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Phenotype-Genotype Correlations in Early-Onset Myelin Protein Zero–Related Neuropathies

Christian Laurini,[1,2,3] Federica Rachele Danti,[3,4] Massimo Russo,[5] Stefano Tozza,[6] Sara Massucco,[3,7] Alessandro Bertini,[3,4] Chiara Pisciotta,[3,4] Federica D'Arma,[5] Luca Gentile,[5] Yuri Matteo Falzone,[1,3] Adele Ratti,[1,2] Michela Catteruccia,[3,8] Chiara Fiorillo,[3,9,10] Gianpaolo Cicala,[11] Marco Luigetti,[3,11] Stefania Magri,[4] Emilia Bellone,[7,12] Gian Maria Fabrizi,[13] Fiore Manganelli,[6] Marina Grandis,[3,7,12] Anna Mazzeo,[5] Davide Pareyson,[3,4] Isabella Moroni,[3,4] and Stefano Carlo Previtali[1,2,3]

Correspondence
Prof. Previtali
previtali.stefano@hsr.it

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Abstract

Background and Objectives

Myelin protein zero (MPZ)–related neuropathies include demyelinating CMT1B and later-onset axonal forms (CMT2I/J). CMT1B pathogenic variants act through gain-of-function, destabilizing MPZ and activating the unfolded protein response (UPR), or loss-of-function, disrupting MPZ homomeric interactions in myelin. This study investigates early-onset (<18 years) CMT1B due to MPZ variants in a large Italian cohort, shedding light on clinical progression and genotype-phenotype correlations, with relevance to emerging UPR-targeted therapies.

Methods

We analyzed cross-sectional clinical and genetic data from 75 patients across 7 Italian centers. MPZ variants were categorized as destabilizing or non-destabilizing based on published data. To explore the relationship between the degree of protein destabilization and clinical severity, missense variants were evaluated for stability alterations using the DUET online tool, with negative ΔΔG values indicating greater destabilization. Statistical correlations between clinical and molecular features were assessed.

Results

This study presents a comprehensive clinical characterization of one of the largest cohorts of early-onset MPZ-related neuropathies reported to date. The Charcot-Marie-Tooth Examination Score (CMTES) correlated with age at assessment (r_s = 0.56; p < 0.001), suggesting disease progression in this cohort. Destabilizing variants were associated with earlier onset (p < 0.001), upper limb involvement (p < 0.001), impaired ambulation (p < 0.01), scoliosis (p < 0.01), and faster progression as measured by CMTES/age (0.24 vs 0.11 CMTES/year). After excluding the c.245A>C, p.Y82S outlier (highly destabilizing but not UPR-activating), ΔΔG values showed significant correlations with age at onset (r_s = 0.54, p = 0.030), CMTES (r_s = −0.82, p < 0.001), and progression (r_s = −0.60, p = 0.032).

Discussion

Stratification of MPZ variants into destabilizing and non-destabilizing is crucial for predicting severity and prognosis and tailoring therapeutic strategies. This approach has significant implications for upcoming UPR-modulating treatments and underscores the necessity of integrating molecular and clinical insights for optimal patient care.

[1]IRCCS San Raffaele Scientific Institute, Milan, Italy; [2]Vita-Salute San Raffaele University, Milan, Italy; [3]Inherited Neuropathy Group of the National Virtual Institute for Rare Diseases of the RIN, Bologna, Italy; [4]Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; [5]Unit of Neurology and Neuromuscular Diseases, Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy; [6]University of Naples "Federico II", Naples, Italy; [7]IRCCS Ospedale Policlinico San Martino, Genoa, Italy; [8]Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [9]Unit of Child Neuropsychiatry, IRCCS Istituto Giannina Gaslini, Genoa, Italy; [10]Department of Neuroscience, University of Genoa, Genoa, Italy; [11]Fondazione Policlinico Universitario A. Gemelli IRCCS, UOC Neurologia, Rome, Italy; [12]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), University of Genoa, Italy; and [13]Department of Neurosciences, Biomedicine, and Movement Sciences, University of Verona, Italy.

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Glossary

AFOs = ankle-foot orthoses; **CMT** = Charcot-Marie-Tooth; **CMTES** = Charcot-Marie-Tooth Examination Score; **MPZ** = myelin protein zero; **MRC** = Medical Research Council; **UPR** = unfolded protein response.

Introduction

Charcot-Marie-Tooth (CMT) disease is the most prevalent hereditary neurologic disorder, with recent studies indicating a pooled prevalence of approximately 1 in 5,500 individuals.[1] Pathogenic variants in over 100 genes have been identified as causes of CMT disease, reflecting the intricate pathophysiology of peripheral nerves.[2]

Myelin protein zero (MPZ), which constitutes approximately half of the protein content in peripheral nerves,[3] plays a crucial mechanical role in the compaction of adjacent myelin sheets. Variants in the *MPZ* gene account for 2–10% of CMT cases and give rise to a broad spectrum of clinical phenotypes, ranging from early-onset hypomyelinating/dysmyelinating neuropathy (Déjèrine-Sottas syndrome) to childhood-onset demyelinating CMT1B, late-onset axonal CMT2I/J, and milder forms with NCVs in the intermediate range.[4]

Through a clever combination of in vitro and in silico analyses, recent work has mapped the MPZ protein surfaces involved in "cis" tetramerization (within the same membrane) and "trans" dimerization (across the intraperiod line), effectively distinguishing residues essential for efficacious myelin compaction from those necessary for proper protein folding.[5] Within this framework, the pathogenic mechanisms underlying MPZ-related demyelinating CMT1B appear to involve at least 2 distinct processes: "destabilizing" pathogenic variants located within the MPZ protein core lead to protein misfolding and accumulation in the endoplasmic reticulum (ER), triggering the unfolded protein response (UPR) in Schwann cells[5]; by contrast, "non-destabilizing" variants situated on the surface of the extracellular immunoglobulin-like domain (Ig^{MPZ}) impair MPZ oligomerization, exerting a dominant-negative effect on the wild-type allele[5] and disrupting myelin compaction.

