Genetic spectrum of Charcot–Marie–Tooth disease associated with myelin protein zero gene variants in Japan

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Abstract
We aimed to reveal the genetic features associated with MPZ variants in Japan. From April 2007 to August 2017, 64 patients with 23 reported MPZ variants and 21 patients with 17 novel MPZ variants were investigated retrospectively. Variation in MPZ variants and the pathogenicity of novel variants was examined according to the American College of Medical Genetics standards and guidelines. Age of onset, cranial nerve involvement, serum creatine kinase (CK), and cerebrospinal fluid (CSF) protein were also analyzed. We identified 11 novel pathogenic variants from 13 patients. Six novel MPZ variants in eight patients were classified as likely benign or uncertain significance. Cranial nerve involvement was confirmed in 20 patients. Of 30 patients in whom serum CK levels were evaluated, eight had elevated levels. Most of the patients had age of onset >20 years. In another subset of 30 patients, 18 had elevated CSF protein levels; four of these patients had spinal diseases and two had enlarged nerve root or cauda equina. Our results suggest genetic diversity across patients with MPZ variants.

KEYWORDS
cerebrospinal fluid protein, Charcot–Marie–Tooth disease, cranial nerve involvement, creatine kinase, myelin P0 protein
1 | INTRODUCTION

Myelin protein zero (MPZ) protein is a major structural component of myelin and encoded by MPZ gene, which is expressed by Schwann cells. MPZ protein is classified as a member of immunoglobin superfamily and an essential membrane protein comprising 248 amino acids. The final structure of MPZ protein consists of three domains: extracellular domain comprising 124 amino acids, transmembrane domain comprising 26 amino acids, and intracellular domain comprising 69 amino acids located at the C-terminus.

Charcot–Marie–Tooth disease (CMT) is the most common inherited peripheral neuropathy, CMT is commonly divided into two groups: demyelinating type with slower median nerve conduction velocity (<38 m/s) and axonal type with maintained median nerve conduction velocity (>38 m/s).

MPZ variants contribute to the cause of demyelinating neuropathy CMT1B (OMIM 118200) or axonal neuropathy CMT2I/J (OMIM 607677/607736) and also the more severe, juvenile-onset Dejerine-Sottas syndrome (OMIM 145900) and hypomyelinating neuropathy, congenital 2 (OMIM 618184). Moreover, MPZ variants are associated with dominant intermediate Charcot–Marie–Tooth disease D (CMTDID) (OMIM 607791). The phenotype of CMT caused by MPZ variants varies from severe pediatric onset to mild adult onset.

To date, about 250 variants of this gene have been described as the cause of inherited peripheral neuropathy (https://portal.biobaseinternational.com/hgmd/pro/). There are limited studies that analyzed large number of patients with MPZ variants. Our laboratory analyzed the genetic spectrum of Japanese patients with CMT.

In this study, we investigated 85 patients to clarify the genetic spectrum of inherited peripheral neuropathy associated with MPZ variants in Japan. In addition, we also investigated the age of onset, cranial nerve involvement, serum creatine kinase (CK), and cerebrospinal fluid (CSF) protein in 77 patients with reported and novel pathogenic variants.

2 | MATERIALS AND METHODS

2.1 | Subjects

We examined 1657 Japanese patients who were considered to have inherited peripheral neuropathy from April 2007 to August 2017. All patients and family members provided written informed consent to participate in the study. Before starting this study, patients suspected to have demyelinating CMT with median motor nerve conduction velocity (median MCV) below 38 m/s were checked for duplication or deletion of PMP22 using fluorescence in situ hybridization or multiplex ligation-dependent probe amplification, and patients with duplication or deletion of PMP22 were excluded. Clinical information and blood/DNA samples were collected by neurologists or pediatricians and referred to our genetic laboratory at Kagoshima University Hospital. Using the Gentra Puregene Blood kit (QIAGEN), genomic DNA derived from patients and their families was extracted from peripheral blood cells according to the manufacturer’s instructions.

2.2 | Microarray sequencing and whole-exome sequencing

From April 2007 to April 2012, variant screening was conducted in 417 patients using customized MyGeneChip, CustomSeq, Resequencing Array (Affymetrix, Inc.), targeting 28 disease-causing or related genes of CMT. We have described the procedure of sequencing and data analysis previously. However, this methodology could not identify some variants due to the false negative hybridization and a low-detection efficiency of the DNA microarray in our laboratory. Thus, we combined whole-exome sequencing to overcome these issues. Whole-exome sequencing was performed by HiSeq2000 (Illumina Inc., San Diego). Using the Burrows-Wheeler Aligner, we aligned the sequences to human genome reference (NCBI37/hg19) and used SAM tools (http://www.htslib.org) for calling the variants. The called variants annotation was performed using CLC Genomic Workbench software program (Qiagen, Hilden, Germany) and an in-house script. Whole-exome sequencing was performed as indicated in the previous study.

2.3 | Targeted resequencing

In May 2012, we introduced the Illumina MiSeq platform (Illumina Inc.), targeting all coding exons and exon–intron junctions of 60 disease-causing or candidate genes of inherited peripheral neuropathies. We have described this system previously. We performed variant screening in 437 patients using this sequencing platform, until July 2014. In September 2014, we introduced the Ion Proton System, applying the Ion PI Chip kit v2/v3 BC (Thermo Fisher Scientific, Carlsbad) and began using the Ion AmpliSeq gene panel to target 72 inherited peripheral neuropathy disease-causing or candidate genes consisting of 1800 amplicons divided into two primers. Variant screening was conducted in 803 patients using this platform, until August 2017.

To analyze the copy number variations of MPZ, we screened the 803 patients using CovCopCan software.

2.4 | Data analysis and variant interpretation

All MPZ variants were checked against the Human Gene Mutation Database (https://portal.biobaseinternational.com/hgmd/pro/gene). We then confirmed all variants by checking each variant against the gnomAD browser (https://gnomad.broadinstitute.org) as a global control database and the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp) and the Japanese Multi Omics Reference Panel (https://jmorp.megabank.tohoku.ac.jp/ijgvd/) as Japanese databases to assess whether they were normal variants. We
also checked variants against our in-house database. A series of in silico analyses were executed to predict the pathogenicity of variants using POLYPHEN2 (http://genetics.bwh.harvard.edu/pph2, cut-off >0.9), SIFT (http://sift.jcvi.org, cut-off <0.05), PROVEAN (http://provean.jcvi.org/index.php, cut-off <-2.5), Mutation Taster (http://mutationtaster.org, scores ranging between 0 and 215, variant suspected of pathogenicity is classified as “disease causing” and variant suspected of less pathogenicity is classified as “polymorphism”). We then used Sanger sequencing to validate the suspected variants, and segregation analysis was conducted where possible. Variants were classified according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines published in 2015.15

The types and frequency of reported MPZ variants in our study were compared with previous studies. We also referred to the reports of MPZ variants in Human Genome Mutation Database (HGMD, https://portal.biobaseinternational.com/hgmd/pro/gene). Reports without information regarding the number of patients and related MPZ variants were excluded. Patients with MPZ variants described in the referenced data were aggregated. To analyze the worldwide mutational distribution of MPZ variants, we checked the previous reports described in HGMD. Further, we studied the types of MPZ variants and number of patients. Data of MPZ variants reported from the same country were also compiled. According to the origin of patients with MPZ variants or the country from which the MPZ report originated, we classified patients with MPZ variants into five regions (Africa, America, Asia, Europe, and Oceania). Our data were classified and aggregated into Asian data. Referenced data in HGMD are (Africa, America, Asia, Europe, and Oceania). Our data were classified originated, we classified patients with MPZ variants published in 2015,8 the age of onset of MPZ disease causing variants in our study

| 2.5 | Clinical assessment and statistical analysis |

Clinical findings and laboratory data of all patients with MPZ variants were based on their currently available information. As the large cohort study of MPZ variants published in 2015,8 the age of onset of patients aged <6, 6–20 and >20 years was classified as infantile, child and adult onset, respectively.

Patients with median MCV of <38 m/s were classified as demyelinating CMT, and those with median MCV ≥38 m/s were classified as axonal CMT. Patients with deficient electrophysiological findings were designated as unclassified. Serum CK and CSF protein levels were evaluated via blood and CSF tests. We defined elevated CK as serum CK levels >250 IU/L and elevated CSF proteins as CSF protein levels >50 mg/dL. The relationships among age of onset, variant type, CMT type (demyelinating/axonal CMT), CK levels, and CSF protein levels were evaluated. We also evaluated relationship between CSF protein levels and spine MRI findings. Fisher’s exact test was used to compare the proportion of patients with adult onset in the elevated and normal CK groups. Proportion of axonal CMT in elevated and normal CK group was also compared using Fisher’s exact test. The difference between the proportion of patients with demyelinating and axonal CMT in the elevated and normal CSF protein groups was also evaluated using Fisher’s exact test. We considered p-value of <0.05 as statistical significance. Statistical analysis was performed using R (version 3.6.1 [2019-07-05] Copyright 2019). The study protocol was reviewed and approved by the Institutional Review Board of Kagoshima University. Figure S1 shows the schematic diagram of this study.

