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Genotype–phenotype characteristics and baseline natural history of heritable neuropathies caused by mutations in the *MPZ* gene

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We aimed to characterize genotype–phenotype correlations and establish baseline clinical data for peripheral neuropathies caused by mutations in the myelin protein zero (*MPZ*) gene. *MPZ* mutations are the second leading cause of Charcot–Marie–Tooth disease type 1. Recent research makes clinical trials for patients with *MPZ* mutations a realistic possibility. However, the clinical severity varies with different mutations and natural history data on progression is sparse. We present cross-sectional data to begin to define the phenotypic spectrum and clinical baseline of patients with these mutations. A cohort of patients with *MPZ* gene mutations was identified in 13 centres of the Inherited Neuropathies Consortium - Rare Disease Clinical Research Consortium (INC-RDCRC) between 2009 and 2012 and at Wayne State University between 1996 and 2009. Patient phenotypes were quantified by the Charcot–Marie–Tooth disease neuropathy score version 1 or 2 and the Charcot–Marie–Tooth disease paediatric scale outcome instruments. Genetic testing was performed in all patients and/or in first- or second-degree relatives to document mutation in *MPZ* gene indicating diagnosis of Charcot–Marie–Tooth disease type 1B. There were 103 patients from 71 families with 47 different *MPZ* mutations with a mean age of 40 years (range 3–84 years). Patients and mutations were separated into infantile, childhood and adult-onset groups. The infantile onset group had higher Charcot–Marie–Tooth disease neuropathy score version 1 or 2 and slower nerve conductions than the other groups, and severity increased with age. Twenty-three patients had no family history of Charcot–Marie–Tooth disease. Sixty-one patients wore foot/ankle orthoses, 19 required walking assistance or support, and 10 required wheelchairs. There was hearing loss in 21 and scoliosis in 17. Forty-two patients did not begin walking until after 15 months of age. Half of the infantile onset patients then required ambulation aids or wheelchairs for ambulation. Our results demonstrate that virtually all *MPZ* mutations are associated with specific phenotypes. Early onset (infantile and childhood) phenotypes likely represent developmentally impaired myelination, whereas the adult-onset phenotype reflects axonal degeneration without antecedent demyelination. Data from this cohort of patients will provide the baseline data necessary for clinical trials of patients with Charcot–Marie–Tooth disease caused by *MPZ* gene mutations.

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Abbreviations: CMT = Charcot–Marie–Tooth disease; CMTNS = CMT neuropathy score; INC-RDCRC = Inherited Neuropathies Consortium - Rare Disease Clinical Research Consortium; NCV = nerve conduction velocity

Introduction

Charcot–Marie–Tooth disease (CMT), or hereditary motor sensory neuropathy (HMSN), is the most common inherited neuromuscular disorder, affecting 1 in 2500 people (Skre, 1974). CMT type 1B (CMT1B) is caused by mutations in the myelin protein zero (*MPZ*) gene and is the second most common form of the autosomal dominant hereditary demyelinating neuropathy, collectively called CMT1 (Nelis *et al.*, 1996; Saporta *et al.*, 2011). More than 200 different disease-causing mutations in *MPZ* have been identified (Timmerman *et al.*, 2014). Most patients with CMT1B are thought to present with one of two distinct phenotypes: one with extremely slow nerve conduction velocities and onset of symptoms during the period of motor development; the other with normal or near-normal nerve conduction velocities and the onset of symptoms as adults (Shy *et al.*, 2004). Because patients in the latter group can clinically appear to have an axonal neuropathy, they are also classified as having CMT2I (OMIM), despite the fact that *MPZ* is only expressed by myelinating Schwann cells and not by neurons (Trapp *et al.*, 2003).

The natural history and genotype–phenotype correlations of CMT1B are poorly understood. We have followed patients with CMT1B at Wayne State University and the Inherited Neuropathy Consortium (INC), which is a member of the Rare Disease Clinical Research Network (RDCRN) (http://www.rarediseasesnetwork.org/). The INC currently includes 17 sites that evaluate patients using standardized clinical, electrophysiological and genetic measures. Our goal was to collect cross-sectional data to characterize the phenotype of patients with different *MPZ* mutations that can also be used as baseline data for longitudinal natural history studies.

Materials and methods

Observational studies were performed on all patients with *MPZ* mutations evaluated at Wayne State University between 1996 and 2011 as well as patients enrolled in the initial 13 centres comprising the INC between 2009 and 2012. History, neurological examination and nerve conduction studies were evaluated. Genetic testing was performed in all patients and/or in first- or second-degree relatives to document mutation in *MPZ* gene indicating diagnosis of CMT1B. First- or second-degree relatives of genetically-defined CMT1B patients with a CMT phenotype were assumed to have the same mutation.

CMT outcome assessment measures

The severity of peripheral neuropathy was evaluated in all adult patients by the CMT neuropathy score (CMTNS) version 1 or 2 (Shy *et al.*, 2005*b*; Murphy *et al.*, 2011*a*). Both have been validated for composite measurement of impairment and assess symptoms, signs and neurophysiology of CMT patients. CMTNSv1 and CMTNSv2 are both composed of nine assessments: symptoms (three items), signs (four items), and neurophysiology (two items). Each measurement is scored on a 0–4 point scale for a total possible score of 36. To compare impairment for patients evaluated prior to 2011, we converted features of the CMTNSv1 score into CMTNSv2 by using available data for fine and gross motor abilities, neurological examinations, and physiological studies. We were unable to directly convert tuning fork data as only CMTNSv2 uses a Rydel-Seiffer tuning fork. The CMT examination score was used for patients who did not undergo electrophysiological testing. The CMT examination score is calculated by the sum of the symptoms plus the signs

in the CMTNS; it is therefore the CMTNS without the electrophysiological testing. CMT paediatric scale is a reliable, valid, and sensitive global measure of disability for children with CMT from the age of 3 years (Burns *et al.*, 2012). CMT paediatric scale measures seven areas: strength, hand dexterity, sensation, gait, balance, power, and endurance, and has a total score of 44. As with the CMTNS, higher scores indicate greater disability.