Although UPR activation has been directly assessed in vitro for several pathogenic MPZ variants,[6] an alternative approach to indirectly estimate the destabilizing potential of missense variants involves in silico prediction tools that calculate changes in Gibbs free energy (ΔG) of the wild-type protein, or $\Delta\Delta G$ values (in kcal/mol). More negative $\Delta\Delta G$ values indicate greater structural destabilization and, theoretically (although not invariably), increased UPR activation. It is important to note that no definitive correlation between UPR activation or predicted $\Delta\Delta G$ values and clinical severity has been established to date.

Prolonging the duration and enhancing the efficacy of the UPR to restore proteostasis represents a promising therapeutic strategy for CMT1B.[7] Preclinical studies of sephin1, a small molecule that inhibits the GADD34/PPP1R15A phosphatase complex and prolongs the integrated stress response, have demonstrated significant benefits in animal models of CMT1B, including improved motor function, reduced ER stress, and enhanced myelination.[8,9] Translating these findings into clinical trials, however, requires a detailed understanding of the patient population, including which variants activate UPR and the selection of appropriate clinical outcome measures to ensure robust and reliable evaluation of investigational therapies.

This study focuses on a large cohort of Italian patients with early-onset CMT1B due to MPZ variants, with the aim of elucidating genotype-phenotype correlations and informing therapeutic strategies. By combining clinical, genetic, and in silico analyses, we provide insights into disease progression and its molecular underpinnings, with significant implications for the development of UPR-modulating therapies.

Methods

We conducted a retrospective review of clinical and genetic data from patients with early-onset CMT disease associated with MPZ variants. Patients were evaluated across 7 referral centers in Italy. Inclusion criteria required a genetically confirmed diagnosis of MPZ-related CMT and disease onset prior to 18 years of age.

Clinical data collected, as recorded at each patient's most recent evaluation, included sex, age at assessment, age at disease onset, presenting symptoms, walking ability, and the use of orthotic aids. Proximal and distal muscle strengths in both upper and lower limbs were evaluated using the Medical Research Council (MRC) scale, which ranges from 0 (no movement) to 5 (normal strength).[10] Muscle strength graded as MRC 4 was categorized as "mild" weakness while grades of MRC 3 or lower were classified as "severe" weakness. Additional parameters included hand motor skills, muscle stretch reflexes, sensory symptoms, and the presence of hearing loss.

Disease severity was determined by experienced neurologists using the Charcot-Marie-Tooth Examination Score (CMTES).[11] When available, additional clinical features such as tremor, cerebellar signs, autonomic or respiratory involvement, and cranial nerve function (including visual deficits and pupillary reflexes) were documented.

The age at onset was defined as the age at which the first neuropathy-related symptom manifested. For patients with hypotonia at birth, the age at onset was recorded as 0. For those with delayed acquisition of motor milestones, the age at onset was recorded as 1.

For every patient, we collected the specific MPZ variant and the mode of inheritance (autosomal dominant or sporadic). We applied the aforementioned MPZ residue classification[5] to categorize missense variants in our patients as either destabilizing or non-destabilizing. For non-missense variants, a review of the literature was conducted to identify experimental evidence of UPR activation, enabling the inclusion of c.188_190del, p.S63del in the destabilizing group.[12]

For missense variants, we used the DUET online tool to quantitatively assess their destabilizing impact on the MPZ protein structure (PDB: 1NEU). DUET is an online server that predicts the effect of missense variants on protein stability, using a combined approach based on structural and evolutionary information, integrated through a support vector machine regression model.[13] This analysis provided the predicted change in protein stability (ΔΔG value, expressed in kcal/mol), where negative values indicated a greater degree of destabilization.

Statistical Analysis and Visualization

All data were analyzed and visualized using R software (version 4.3.1) and the ggplot2 package for graphs. Continuous variables were expressed as mean ± SD while categorical variables were summarized as percentages and absolute counts.

For comparative analyses between subgroups, we used *t* tests for parametric continuous data, Mann-Whitney U tests for nonparametric continuous data, and chi-square tests (or Fisher exact tests when appropriate) for categorical data. To assess correlations between continuous variables, we used the Spearman rank correlation coefficient (r_s).

For variants observed in multiple patients, the average value of the corresponding clinical parameter was calculated and used in the analysis. Outlier detection was conducted using the Cook distance, with a threshold of >4/n (where n represents the sample size) to identify influential data points.

The threshold for statistical significance was set at $p < 0.05$.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by an ethics committee (Comitato Etico Territoriale Lombardia 4, INT. 26/24), and informed consent was obtained from all patients or their legal guardians.

Data Availability

The Principal Author had full access to all the data and takes full responsibility for the integrity of the analyses and the decision to publish. Deidentified participant data may be made available on reasonable request from qualified investigators, subject to approval and a data use agreement.