3 | RESULTS

3.1 | Analysis of variants

In 1657 Japanese patients with suspected inherited peripheral neuropathy, we identified 23 known and 17 novel MPZ variants in 85 unrelated patients. We confirmed 23 previously reported variants in MPZ gene from 64 patients with inherited peripheral neuropathy from different families. The inheritance pattern of the cases was autosomal dominant or sporadic, with 29 (45.3%) patients considered as sporadic cases. The common MPZ variants found in our case series were p.Arg98His, p.Thr124Met, p.Asp75Val, p.Arg98Cys, p.Asn35Tyr, and p.Ser78Leu. CNV in the MPZ gene have been reported as the cause of inherited peripheral neuropathy,16,17 however, none of the patient were confirmed with CNV in MPZ gene in the present study.

Next, we analyzed the differences in worldwide variant distribution. MPZ variants observed in more than three regions were considered to be variants distributed worldwide.

Major MPZ variants reported in patients from countries other than Japan were p.Ser78Leu, p.His39Pro, p.Ser44Phe, p.Arg98His, p.Thr124Met, p.Asp134Glu, p.Ser63del, p.Arg98Cys, and p.Tyr82His. Patients with p.His39Pro, p.Ser44Phe, p.Ser63del, p.Tyr82His, and p.Asp134Glu were not detected in our study (Figure 1). We confirmed that 22 variants (p.Arg36Trp, p.Ser44Phe, p.Leu63del, p.Ser63Phe, p.Thr65Ala, p.Ser78Leu, p.Tyr82Cys, p.Arg98Cys, p.Arg98His, p.Gln103Glu, p.Asp104Tyrfs*14, p.Ile114Thr, p.Asp128Asn, p.Lys130Arg, p.Ile135Thr, p.Gly137Ser, p.Ser140Thr, p.Gly163Arg, p.Gly167Arg, p.Gln215*, and p.Arg227Ser) were distributed worldwide, whereas six variants (p.Ser44Phe, p.Ser63Phe, p.Thr65Ala, p.Ile135Thr, p.Ser140Thr, and p.Arg227Ser) were not detected in Japan. Moreover, 13 variants (p.Val32Phe, p.Leu48Val, p.Leu62Phe, p.Phe64del, p.Asp75Val, p.Gly93Glu, p.Lys96Glu, p.Asp118_Tyr119insPheTyr, p.Asn131Ser, p.Val146Phe, p.Leu170Arg, p.Ala189Glyfs*47, and p.Arg227Gly) were detected only in Japan (Table 1, Table S1).

3.2 | Analyses of novel MPZ variants

We found 17 novel MPZ variants from 21 patients and assessed each variant based on ACMG/AMG guidelines. The pedigree trees of 21 patients with 17 novel variants are described in Figure S2. Ten of these (p.Phe19Ser, p.Phe19Ser/p.Asp75Val, p.Ser54Tyr, p.Asp75Gly, p.His81Asp, p.Trp101Arg, p.Ser111Tyr, p.Leu112Val, p.Asn122Asp, and p.Val142Asp) fulfilled three categories of moderate pathogenic
evidence. One variant (p.Glu37Lys) fulfilled two categories of moderate pathogenic evidence and two supportive pathogenic evidences. Thus, 11 variants were classified as likely pathogenic and considered as novel pathogenic variants. The six remaining variants (p.Ala5Glyfs*52, p.Val31Leu, c.234 + 1G > A, p.Val102Gly, p.Tyr119dup, and c.646-3C > G) were classified as uncertain significance or likely benign (Table 2). The mutation sites associated with novel missense variants of likely pathogenic were preserved among mammals and located in mutational hot spots (Figure S3). p.Phe19Ser, p.Glu37Lys, p.AsplGly, p.Ile112Val, p.Asn122Asp, and p.Val142Asp were associated with adult onset, whereas p.Ser54Tyr, p.His81Asp, p.Tyr101Arg, and p.Ser111Tyr were associated with child and infantile onset, respectively. p.His81Asp, p.Ser111Tyr, and p.Val142Asp were classified as demyelinating CMT, whereas p.Phe19Ser, p.Glu37Lys, p.AsplGly, and p.Ile112Val were classified as axonal CMT (Table 3). We compared the clinical features of 17 novel variants with the reported variants that occurred near or at the same codon. The clinical features of p.Ala5Glyfs*52 and p.Tyr119dup were compared with the reported small insertions. c.234 + 1G > A and c.646-3C > G were compared with variants occurring at intron. We observed that some
| Variants     | Amino acid change | Europe | America | America, Europe and Oceania | Africa | Asia | Japan |
|--------------|-------------------|--------|---------|-----------------------------|--------|------|-------|
| c.94G > T    | p.Val32Phe        | –      | –       | –                           | –      | –    | 1     |
| c.103G > T   | p.Asp35Tyr        | –      | –       | 10 (Australia)              | –      | –    | 3     |
| c.103G > A   | p.Asp35Asn        | 5 (Norway) | –      | –                           | –      | –    | –     |
| c.106A > T   | p.Arg36Trp        | 1 (UK) | 2 (USA) | 1 (America, Europe, and Oceania) | –      | –    | –     |
| c.116A > C   | p.His39Pro        | –      | 15 (USA) | 21 (America, Europe, and Oceania) | –      | –    | –     |
| c.131C > T   | p.Ser44Phe        | 26 (Italy) | 1 (USA) | 4 (America, Europe, and Oceania) | –      | –    | –     |
| c.143 T > A  | p.Leu48Gln        | 10 (Czech) | –      | –                           | –      | –    | –     |
| c.143 T > C  | p.Leu48Pro        | 5 (Hungary) | –      | –                           | –      | –    | –     |
| c.142C > G   | p.Leu48Val        | –      | –       | –                           | –      | –    | 2     |
| c.178G > C   | p.Asp60His        | 9 (Austria) | –      | –                           | –      | –    | –     |
| c.184A > T   | p.Ile62Phe        | –      | –       | –                           | –      | –    | 4     |
| c.188_190delCCT | p.Ser63del   | 14 (Netherlands 13, Belgium 1) | –      | 4 (America, Europe, and Oceania) | –      | –    | 1     |
| c.188C > T   | p.Ser63Phe        | 5 (France) | –       | 2 (America, Europe, and Oceania) | 1 (Algeria) | 1 (Taiwan) | –     |
| c.190_192delTTC | p.Phe64del     | –      | –       | –                           | –      | –    | 4     |
| c.193A > G   | p.Thr65Ala        | 1 (Poland) | 1 (USA) | 3 (America, Europe, and Oceania) | –      | –    | –     |
| c.224A > T   | p.Asp75Val        | –      | –       | –                           | –      | –    | 10    |
| c.233C > T   | p.Ser78Leu        | 30 (Belgium 3, Italy 6, France 4, Finland 5, Serbia 6, Spain 1, Switzerland 1, UK 4) | 2 (USA) | 7 (America, Europe, and Oceania) | –      | –    | 3     |
| c.233C > G   | p.Ser78Trp        | –      | –       | –                           | 1 (Nigeria) | –      | –     |
| c.242A > G   | p.His81Arg        | 10 (UK) | –       | –                           | –      | –    | –     |
| c.243C > G   | p.His81Gln        | –      | –       | –                           | 7 (Korea) | –      | –     |
| c.245A > G   | p.Tyr82Cys        | 1 (Finland) | 3 (USA) | 2 (America, Europe, and Oceania) | –      | –    | 5     |
| c.244 T > C  | p.Tyr82His        | 13 (Netherlands) | –      | –                           | –      | –    | –     |
| c.270C > A   | p.Asp90Glu        | 4 (Cyprus 3, Spain 1) | –      | –                           | –      | –    | 8     |
| c.278G > A   | p.Gly93Glu        | –      | –       | –                           | –      | –    | 3     |
| c.286A > C   | p.Lys96Glu        | –      | –       | –                           | –      | –    | 12    |
| c.292C > T   | p.Arg98Cys        | 6 (Belgium 2, Austria 1, France 1, Italy 1, Spain 1) | 4 (USA) | 4 (America, Europe, and Oceania) | –      | 1 (Taiwan) | 4     |
| c.293G > A   | p.Arg98His        | 10 (France 3, Switzerland 2, Belgium 1, Italy 1, Russia 1, European countries 2) | 1 (USA) | 15 (America, Europe, and Oceania) | 2 (Algeria) | 1 (China) | 31    |
| c.293G > C   | p.Arg98Pro        | 7 (France) | –      | –                           | –      | –    | –     |
| c.296T > C   | p.Ile99Thr        | 10 (UK) | –      | 2 (America, Europe, and Oceania) | –      | –    | –     |
| c.308G > A   | p.Gly103Glu       | 3 (UK) | –      | 2 (America, Europe, and Oceania) | –      | –    | 1     |

