0.001) and p.Thr124Met (rs=0.43, p=0.024) variants (figure 2).

Notably, when analysing the progression rate, the CMTES change (worsening) per year was higher in the three axonal forms, particularly in p.Pro70Ser cohort, as compared with p.Ser78Leu patients (Δ CMTES/year p.Pro70Ser=0.8±1.0; p.Ser44Phe=0.7±0.4; p.Thr124Met=0.4±0.5; p.Ser78Leu=0.2±0.4; p<0.001). Disease progression was

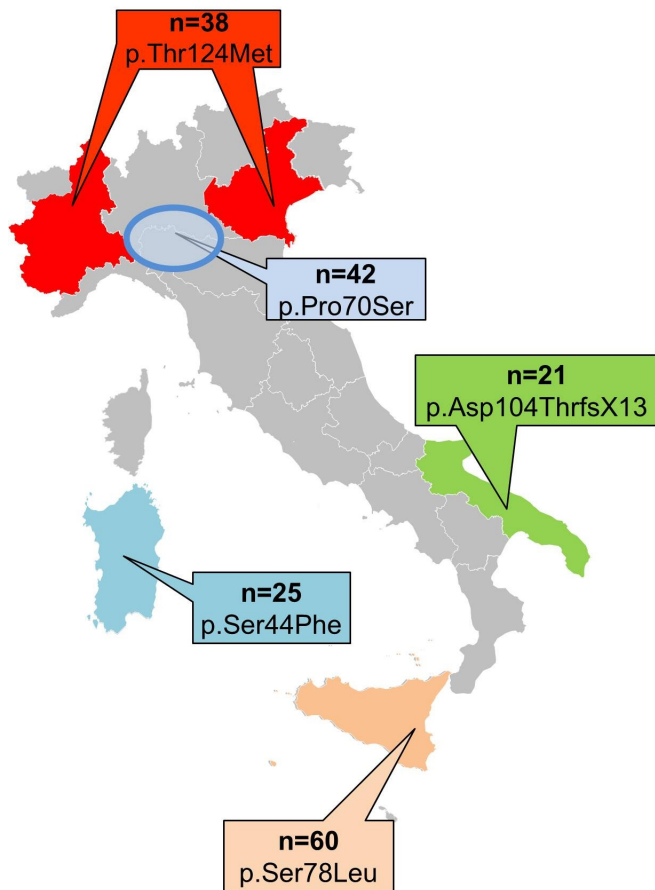


Figure 1 Distribution of the five myelin protein zero mutation clusters across Italy and the number of subjects for each variant.

negligible in the p.Asp104ThrfsX13 (Δ CMTES/year=0.1 \pm 0.4; rs=0.21, p=0.438, [figure 2](#)) cohort.

As far as regards clinical features, none of the patients with the p.Asp104ThrfsX13 amino acid change reported walking difficulties (p<0.001), AFO use (p<0.001), walking support (unilateral/bilateral) or wheelchair need. Three p.Asp104ThrfsX13 patients with pes cavus used shoe inserts to relieve foot pain during walking. In the other four clusters, walking difficulties were reported by 69–85% of patients, orthotic aid use ranged between 40% and 62% (24% and 52% for AFOs), while walking support devices were used by 16–28% of subjects, with higher frequency in those carrying the p.Pro70Ser (28%) and p.Ser44Phe (26%) mutations. Three patients became wheelchair users, in particular two (6%) with the p.Thr124Met amino acid change at the age of 62 and 65 (disease duration 22 and 32 years, respectively) and one (2%) with the p.Ser78Leu mutation at the age of 46 years (disease duration 26 years). Delayed walking (beyond 15 months of age) occurred only in the p.Ser78Leu cohort, specifically in 16% of cases. Upper limb involvement was observed in 12–47% of patients. Notably, in comparison to other clusters, patients with the p.Asp104ThrfsX13 mutation exhibited a significantly higher occurrence (78%, p<0.001) of neuropathic pain/positive sensory symptoms (eg, burning, tingling), which were arguably the most frequent presenting symptoms.