Clinical electrophysiology

Motor and sensory nerve conduction velocities (NCVs) were performed by standard techniques. Temperature was maintained at 32°C in the hands and feet for all visits. Surface electrodes were used in all studies. The amplitudes of the compound muscle action potential and sensory nerve action potential were recorded.

Statistical analysis

Patient characteristics, data from clinical examination, electrophysiological examination and physical disability were analysed using descriptive statistics.

The institutional review board (IRB) at Wayne State University and at each of the 13 centres comprising the INC, approved the study.

Results

Characterization of cohort

We identified 103 patients (from 71 different families) with CMT caused by mutations in the *MPZ* gene (39 males, 64 females). Baseline characteristics and clinical features of all patients are summarized in Table 1. The age of patients ranged from 3 to 84 years, with a mean age of 40 years. Pedigrees were obtained in all patients. One family had five members, three families had four members, four families had three members, 11 families had two members, and 52 families had a single affected member evaluated although 29 of these had other affected members that we did not see. Twenty-three families had only a single affected individual (sporadic case). Phenotypes and ages of onset in individuals within the same family were similar. The majority of patients were ambulatory, although most required orthoses or walking aids. About 10% required the use of wheelchair for ambulation. Optic nerve atrophy was noted in two patients with G137S and I135T *MPZ* mutations. Hip dysplasia was noted at birth in two patients with R98H and I135T *MPZ* mutations. Those four patients were the only members of their affected family members with optic atrophy or hip dysplasia. Scoliosis was found in 17 patients. Hearing loss was found in 21 patients; seven were infantile-onset (R98C, R98H, S111C, S111P, I135T, G137S) and 14 were adult-onset (R36W, H39P, I114fs, Y119C, T124M, R227S, c.614 + 2T > G splice site mutation). The age of symptom onset varied from 1 to 67 years old and the severity varied widely (CMTNS from 1 to 33). Patients were separable into infantile, childhood and adult onset groups clinically and by electrophysiology (Table 2).

Table 1 Demographic and clinical characteristics of 103 patients with CMT caused by mutations in the *MPZ* gene

Characteristics	Values
Age; mean ± SD (range in years)	40 ± 20 (3–84)
Age of symptom onset (range in years)	23 ± 20 (1–67)
CMTES (*n* = 87)	9.4 ± 5 (1–25)
CMTNS (*n* = 81)	13 ± 7 (1–33)
CMTPedS (*n* = 12)	23 ± 7 (13–42)
Orthoses (shoe insert/ankle bracing; *n*)	61 (25/36)
Walking assistance (*n*)	19
Wheelchair-dependent (*n*)	10
Foot, ankle, or toe surgery (*n*)	21

Data are mean ± SD and range; CMTES = CMT examination score; CMTPedS = CMT paediatric score.

The infantile onset group was characterized by a delayed onset of walking independently until at least 15 months of age (18–48 months), and the development of symptoms before 5 years of age. Forty per cent of all patients fell into this group; one-third were sporadic cases. Hip dysplasia and optic nerve atrophy were observed only in this group; scoliosis was found in 36% of infantile onset patients. The mean CMTNS at the initial visit was 18. CMT paediatric scale was performed in nine of the infantile onset children, with a mean score of 23. Nineteen per cent of infantile onset patients (eight patients) were in a wheelchair and 29% (12 patients) needed walking aids beyond ankle–foot orthoses. Three patients required a wheelchair at ages 7, 50 and 55; the age for wheelchair use was not recorded for the additional five. The age at which 9 of 12 patients needed ambulation aids in addition to ankle–foot orthoses were 4, 10, 20, 20, 26, 27, 48, 49 and 55 with a mean of 29 years. The mean ulnar motor NCV was very slow (12 m/s) with moderately reduced compound muscle action potential amplitude. Approximately 80% of patients in the infantile onset group had ulnar motor NCV $\leqslant$ 15 m/s with the remaining values all in the 16–25 m/s range. All patients in the infantile onset group had absent radial sensory nerve action potential.

The childhood onset group was characterized by developmentally normal children who developed their initial symptoms between the ages of 6 and 20 years; the mean age of symptom onset was 10 years old. Only 7% of patients fell into this group. Their mean CMTNS was 12 at the time of initial evaluation, typically in their late twenties. A CMT paediatric scale was performed in three of the childhood onset children with a mean score of 20. No patient in the childhood onset group required a wheelchair or walking aid. The mean ulnar motor NCV was 28 m/s, all within the slow range (15–35 m/s), with mildly reduced compound muscle action potential amplitudes. The mean radial sensory nerve action potential was 2.6 mV.