(Continues)
| Variants | Amino acid change | Europe | America | America, Europe and Oceania | Africa | Asia | Japan |
|----------|-------------------|--------|---------|-----------------------------|--------|------|-------|
| c.306delA  | p.Asp104Thrfs*14 | 1 (Italy) | 6 (USA) | 2 (America, Europe, and Oceania) | - | - | - |
| c.341 T > C | p.Ile114Thr | 1 (USA) | - | 2 (America, Europe, and Oceania) | - | - | 2 |
| c.355-356insTCTACT | p.Asp119Thr119InsPheTyr | - | - | - | - | 1 |
| c.367A > G | p.Tyr119Cys | 5 (Germany 3, European countries 2) | - | 6 (America, Europe, and Oceania) | - | - | - |
| c.361G > A | p.Asp121Asn | - | - | - | 5 (China) | - | - |
| c.362A > C | p.Glu123Ser | - | - | 2 (America, Europe, and Oceania) | - | 9 (Taiwan) | - |
| c.371C > T | p.Asp118_Tyr119InsPheTyr | 1 (Italy) | 6 (USA) | 2 (America, Europe, and Oceania) | - | - | - |
| c.382G > A | p.Asp122Asn | 2 (UK) | 1 (USA) | - | - | 1 |
| c.389A > G | p.Lys130Arg | 2 (Belgium) | 1 (UK) | 2 (America, Europe, and Oceania) | - | 1 (China) | 7 |
| c.392A > G | p.Asn131Ser | - | - | - | - | - |
| c.400G > A | p.Asp132Asn | 6 (Belgium) | - | - | - | - |
| c.402C > A | p.Asp134Glu | 20 (Belgium 19, Russia 1) | - | 2 (America, Europe, and Oceania) | - | - | - |
| c.404T > C | p.Ile135Thr | 2 (UK 1, Russia 1) | 2 (USA) | 5 (America, Europe, and Oceania) | - | - | - |
| c.409G > A | p.Glu137Ser | 1 (UK) | 4 (USA) | 5 (America, Europe, and Oceania) | - | - | 2 |
| c.418T > A | p.Asp140Thr | 1 (Europe country) | 2 (USA) | 2 (America, Europe and Oceania) | - | - | - |
| c.434A > C | p.Asp145Ser | - | 6 (Costa Rica) | 3 (America, Europe, and Oceania) | - | - | - |
| c.436G > T | p.Val147Phe | - | - | - | - | 1 |
| c.487G > A | p.Glu163Arg | 1 (Belgium) | 1 (USA) | - | - | 1 |
| c.495G > A | p.Glu167Arg | 2 (UK) | 1 (USA) | 2 (America, Europe, and Oceania) | 1 (Kenya) | - | 1 |
| c.509T > G | p.Leu170Arg | - | - | - | - | 2 |
| c.560_563dupAGGC | p.Ala189Glyfs*47 | - | - | - | - | 1 |
| c.611A > T | p.Lys204Met | 7 (Spain) | - | - | - | - |
| c.643C > T | p.Gln215* | 2 (Italy) | 2 (USA) | 2 (America, Europe, and Oceania) | - | - | 1 |
| c.670G > T | p.Asp224Glu | 9 (Germany 4, Italy 4, Austria 1) | - | - | - | - |
| c.674dupA | p.His225Glnfs*10 | - | - | - | 5 (China) | - | - |
| c.679A > G | p.Arg227Gly | - | - | - | - | - |
| c.681A > T | p.Arg227Ser | 2 (Serbia) | 1 (USA) | 2 (America, Europe, and Oceania) | - | - | 1 |
| c.699_702delTGAG | p.Ser233Argfs*18 | 5 (Italy) | - | - | 1 (Taiwan) | - | - |
| c.706_708delGGAG | p.Lys236del | - | 2 (USA) | 6 (America, Europe, and Oceania) | - | - | - |
| Duplication118 kb inclentire gene(5 copies) | - | - | - | 6 (Taiwan) | - | - | - |
| Duplication4172 bp inclentire gene | - | 12 (Norway) | - | - | - | - | - |
| Patients with other variants | 192 | 62 | 45 | 0 | 15 | 17 |

Abbreviations: n, number of patients with inherited peripheral neuropathy having MPZ variants.
| ID    | Nucleotide change | Amino acid change  | Control database | Japanese and in-house database | In-silico analysis | Mutation taster | ACMG standard and guidelines | Pathogenicity | Benign impact | Criteria | Number of patients |
|-------|------------------|--------------------|------------------|-----------------------------|-------------------|----------------|-----------------------------|----------------|----------------|----------|-----------------|
| 7689  | c.13dup          | p.Ala5Glyfs*52     | –                | –                           | –                 | –              | Disease causing            | PM2, 4         |              | Uncertain significance | 1               |
| 7484  | c.56 T > C       | p.Phe19Ser         | –                | –                           | –                 | –              | Disease causing            | PS4-moderate, PM1, 2, PP3 | BP4            | Likely pathogenic (iv) | 1               |
| 7344  | c.56 T > C       | p.Phe19Ser         | –                | –                           | –                 | –              | Disease causing            | PS4-moderate, PM1, 2, PP3 | BP4            | Likely pathogenic (iv) | 1               |
| 391  | c.224A > T       | p.Asp75Val         | Reported variant  | (Misu K et al. 2000)       | –                 | –              | Disease causing            | PS4-moderate, PM1 | BS1, BP4       | Likely benign (i) | 3               |
| 5527  | c.109G > A       | p.Glu37Lys         | –                | –                           | –                 | –              | Disease causing            | PM1, 2, PP1, 3 |              | Likely pathogenic (v) | 1               |
| 8160  | c.161C > A       | p.Ser54Tyr         | –                | –                           | –                 | –              | Disease causing            | PM1, 2, 5, 6, PP3 |              | Likely pathogenic (iv) | 1               |
| 6181  | c.224A > G       | p.Asp75Gly         | –                | –                           | –                 | –              | Disease causing            | PM1, 2, 5, PP1, 3 | BP4            | Likely pathogenic (iv) | 1               |
| 6621  | c.234 + 1 G > A  | p.Asp75Val         | –                | –                           | –                 | –              | Disease causing            | PM2, PP1, 4    |              | Uncertain significance | 1               |
| 6132  | c.241 C > G      | p.His81Asp         | –                | –                           | –                 | –              | Disease causing            | PS4-moderate, PM1, 2, 5, PP3 | Likely pathogenic (iv) | 2               |
| 4407  | c.301 T > C      | p.Trp101Arg        | –                | –                           | –                 | –              | Disease causing            | PM1, 2, 5, PP1, 3 | 4              | Likely pathogenic (iv) | 1               |
| 6125  | c.305 T > G      | p.Val102Gly        | –                | –                           | –                 | –              | Disease causing            | PM1, 2, 5, 6, PP3 |              | Likely pathogenic (iv) | 1               |
| 7389  | c.332C > A       | p.Ser111Tyr        | –                | –                           | –                 | –              | Disease causing            | PM1, 2, 5, 6, PP3 |              | Likely pathogenic (iv) | 1               |
| 5231  | c.334A > G       | p.Ile112Val        | –                | –                           | –                 | –              | Disease causing            | PM1, 2, 5, PP3 | BP4            | Likely pathogenic (iv) | 1               |
| 5038  | c.355_6insACT    | p.Tyr119dup        | –                | –                           | –                 | –              | Polymorphism               | PM2, 4, PP1    |              | Uncertain significance | 1               |
| 4347  | c.364A > G       | p.Asn122Asp        | –                | –                           | –                 | –              | Disease causing            | PS4-moderate, PM1, 2, 5, PP3 | Likely pathogenic (iv) | 2               |
| 7163  | c.425 T > A      | p.Val142Asp        | –                | –                           | –                 | –              | Disease causing            | PM1, 2, 5, PP3 |              | Likely pathogenic (iv) | 1               |
| 7393  | c.646–3 C > G   | 2/250518           | –                | –                           | –                 | –              | PP1, 4                      |                  |              | Uncertain significance | 1               |