Hearing loss and pupillary abnormalities were almost exclusive of patients with the p.Thr124Met mutation (47% and 74%, respectively; p<0.001 for both).

Nerve conduction studies were available for 128 patients. Values of motor conduction velocity for ulnar nerve were in

the demyelinating range in patients with the p.Ser78Leu amino acid change (23.4 \pm 6.7 m/s), intermediate range in those with the p.Asp104ThrfsX13 (39.8 \pm 5.6) variant and axonal range in the p.Thr124Met (48.3 \pm 7.6), p.Pro70Ser (54.8 \pm 5.2) and p.Ser44Phe (50.1 \pm 4.8) cohorts ([table 2](#)).

DISCUSSION

In this nationwide, multicentre, retrospective study, we investigated the largest MPZ-related CMT cohort ever collected to specifically assess the clinical features of the five most frequent MPZ mutations in Italy, as formerly reported by Pisciotta and colleagues.¹² The two previous largest studies on MPZ-related CMT included 103 (Sanmaneechai *et al*¹⁵) and 139 (Fridman *et al*³) patients recruited within the International Neuropathy Consortium. In particular, we characterised the largest (n=96) late-onset axonal MPZ-related neuropathy (and late-onset CMT2 in general) series to date.

We found a notable cluster of 60 patients with the p.Ser78Leu mutation in Eastern Sicily. Part of this cluster was previously described by Mazzeo and coauthors.¹⁶ In Northern Italy, 42 patients from Lombardy and Emilia carried the p.Pro70Ser amino acid change which is related to a late-onset axonal form.¹⁷ The p.Ser44Phe amino acid mutation was typical of Sardinia, as previously reported by Loreface and colleagues,¹⁸ while a cluster of 21 patients with the p.Asp104ThrfsX13 mutation, associated with a mild phenotype,^{13 19–21} occurred in Apulia. Remarkably, these four mutations have never been reported outside Italy, as they are likely due to founder effects. Conversely, the p.Thr124Met amino acid change, which was found in 36 patients from Veneto and 2 from Piedmont, has been reported from different populations worldwide.^{22–25}

The presence of these five large clusters is the main reason for the higher relative frequency of MPZ mutation (10.2% of all CMTs¹²) in Italy, as compared with other national and international series. Indeed, among CMTs, MPZ mutation accounted for 2–4.5% of cases in previous national European series (Germany,^{8 9} UK,⁶ Spain,¹¹ Hungary¹⁰), 5.7% in the USA,⁵ 5.1% in Japan,²⁶ 4.9% in the International Neuropathy Consortium series⁷ and 2% in Turkey²⁷ ([table 1](#)).

Remarkably, our study allowed us to compare three different MPZ-related CMT forms, namely ‘classical’ demyelinating with childhood-onset (p.Ser78Leu), mild with slight NCV decrease (p.Asp104ThrfsX13) and axonal with late-onset (p.Pro70Ser, p.Ser44Phe, p.Thr124Met).

As expected,^{3 15} disease onset, with delayed autonomous walking reported in 16% of cases, occurred earlier in the demyelinating p.Ser78Leu cohort as compared with axonal forms. Accordingly, patients with the axonal forms came to medical attention much later, around the sixth/seventh decade.

As far as disease burden is concerned, mean CMTES was higher, indicating more severe disease, in the three axonal forms, namely 9.2 points for p.Thr124Met, 7.8 for p.Ser44Phe and 7.6 for p.Pro70Ser as compared with 6.1 in p.Ser78Leu patients. This differs from previous findings by Fridman and colleagues³ who conversely reported a higher disease severity in demyelinating forms (CMTES 11.8 demyelinating vs 9.6 axonal). However, as acknowledged by the authors, this could reflect the high phenotypic heterogeneity within their demyelinating cohort, which also encompassed patients with infantile-onset hypomyelinating/dysmyelinating neuropathy, characterised by extremely severe disease.