Results

Clinical Picture of the Overall Cohort

The study included 75 patients with early-onset demyelinating CMT1B. Table 1 provides a summary of the main clinical findings, including a subgroup analysis stratified by variant status (destabilizing vs non-destabilizing). The mean age at onset was 4.7 ± 4.8 years, and the mean age at assessment was 28.1 ± 20.7 years. Walking difficulties were reported in 83.1% of patients, with a mean onset at 11.3 ± 15.8 years. Approximately 52% of patients began walking after 15 months of age, and 75.4% reported balance difficulties, with a mean onset at 13.9 ± 18.2 years.

Distal lower limb weakness was observed in 71.7% of patients, with 43.3% classified as mild and 28.4% as severe, while proximal weakness affected 20.6%. In the upper limbs, distal weakness was noted in 73.2% of patients, with 49.3% exhibiting mild and 23.9% severe weakness, whereas proximal weakness was present in 11.4%. Difficulties with fine motor skills in the hands affected 61.8% of the cohort, with a mean onset at 29.6 ± 22.9 years. The mean CMTES was 9.5 ± 5.4, with a significant positive correlation observed between the CMTES and age at assessment ($r_s = 0.47$, $p < 0.001$). This finding suggests mild disease progression across the cohort, with an average increase of 0.11 points per year (Figure 1).

Assistive device use was common: 27.8% required ankle-foot orthoses (AFOs), and 23.6% needed walking support. Wheelchair dependence was noted in 6.9% of patients. Scoliosis and neuropathic pain were present in 39.7% and 26.4% of patients, respectively. A positive Romberg test was seen in 67.7% and tremor in 18.6%. Pallesthesia was preserved in 21.7% of patients, mildly impaired (reduced at the ankle or wrist or more distally) in 43.3% and severely impaired (reduced at the knee or elbow or more proximally) in 35%.

The pupillary response to light was absent in a single patient with a severe, early-onset R98C variant, whereas it was normal in all other patients. Only 5 patients (6.7%) exhibited cranial nerve dysfunction, primarily affecting extraocular motility and bulbar function, with strabismus observed in 3 patients, dysarthria in 2, and hypophonia in one. In addition, 3 patients exhibited cerebellar signs, including nystagmus and dysmetria. There were no reports of autonomic involvement in any patient. Only 1 patient, carrying the c.233C>T, p.S78L variant and presenting with a mild, sensory-predominant phenotype, exhibited respiratory involvement (obstructive sleep apnea syndrome), which might be unrelated to neuropathy.

Genetic Description of the Cohort

The cohort included a diverse array of variants in the MPZ gene, with 28 distinct variants identified among the 75 patients (Figure 2). The most prevalent variant was c.233C>T, p.S78L, observed in 32 patients, representing 42.7% of the cohort. Other recurrent variants included c.292C>T, p.R98C (6 patients); c.389A>G, p.K130R (4

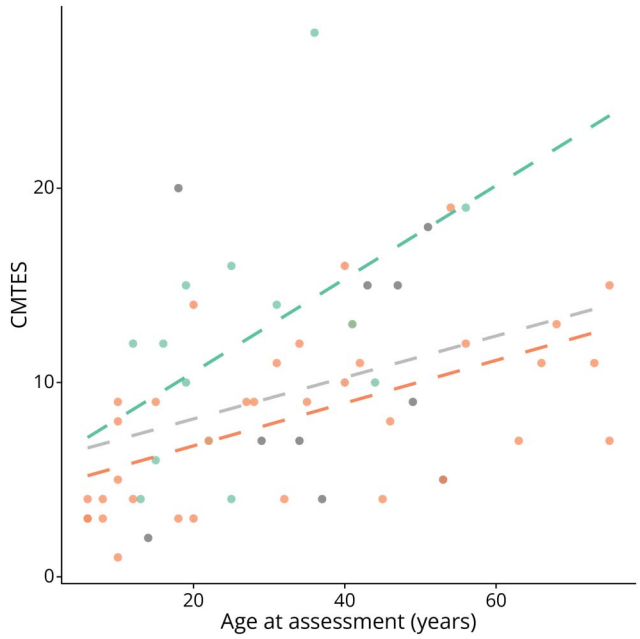
Table 1 Clinical Characteristics of 75 Patients With Early-Onset CMT1B, Stratified by Variant Type (Destabilizing vs Non-Destabilizing)

