Genetic Profile and Onset Features of 1005 Patients with Charcot-Marie-Tooth disease in Japan

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ABSTRACT

Objective To identify the genetic characteristics in a large-scale of patients with Charcot-Marie-Tooth disease (CMT) patients.

Methods From May 2012 to August 2016, we collected 1005 cases with suspected CMT throughout Japan, whereas *PMP22* duplication/deletion were excluded in advance for demyelinating CMT cases. We performed next generation sequencing targeting CMT-related gene panels using Illumina MiSeq or Ion Proton, then analyzed the gene-specific onset age of the identified cases and geographical differences in terms of their genetic spectrum.

Results From 40 genes, we identified pathogenic or likely pathogenic variants in 301 cases (30.0%). The most common causative genes were *GJB1* (n = 66, 21.9%), *MFN2* (n = 66, 21.9%), and *MPZ* (n = 51, 16.9%). In demyelinating CMT, variants were detected in 45.7% cases, and the most common reasons were *GJB1* (40.3%), *MPZ* (27.1%), *PMP22* point mutations (6.2%), and *NEFL* (4.7%). Axonal CMT yielded a relatively lower detection rate (22.9%), and the leading causes, occupying 72.4%, were *MFN2* (37.2%), *MPZ* (9.0%), *HSPB1* (8.3%), *GJB1* (7.7%), *GDAP1* (5.1%), and *MME* (5.1%). First decade of life was found as the most common disease onset period, and

early-onset CMT cases were most likely to receive a molecular diagnosis. Geographical distribution analysis indicated distinctive genetic spectrums in different regions of Japan.

Conclusions Our results updated the genetic profile within a large-scale of Japanese CMT cases. Subsequent analyses regarding onset age and geographical distribution advanced our understanding of CMT, which would be beneficial for clinicians.

INTRODUCTION

Charcot–Marie–Tooth disease (CMT) is the most common phenotype of inherited peripheral neuropathy (IPN), the latter of which also encompass hereditary sensory and autonomic neuropathy, hereditary neuropathy with liability to pressure palsy and hereditary motor neuropathy (HMN). [1] In terms of median motor nerve conduction velocity (MNCV), CMT can be further classified into demyelinating CMT (MNCV < 38 m/s) and axonal CMT (MNCV ≥ 38 m/s).

CMT is typically characterized by progressive motor and sensory polyneuropathy, but it may also present with significant clinical heterogeneity. CMT

disease-causing genes, such as *GARS* (CMT2D and distal HMN5A), *HSPB1* (CMT2F and distal HMN2B), or *IGHMBP2* (CMT2S and spinal muscular atrophy with respiratory distress type 1), often produce other IPN phenotypes. [2 3 4] To date, approximately 100 different genes have been linked to CMT-like phenotypes (https://neuromuscular.wustl.edu/). Owing to its clinical complexity and genetic diversity, the clinical subtyping of CMT is always laborious and difficult.

The development of next-generation sequencing (NGS) technology allows us to conduct gene panel sequencing simultaneously the targeting of numerous genes. Within approximately 4 years, using two NGS systems successively, we have completed genetic assessment in more than 1,000 Japanese cases with suspected CMT, which enables us to describe the genetic and clinical features of these cases.

MATERIALS AND METHODS

From May 2012 to August 2016, we collected blood or DNA samples from 1005 apparently unrelated patients throughout Japan with suspected CMT. These cases were examined by their local neurologists or pediatricians, and were referred to our genetic laboratory for diagnostic genetic test. Duplication/deletion mutation of *PMP22* was

pre-excluded in all cases suspected with demyelinating CMT, using fluorescence *in situ* hybridisation or multiplex ligation-dependent probe amplification.

On the basis of their family history, the included cases were grouped into sporadic (n = 570, 56.7%), autosomal dominant (AD) or X-linked (n = 341, 33.9%), autosomal recessive (AR; n = 72, 7.2%), or with an unknown inheritance pattern (no clinical data, n = 22). All cases were further categorized as demyelinating CMT (n = 282), axonal CMT (n = 682) or unclassified type with no MNCV data or MNCV = 0 (n = 41) referring to their records of electrophysiological examination.