Note: Likely Pathogenic (iv), Variants fulfill three categories of moderate pathogenic evidence; Likely Pathogenic (v), Variants fulfill two categories of moderate pathogenic evidence and two supportive pathogenic evidences; Likely Benign (i), Variants fulfill one category of strong benign evidence and one category of supporting benign evidence.
| MPZ variants | Age | Onset | Inheritance pattern | Median MCV (m/s) | Demyelinating/axonal CMT | Clinical and laboratory findings | Reference |
|-------------|-----|-------|---------------------|-----------------|-------------------------|-------------------------------|-----------|
| p.Phe19Ser  | 56  | 51    | Sp                  | 40.5            | A                       | Elevated CSF protein (52 mg/dl) | This report (ID 7484) |
| p.Phe19Ser  | 77  | 30    | AD                  | 31.1            | D                       | Moderate weakness of lower limbs | This report (ID 7344) |
| p.Asp75Val  |     |       |                     |                 |                         |                               |           |
| p.Ser20Phe  | 64  | 59    | AD                  | Normal          | A                       | Weakness of lower limbs         | Finsterer J, et al Eur J Neurol. 2006; 13: 1149–1152 |
| p.Ser20Phe  | 77  | 30    | AD                  |                 | D                       | Weakness of lower limbs         | Milley GM, et al Neuromuscul disord 2018; 28:38–43 |
| p.Val31Leu  | 16  | 14    | AD                  | 20              | D                       | Mild weakness of all limbs      | This report (ID 3918) |
| p.Val31Leu  | 73  | 63    | Sp                  | 20.8            | D                       | Mild weakness of all limbs      | This report (ID 7420) |
| p.Val31Leu  | 58  | 12    | –                   | 29.5            | D                       | Mild weakness of all limbs      | This report (ID 8166) |
| p.Ile30Phe  | –   | 2     | AD                  | –               | U                       | Delayed motor milestones        | Niermeijer JMF, et al Neuromuscul Disort. 2011; 26: 688 |
| p.Ile30Met  | 39  | <3    | AD                  | 25              | D                       | Weakness of upper and lower limb muscles Atrophy of all limbs Absent deep tendon reflexes Sensory disturbance Romberg's sign positive | Hayasaka K, et al Hum Mol Genet 1993; 2: 1369 |
| p.Ile30Met  | –   | early onset | AD               | –               | U                       | Distal weakness of the limbs    |           |
| p.Ile30Met  | –   | early onset | AD               | –               | U                       | Distal weakness of the limbs    |           |
| p.Ile30Ser  | 9   | 2     | AD                  | 4               | D                       | Weakness of all limbs Sensory disturbance Pes cavus | Miltenberger-Miltenyi G, et al Eur J Hum Genet 2009; 17: 1154–1159 |
| p.Ile30Thr  | 55  | 3     | AD                  | –               | U                       | Severe weakness and atrophy of all limbs Romberg’s sign positive Claw hands. Pes cavus. Hammer toes | Floroskufi P, et al Muscle Nerve 2007; 35: 667–669 |
| p.Ile30Thr  | –   | 0     | AD                  | –               | U                       | Delayed motor milestonesSevere weakness and atrophy Impaired deep sense. Steppage gait | Yoshihara T, et al Hum Mutat 2000; 16: 177–178 |
| p.Val32Phe  | 26  | –     | –                   | 15.6            | D                       | –                             |           |
| p.Glu37Lys  | 59  | 39    | AD                  | 55.9            | A                       | Severe weakness of lower limbs  | This report (ID 5527) |
| p.Arg36Gly  | 77  | 73    | AD                  | 50              | A                       | Weakness of lower limbs         | Dacci P, et al J Peripher Nerv Syst 2012; 17: 422–425 |
| p.Arg36Gly  | 47  | –     | AD                  | –               | U                       | Sensory disturbance             |           |
| p.Arg36Trp  | 47  | 44    | AD                  | 45.6            | A                       | Impaired superficial and deep sense in feet Pes planus Elevated CSF protein (60 mg/dl) | Burs TM, et al Neuromuscul Disord 2006; 16: 308–310 |
| p.Ser54Tyr  | 2   | 0     | Sp                  | –               | U                       | Mild weakness of upper limbs Elevated CSF protein | This report (ID 8160) |
| MPZ variants | Age | Onset | Inheritance pattern | Median MCV (m/s) | Demyelinating/axonal CMT | Clinical and laboratory findings | Reference |
|--------------|-----|-------|---------------------|-----------------|--------------------------|---------------------------------|-----------|
| p.Ser54Cys   | -   | -     | -                   | -               | D                        | Classified as CMT1              | Hoyer H, et al Biomed Res Int 2014: 13 |
| p.Ser54Pro   | 26  | -     | Sp                  | 22              | D                        | Distal weakness Impaired vibratory sensation in lower limbs Pes cavus. Hammer toes | Baissar-Tadmouri N, et al Hum Mutat 1999; 14: 199 |
| p.Asp75Gly   | 59  | 57    | Sp                  | 47              | A                        | Mild weakness of lower limbs Elevated CK | This report (ID 6181) |
| p.Asp75Val   | 74  | 60    | -                   | 52              | A                        | Moderate weakness of upper limbs Severe weakness of lower limbs Sensory disturbance | Misu K, et al J Neurol Neurosurg Psychiatry 2000; 69: 806–811 |
|              | 66  | 61    | -                   | 44              | A                        | Severe weakness of lower limbs Sensory disturbance. Elevated CK |           |
|              | 64  | 61    | -                   | 50              | A                        | Mild weakness of lower limbs Sensory disturbance |           |
| p.His81Asp   | 14  | 0     | AD                  | 11.3            | D                        | Moderate weakness of lower limbs Elevated CSF protein | This report (ID 6132) |
| p.His81Asp   | 12  | 0     | AD                  | 9.1             | D                        | Mild weakness of lower limbs | This report (ID 7199) |
| p.His81Arg   | -   | -     | AD                  | -               | U                        | Severe muscle atrophy. Ataxia Adie's pupil. Tremor | Sorour E, et al Hum Mutat 1997; 9: 74–77 |
| p.His81Arg   | 28  | -     | AD                  | 11.1            | D                        | Walking difficulties. Sensory ataxia Foot deformities | Jonathan Beats et al Brain 2011; 134: 2665–2676 |
| p.His81Gln   | 44  | 8     | AD                  | N.D             | U                        | Severe muscle atrophy. Ataxia Adie's pupil | Choi BO et al Int J Mol Med 2011; 28: 389–396 |
|              | 40  | 9     | AD                  | 12.2            | D                        | Moderate muscle atrophy Ataxia. Adie's pupil |           |
|              | 12  | 5     | AD                  | 9.1             | D                        | Mild muscle atrophy Ataxia. Adie's pupil |           |
|              | 8   | 2     | AD                  | 8.2             | D                        | Moderate muscle atrophy Ataxia. Adie's pupil |           |
|              | 3   | 2     | AD                  | 6.2             | D                        | - |           |
|              | 13  | 5     | AD                  | 11.9            | D                        | Moderate muscle atrophy Ataxia. Adie's pupil |           |
|              | 5   | 4     | AD                  | 9.2             | D                        | Mild muscle atrophy. Ataxia |           |
| p.His81Leu   | 56  | 48    | AD                  | 42              | A                        | Weakness and atrophy of all limbs Sensory disturbance Gait disturbance. Pes cavus | Liu L, et al J Peripher Nerv Syst 2013; 18: 256–260 |
|              | 63  | 52    | AD                  | 40              | A                        | Weakness and atrophy of all limbs Sensory disturbance Gait disturbance. Pes cavus |           |
| p.Trp101Arg  | 22  | 6     | AD                  | -               | U                        | Severe weakness of all limbs Elevated CSF protein | This report (ID 4407) |