Variable	Overall (n = 75)	Destabilizing (n = 21)	Non-destabilizing (n = 41)	*p* Value
Male sex, % (n/tot)	46.7 (35/75)	52.4 (11/21)	48.8 (20/41)	1
Sporadic, % (n)/autosomal dominant, % (n)	25 (16)/75 (48)	57.1 (12)/42.9 (9)	6.3 (2)/93.7 (30)	<0.001
Age at onset, y, mean (SD)	4.7 (4.8)	1.4 (1.5)	6.7 (5.1)	<0.001
Age at assessment, y, mean (SD)	28.1 (20.7)	19.1 (15.4)	30.6 (22.3)	0.058
Difficulty walking, % (n/tot)	83.1 (59/71)	80 (16/20)	81.6 (31/38)	1
Difficulty walking—age at onset, y, mean (SD)	11.3 (15.8)	3.6 (7.3)	15.2 (18)	<0.001
Started walking >15 mo of age, % (n/tot)	52 (39/75)	81 (17/21)	39 (16/41)	0.004
Difficulty with balance, % (n/tot)	75.4 (52/69)	77.8 (14/18)	73.7 (28/38)	1
Difficulty with balance—age at onset, y, mean (SD)	13.9 (18.2)	3.9 (7.9)	21 (21)	<0.001
CMTES, mean (SD)	9.5 (5.4)	12.1 (6.4)	8.2 (4.4)	0.049
Use of AFOs, % (n/tot)	27.8 (20/72)	47.4 (9/19)	14.6 (6/41)	0.010
Age at AFO use, y, mean (SD)	21.1 (19.6)	9.3 (8.7)	33.2 (20)	0.005
Need for walking support, % (n/tot)	23.6 (17/72)	47.4 (9/19)	12.2 (5/41)	0.007
Age at walking support use, y, mean (SD)	27.7 (22.7)	19.6 (18.8)	51.3 (11.8)	0.037
Wheelchair, % (n/tot)	6.9 (5/73)	20 (4/20)	0 (0/41)	0.009
Independent walking, % (n/tot)	57.5 (42/73)	25 (5/20)	75.6 (31/41)	<0.001
Difficulties with hand motor skill, % (n/tot)	61.8 (42/68)	94.4 (17/18)	44.7 (17/38)	0.001
Difficulties with hand motor skill—age at onset, y, mean (SD)	29.6 (22.9)	21.3 (19.6)	32.8 (24.5)	0.460
Distal lower limb weakness, % none/% mild/% severe (n none/n mild/n severe)	28.4/43.3/28.4 (19/29/19)	44.4/27.8/27.8 (8/5/5)	23.7/50/26.3 (9/19/10)	0.210
Proximal lower limb weakness (MRC score <5), % (n/tot)	20.6 (13/63)	43.8 (7/16)	8.1 (3/37)	0.005
Distal upper limb weakness, % none/% mild/% severe (n none/n mild/n severe)	26.9/49.3/23.9 (18/33/16)	11.1/44.4/44.4 (2/8/8)	34.2/55.3/10.5 (13/21/4)	0.001
Proximal upper limb weakness (MRC score <5), % (n/tot)	11.4 (8/70)	15.8 (3/19)	5.3 (2/38)	0.320
Pallesthesia, % normal/% mildly affected/% severely affected (n normal/n mildly affected/n severely affected)	21.7/43.3/35 (13/26/21)	7.7/30.8/61.5 (1/4/8)	27.8/50/22.2 (10/18/8)	0.041
Neuropathic pain, % (n/tot)	26.4 (19/72)	10.5 (2/19)	34.2 (14/41)	0.110
Positive Romberg, % (n/tot)	67.7 (46/68)	64.7 (11/17)	68.4 (26/38)	1
Ataxia, % (n/tot)	53.5 (23/43)	60 (3/5)	54.6 (18/33)	1
Burning or tingling sensation, % (n/tot)	23.5 (16/68)	22.2 (4/18)	23.7 (9/38)	1
Tremor, % (n/tot)	18.6 (13/70)	21.1 (4/19)	17.1 (7/41)	0.730
Scoliosis, % (n/tot)	39.7 (23/58)	61.1 (11/18)	17.9 (5/28)	0.007
Hearing loss, % (n/tot)	11.3 (7/62)	11.8 (2/17)	12.1 (4/33)	1

Abbreviations: MRC = Medical Research Council; tot = total.

patients); and c.293G>T, p.R98L (3 patients). Notably, R98 emerged as a mutational hotspot, with additional variants such as c.292_294delCGCinsTGG, p.R98W (1 patient) and c.293G>A, p.R98H (1 patient) identified, bringing the total number of patients with R98 variants to 11. Less common variants included c.90C>G, p.I30M; c.94G>T, p.V32F; c.166G>A, p.E56K; c.242A>G, p.H81R; c.245A>C, p.Y82S; c.270C>G, p.D90E; c.308G>A, p.G103E; c.332C>G, p.S111C; c.382G>T, p.D128Y; c.393C>A, p.N131K; c.397C>A, p.P133T; c.410G>T, p.G137V; and c.499G>C,

Figure 1 Scatter Plot Depicting the Correlation Between CMTES and Age at Assessment



Green points and the green dotted regression line represent patients with destabilizing variants while red points and the red dotted regression line correspond to patients with non-destabilizing variants. Dark gray points indicate patients with uncategorized variants, and the light gray dotted regression line represents the overall population. CMTES = Charcot-Marie-Tooth Examination Score.

p.G167R, each appearing in 1 or 2 patients. We identified 4 novel variants in this cohort: 2 missense variants, c.154T>C, p.F52L and c.404T>A, p.I135K (the latter observed in a patient who also carried the P133T variant, although segregation data were unavailable), and 2 frameshift variants, c.513dup, p.L172Afs*63 and c.502del, p.V168Wfs (2 patients). Other frameshift variants included c.306del, p.D104Tfs*14; c.646dup, p.T216fs; and c.699_702del, p.S233fs (2 patients). Of interest, the only frameshift variant predicted to undergo nonsense-mediated decay (NMD) according to the "50–55 nucleotide rule"[14] was D104Tfs*14, which has been associated with a mild, nonprogressive phenotype.[4,15] By contrast, all other frameshift variants were located near the C-terminal domain and introduced premature termination codons beyond the final intron-exon junction, suggesting that these transcripts might evade NMD and result in the production of nonfunctional or UPR-activating protein. The cohort also included 2 nonsense variants (c.171G>A, p.W57ter and c.742A>T, p.K248ter) and a one–amino acid deletion (c.188_190del, p.S63del). Table 2 summarizes the MPZ variants identified in our cohort, their predicted effects on protein stability, and the associated clinical features.