The adult onset group developed symptoms after the age of 20 years. Approximately half of all patients were in this group. The mean age of symptom onset was 40 years. Their mean CMTNS was 10 at the time of evaluation,

Table 2 Clinical and electrophysiological characteristics classified by age of symptom onset of 103 patients with CMT caused by mutations in the *MPZ* gene

Characteristics	Infantile onset 0–5 years	Childhood 6–20 years	Adult ⩾21 years	*P*-value
Number of patients	42	8	53	
Age at first visit (years)	28 ± 18	29 ± 12	50 ± 16	
Sporadic case, *n* (%)	14 (33)	2 (25)	7 (13)	
Age of symptom onset (years)	3.4 ± 4	10.4 ± 2.7	40 ± 14	
Dexterity problems, *n* (%)	31 (74)	5 (63)	26 (49)	0.09
Orthoses, *n* (%)	30 (71)	2 (25)	29 (56)	0.03
Walking assistance, *n* (%)	12 (29)	0	7 (13)	0.08
Wheelchair-dependent, *n* (%)	8 (19)	0	2 (3.8)	0.04
Foot surgery, *n* (%)	32 (76)	8 (100)	31 (59)	0.1
Optic nerve atrophy, *n* (%)	2 (4)	0	0	
Hip dysplasia; *n* (%)	2 (4)	0	0	
Hearing loss, *n* (%)	7 (17)	0	14 (26)	0.2
Scoliosis, *n* (%)	15 (36)	0	2 (3.8)	<0.0001
CMTES (*n* = 87)	11.7 ± 6	7.4 ± 3.5	7.9 ± 4.3	0.001
CMTNS (*n* = 81)	18 ± 6.3	11.9 ± 4.4	9.9 ± 5.6	<0.0001
CMTPedS (*n* = 12)	23.4 ± 8.4 (*n* = 9)	19.5 ± 0.7 (*n* = 3)		0.04
Ulnar MNCV (m/s)	12 ± 5.9	28 ± 9.4	44 ± 9.6	
Ulnar CMAP amplitude (mV)	2.7 ± 3	5.6 ± 3	6.8 ± 2.4	
Ulnar SNAP amplitude (mV)	0.08 ± 0.3	1.85 ± 2.3	8.36 ± 9.0	
Radial SNAP amplitude (mV)	0	2.6 ± 4.2	11.8 ± 7	
Patients with absent SNAP	100%	57% (4/7)	7% (3/43)	

Data are mean ± SD; CMTES = CMT examination score; CMTPedS = CMT paediatric score; MNCV = motor NCV; SNAP = sensory nerve action potential.

which was usually in their fifties. Four per cent were wheelchair-dependent, and 13% needed walking aids in addition to ankle–foot orthoses. The mean ulnar motor NCV was 44 m/s, with normal compound muscle action potential amplitudes. About half of patients in this group had been previously diagnosed as CMT type 2 due to reduced compound muscle action potential amplitudes in their legs and normal conduction studies. The mean radial sensory nerve action potential was 11.8 mV.

Overall, the CMTNS was performed in 81 patients with mean score of 13. Thirty-seven per cent had mild impairment (CMTNS ⩽ 10), 50% had moderate impairment (CMTNS 11–20) and 13% had severe impairment (CMTNS >20). The CMTNS was plotted against age of initial visit for each of the three clinical groups (Fig. 1). Derived data demonstrated a linear correlation between CMTNS and age of the patient for each clinical group. Thus, within each group, older patients had higher CMTNS. At any age, infantile onset patients tended to be the most severely affected type. The CMT paediatric scale was performed in 12 patients with mean of 23. Individual CMT paediatric scale at the age of initial visit behaved in a similar fashion as CMTNS, although the numbers of patients are small.

Electrophysiological findings

We investigated whether the ulnar motor NCV would be useful in characterizing the aforementioned three groups of patients. As shown in Table 3, patients were separated into four groups according to motor NCV: very slow (⩽15 m/s); slow (16–35 m/s); intermediate slow (36–45 m/s); and normal (>45 m/s). Each group consists of ~25% of all patients. Neither temporal dispersion nor conduction block was recorded in any patient. Different electrophysiological findings were observed in different age of onset groups. Very slow motor NCV was only observed in patients with infantile onset. Intermediate and normal motor NCV was only observed in patients with adult onset. Patients who had slowing of conduction velocity were more severely affected when compared to patients who had intermediate or normal conduction velocity. Figure 2 shows a linear correlation between age of symptom onset and ulnar motor NCV: patients who had slowing of conduction velocity were more severely affected in early life when compared to patients who had intermediate or normal conduction velocity. Absent radial sensory responses were observed in all patients in infantile onset group, 57% in childhood onset group and only 7% in adult onset group. The mean radial sensory response was 2.6 mV in childhood onset and 11.8 mV in adult onset patients (Table 2).

Genotype

We found 47 different *MPZ* mutations in 71 kindreds. Of these, 15 were new mutations compared to the ones summarized in the Inherited Peripheral Neuropathies mutation database http://www.molgen.ua.ac.be/CMTMutations and reviewed by us previously (Shy *et al.*, 2004). Table 4 lists the particular *MPZ* mutations, according to the infantile, childhood and adult onset phenotypes. Affected individual