We found that disease progression (mean Δ CMTES/year) was much faster in the axonal forms, ranging from 0.4 (p.Thr124Met)

Table 2 Clinical features and disease severity of the neuropathy associated with the five myelin protein zero mutation clusters across Italy

	p.Pro70Ser (n=38)*	p.Thr124Met (n=37)*	p.Ser44Phe (n=22)*	p.Ser78Leu (n=59)*	p.Asp104ThrfsX13 (n=21)	P value
Males	45% (17/38)	59% (22/37)	41% (9/22)	53% (31/59)	48% (10/21)	0.614
Females	55% (21/38)	41% (15/37)	59% (13/22)	47% (28/59)	52% (11/21)	
Age at last evaluation	67.6±15.6 (34–88)	60.2±11.4 (42–84)	57.8±11.4 (40–78)	49±21.1 (9–85)	53.8±11.9 (36–75)	0.001
N of families with more than one familiar affected (n=patients)	12 (n=32)	10 (n=30)	8 (n=16)	18 (n=50)	5 (n=13)	n/a
Age at first evaluation (years)	62.0±10.3 (37–82)	53.5±12.8 (32–80)	51.6±10.1 (38–70)	39.6±19.6 (5–72)	46.6±12.5 (28–72)	0.001
CMTES at baseline	7.6±4.8 (0–16)	9.2±6.6 (0–19)	7.8±5.7 (0–22)	6.1±3.5 (0–13)	1.2±1.5 (0–4)	0.001
Rs age evaluation-CMTES	0.81 (p<0.001)	0.43 (p=0.024)	0.72 (p=0.003)	0.56 (p<0.001)	0.21 (p=0.438)	n/a
ΔCMTES/year†	0.8±1.0 (0–3.3)	0.4±0.5 (0–1.5)	0.7±0.4 (0.5–1.4)	0.2±0.4 (0–1.7)	0.1±0.4 (0–1.0)	0.001
Time from onset to first evaluation (years)‡	9.4±6.5 (1–24)	11.3±10.4 (0–40)	12.3±9.7 (2–28)	10.2±10.1 (0–42)	/	0.761
Walked after 15 months	0% (0/37)	0% (0/34)	0% (0/23)	16% (6/37)	0% (0/20)	0.009
Walking difficulties	85% (28/33)	74% (25/34)	84% (16/19)	69% (37/54)	0% (0/20)	0.001
Age of onset (years)	56.4±5.8 (47–69)	45.2±9.4 (30–65)	41.4±10.9 (21–58)	34.7±20.3 (2–65)	/	0.001
Orthotic aids	61% (20/33)	47% (14/30)	62% (13/21)	40% (20/50)	15% (3/20)	0.006
Shoe inserts	33% (11/33)	10% (3/30)	19% (4/21)	34% (17/50)	15% (3/20)	0.074
AFOs/orthopaedic shoes	52% (17/33)	37% (11/30)	52% (11/21)	24% (12/50)	0% (0/20)	0.001
Age (years)	62.5±5.8 (55–72)	43.9±8.4 (32–55)	52.1±8.2 (38–65)	43.7±21.8 (6–70)	/	0.006
Walking support need	28% (9/32)	17% (5/30)	28% (5/18)	16% (8/51)	0% (0/20)	0.090
Wheelchair use required	0% (0/31)	6% (2/34)	0% (0/17)	2% (1/49)	0% (0/20)	0.402
Difficulties with buttons	47% (14/30)	41% (13/32)	40% (8/20)	29% (14/49)	12% (2/17)	0.115
Neuropathic pain/positive sensory symptoms	50% (15/30)	56% (18/32)	38% (8/21)	14% (7/50)	78% (14/18)	0.001
Pupillary abnormalities	9% (3/32)	74% (20/27)	0% (0/21)	0% (0/50)	0% (0/20)	0.001
Hearing Loss	13% (4/32)	47% (15/32)	0% (0/21)	4% (2/50)	0% (0/20)	0.001
Ulnar CMAP (mV)	12.2±4.0 (4.2–19.4) (n=29)	12.7±4.4 (4.1–20.3) (n=27)	14.1±4.2 (8.7–22.4) (n=15)	8.4±4.5 (2.1–20.2) (n=42)	11.6±2.7 (8.8–17) (n=14)	0.015
Ulnar NCV (m/s)	54.8±5.2 (47–66) (n=29)	48.3±7.6 (39–66) (n=27)	50.1±4.8 (41–56) (n=15)	23.4±6.7 (13.6–32.6) (n=42)	39.8±5.6 (29.6–48.2) (n=14)	0.001