Comparison of Destabilizing vs Non-Destabilizing Variants

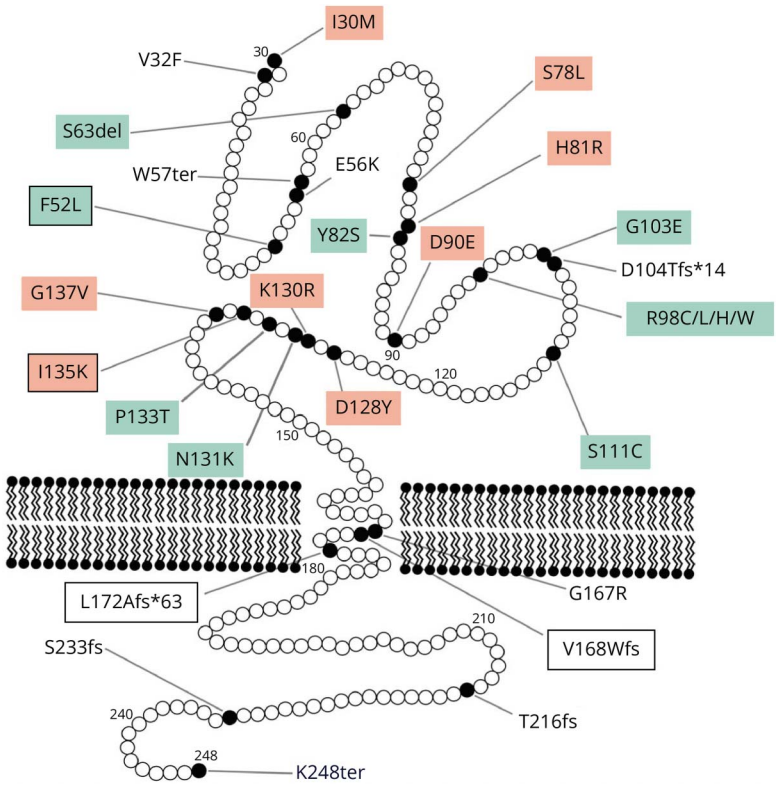
Patients with destabilizing variants had an earlier age at onset (mean 1.4 vs 6.7 years, $p < 0.001$) and were last assessed at a younger age (mean 19.1 vs 30.6 years), although this difference was not significant ($p = 0.058$). When onset was categorized according to previously established definitions,[16] nearly all patients with destabilizing variants showed an infantile onset (0–5 years, 95%), whereas non-destabilizing variants were more evenly distributed between infantile (43.9%) and childhood (6–18 years, 57.1%) onset. This difference was significant ($p < 0.001$). Among patients with destabilizing variants, 57.1% were sporadic while 42.9% had autosomal dominant inheritance. By contrast, only 6.3% of patients with non-destabilizing variants were sporadic whereas 93.7% inherited the variant from a parent ($p < 0.001$). This suggests that destabilizing variants may arise de novo more frequently, potentially due to stronger negative selection against transmission. Walking difficulties were reported to begin significantly earlier in patients harboring destabilizing variants (mean 3.5 vs 15.2 years, $p < 0.001$), and a greater proportion of patients exhibited delayed achievement of independent ambulation (defined as walking independently after 15 months of age) at 77.3% compared with 39% ($p = 0.009$). Balance difficulties were similarly prevalent between the 2 groups (79% vs 73.7%, $p = 0.750$) but began earlier in patients with destabilizing variants (mean 3.9 vs 21 years, $p < 0.001$).

Destabilizing variants were associated with greater use of AFOs (45% vs 14.6%, $p = 0.024$) and walking support (45% vs 12.2%, $p = 0.008$), as well as wheelchair dependence (19.1% vs 0%, $p = 0.010$). Independent walking, defined as the ability to walk without the use of AFOs or walking aids, was significantly less frequent (28.6% vs 75.6%, $p < 0.001$). These patients were also significantly more likely to report difficulties with hand motor skills (94.7% vs 44.7%, $p < 0.001$).

Lower limb proximal weakness was significantly more common in patients with destabilizing variants compared with non-destabilizing ones (41.2% vs 8.1%, $p = 0.007$). Proximal weakness in the upper limbs was also more frequent in those with destabilizing variants, although this difference did not reach significance (15% vs 5.3%, $p = 0.330$). Distal weakness in the lower limbs was comparable between the 2 groups ($p = 0.140$), whereas distal weakness in the upper limbs was more severe in patients with destabilizing variants ($p = 0.012$). Vibration sense was also more markedly impaired in these patients (normal in 7.7%, mildly affected in 30.8%, severely affected in 61.5% vs 27.8%, 50%, and 22.2% in patients with non-destabilizing variants, respectively, $p = 0.41$). Patients with destabilizing variants also had higher rates of scoliosis (63.2% vs 17.9%, $p = 0.004$). By contrast, neuropathic pain was less common in those with destabilizing variants, although this difference was not significant (10% vs 34.2%, $p = 0.089$).

The mean CMTES in the destabilizing group was 12.1 ± 6.4, while in the non-destabilizing group, it was 8.2 ± 4.4. The difference between the 2 groups was significant ($p = 0.049$). In the non-destabilizing group, CMTES demonstrated

Figure 2 Schematic Representation of the MPZ Protein Structure, Illustrating the Location of Identified Variants



The extracellular, transmembrane, and intracellular domains are depicted, with variants mapped accordingly. Black-filled circles denote affected residues identified in our cohort. Variants are color-coded as follows: green represents destabilizing variants while red indicates non-destabilizing variants. Variants with black borders correspond to previously unreported variants. MPZ = myelin protein zero.

a significant correlation with age at assessment ($r = 0.61$, $p < 0.001$), while in the destabilizing group, it only approached significance ($r = 0.49$, $p = 0.075$). However, patients with destabilizing variants exhibited a higher slope of 0.24 points per year, more than double the 0.11 points per year observed in the non-destabilizing group (Figure 1), suggesting steeper progression.