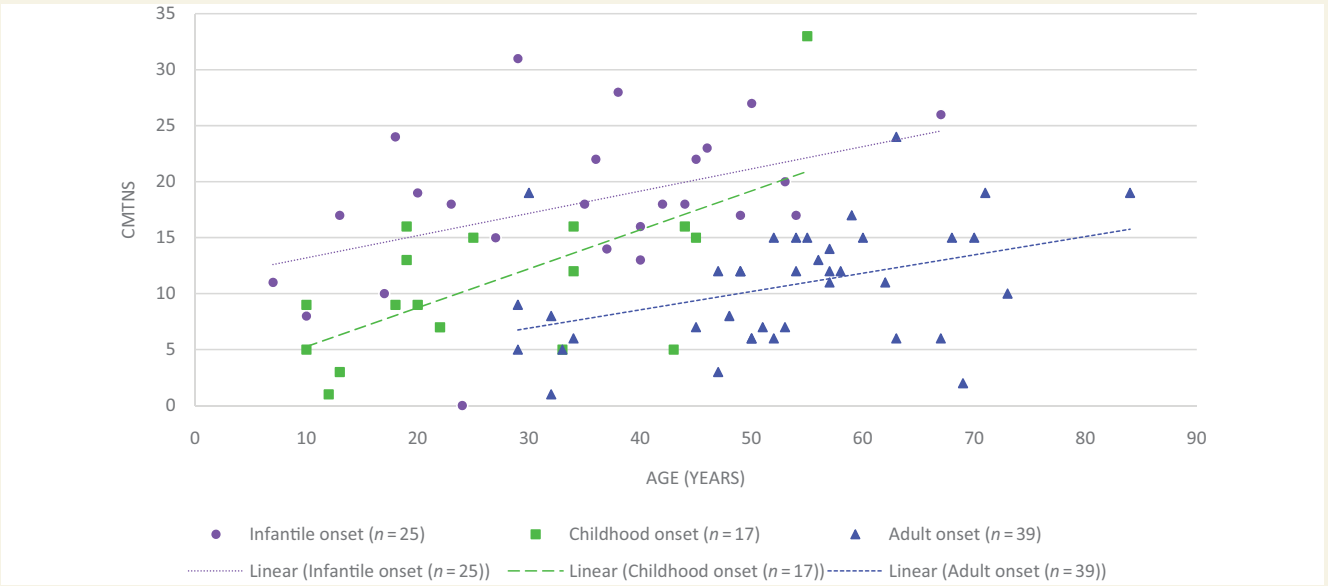

Figure 1 CMTNS at initial visit in 81 patients with CMT caused by mutations in the *MPZ* gene.

Table 3 Clinical manifestation and severity classified by ulnar motor NCV of 76 patients with CMT caused by mutations in the *MPZ* gene

	Ulnar MNCV (m/s)				
	Very slow ⩽ 15	Slow 16–35	Intermediate 36–45	Normal >45	*P*-value
n (%)	20 (26)	17 (22)	17 (23)	22 (29)	
Mean age (years)	32	35	39	58	<0.0001
Mean age onset (years)	4.9 ± 4	18.8 ± 17	29 ± 13	45 ± 12	<0.0001
Delay walking	20	6	0	0	<0.0001
Age of onset					
Infantile (n = 25) (%)	20 (80)	5 (20)	0	0	<0.0001
Childhood (n = 6) (%)	0	6 (100)	0	0	
Adult (n = 45) (%)	0	6 (13)	17 (38)	22 (49)	
CMTNS (n = 66)	17.4 ± 5.6	16 ± 12.7	8.5 ± 5	10 ± 6.2	<0.0001
CMTPedS (n = 6)	26 ± 2.8	16 ± 5	21 ± 2.8	—	0.16

Data are mean ± SD or *n* = number; CMTES = CMT examination score; CMTPedS = CMT paediatric score; MNCV = motor NCV.

members within the same family always had a similar phenotype based on clinical presentation, age of onset, clinical severity, disease progression, and nerve conductions. Patients from different families with the same mutations also presented with similar phenotypes with the exception of two mutations: Ser78Leu and Arg98His. A schematic diagram of the amino acid sequence of MPZ protein and its putative secondary structure with the mutations known to cause neuropathy is shown in Fig. 3, updated from our earlier version (Shy *et al.*, 2004).

Discussion

We found 47 different *MPZ* mutations that cause either infantile, childhood or adult onset phenotypes in 103 patients. Only two of the 47 mutations were found in more than one of these groups. Thus, there appears to be characteristic genotype–phenotype correlations in CMT1B, confirming and extending our previous study of 13 patients with eight mutations (Shy *et al.*, 2004). In that paper, we also reviewed the literature and found many mutations caused either an infantile or adult onset phenotype (Shy *et al.*, 2004). However, our present study also identifies a smaller group of mutations that cause a 'classic' CMT1 phenotype, with childhood onset phenotype and demyelinating physiology. Because we have now evaluated the patients with standardized outcome measures, we are able to comment on the severity of neuropathy caused by the different mutations. Infantile onset patients were typically the most severely affected and the age of onset correlated with severity. Overall age also correlated with severity in this cross-sectional analysis, suggesting that CMT1B is progressive in all three phenotypic groups.