(Continues)
| MPZ variants | Age | Onset | Inheritance pattern | Median MCV (m/s) | Demyelinating/axonal CMT | Clinical and laboratory findings | Reference |
|--------------|-----|-------|---------------------|----------------|--------------------------|---------------------------------|-----------|
| p.Trp101Cys  | 32  | early onset | AD | 10 | D | Atrophy of the distal leg muscles | Latour P, et al Hum Mutat 1995; 6: 50–54 |
| p.Trp101Cys  | 7   | –     | AD | – | U | Walking difficulties. Pes cavus | |
| p.Val102Gly  | 67  | 59    | AD | 40 | A | Mild weakness of lower limbs | This report (ID 6125) |
| p.Gly103 Ala | 20  | –     | AD | – | U | – | Sun Y, et al. Sci Rep 2018; 8: 1–9 |
| p.Gly103Arg  | –   | –     | AD | – | U | – | Dohrn MF, et al Journal of Neurochemistry 2017; 143: 507–522 |
| p.Gly103Trp  | 20  | Infancy | AD | – | U | Delayed motor milestones | Brožková D et al Clin Genet 2010; 78: 81–87 |
| p.Gly103Glu  | 14  | 0     | AD | 7 | D | Delayed motor milestones | Fabrizi GM, et al Neurology 2001; 57: 101–105 |
|              | 10  | 1     | AD | slowing NCV | U | Weakness of all limbs | |
| p.Ser111 Tyr | 23  | 0     | Sp | 3.8 | D | – | This report (ID 7389) |
| p.Ser111 Cys | 32  | <10   | AD | 14.6 | D | Severe weakness in distal lower limbs | Mandich P, et al Eur J Hum Genet 2009; 17: 1129–1134 |
| p.Ser111 Phe | 4   | 0     | – | 2.9 | D | Hypotonia. Weak crying | Sevilla T, et al J Peripher Nerv Syst 2011; 16: 347–352 |
| p.Ser111 Pro | –   | Infancy | – | – | U | – | Sanmaneechai O, et al Brain 2015; 138: 3180–3192 |
| p.Ile112 Val | 78  | 58    | Sp | 51.3 | A | Severe weakness of all limbs | This report (ID 5231) |
| p.Ile112 Thr | –   | –     | AD | – | U | Severe weakness of all limbs | Sorour E et al Hum Mutat 1998; 51: 5242-5247 |
| p.Asn122 Asp | 68  | 43    | AD | 31 | D | Elevated CK (297 U/L) and CSF protein (108 mg/dL) | This report (ID 4347) |
| p.Asn122 Asp | 47  | 42    | AD | 39 | A | – | This report (ID 5037) |
| p.Asn122 Ser | 53  | 44    | Sp | 32 | D | Severe scoliosis | Blanquet-Grossard F, et al Hum Mutat 1996; 8: 185–186 |
| MPZ variants          | Age | Onset | Inheritance pattern | Median MCV (m/s) | Demyelinating/axonal CMT | Clinical and laboratory findings                                                                 | Reference                                                                 |
|-----------------------|-----|-------|---------------------|-----------------|--------------------------|----------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| p.Val142Asp           | 53  | 43    | AD                  | 16              | D                        | Severe weakness of all limbs                                                                                 | This report (ID 7163)                                                      |
| p.Val142Phe           | –   | –     | –                   | –               | U                        | –                                                                                                            | Sanmaneechai O, et al Brain 2015; 138: 3180–3192                             |
| c.234 + 1 G > A       | 51  | 36    | AD                  | 29.5            | D                        | Severe weakness of all limbs                                                                                 | This report (ID 6621)                                                      |
| c.646-3C > G          | 44  | 36    | AD                  | 31.1            | D                        | –                                                                                                            | This report (ID 7393)                                                      |
| c.235-2A > C          | –   | –     | –                   | –               | U                        | –                                                                                                            | DiVincenzo C, et al Mol Genet Genomic Med. 2014; 2(6): 522–529              |
| c.449-9C > T          | 46  | 26    | –                   | –               | U                        | Distal weakness Sensory disturbance                                                                         | Kecharevic-Markovic M, et al J Peripher Nerv Syst 2009; 14: 125–136        |
| c.449-1G > A          | 50  | 50    | –                   | 22              | D                        | Weakness of distal legs Impaired deep sense Romberg’s sign                                                 | Lancaster E, et al Muscle Nerve 2010; 41: 555–558                          |
| c.449-1G > C          | –   | –     | –                   | –               | U                        | –                                                                                                            | Bort S, et al Hum Genet 1997; 99: 746–754                                  |
| c.449-1G > C          | 52  | 42    | –                   | –               | U                        | Sensory disturbance Elevated CSF protein (70 mg/dl)                                                        | Campagnolo M, et al J Peripher Nerv Syst 2020; 25: 19–26                   |
| c.449-1G > T          | –   | –     | –                   | –               | U                        | –                                                                                                            | Choi BO, et al Hum Mutat 2004; 24: 185–186                                  |
| c.448 + 1G > A        | –   | –     | –                   | –               | U                        | –                                                                                                            | DiVincenzo C, et al Mol Genet Genomic Med. 2014; 2(6): 522–529              |
| c.448 + 2T > G        | –   | –     | –                   | –               | U                        | –                                                                                                            | DiVincenzo C, et al Mol Genet Genomic Med. 2014; 2(6): 522–529              |
| c.584 + 2T > G        | 55  | 20s   | AD                  | –               | U                        | Mild weakness of all limbs Sensory disturbance, Hammer toes                                               | Sabet A, et al Neurology 2006; 67: 1141–1146                               |
|                       | 33  | 29    | AD                  | –               | U                        | Mild weakness of distal arms Sensory disturbance                                                           |                                                                           |
|                       | 23  | 0     | AD                  | –               | U                        | Mild weakness of distal lower limbs Sensory disturbance, Pes cavus                                        |                                                                           |
|                       | 36  | –     | –                   | –               | U                        | –                                                                                                            |                                                                           |
| c.645 + 1G > T        | –   | 42    | –                   | –               | U                        | Atrophy of hands Sensory disturbance, Pes cavus                                                           | Kleffner L, et al J Neurol 2010; 257: 1864–1868                            |
| p.Ala5Glyfs*52        | 63  | 48    | AD                  | 25.5            | D                        | Severe weakness of upper limbs Elevated CK (447 U/L) and CSF protein (116 mg/dl)                           | This report (ID 7689)                                                      |
| p.Tyr119dup           | 49  | 6     | AD                  | 38.7            | A                        | Elevated CSF protein (199 mg/dl)                                                                           | This report (ID 5038)                                                      |
| c.10 _6dupTGCCC       | 39  | 30    | AD                  | 54              | A                        | Mild phenotype                                                                                             | Sivera R, et al Neurology 2013; 81: 1617–1625                               |
| p.Tyr88*              | –   | –     | –                   | –               | U                        | –                                                                                                            | DiVincenzo C, et al Mol Genet Genomic Med. 2014; 2(6): 522–529              |
| p.Asp118_Tyr119insPheTyr| 0   | 0     | –                   | –               | U                        | Clinically severe Diaphragmatic weakness Died at the age of 10 months                                       | Ikekami T, et al Hum Mutat 1998; 51: 5103–105                               |

(Continues)
| MPZ variants          | Age | Onset | Inheritance pattern | Median MCV (m/s) | Demyelinating/axonal CMT | Clinical and laboratory findings | Reference                      |
|----------------------|-----|-------|---------------------|-----------------|---------------------------|--------------------------------|--------------------------------|
| p.Tyr145Leufs*4      | 41  | 40    | Sp                  | 38              | A                         | Weakness                        | Mandich P, et al             |
|                      |     |       |                     |                 |                           | Muscle wasting in lower legs    | Eur J Hum Genet 2009; 17: 1129–1134 |
|                      |     |       |                     |                 |                           | Pes cavus                       |                                |
| p.Pro151Alafs*3      | 34  | 27    | AD                  | 36.8            | D                         | Pes cavus                       | Piazza S, et al               |
|                      |     |       |                     |                 |                           |                                | Neuromuscul Disord 2010; 20: 817–819 |
|                      |     |       |                     |                 |                           |                                |                                |
| p.Leu184Alafs*51     | 0   | 0     | AD                  | 4               | D                         | Hypotonia                       | Smit LS, et al                |
|                      |     |       |                     |                 |                           | Arthrogrypesis of hands and feet | Neuromuscular disorder 2008; 18: 56–62 |
|                      |     |       |                     |                 |                           |                                |                                |
| p.Ala189Glyfs*47     | 8   | 0     | Sp                  | –               | U                         | Delayed motor milestones        | Tachi N, et al                |
|                      |     |       |                     |                 |                           | Gait disturbance                | J Neurol Sci 1998; 156: 167–171 |
|                      |     |       |                     |                 |                           |                                |                                |
| p.Met197Tyrfs*38     | 35  | 0     | Sp                  | –               | U                         | Delayed motor milestones        | Zschuntzsch J, et al          |
|                      |     |       |                     |                 |                           | Weakness of lower limbs          | J Neurol Sci 2009; 281: 113–115 |
|                      |     |       |                     |                 |                           | Sensory disturbance              |                                |
| p.Lys207Asnfs*51     | –   | –     | –                   | –               | U                         | –                              | Bort S, et al                 |
|                      |     |       |                     |                 |                           |                                | Hum Genet 1997; 99: 746–754     |
|                      |     |       |                     |                 |                           |                                |                                |
| –                    | 26  | –     | AD                  | 36.9            | D                         | Sensory disturbance              | Crehalet H, et al             |
|                      |     |       |                     |                 |                           |                                | Neurogenetics 2010; 11: 13–19   |
| p.Thr216Asnfs*19     | –   | –     | –                   | –               | U                         | –                              | D’Vincenzo C, et al           |
|                      |     |       |                     |                 |                           |                                | Mol Genet Genomic Med. 2014; 2(6): 522–529 |
| p.His225Glnfs*10     | –   | 25    | AD                  | –               | U                         | Weakness and atrophy of distal limbs | He J, et al                   |
|                      |     |       |                     |                 |                           | Sensory disturbance              | J Peripher Nerv Syst.2018; 23: 216–226 |
|                      |     |       |                     |                 |                           | Pes cavus                       |                                |