ΔΔG Values Correlate With Time of Onset, Progression, and Severity

Using the DUET online tool,[13] we calculated the ΔΔG value (kcal/mol) to quantitatively predict the destabilizing impact of missense variants on the crystal structure of the extracellular domain of MPZ (PDB: 1NEU). A prediction could not be obtained for amino acid changes occurring outside the extracellular domain or within poorly modeled regions of the PDB structure. Consequently, variants P133T, G137V, and G167R were excluded from the analysis.

After plotting ΔΔG values against age at onset, CMTESs, and CMTES/age at assessment (as a surrogate for progression speed), Y82S consistently emerged as a clear outlier in all 3 analyses (Figure 3). This observation was further validated by the Cook distance analysis, as detailed in Methods. Notably, variants at position Y82 are predicted to be highly destabilizing in silico[5] but do not activate the UPR in vitro.[6] Moreover, a different variant at the same position (Y82H) has been previously reported in a family with a late-onset, mild, axonal form of CMT disease.[17]

After exclusion of Y82S from the analysis, ΔΔG values showed moderate positive correlation with age at onset, strong negative correlation with CMTES, and moderate negative correlation with CMTES/age at assessment (Figure 3).

Discussion

This study presents a comprehensive clinical characterization of one of the largest cohorts of early-onset MPZ-related neuropathies reported to date, detailing key phenotypic features and genotype-phenotype correlations. Conducted as a multicentric effort across 7 reference centers in Italy, the study captures the broad clinical spectrum of the disease, ranging from mild to severe forms, with variability in disease onset, motor impairment, and the need for assistive devices. This heterogeneity underscores the complex genotype-phenotype relationships in MPZ-related neuropathies.

Of interest, when stratifying patients based on variant status (destabilizing vs non-destabilizing), we observed a segregation of clinical features that closely mirrors the differences previously described[16] between infantile-onset and childhood-onset CMT1B cases. While dexterity impairment, use of orthoses, walking aids, wheelchair dependence, and scoliosis were more prevalent in their infantile-onset group compared with their childhood-onset group, our stratification by variant status resulted in a similarly clear

Table 2 Summary of MPZ Variants Identified in Our Cohort, Including Their Predicted Impact on Protein Stability, the Number of Patients Carrying Each Specific Variant, and Key Clinical Parameters Associated With That Variant

Nucleotide change	Amino acid change	Category	ΔΔG (kcal/mol)	Patients	CMTES, mean (SD; min-max)	Mean assessment (y), mean (SD, min-max)	Mean onset (y), mean (SD, min-max)
c.90C>G	p.I30M	Non-destabilizing	−0.547	1	12	34	4
c.94G>T	p.V32F	Uncategorized	−1.626	1	20	18	2
c.154T>C	p.F52L	Destabilizing	−1.294	1	NA	6	1
c.166G>A	p.E56K	Uncategorized	0.650	1	9	49	12
c.171G>A	p.W57ter	Uncategorized	NA	1	7	29	3
c.188_190del	p.S63del	Destabilizing	NA	1	4	13	2
c.233C>T	p.S78L	Non-destabilizing	0.597	32	7.5 (4.1; 1–16)	29.2 (21.6; 6–75)	7.4 (5.4; 1–18)
c.242A>G	p.H81R	Non-destabilizing	−0.752	1	19	54	1
c.245A>C	p.Y82S	Destabilizing*	−3.473	1	4	25	7
c.270C>G	p.D90E	Non-destabilizing	−0.171	1	NA	12	7
c.292C>T	p.R98C	Destabilizing	−1.301	6	19.3 (6.2; 14–28)	37 (13.4; 25–56)	0.5 (0.5; 0–1)
c.293G>A	p.R98H	Destabilizing	−1.49	1	NA	2	2
c.293G>T	p.R98L	Destabilizing	−0.097	3	10 (1 pt with available data)	19 (1 pt with available data)	1 (0; 1-1)
c.292_294delCGCinsTGG	p.R98W	Destabilizing	−0.433	1	15	19	1
c.308G>A	p.G103E	Destabilizing	−0.861	1	13	41	1
c.306del	p.D104Tfs*14	Uncategorized	NA	1	2	14	2
c.332C>G	p.S111C	Destabilizing	−0.041	1	10	44	4
c.382G>T	p.D128Y	Non-destabilizing	−0.158	1	5	53	8
c.389A>G	p.K130R	Non-destabilizing	0.240	4	11.7 (1.2; 11–13)	47 (19; 31–68)	3.5 (2.4; 1–6)
c.393C>A	p.N131K	Destabilizing	0.629	2	6.5 (0.7; 6–7)	18.5 (4.9; 15–22)	1.3 (0.4; 1–1.5)
c.397C>A	p.P133T	Destabilizing	NA	1	12	12	1
c.397C>A + c.404T>A	p.P133T + p.I135K	Destabilizing + non-destabilizing	NA	1	NA	4	1
c.410G>T	p.G137V	Non-destabilizing	NA	1	7	75	1
c.499G>C	p.G167R	Uncategorized	NA	1	12	16	1
c.502del	p.V168Wfs	Uncategorized	NA	2	18 (1 pt with available data)	51 (1 pt with available data)	1 (0; 1-1)
c.513dup	p.L172Afs*63	Uncategorized	NA	1	NA	4	0
c.646dup	p.T216fs	Uncategorized	NA	1	4	37	1
c.699_702del	p.S233fs	Uncategorized	NA	2	15	45	5
c.742A>T	p.K248ter	Uncategorized	NA	1	5	53	11