Genomic DNA was extracted from peripheral blood using a Gentra Puregene Blood kit (QIAGEN, Valencia, California, USA), according to the manufacturer's instructions. The protocol was reviewed and approved by the Institutional Review Board of Kagoshima University. All cases and their family members provided written informed consent to participate in this study.

Targeted Gene panel sequencing

Primers were designed to cover the coding regions and exon/intron junctions of genes in our CMT panel. Beginning in May 2012, we conducted mutation screening targeting 60

genes (Supplementary table 1) with the Illumina MiSeq platform (Illumina, San Diego, California, USA). We used the same methodology as the one employed in a previous study. [5] We completed genetic analysis in 437 cases with this system, concluding in July 2014.

In September 2014, a custom Ion AmpliSeq gene panel targeting 72 IPNs disease-causing or candidate genes (Supplementary table 1) was designed and introduced. This panel consisted of 1,800 amplicons divided into 2 primer pools. Library and template preparation was performed according to the manufacturer's instructions, and then run on the Ion Proton (Thermo Fisher Scientific, Waltham, Massachusetts, USA) applying the Ion PI Chip kit v2/v3 BC (Thermo Fisher Scientific, Carlsbad, California, USA). We used the same methodology as the one employed in a previous study. [6] Using this platform, we executed genetic assessment in 568 cases until August 2016.

Data analysis and variant interpretation

We confirmed all previously reported pathogenic mutations with reference to the Human Gene Mutation Database Professional 2017.3 (https://portal.biobase-international.com/hgmd/pro). Moreover, we checked all variants against global databases, including the 1000 Genomes (http://www.internationalgenome.org), the Exome Sequencing Project (http://evs.gs.washington.edu/EVS) and the Exome Aggregation Consortium (http://exac.broadinstitute.org/), as well as against Japanese databases, including the integrative Japanese Genome Variation Database (https://ijgvd.megabank.tohoku.ac.jp) and the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp). We also checked the variants against our in-house whole-exome sequencing database of individuals with non-IPNs. In silico analyses of variants were performed using SIFT (http://sift.jcvi.org), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2), PROVEAN (http://provean.jcvi.org/index.php), Mutation Assessor (http://mutationassessor.org) and Condel (http://bg.upf.edu/fannsdb). We completed the annotation process using the CLC Genomic Workbench software and an in-house R script. All suspected variants were validated using Sanger sequencing and interpreted according to the American College of Medical Genetics and Genomics standards and guidelines.[7]

RESULTS

Genetic Profile

Among the 1005 cases with suspected CMT, we detected pathogenic or likely pathogenic variants in 301 cases (30.0%). The most common genetic causes in the mutation-positive cases were *GJB1* and *MFN2*, and each accounted for 21.9% (66 cases). Within *MFN2*, 40 types of reported and 3 novel variants (two pathogenic and 1 likely pathogenic) were identified. The following genetic causes were *MPZ* (n = 51, 16.9%), *HSPB1* (n = 14, 4.6%), *PMP22* point mutations (n = 13, 4.3%), *GDAP1* (n = 9, 3.0%), *NEFL* (n = 9, 3.0%), *MME* (n = 8, 2.7%), *BSCL2* (n = 6, 2.0%), *MARS* (n = 6, 2.0%), *DNM2* (n = 5, 1.7%), *SETX* (n = 5, 1.7%), *SH3TC2* (n = 5, 1.7%), *PRX* (n = 4), *GARS* (n = 3), *IGHMBP2* (n = 3), *LRSAM1* (n = 3), *AARS* (n = 2), *ARHGEF10* (n = 2), *FGD4* (n = 2), *SACS* (n = 2), *SBF2* (n = 2), *TRPV4* (n = 2), and *TTR* (n = 2). Pathogenic or likely pathogenic variants were also detected in *COA7*, *DCTN1*, *DHTKD1*, *EGR2*, *FBLN5*, *GALC*, *GAN*, *HARS*, *HSPB3*, *HSPB8*, *INF2*, *KARS*, *MTMR2*, *PRPS1*, *RAB7A* and *SOX10* in single cases. (Figure 1) Additionally, digenic variants were identified in