Significant p values refer to differences among the five groups (highest and lowest values in bold).

*Asymptomatic patients excluded.

†Disease progression is expressed as CMTES variation from baseline to the last available visit/time elapsed (ΔCMTES/year).

‡Time from onset to first evaluation was calculated only for patients whose motor involvement antedated the first evaluation.

AFO, ankle foot orthose ; CMAP, Compound Motor Action Potential; CMTEs, Charcot-Marie-Tooth Examination Score ; n/a, not applicable; NCV, nerve conduction velocities ; Rs, Spearman's rank correlation coefficient.

to 0.8 (p.Pro70Ser) points per year and in keeping with the CMTEs 1.30 points progression over a 2-year period estimated by Fridman and colleagues.³ Remarkably, it is noteworthy that p.Ser78Leu patients still showed detectable worsening, although slight, over time, which diverges from previous findings (Fridman *et al*³) of a null progression of demyelinating patients in adulthood. Interestingly, as for disease severity, the explanation might reside in the heterogeneity of the demyelinating cohort collected in this important study. Indeed, as a potential bias, they included patients with the p.Asp104ThrfsX13 and p.Lys236del loss of function (LOF) mutations, associated with mild CMT1B with almost negligible progression, as previously reported.^{19–21 28} Moreover, as explained above, their cohort also encompassed infantile-onset hypomyelinating/dysmyelinating MPZ neuropathies which, in spite of the extreme severity, arguably display lower progression in the adulthood, as worsening takes place mainly in the first years of life. In addition, the intrinsic poor sensitivity of CMTEs in detecting disease progression in severe stages of disease is well-recognised.^{3 29}

As far as the p.Asp104ThrfsX13 mutation is concerned, we confirmed its mild phenotype, typical of MPZ LOF mutations, characterised by the absence of disabling motor involvement

and by a negligible progression over time. Nonetheless, subjects harbouring this mutation frequently (78%) reported annoying positive sensory symptoms including neuropathic pain, which justified their relative early presentation to medical attention, despite mild impairment.

Among the three CMT2I/J clusters, the p.Thr124Met mutation was related to the highest disease severity (CMTEs). Indeed, two out of three of the chairbound patients in our series carried the p.Thr124Met variant. However, the analyses of the cross-sectional correlation between CMTEs and age and longitudinal progression of the CMTEs per year (mean ΔCMTEs/year) showed that disease worsening was faster in subjects carrying the p.Ser44Phe (0.7 points/year) and, as previously emphasised,³ the p.Pro70Ser (0.8 points/year) amino acid change. Moreover, the time interval from the onset of walking difficulties to the first evaluation was shorter in patients carrying the p.Pro70Ser mutation, indirectly supporting a faster disease progression. Accordingly, despite the latest onset, we found that the p.Pro70Ser mutation was associated with a high frequency of walking difficulties (85%), AFOs use (52%) and upper limb involvement (47%). Notably, juvenile-onset hearing loss and pupillary abnormalities were highly suggestive of the p.Thr124Met (CMT2J) mutation.²³