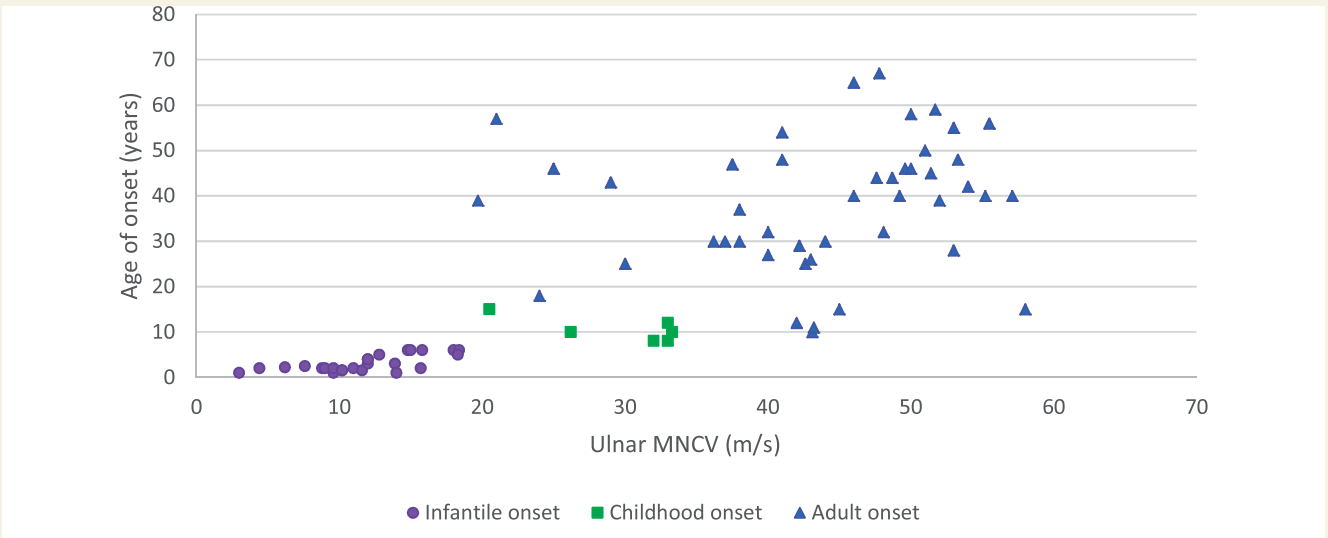

Figure 2 Correlation between age of symptom onset and ulnar motor NCV (MNCV) in 76 patients with CMT caused by mutations in the *MPZ* gene.

The current nomenclature for MPZ mutations remains confusing as patients with NCV <38 m/s are classified as CMT1B and those with NCV >38 m/s are classified as CMT2I by OMIM. Moreover, cases that present in infancy have been said to have Dejerine–Sottas syndrome whereas others have been diagnosed with congenital hypomyelination. Dejerine–Sottas syndrome was originally used to diagnose severely affected children with autosomal recessive CMT (Dejerine, 1893; Martin *et al.*, 1999). Harding and Thomas (1980) subsequently noted that severely affected infants represented a heterogeneous group and many such patients have turned out to have *de novo* mutations in dominantly inherited genes such as *MPZ* (reviewed in Shy *et al.*, 2005*a*). Alternatively, congenital hypomyelination is a pathologically based term originally used to describe peripheral nerves with absent or severely disrupted myelin suggesting a developmental failure of peripheral nervous system myelination (Lyon, 1969; Karch and Urich, 1975; Kennedy *et al.*, 1977). Similar pathological features have been reported from sural nerve biopsies of patients with congenital hypomyelination or Dejerine–Sottas syndrome. Moreover the same patient has been diagnosed with congenital hypomyelination in one publication (Becker, 1978) and Dejerine–Sottas syndrome in another (Bell, 1935). Ultimately, we believe that all of these classifications make it harder to focus on the pathogenic processes underlying these neuropathies. Perhaps the most useful way to characterize phenotypes associated with *MPZ* mutations is to simply classify them according to the age of presentation, severity of disease and pathological features.

Our data add to the evidence that *MPZ* mutations act in different ways to cause infantile, childhood, or adult onset neuropathy. Mutations that cause infantile phenotypes likely disrupt the developmental process of myelination to the extent that normal myelin sheaths are never formed. This idea is supported by morphological observations of nerve biopsies from patients with infantile onset mutations (Warner *et al.*, 1996; Gabreels-Festen *et al.*, 1999; Mandich *et al.*, 1999; Nelis *et al.*, 1999; Mersiyanova *et al.*, 2000; Eggers *et al.*, 2004) and the very slow motor NCV in infantile onset cases (often <10 m/s). In childhood onset cases, myelin sheaths are more fully formed, although still abnormal, as evidenced by NVCs that are in the 20–25 m/s range (Miller *et al.*, 2012). Finally, myelin sheaths are normally formed in adult onset cases, and the degeneration of myelinated axons causes neuropathy in adults (De Jonghe *et al.*, 1999; Li *et al.*, 2006). Why particular mutations cause these phenotypes is not understood. Mutations within specific cellular domains of the *MPZ* gene have no apparent prediction of phenotypic severity.

Reviewing the various mutations listed in Table 4 and Fig. 3 can provide some guidance as to which particular mutations will cause infantile or adult onset neuropathy. Mutations that introduce a cysteine into the extracellular loop of MPZ would be predicted to disrupt disulphide bridging that would be essential for its 3D structure (Shapiro *et al.*, 1996) and would therefore prevent myelination. Indeed when the Ser63Cys mutation is introduced into mice, ectopic disulphide bonds *in trans* retard initial wrapping of myelin (Avila *et al.*, 2010). In our current manuscript, Tyr82Cys, Arg98Cys, Ser111Cys and Ser123Cys all cause infantile onset disease. However, Tyr119Cys allows myelination to develop and causes an adult onset neuropathy. Moreover, while Arg98Cys causes infantile disease it appears to do so by activating an intracellular process called the unfolded protein response rather than by disrupting myelin wrapping (Patzko *et al.*, 2012; Saporta *et al.*, 2012). Therefore, how a novel cysteine would cause neuropathy in CMT1B is more complicated than initially perceived. Pathogenic pathways in

Table 4 Forty-seven different *MPZ* mutations in 103 patients.