For variants found in more than one patient, mean, SD, minimum, and maximum values are reported for the CMT Examination Score (CMTES), age at assessment (corresponding to the CMTES evaluation), and age at disease onset. *Y82 variants are predicted to destabilize MPZ in silico but do not elicit unfolded protein response (UPR) activation in vitro.[6] NA = not available; pt = patient.

segregation of these clinical characteristics. Notably, in their study, both subgroups included a mix of destabilizing and non-destabilizing variants (half of infantile-onset patients and 5 of 8 childhood-onset patients carried destabilizing variants), indicating that the parallelism in clinical patterns between the 2 classification strategies does not merely reflect redundancy. Indeed, when we applied the infantile vs childhood-onset categorization to our cohort, several key clinical variables

Figure 3 Correlation Between Predicted Destabilizing Effect of Missense Variants and Clinical Parameters

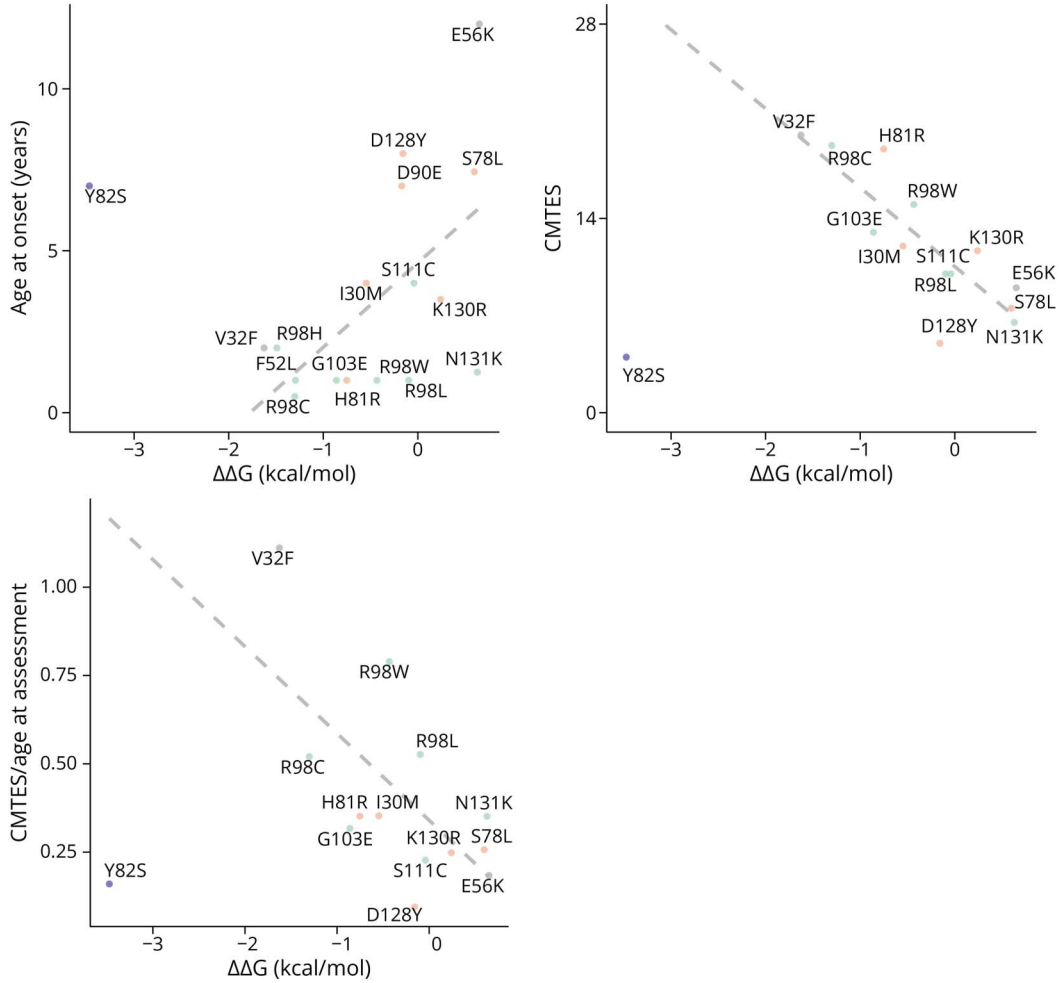


Figure shows the correlation between the predicted destabilizing effect of missense variants (ΔΔG, kcal/mol) and clinical parameters, including age at onset (left panel, $r_s = 0.54$, $p = 0.030$), CMTES (middle panel, $r_s = -0.82$, $p < 0.001$), and CMTES/age at assessment ratio (right panel, $r_s = -0.60$, $p = 0.032$). Each point represents an individual variant, labeled accordingly. Green points correspond to destabilizing variants, red points indicate non-destabilizing variants, and gray points represent uncategorized variants. The purple point represents Y82S, a biological and mathematical outlier that was removed from the analysis. CMTES = Charcot-Marie-Tooth Examination Score.

failed to reach statistical significance. For example, differences in CMTES (10.1 in infantile-onset vs 8.5 in childhood-onset, $p = 0.46$), vibration sense ($p = 0.21$), difficulty with hand motor skills (68.8% vs 48%, $p = 0.18$), use of walking aids (28.3% vs 15.4%, $p = 0.34$), and wheelchair dependence (10.7% vs 0%, $p = 0.15$) did not show statistically significant separation between the 2 groups. These findings reinforce the existence of a strong genotype-phenotype correlation in MPZ neuropathies, whereby the nature of the variant determines the pathogenic mechanism and consequently the clinical manifestations.