Abbreviations: A, axonal CMT; AD, autosomal dominant; CK, creatine kinase; CSF, cerebrospinal fluid; D, demyelinating CMT; −, Not available or not evoked; NCV, nerve conduction velocity; Sp, Sporadic; U, unclassified type.
variants had similar phenotype as the reported variants, which are occurred near or at the same codon. Patients with p.Glu37Lys, p.Arg36Gly, or p.Arg36Trp had adult onset and they were classified as axonal CMT. Patients with p.Asp75Gly or p.Asp75Val also had the similar phenotype. Although, patients with p.Asn122Asp or p.Asn122Ser were associated with adult onset, the electrophysiological classification varied in each patient. Meanwhile, patients with p.Ser111Tyr, p.Ser111Cys, p.Ser111Pro had infantile or child onset (Table 3).

A patient had compound heterozygous variant (p.Asp75Val/p.Phe19Ser) with one reported variant (p.Asp75Val) and one novel variant (p.Phe19Ser). This patient had numbness and muscle weakness in legs since his 30s. The brother and nephew of the patient also had difficulty in walking. The nerve conduction velocity of the right median nerve was 31.1 m/sec, which indicated demyelinating CMT. The age of onset in our case series was earlier than in patients with p.Asp75Val in our case series (30 years old vs the average age of 48 years). The daughter and nephew of the patient had one variant (p.Asp75Val). The nephew had weakness, atrophy of all limbs, hypo-reflexia of tendon reflexes, pes cavus, and walked with a cane support. The patient's daughter did not have weakness, sensory impairment and decreased tendon reflexes, and denied to undergo electrophysiological examination. Presently, the age of the daughter is 50 years old and she does not have symptoms associated with neuropathy. The nephew's age of onset (56 years) was older than that of the patient (56 years old vs 30 years old; Figure S4).

### 3.3 Clinical and laboratory findings

We assessed 77 patients with inherited peripheral neuropathy comprising 64 with reported MPZ variants and 13 with novel pathogenic MPZ variants (Table S2). The onset age of these patients indicated a bimodal distribution (Figure 2(A)). Prominent clustering in the first decade and slight clustering between the third and fifth decade were evident, in line with large genetic profiles of Japanese CMT patients.\(^9\)

Cranial nerve involvement was confirmed in 20 patients (Table S3). Dysarthria was detected in seven patients, while dysphagia was confirmed in four patients. Hearing loss was also detected in four patients (Table 4). The most common MPZ variant in patients...
presenting with cranial nerve involvement was p.Arg98His. Furthermore, patients with p.Arg98Cys, p.Asp35Tyr, p.Leu48Val, p.Asp61Asn, p.Asp75Val, p.Phe19Ser/p.Asp75Val, p.Arg98His (2), p.Asp128Asn showed symptoms associated with cranial nerve dysfunction. The clinical information of patient with p.Thr124Met has been described elsewhere.18

We analyzed serum CK levels in 30 patients. Of them, eight (26.7%) showed elevated CK levels, with the levels being <1000 U/L in most cases. Most of the patients with elevated CK levels had neuropathic symptoms in their middle age (Figure 2(B)). The proportion of patients with adult onset was greater in the elevated CK group than in the normal CK group (p = 0.039) (Figure 2(C)). However, patients with elevated CK were not statistically associated with axonal CMT (p = 0.57) (Table S4).

We analyzed CSF protein levels in 30 patients, 18 (60%) of whom had elevated levels. Among patients with elevated CSF protein levels, 11 (61.1%) patients were classified as demyelinating and 2 (11.1%) were classified as axonal CMT. There was no significant difference between the proportion of patients with demyelinating and axonal CMT in the elevated and normal CSF protein groups (p = 0.039) (Figure 2(C)). However, patients with elevated CK were not statistically associated with axonal CMT (p = 0.57) (Table S4).

We analyzed CSF protein levels in 30 patients. Among patients with elevated CSF protein levels, 11 (61.1%) patients were classified as demyelinating and 2 (11.1%) were classified as axonal CMT. There was no significant difference between the proportion of patients with demyelinating and axonal CMT in the elevated and normal CSF protein groups (p = 0.039) (Figure 2(C)). However, patients with elevated CK were not statistically associated with axonal CMT (p = 0.57) (Table S4).

We investigated 85 patients with inherited peripheral neuropathy associated with MPZ variants in Japan. In this study, we focused on the distribution of MPZ variants in the world to compare Japanese patients with known MPZ variants included in our case series. Interestingly, there were differences in the types of MPZ variants between Japan and other countries. In the present study, we confirmed 13 variants, which have been reported only in Japan. However, one of the 13 variants (p.Leu48Val) was reported from Russia.19 Therefore, patients with 12 variants were considered to be concentrated in Japan. Three of the 12 variants (p.Asp75Val, p.Gly93Glu, and p.Leu170Arg) were also detected in our study. Patients with p.Asp75Val were frequently observed and described in a study of axonal CMT in Japan.20,21 p.Gly93Glu was detected in a Japanese CMT1B family with low-nerve conduction velocities.22 p.Leu170Arg was described in a large study analyzing 161 CMT patients for PMP22, GJB1, and MPZ.20 Although not explored in the present study, there may be various factors including founder effect and/or difference of ethnicity.

4 | DISCUSSION

We investigated 85 patients with inherited peripheral neuropathy associated with MPZ variants in Japan. In this study, we focused on the distribution of MPZ variants in the world to compare Japanese patients with known MPZ variants included in our case series. Interestingly, there were differences in the types of MPZ variants between Japan and other countries. In the present study, we confirmed 13 variants, which have been reported only in Japan. However, one of the 13 variants (p.Leu48Val) was reported from Russia.19 Therefore, patients with 12 variants were considered to be concentrated in Japan. Three of the 12 variants (p.Asp75Val, p.Gly93Glu, and p.Leu170Arg) were also detected in our study. Patients with p.Asp75Val were frequently observed and described in a study of axonal CMT in Japan.20,21 p.Gly93Glu was detected in a Japanese CMT1B family with low-nerve conduction velocities.22 p.Leu170Arg was described in a large study analyzing 161 CMT patients for PMP22, GJB1, and MPZ.20 Although not explored in the present study, there may be various factors including founder effect and/or difference of ethnicity.