Nucleotide	Amino acid	Patients	Families	References
Infantile onset				
c.151-171del	p.P50-57 del	1	1	This report
c.156C>G	p.Phe52Leu	1	1	This report
c.188C>T	p.Ser63Phe	1	1	Blanquet-Grossard *et al.*, 1995; Mostacciuolo *et al.*, 2001; Lee *et al.*, 2004, 2005
c.193A>G	p.Thr65Ala	2	1	Kochanski *et al.*, 2004
c.194C>A	p.Thr65Asp	1	1	This report
c.233C>T	p.Ser78Leu*	2	2	Latour *et al.*, 1995; Bort *et al.*, 1997; Fabrizi *et al.*, 2000; Boerkoel *et al.*, 2002; Keckarevic-Markovic *et al.*, 2009
c.245A>G	p.Tyr82Cys	1	1	Himoro *et al.*, 1993; Mitsui *et al.*, 1994; Haites *et al.*, 1998; Silander *et al.*, 1998; Boerkoel *et al.*, 2002; Numakura *et al.*, 2002
c.268G>C	p.Asp90His	1	1	This report
c.292C>T	p.Arg98Cys	2	2	Gabreels-Festen *et al.*, 1996; Kirschner *et al.*, 1996; Meijerink *et al.*, 1996; Rouger *et al.*, 1996; Warner *et al.*, 1996; Bort *et al.*, 1997; Komiyama *et al.*, 1997; Phillips *et al.*, 1999; Hattori *et al.*, 2003; Lee *et al.*, 2004; Bai *et al.*, 2006; Mandich *et al.*, 2009; Baets *et al.*, 2011
c.292C>T	p.Arg98Trp	1	1	This report
c.293G>A	p.Arg98His*	2	2	Hayasaka *et al.*, 1993*b*; Gabreels-Festen *et al.*, 1996; Kirschner *et al.*, 1996; Meijerink *et al.*, 1996; Rouger *et al.*, 1996; Lagueny *et al.*, 1999; Ohnishi *et al.*, 1999; Mersiyanova *et al.*, 2000; Young *et al.*, 2001; Watanabe *et al.*, 2002; Shy *et al.*, 2004; Lee *et al.*, 2005; Mandich *et al.*, 2009
c.308G>A	p.Gly103Glu	1	1	Fabrizi *et al.*, 2001
c.329G>A	p.Gly110Asp	2	1	Ekici *et al.*, 2000; Huehne *et al.*, 2003
c.331T>C	p.Ser111Pro	2	1	This report
c.332C>G	p.Ser111Cys	2	1	Mandich *et al.*, 2009
c.335T>C	p.Ile112Thr	1	1	Haites *et al.*, 1998; Sorour and Upadhyaya, 1998; Murphy *et al.*, 2011*b*
c.341T>C	p.Ile114Thr	1	1	Warner *et al.*, 1997
c.367G>T	p.Gly123Cys	1	1	Boerkoel *et al.*, 2002; Shy *et al.*, 2004
c.389A>G	p.Lys130Arg	1	1	Gabreels-Festen *et al.*, 1996; Tachi *et al.*, 1996; Yoshihara *et al.*, 2000; Shy *et al.*, 2004
c.397C>G	p.Pro133Ala	1	1	This report
c.400G>C	p.Asp134His	1	1	This report
c.402C>G	p.Asp134Glu	1	1	This report; Mersiyanova *et al.*, 2000 reported c.402C>A that also results in p.Asp134Glu
c.404T>C	p.Ile135Thr	4	1	Roa *et al.*, 1996; Tyson *et al.*, 1997; Mersiyanova *et al.*, 2000
c.409G>A	p.Gly137Ser	4	1	Roa *et al.*, 1996
c.410G>A	p.Gly137Asp	2	2	Ostern *et al.*, 2014
c.424G>T	p.Val142Phe	1	1	This report
c.499G>A	p.Gly167Arg	1	1	Hayasaka *et al.*, 1993*a*; Tachi *et al.*, 1994; Takashima *et al.*, 1999; Simonati *et al.*, 2002; Hattori *et al.*, 2003; Shames *et al.*, 2003; Cartwright *et al.*, 2009
c.643C>T	p.Gln215stop	1	1	Warner *et al.*, 1996; Mandich *et al.*, 1999, 2009; Shy *et al.*, 2004
Total		**42**	**32**	
Childhood onset				
c.188_190delCCT	p.Ser63 del	3	1	Kulkens *et al.*, 1993; Gabreëls-Festen *et al.*, 1996; Numakura *et al.*, 2002; Hattori *et al.*, 2003
c.233C>T	p.Ser78Leu*	2	1	Latour *et al.*, 1995; Bort *et al.*, 1997; Fabrizi *et al.*, 2000; Boerkoel *et al.*, 2002; Keckarevic-Markovic *et al.*, 2009
c.293G>A	p.Arg98His*	2	2	Hayasaka *et al.*, 1993*b*; Gabreels-Festen *et al.*, 1996; Kirschner *et al.*, 1996; Meijerink *et al.*, 1996; Rouger *et al.*, 1996; Lagueny *et al.*, 1999; Ohnishi *et al.*, 1999; Mersiyanova *et al.*, 2000; Young *et al.*, 2001; Watanabe *et al.*, 2002; Shy *et al.*, 2004; Lee *et al.*, 2005; Mandich *et al.*, 2009
c.646_647dupA	p.Thr216fs	1	1	This report
Total		**8**	**5**	
Adult onset				
c.106A>T	p.Arg36Trp	1	1	Burns *et al.*, 2006
c.116A>C	p.His39Pro	15	6	Shy *et al.*, 2004; Li *et al.*, 2006; Souayah *et al.*, 2007; Kilfoyle *et al.*, 2006 report a c.117A>C
c.131C>T	p.Ser44Phe	2	2	Marrosu *et al.*, 1998; Shy *et al.*, 2004; Benedetti *et al.*, 2010
c.136delG	p.Val46fs	1	1	This report
c.208C>T	p.Pro70Ser	4	3	Laura *et al.*, 2007; Benedetti *et al.*, 2010

(continued)