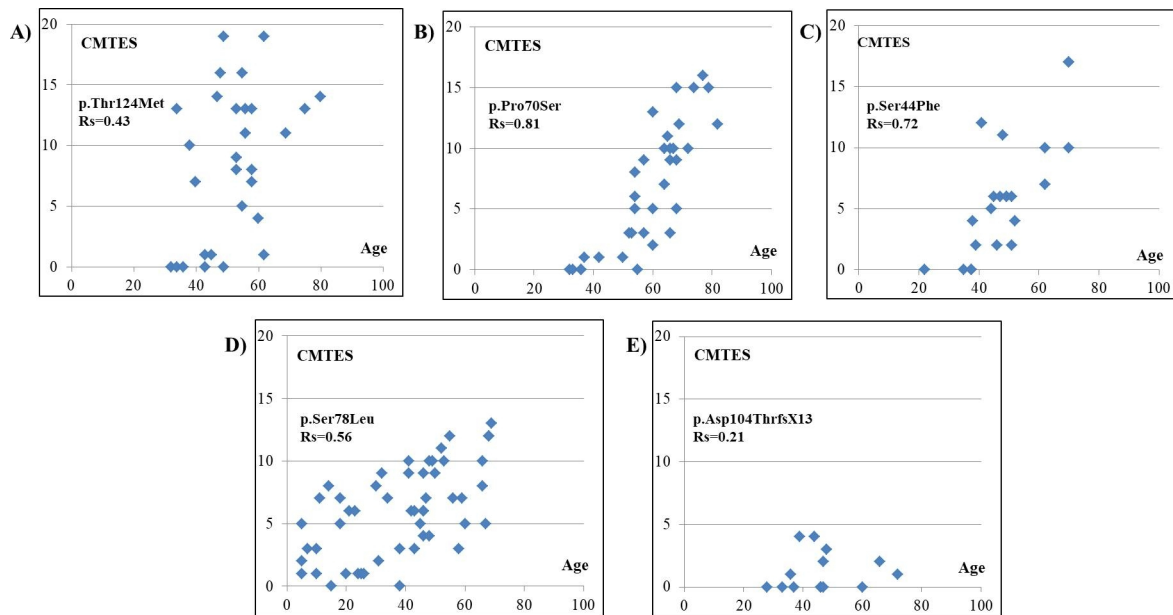


Figure 2 Disease progression of the neuropathy associated with the five myelin protein zero mutation clusters expressed as the correlation between age and CMTES value. CMTES, Charcot-Marie-Tooth Examination Score; Rs, Spearman's rank correlation coefficient.

Importantly, the present work corroborates the wide phenotypic heterogeneity within the *MPZ*-related neuropathies spectrum. Indeed in 2004, Shy and colleagues² emphasised the phenotypic differences between early (CMT1B) and adult (CMT2I/J) onset forms. However, the pathomechanism underlying such heterogeneity remains unclear.

P0 is a transmembrane protein (figure 3), which holds apposing membranes of the myelin sheath together through the single immunoglobulin-like extracellular domain (IgP0) by oligomerisation. There is evidence that variants causing demyelinating forms variably act by disrupting the stability of the protein itself or by preventing homomeric interactions.³⁰ In the first case, as reported by Bai *et al*,³¹ misfolded P0 accumulates in the endoplasmic reticulum activating the unfolded protein

response (UPR) which is an adaptative response to stress eventually leading to cell apoptosis. However, some degree of UPR activation was detected also in a few late-onset *MPZ*-related neuropathies (eg, p.Thr124Met).³¹ Concerning homomeric interaction, mutations such as p.Ser78Leu do not affect protein conformation, but map onto the surface of IgP0, within the region accessible to interactions with other *MPZ* oligomers.^{30 32}

Pathomechanism of late-onset axonal forms is even more uncertain. Recently, Ptak and colleagues³⁰ pointed out that variants causing CMT2I/J mapped to surface residues of IgP0 proximal to the transmembrane domain, where the protein undergoes N-linked glycosylation. However, while the p.Thr124Met variant³¹ is known to clearly alter the glycosylation, this does not occur for the p.Pro70Ser amino acid change.³³ Indeed, how a mutation in a structural myelin protein causes a mainly axonal neuropathy remains an important but unanswered question. In 2015, Brügger and colleagues³⁴ showed that P0 expression was fundamental for the maintenance of paranodal/nodal integrity and axonal function through interaction with the neurofascins, and speculated that this mechanism might be impaired by some *MPZ* mutations related to late-onset axonal neuropathy. These findings were corroborated by Shackleford and coauthors,³⁵ who generated a p.Thr124Met knock-in mouse model that showed prominent alterations in non-compact myelin domains such as paranodes, Schmidt-Lanterman incisures and gap junctions, implicated in Schwann cell-axon communication and axonal metabolic support. Nevertheless, this hypothesis remains to be confirmed and verified in its specificity for *MPZ* mutations.