five cases, which were variants in *SETX* (likely pathogenic) and *ARHGEF10* (likely pathogenic); *SH3TC2* (biallelic, likely pathogenic) and *SACS* (biallelic, likely pathogenic); *LRSAM1* (likely pathogenic) and *MARS* (likely pathogenic); *HARS* (likely pathogenic) and *ARHGEF10* (likely pathogenic); and *MFN2* (reported, pathogenic) and *PMP22* (reported pathogenic).

In terms of sporadic cases, detection rate was 21.9% (125/570), comprising 108 monoallelic and 18 biallelic variants. Molecular diagnosis was accomplished in 44.6% (152/341) cases with AD or X-linked inheritance and in only 25.0% (18/72) of cases with AR. In demyelinating CMT cases, 45.7% (129/282) received a genetic diagnosis, and mutations in *GJB1* (40.3%) and *MPZ* (27.1%) were the most common reasons, accounting for 67.4% of all mutation-positive cases. Among cases with axonal CMT, mutation detection rate was 22.9% (156/682), with *MFN2* as the most frequent causative gene, accounting for 37.2% of all mutation-positive cases, followed by *MPZ* (9.0%), *HSPB1* (8.3%), and *GJB1* (7.7%), *GDAP1* (5.1%) and *MME* (5.1%). (Figure 2)

Onset age analysis

We analyzed the onset age in the mutation-positive CMT cases and attempted to specify their onset features. The CMT onset age of all the patients was determined to be when either of their parents noticed any motor abnormalities in their children, or when the patients themselves began to be aware of their motor or sensory dysfunctions. In cases with demyelinating CMT, 104/282 (36.9%) cases developed clinical symptoms in the first decade of life, and pathogenic or likely pathogenic variants were identified in 57 of these cases (54.8%). In the same age group, 58 out of 190 cases (30.5%) with axonal CMT received a molecular diagnosis, which also yielded the highest diagnostic rate. Unexpectedly, cases with demyelinating CMT with onset in the seventh decade demonstrated the highest diagnostic rate of 66.7% (6/9). (Figure 3A, 3B)

The majority (48/58cases) of cases with *MFN2* variants were manifested with an early-onset (0~20 years) axonal polyneuropathy. Demyelinating neuropathy was predominant in cases with *GJB1* variants, with case numbers decreasing with age. A two-peak pattern was observed in cases with *MPZ* variants, consisting of a peak of demyelinating type with onset age in the first decade, and a second peak of axonal type in cases with disease onset during the fifth and six decades. The most common onset

age of cases with *HSPB1* variants was during the six decade. Most cases with *PMP22* point variants (7/13 cases) developed demyelinating neuropathy in the first decade, and the majority of cases with *NEFL* variants presented with a demyelinating phenotype at a younger age. (Figure 3C)

Regarding two genes linked to AR-CMT, *GDAP1* and *MME*, we noted that cases with biallelic variants of *GDAP1* developed clinical manifestations earlier than those with monoallelic variants; cases with biallelic variants in *MME* commonly presented with late-onset axonal neuropathies. (Figure 3D)

Geographic distribution analysis

We conducted a geographic distribution analysis on the basis of all available medical records, but without further validation of their familial place of origin. Here, Japan was separated into eight regions. Variants in *GJB1*, *MFN2* and *MPZ* were identified to be the top three causative genes in six regions of Japan. Therein, *MFN2* was found as the most common cause of CMT in northern (Hokkaido and Tohoku) and southern Japan (Chugoku and Kyushu/Okinawa), while *GJB1*-related CMT was more prevalent in

middle Japan (particularly in Kanto and Kinki). In Hokkaido region (A), *MFN2* variants accounted for more than half of all mutation-positive cases, and no case with *MPZ* variant was identified. In Shikoku (G), a characteristic high incidence of *NEFL* variants was observed. (Figure 4)