Table 4 Continued

Nucleotide	Amino acid	Patients	Families	References
c.293G>A	p.Arg98His*	4	3	Hayasaka *et al.*, 1993*b*; Gabreels-Festen *et al.*, 1996; Kirschner *et al.*, 1996; Meijerink *et al.*, 1996; Rouger *et al.*, 1996; Lagueny *et al.*, 1999; Ohnishi *et al.*, 1999; Mersiyanova *et al.*, 2000; Young *et al.*, 2001; Watanabe *et al.*, 2002; Shy *et al.*, 2004; Lee *et al.*, 2005; Mandich *et al.*, 2009
c.296T>C	p.Ile99Thr	1	1	Haites *et al.*, 1998; Donaghy *et al.*, 2000
c.306delA	p.Val102fs	1	1	Sghirlanzoni *et al.*, 1992; Warner *et al.*, 1996; De Angelis *et al.*, 2004; Steck *et al.*, 2006; Benedetti *et al.*, 2010
c.341delT	p.Ile114fs	3	1	This report
c.356A>G	p.Tyr119Cys	4	2	Senderek *et al.*, 2000
c.371C>T	p.Thr124Met	5	4	Chapon *et al.*, 1999; De Jonghe *et al.*, 1999; Misu *et al.*, 2000; Senderek *et al.*, 2000; Yoshihara *et al.*, 2000; Hanemann *et al.*, 2001; Numakura *et al.*, 2002; Hattori *et al.*, 2003; Kurihara *et al.*, 2003, 2004; Stojkovic *et al.*, 2003; Baloh *et al.*, 2004; Rajabally and Abbott, 2005; Triggs *et al.*, 2006; Briani *et al.*, 2008; Grandis *et al.*, 2008; Gallardo *et al.*, 2009; Mandich *et al.*, 2009
c.418T>A	p.Ser140Thr	1	1	Street *et al.*, 2002; Shy *et al.*, 2004
c.434A>C	p.Tyr145Ser	2	1	Leal *et al.*, 2003; Starr *et al.*, 2003
c.641G>A	p.Arg214Glu	1	1	This report
c.681A>T	p.Arg227Ser	1	1	Xu *et al.*, 2001; Keckarevic-Markovic *et al.*, 2009
c.706_708del AAG	p.Lys236del	3	3	Street *et al.*, 2002; Choi *et al.*, 2004; Sowden *et al.*, 2005
c.215+1G>C	5'-splice site	1	1	This report
c.614+2T>G	5'-splice site	3	1	Sabet *et al.*, 2006
Total		**53**	**34**	

*The same mutation has been found in patients in more than one clinical group.

the cell also do not tightly correlate with clinical phenotype. Activation of the unfolded protein response correlates with an infantile onset neuropathy with Arg98Cys, but results in a childhood onset neuropathy with Ser63Del (Pennuto *et al.*, 2008; Miller *et al.*, 2012) or adult onset neuropathy with Thr124Met (Shy, unpublished results). Finally, mutations that disrupt amino acids that are particularly important for MPZ's role as an adhesion molecule (Shapiro *et al.*, 1996) do not correlate with particular phenotypes. Variable severity also occurs in CMT1A, the most common form of CMT, but rarely to the extent described in CMT1B (Thomas *et al.*, 1997; Fridman *et al.*, 2015). It has been hypothesized that modifier genes (Brewer *et al.*, 2014), inflammation or other factors may influence disability in CMT1A (Fledrich *et al.*, 2012). However, we think it unlikely that these factors can explain the different phenotypes of CMT1B. Variable disability in CMT1A occurs with one common mutation, the duplication on chromosome 17 (Lupski *et al.*, 1991; Timmerman *et al.*, 1992). In the patients with CMT1B, however, there are different mutations and we believe that it is the specific mutation that determines whether there is an early, childhood or adult onset and whether the patient has profound demyelination or axonal changes on nerve conduction studies. This is supported by our findings that the phenotypes are almost completely mutation-specific. Whether there is additional variability in progression for given mutations will be determined in longitudinal studies. This would enable us to better determine whether modifier genes, inflammation or environmental factors contribute to the ultimate disease course. Determining why particular mutations cause particular phenotypes remains an important question to answer to understand the pathogenesis of CMT1B.

Determining why adult onset mutations cause axonal degeneration with minimal effects on myelin is a particularly important question as its answer may provide clues that explain axonal degeneration in demyelinating neuropathy in general. Virtually all de/dysmyelinating neuropathies have associated axonal degeneration that often correlates more with the patient's disability than the de/dysmyelination itself (reviewed in Scherer and Wrabetz, 2008). MPZ is expressed only in myelinating Schwann cells, not neurons (Lemke and Axel, 1985; Lemke, 1988). Because the adult onset *MPZ* mutations damage axons with only minimal effects on myelin, it suggests that axonal damage from de/dysmyelination can be mechanistically separated from the direct effects of damaged myelin, either by segmental demyelination or developmental dysmyelination. This in turn suggests that signalling pathways between myelin and the axon may be appropriate therapeutic targets no matter what is damaging the myelin. Bird *et al.* (1997) have elegantly demonstrated axonal degeneration extending into the dorsal columns in a family with an early onset case of dysmyelinating CMT1B. They illustrate that axonal degeneration also occurs with dys/demyelination, a well-known finding in most dys/demyelinating neuropathies. The degeneration of anterior horn cells in their study is also noteworthy in that there is debate about whether motor neuron cell bodies are affected along with axons in