In cross-sectional studies, a significant association between age and clinical outcome measures is often used as an indirect indicator of disease progression, with the slope of this association providing an estimate of the rate of progression. Sanmaneechai et al. observed a steeper correlation between age and CMTES in the childhood-onset group compared with the infantile-onset group,[16] suggesting a potentially faster clinical progression in the former. However, the limited sample size of the childhood-onset cohort may have influenced these findings. In our cohort, patients with destabilizing variants exhibited more severe clinical phenotypes despite being assessed at a younger age. The correlation slope between age and CMTES in this group was more than twice as steep as that observed in patients with non-destabilizing variants, indicating a potentially accelerated disease course. A similar association between age and CMTES has been reported in individuals with MPZ-related neuropathy and disease onset before 22 years of age.[18] However, this approach warrants validation through longitudinal studies. Notably, a recent study reported minimal progression in demyelinating forms over a two-year period, whereas patients with axonal forms exhibited an average increase of 1.3 points in CMTES within the same time frame.[19] This apparent discrepancy between cross-sectional and longitudinal findings likely

reflects the inherently slow and nonlinear progression of the disease, with most of the clinical worsening taking place in the first decades for early-onset cases.

This study provides evidence for a clear correlation between variant status—categorized as destabilizing vs non-destabilizing—and patient phenotype in CMT1B. Previous in vitro work showed that a significant proportion of MPZ variants activate the UPR[6], but no link was established between UPR activation and either age at onset or clinical severity. Our results complement previous structural and computational analyses that classified MPZ variants based on their effects on the stability of the Ig domain of MPZ.[5] While that study did not include patient data or directly demonstrate clinical differences between destabilizing and non-destabilizing variants, it provided a mechanistic basis for understanding how protein stability influences MPZ function: variants that disrupt the core integrity of the Ig domain, often involving residues buried deep within the protein structure, are more likely to impair protein folding and trafficking, thereby increasing the likelihood of UPR activation.[5] An alternative pathogenic mechanism involves variants affecting residues located on the surface of the IgMPZ tetramer, which play a crucial role in homomeric interactions necessary for proper myelin adhesion. Disruptions in these interactions may lead to a loss-of-function phenotype due to alterations in MPZ homomeric interactions, distinct from UPR-driven pathogenesis, expanding the spectrum of molecular mechanisms underlying CMT1B. In addition, a milder loss-of-function phenotype can result from pure haploinsufficiency, as observed in families carrying variants such as Y68ter or D104Tfs*14.[15]

From a therapeutic standpoint, this distinction between different pathomechanisms is critical. Strategies aimed at modulating the UPR, such as XBP1s activation[7] or treatment with sephin1—an inhibitor of the GADD34/PPP1R15A phosphatase complex—have shown promise in preclinical models by reducing ER stress and improving myelination.[8,9] Although initial clinical studies have focused on other neurodegenerative conditions such as ALS,[20] future trials may explore its potential in inherited neuropathies, including CMT1B. However, given that not all MPZ variants activate the UPR to the same extent, patient stratification based on mutational effects on protein stability may be essential to optimizing therapeutic responses. In patients with CMT1B harboring variants that do not significantly activate the UPR and result in membrane-expressed MPZ protein, alternative therapeutic approaches may be required, such as variant-specific allele editing or complete silencing of *MPZ* expression, followed by replacement with a healthy allele.[21]

Our study underscores the necessity of integrating in silico structural modeling with clinical and genetic data to refine genotype-phenotype correlations in CMT1B. Notably, our data suggest that destabilizing, UPR-activating variants tend to result in an early-onset, more severe phenotype with a steeper disease progression. This accelerated progression could facilitate the transition to clinical trials, as fewer patients would be required to demonstrate a treatment effect over a shorter time span. However, these trials will need to carefully select the appropriate patient population, likely including children with initial clinical signs or even presymptomatic individuals harboring variants predicted to affect MPZ stability and activate the UPR. By bridging computational predictions with patient outcomes, we gain a clearer picture of MPZ-related neuropathies and contribute to a more tailored approach to treatment in inherited neuropathies.

Authors Contributions

C. Laurini: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. F.R. Danti: major role in the acquisition of data. M. Russo: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. S. Tozza: major role in the acquisition of data. S. Massucco: major role in the acquisition of data. A. Bertini: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. C .Pisciotta: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. F. D'Arma: major role in the acquisition of data. L. Gentile: major role in the acquisition of data. Y.M. Falzone: major role in the acquisition of data. A. Ratti: drafting/revision of the manuscript for content, including medical writing for content. M. Catteruccia: major role in the acquisition of data. C. Fiorillo: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. G. Cicala: major role in the acquisition of data. M. Luigetti: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. S. Magri: major role in the acquisition of data. E. Bellone: major role in the acquisition of data. G.M. Fabrizi: major role in the acquisition of data. F Manganelli: major role in the acquisition of data. M. Grandis: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. A. Mazzeo: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. D. Pareyson: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. I. Moroni: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. S.C. Previtali: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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