Herein, we detected 11 confirmed novel variants that are likely to induce a pathogenic phenotype. Remarkably, p.Glu37Lys, p.Asp75Gly, and p.Ser111Tyr were associated with a similar phenotype as the reported variants that occurred at and near the same codon. Therefore, the confirmed novel variants likely induced a pathogenic phenotype, especially in these missense variants. Furthermore, in one of the

| Cranial nerve involvement | Variants | Number of patients |
|---------------------------|----------|--------------------|
| Dysarthria                | p.Leu48Val, p.Asp75Val, p.Phe19Ser/p.Asp75Val, p.Arg98Cys, p.Arg98His (2), p.Asp128Asn | 7 |
| Dysphagia                 | p.Asp75Val, p.Arg98His (2), p.Asp128Asn | 4 |
| Hearing loss              | p.Leu48Val, p.Gly103Glu, p.Lys130Arg, p.Leu170Arg | 4 |
| Anisocoria                | p.Leu48Val, p.Phe19Ser/p.Asp75Val, p.Thr124Met | 3 |
| Weakness of facial muscle | p.Asp61Asn, p.Arg98His (2) | 3 |
| Deviation of tongue protrusion | p.Arg98His (2), p.Leu170Arg | 3 |
| Nystagmus                 | p.Phe19Ser/p.Asp75Val, p.Arg98His | 2 |
| Strabismus                | p.Lys130Arg, p.Leu170Arg | 2 |
| Atrophy of facial muscle  | p.Asp35Tyr, p.Arg98His | 2 |
| Atrophy of tongue         | p.Asp128Asn, p.Leu170Arg | 2 |
| Adie's pupil              | p.Thr124Met | 1 |
| Ptosis                    | p.Ile112Val | 1 |
| Sluggish light reflex     | p.Leu48Val | 1 |
| Trigeminal neuralgia      | p.Ile114Thr | 1 |
| Facial nerve paralysis    | p.Leu170Arg | 1 |
| Tinnitus                  | p.Arg98His | 1 |
| Atrophy of trapezius and sternocleidomastoid | p.Asp61Asn | 1 |
| Involuntary movement of tongue | p.Arg98Cys | 1 |
| Tongue fasciculation      | p.Asp128Asn | 1 |

Abbreviations: p.Arg98His (2), Two patients with MPZ p.Arg98His variant.
patients with a novel pathogenic variant, a compound heterozygous variant of p.Asp75Val and p.Phe19Ser was confirmed. The patients with p.Asp75Val are often classified as axonal CMT with late onset of neuropathic symptoms.21 Compound heterozygous variants have been previously observed in some genes associated with autosomal dominant CMT (PMP22, MFN2, GDAP1, etc.) and have contributed to unusual phenotype.23,24 The cumulative effect of two mild variants was hypothesized in a CMT family with simultaneous MFN2 and GDAP1 variants.21 To the best of our knowledge, there have been a few compound heterozygous variants of MPZ.25-27 Regarding the compound heterozygous variant in our study, this patient was classified as demyelinating CMT. In addition, he had younger age of onset than those with p.Asp75Val and showed demyelinating neuropathy on nerve conduction studies. The clinical findings of compound heterozygous variant of p.Asp75Val and p.Phe19Ser seem to be different from those of p.Asp75Val. Thus, the patient with p.Asp75Val and p.Phe19Ser had atypical phenotype compared to patient with p.Asp75Val. However, the pedigree tree for this compound heterozygous variant indicated the possibility that p.Asp75Val and p.Phe19Ser was located in each allele. Thus, the pathogenicity of p.Phe19Ser in this patient was unclear and p.Phe19Ser may not contribute to atypical phenotype of the patient. Accumulation of the clinical information about the same variant in more patients and functional studies to prove the pathogenicity of p.Phe19Ser must be performed. Variants of two genes related to the protein that are synergetic in the same pathway can cause overlapping disease phenotype, which may contribute to the atypical phenotype of mendelian disorder.28 Thus, the factor such as other genes related to the protein interacting MPZ protein should be considered if the pathogenicity of p.Phe19Ser is denied.

Cranial nerve involvement is rarely seen in CMT.29 However, hearing loss should be carefully discussed considering the involvement of the cranial nerve in MPZ variants. The frequency of hearing loss in 66 CMT patients with MPZ variants was reported as 3.33%, suggesting that the frequency of hearing loss is the same as that in the normal population. Thus, hearing loss may not be associated with MPZ variants.30 However, in addition to hearing loss, papillary abnormalities, trigeminal neuralgia, hemifacial spasm have been observed in CMT patients with MPZ variants.31-33 Further, papillary abnormalities such as Adie’s pupil have been described in association with MPZ variants and autonomic nervous dysfunction.34-36 Moreover, the number of patients with cranial nerve involvement except hearing loss detected in the present study was 19. Eleven patients had more than two symptoms related to cranial nerve involvement. These findings suggest that various cranial nerve involvement can be observed in some CMT patients with MPZ variants. Therefore, these cranial nerve symptoms may provide clues for examining the MPZ variants.

In the present study, we also focused on serum CK and CSF protein levels. Serum CK level elevation has also been detected among demyelinating and axonal CMT patients with MPZ variants.21,37,38 In our case series, patients with elevated CK levels were more likely to have adult onset than those with normal CK levels. This result is in line with previous studies.21 Samanechai et al. have shown that degeneration of myelinated axons causes peripheral neuropathy in adult onset (aged >20 years) patients.8 Moreover, it has been suggested that impaired muscle membrane integrity caused by denervation deriving from impaired axons is involved in CK level elevation.37 Therefore, degeneration of myelinated axons and associated denervation may have contributed to CK level elevation.

Elevated CSF protein levels have previously been described in CMT with MPZ variants.21,38-40 Various factors have been considered for elevated CSF protein levels. Half of patients with elevated CSF protein levels had spinal diseases such as spinal canal stenosis and cervical spondylitis. It is unclear whether these spinal diseases are associated with MPZ variants. However, these spinal diseases can interrupt CSF flow and increase CSF protein levels.41 In the present study, not only patients with demyelinating CMT but also those with axonal CMT had elevated CSF protein levels. In our case series, one axonal CMT patient with an elevated CSF protein had slight enlargement of cauda equina. This finding was described in Italian patient with MPZ p.Gly167Arg variant.42 It has been reported that the leakage of blood protein caused by the partial impairment of CSF circulation and blood-nerve barrier injury at enlarged nerve root site can contribute to elevated CSF protein levels.43-45 Thus, enlargement of cauda equina may be associated with CSF circulation and CSF protein elevation in this case. In contrast, conditions such as spinal diseases, enlarged nerve root or cauda equina were not observed in other axonal CMT patient with an elevated CSF protein. An elevated CSF protein level has been reported in axonal CMT patients with MPZ p.Thr124Met variants.21 Thus, some MPZ variants may be associated with an elevated CSF protein level even in axonal CMT patients. Axonal CMT patients with an elevated CSF protein in this study had novel variants (p.Phe19Ser and p.Ile112Val). These MPZ variants may be associated with elevated CSF protein, while the pathogenicity of these variants and association with elevated CSF protein should be analyzed.

There are several points to consider in this study. First, we analyzed the patients with novel variants and assessed their pathogenicity in accordance with the ACMG guidelines. Although the exact pathogenicity of novel variants should be assessed by functional studies, we were unable to perform functional studies for novel MPZ variants. Also, we were unable to perform the clinical assessment for severity such as CMT neuropathy score. Further, we were able to analyze serum CK, CSF protein levels and MRI findings only in limited patients. Due to the design of this study, these data were insufficient in this study. These points will be addressed in future studies.

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CONFLICT OF INTEREST

The authors declare no financial or other conflicts of interest.

ETHICAL STATEMENT

The study protocol was reviewed and approved by the Institutional Review Board of Kagoshima University. All patients and family members provided written informed consent to participate in the study.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.13881.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article and its supplementary information files.