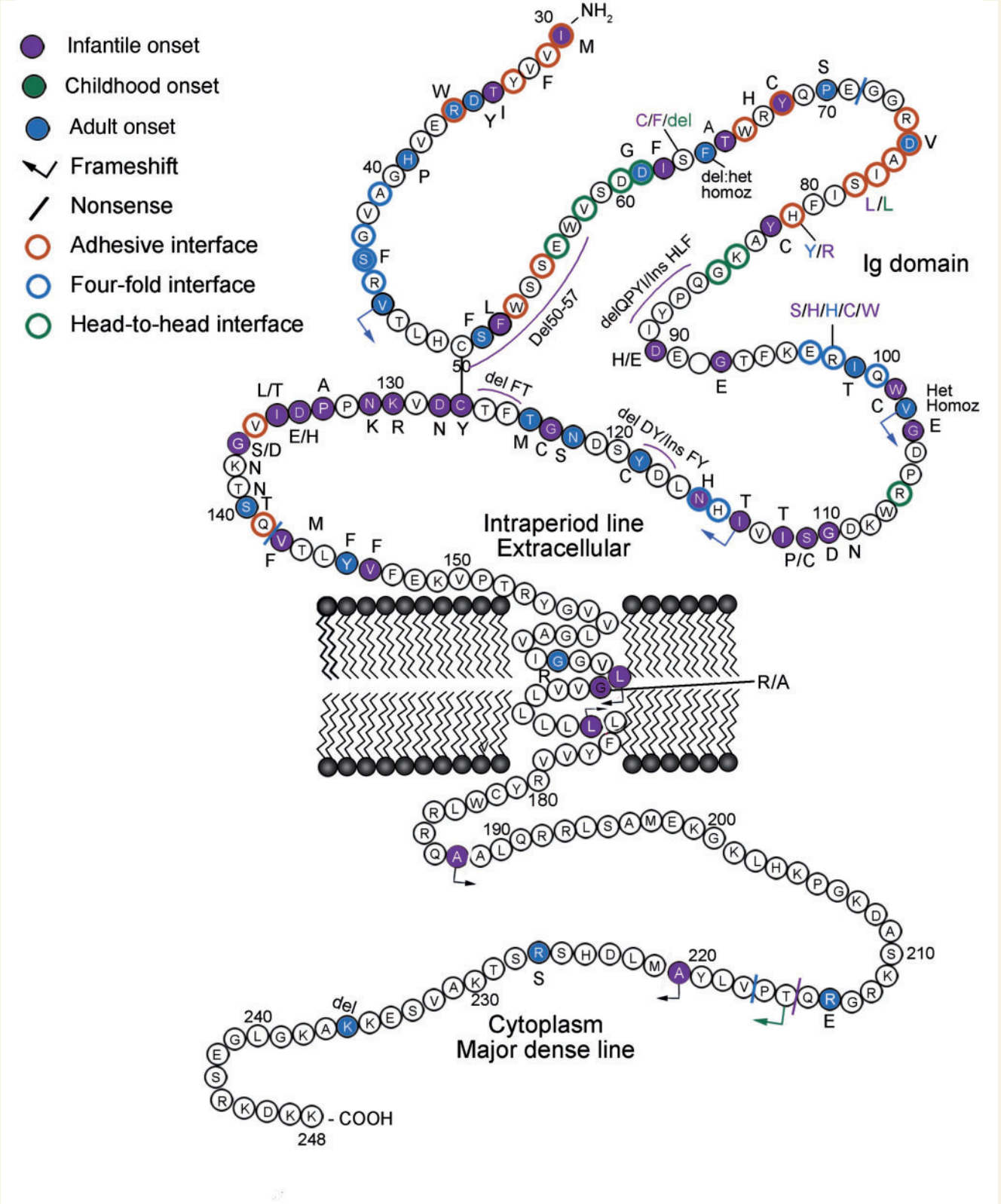

Figure 3 Mutations in the *MPZ* gene associated with inherited neuropathies. Adhesive interface, 4-fold interface and head-to-head interface, marked with colour to the border of circle, refer to amino acid residues deemed essential for *cis* and *trans* adhesion between adjacent myelin wraps. The numbering system for MPZ mutations includes the 29 amino acid leader peptide cleaved before insertion in the myelin sheath. Mutations demonstrated by adding the letter represent amino acid change, arrows represent frameshift mutation and line represent nonsense mutation. Mutations causing early onset phenotype are filled or noted with red colour, while those causing childhood onset phenotype are in green, and those causing late onset phenotype are in blue (updated from Shy *et al.*, 2004).

these cases (Bird *et al.*, 1997). However, patients from this report differ from patients in our late 'axonal' group in that the late onset group show little if any demyelination and typically do not develop symptoms until adulthood. Whether axonal damage in this later onset group occurs by similar mechanisms to that in the early dysmyelinating group is unknown.

We are presently in an era where rational therapies for inherited neuropathies are possible and practical for patients with mutations in *MPZ* (Jang *et al.*, 2012; Johnson *et al.*, 2012; Martinelli *et al.*, 2013; Foley *et al.*, 2014). Curcumin derivatives have improved neuropathy in Arg98Cys MPZ mice (Patzko and Shy, 2012; Patzko *et al.*, 2012) and pharmacological inhibition of Gadd34 (encoded by *Ppp1r15a*) improves the neuropathy of MPZ Ser63del mice (D'Antonio *et al.*, 2013). Organizations such as the Charcot–Marie–Tooth Association (CMTA) are developing specific strategies to develop clinical trials for CMT1B (http://www.cmtausa.org/). However, testing therapies requires detailed, carefully developed natural history studies using validated outcome instruments performed by trained personnel. Cohorts such as the participants in the current manuscript can provide the baseline for longitudinal natural history investigations using established and novel outcome measures.

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