Eventually, mild *MPZ*-related neuropathies are related to variants acting by haploinsufficiency instead of toxic gain of function mechanism. Specifically, the p.Asp104ThrfsX13 mutation results in the synthesis of a shorter protein devoid of the transmembrane domain that cannot be inserted in the membrane and thus likely undergoes non-sense-mediated decay preventing the formation of P0 from the mutant allele.^{19 20} This hypothesis was confirmed by *MPZ* knock-out mouse models showing a mild phenotype with slight NCV slowing and little axon loss.^{36 37} Further similar examples resulting in haploinsufficiency are the p.Tyr68Ter¹⁹ and Lys236del²⁸ mutations.

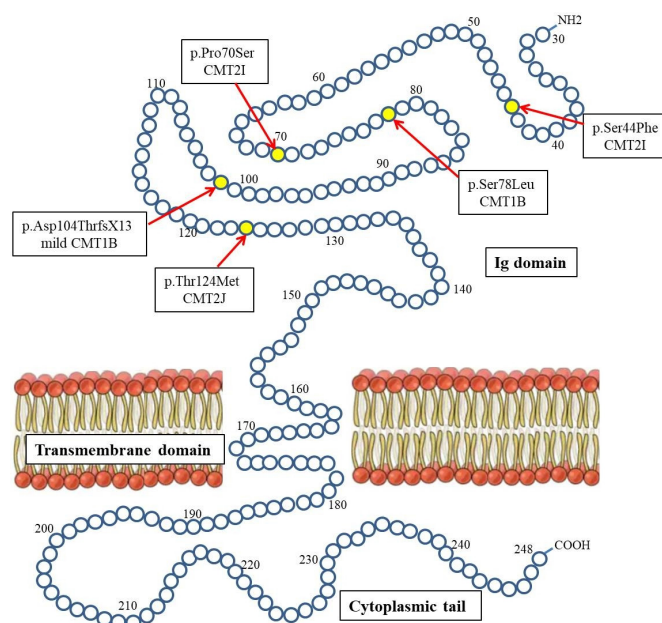


Figure 3 Localisation of the five mutations associated with the different CMT phenotypes in the P0 mature protein. CMT, Charcot-Marie-Tooth.

In conclusion, this is the largest study on MPZ-related neuropathies ever performed, reporting the clinical features and disease progression of five different clusters across Italy, namely p.Thr124Met in Veneto and Piedmont, p.Pro70Ser in Southern Lombardy and Emilia, p.Ser44Phe in Sardinia, p.Asp104ThrfsX13 in Apulia and p.Ser78Leu in Eastern Sicily. Such geographical distribution underlines the importance of always asking patients about the origin of their ancestry, to properly address genetic testing.

We took advantage of a single-mutation-based approach to maximise sample homogeneity, which allowed a better characterisation of the different forms within the MPZ-related neuropathy spectrum. Our findings confirm the broad phenotypic heterogeneity of MPZ mutations. Such variability relies on different pathogenetic mechanisms. In our series, we characterised patients affected by at least three different pathomechanisms, including loss of adhesion properties for p.Ser78Leu, altered axo-glia interaction for late-onset MPZ-related neuropathies and haploinsufficiency for p.Asp104ThrfsX13. This suggests the importance of clearly differentiating between infantile-onset hypomyelinating/dysmyelinating, ‘classical’ childhood-onset demyelinating, late-onset axonal and mild MPZ-related neuropathies in clinical prospective studies and in view of different therapeutic targets.

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