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REFERENCES

1. Corrado L, Margi S, Bagarotti A, et al. A novel synonymous mutation in the MPZ gene causing an aberrant splicing pattern and Charcot-Marie-Tooth disease type 1b. *Neuromuscul Disord*. 2016;26:516-520.
2. Nelse H, Haites N, Van Broeckhoven C. Mutations in the peripheral myelin genes and associated genes in inherited peripheral neuropathies. *Hum Mutat*. 1999;13:11-28.
3. Mandich P, Fossa P, Capponi S, et al. Clinical features and molecular modelling of novel MPZ mutations in demyelinating and axonal neuropathies. *Eur J Hum Genet*. 2009;17:1129-1134.
4. Lemke G, Axel R. Isolation and sequence of a cDNA encoding the major structural protein of peripheral myelin. *Cell*. 1985;40:501-508.
5. Harding AE, Thomas PK. The clinical feature of hereditary motor and sensory neuropathy types I and II. *Brain*. 1980;103:259-280.
6. Shy ME, Jani A, Krajewski K, et al. Phenotype clustering in MPZ mutations. *Brain*. 2004;127:371-384.
7. Mastaglia FL, Nowak KJ, Stell R, et al. Novel mutation in the myelin protein zero gene in a family with intermediate hereditary motor and sensory neuropathy, *J Neurol Neurosurg Psychiatry*. 1999;67:174-179.
8. Samaninichedi O, Feely S, Scherer SS, et al. Genotype–phenotype characteristics and baseline natural history of heritable neuropathies caused by mutations in the MPZ gene. *Brain*. 2015;138:3180-3192.
9. Yoshimura A, Yuan JH, Hashiguchi A, et al. Genetic profile and onset features of 1005 patients with Charcot-Marie-Tooth disease in Japan. *J Neurol Neurosurg Psychiatry*. 2019;90:195-202.
10. Hashiguchi A, Higuchi Y, Nomura M, et al. Neurofilament light mutation causes hereditary motor and sensory neuropathy with pyramidal sings. *J Peripher Nerv Syst*. 2014;19:311-316.
11. Yoshimura A, Yuan JH, Hashiguchi A, et al. Clinical and mutational spectrum of Japanese patients with Charcot-Marie-Tooth disease caused by GDAP1 variants. *Clin Genet*. 2017;92:274-280.
12. Hashiguchi Y, Hashiguchi A, Yuan J, et al. Mutations in MME cause an autosomal-recessive Charcot-Marie-Tooth disease type 2. *Ann Neurol*. 2016;79:659-672.
13. Maeda K, Idehara R, Hashiguchi A, Takashima H. A family with distal hereditary motor neuropathy and a K141Q mutation of small heat shock protein HSPB1. *Intern Med*. 2014;53:1655-1658.
14. Derouault P, Chauzel J, Rizzo D, et al. CovCopCan: an efficient tool to detect copy number variation from amplicon sequencing data in inherited diseases and cancer. *PLoS Comput Biol*. 2020;16:e1007503.
15. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405-424.
16. Maeda MH, Mitsui J, Soong BW, et al. Increased gene dosage of myelin protein zero causes Charcot-Marie-Tooth disease. *Ann Neurol*. 2012;71:84-92.
17. Hoyer H, Braathen GJ, Eek AK, Skjeibred CF, Russell MB. Charcot-Marie-Tooth caused by a copy number variation in myelin protein zero. *Eur J Med Genet*. 2011;54:e580-e583.
18. Nakamura N, Kawanuma N, Tateishi T, Doi H, Ohyagi Y, Kira JI. Predominant parasympathetic involvement in a patient with Charcot-Marie-Tooth disease caused by the MPZ Thr124Met mutation. *Rinsho Shinkeigaku*. 2009;49:582-585.
19. Milovidova TB, Dadadi EL, Fedotov VP, Shchagina OA, Poliakov AV. Clinical-genetic correlations in the hereditary motor-sensor neuropathy caused by mutations in the MPZ (P0) gene. *Zh Nevrol Psihiatr Im S S Korshakova*. 2011;111:48-55.
20. Numakura C, Changqiang L, Ikemati T, Guldberg P, Hayasaka K, et al. Molecular analysis in Japanese patients with Charcot-Marie-Tooth disease: DGGE analysis for PMP22, MPZ, and Cx32/GJB1 mutations. *Hum Mutat*. 2002;20:392-398.
21. Misu K, Yoshihara T, Shikama Y, et al. An axonal form of Charcot-Marie-Tooth disease showing distinctive features in association with mutations in the peripheral myelin protein zero gene (Thr124Met or Asp75Val). *J Neurol Neurosurg Psychiatry*. 2000;69:806-811.
22. Ikemati T, Ikeda H, Mitsui T, Hayasaka K, Ishii S. Novel mutation of the myelin P0 gene in a pedigree with Charcot-Marie-Tooth disease type 1B. *Am J Med Genet*. 1997;71:246-248.
23. Gouvea SP, Borghetti VHS, Bueno KC, et al. Compound Charcot-Marie-Tooth disease may determine unusual and milder phenotypes. *Neurogenetics*. 2010;11:135-138.
24. Vital A, Latour P, Sole G, et al. A French family with Charcot-Marie-Tooth disease related to simultaneous heterozygous MNF2 and GDAP1 mutations. *Neuromuscul Disord*. 2012;22:735-741.
25. Bienfait HM, Baas F, Gabreels-Festen AA, Koelman JH, Langerhorst CT, de Visser M. Two amino-acid substitutions in the myelin protein zero gene of a case of Charcot-Marie-Tooth disease associated with light-near dissociation. *Neuromuscul Disord*. 2002;12:281-285.
26. Drac H, Kabačinska D, Moszyńska I, Strugalska-Cynowska H, Hausmanowa-Petrusewicz I, Kochański A. Dysmyelinating and demyelinating Charcot-Marie-Tooth disease associated with two myelin protein zero gene mutations. *J Appl Genet*. 2011;52:177-183.
27. Plante-Bordeneuve V, Parman Y, Guichon-Mantel A, et al. The range of chronic demyelinating neuropathy of infancy: a clinico-pathological and genetic study of 15 unrelated cases. *J Neurol*. 2001;248:795-803.
28. Posey JE, Harel T, Liu P, et al. Resolutions of disease phenotypes resulting from multilocus genomic variation. *N Engl J Med*. 2017;376:21-31.

29. Pareyson D, Scialom V, Laura M. Clinical and electrophysiological aspects of Charcot-Marie-Tooth disease. *Neuromolecular Med*. 2006;8:3-22.

30. Lerat J, Magdelaine C, Roux AF, et al. Hearing loss in inherited peripheral neuropathies: molecular diagnosis by NGS in a French series. *Mol Genet Genomic Med*. 2019;7:e839.

31. Kurihara S, Adachi Y, Wada K, Adachi A, Ohama E, Nakashima K. Axonal and demyelinating forms of the MPZ Thr124Met mutation. *Acta Neurol Scand*. 2003;108:157-160.

32. Seeman P, Mazanec R, Huehne K, Suslikova P, Kellar O, Rautenstrauss B. Hearing loss as the first feature of late-onset axonal CMT disease due to a novel P0 mutation. *Neurology*. 2004;63:733-735.

33. Caress JB, Lewis JA, Pinyan CW, Lawson VH. A Charcot-Marie-Tooth type 1B kindred associated with hemifacial spasm and trigeminal neuralgia. *Muscle Nerve*. 2019;60:62-66.

34. Baloh RH, Jen HC, Kim G, Baloh RW. Chronic cough due to Thr124Met mutation in the peripheral myelin protein zero (MPZ gene). *Neurology*. 2004;62:1905-1906.

35. Stojkovic T, de Seze J, Dubourg O, et al. Autonomic and respiratory dysfunction in Charcot-Marie-Tooth disease due to Thr124Met mutation in the myelin protein zero gene. *Clin Neurophysiol*. 2003;114:1609-1614.

36. Tokuda N, Noto Y, Kitani-Morii F, et al. Parasympathetic dominant autonomic dysfunction in Charcot-Marie-Tooth disease type 2J with the MPZ Thr124Met mutation. *Intern Med*. 2015;54:1919-1922.

37. Luigetti M, Modoni A, Rema R, et al. A case of CMT 1B due to Val 102/fs null mutation of the MPZ gene presenting as hyperCKemia. *Clin Neuropathol*. 2010;112:794-797.

38. Hattori N, Yamamoto M, Yoshit часа T, et al. Demyelinating and axonal features of Charcot-Marie-Tooth disease with mutations of myelin-related proteins (PMP22, MPZ and Cx32): a clinicopathological study of 205 Japanese patients. *Brain*. 2003;126:134-151.

39. Ohnishi A, Aoki A, Yamamoto T, Tsuji S. A case of Charcot-Marie-Tooth disease 1 B with Val 146Phe mutation of myelin protein zero showing a severe clinical phenotype. *Rinsho Shinkeigaku*. 2000;30:268-270.

40. Donaghy M, Misodiya SM, Kennett R, McDonald B, Hautes N, Bell C. Steroid responsive polynuropathy in a family with a novel myelin protein zero mutation. *J Neurol Neurosurg Psychiatry*. 2000;69:799-805.

41. Seyfert S, Kunzmann V, Schwertfeger N, Koch HC, Faulstich A. Determinants of lumbar CSF protein concentration. *J Neurol*. 2002;249:1021-1026.

42. Simonati A, Frabrizi GM, Taioli F, Polo A, Cerini R, Rizzuto N, Dejerine-Sottas neuropathy with multiple nerve roots enlargement and hypomyelination associated with a missense mutation of the transmembrane domain of MPZ/P0. *J Neurol*. 2002;249:1298-1302.

43. Ishigami N, Kondo M, Nakagawa M. A case of Charcot-Marie-Tooth disease type 1A with increased cerebrospinal fluid proteins and nerve root hypertrophy. *Rinsho Shinkeigaku*. 2008;48:419-421.

44. Pareyson D, Testa D, Morbin M, et al. Does CMT1A homozygosity cause more severe disease with root hypertrophy and higher CSF proteins? *Neurology*. 2003;60:1721-1722.

45. Neuen E, Seitz RJ, Langenbach M, Wechsler W. The leakage of serum proteins across the blood-nerve barrier in hereditary and inflammatory neuropathy. *Acta Neuropathol*. 1987;73:53-61.

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