DISCUSSION

Using two targeted gene panel sequencing systems, we genetically analyzed 1005 cases with suspected CMT to demonstrate their genetic profile. To the best of our knowledge, this is the largest Asian study to date. The total diagnostic rate of our study was 30.0% (301 cases), and remarkable genetic heterogeneity was recognized that pathogenic or likely pathogenic variants were detected from 40 genes. We diagnosed 27.7% cases with the Illumina MiSeq targeting 60 genes, and 31.7% cases with the Ion Proton targeting 72 genes. Cases with demyelinating CMT received a much higher diagnostic rate (45.7% vs. 22.9%) and lower genetic diversity than axonal type.

To date, genetic spectrum studies of CMT have been completed in multiple countries (Table 1). The *PMP22* duplication/deletion mutations, accounting for 23.3%

of demyelinating CMT in Japan, [8] were not involved in this study and were removed from the original data of previous studies to facilitate comparison. Consequently, we found that three genes, *GJB1*, *MFN2*, and *MPZ*, were the leading reasons in the present study, using data from Germany, [9] USA, [10] UK, [11] Norway, [12] and Denmark [13] studies; however, these results differ from previous reports from Japan, [8] Spain, [14]Italy,[15] Korea[16] and a cross-country study.[17] Particularly, in the other Japanese study, [8] regarding other genes with mutation frequency higher than 1%, *PMP22* (3.3%), *NEFL* (2.7%) and *PRX* (1.7%) have been reported, whereas we detected *HSPB1* (1.4%) and *PMP22* (1.3%) in the present study. A sampling bias should be considered to have contributed to these differences.

In cases with monoallelic variants, *GJB1* (n = 66), *MFN2* (n = 65), and *MPZ* (n = 51) were the top three genes, accounting for 68.7% of all mutation-positive cases. Clinically, the majority of cases with *MFN2* variants (n = 58) showed axonal phenotype, making *MFN2* as the most common reason of axonal CMT. Twelve cases with *GJB1* variants exhibited axonal phenotype, whereas the other 52 cases exhibited the

demyelinating phenotype. Cases with *MPZ* variants also exhibited both axonal (n = 14) and demyelinating (n = 35) phenotypes.

In cases with biallelic variants, *MME* accounted for 22.2% (n = 8) of all mutation-positive cases, followed by *SH3TC2* (n = 5, 13.9%). In 2016 we have reported that *MME* gene, which encodes neprilysin, is responsible for a late-onset AR-CMT type 2T. [6] Shortly thereafter, monoallelic rare variants have been reported to be associated with axonal polyneuropathies or dominant spinocerebellar ataxia with neuropathy. [18 19] In the present study, monoallelic variants were detected in family members of four cases with AR-CMT2T, none of whom developed notable clinical manifestations. We also identified digenic variants from five cases (approximately 0.5%), the majority of which were likely pathogenic (8/10); further study is required to identify whether the combinatorial effect of these variants contributes to the phenotypic variability as disease burden.

The onset age distribution of our CMT cases suggested an evident clustering at the first decade, regardless of axonal or demyelinating phenotype. Our targeted gene panel sequencing yielded a significantly high diagnostic rate of axonal (30.5%) and

demyelinating (54.8%) CMT for individuals diagnosed within the first decade of life. This result is not surprising given that genetic factors are more common than acquired factors in cases with early-onset polyneuropathies. Early onset is also a typical feature of cases with *GJB1*, *MFN2*, *MPZ* (demyelinating type), *PMP22* (point variants), *NEFL*, and *GDAP1* (biallelic) variants. In contrast, *MPZ* (axonal type) and *HSPB1* variants always produce a late-onset phenotype. For patients with late-onset demyelinating CMT with an onset age between the fifth and sixth decade, a significant decline of diagnostic rate was observed, which might be due to noninherited factors or to undiscovered genetic causes. Interestingly, however, demyelinating CMT cases with onset at the seventh decade, yielded the highest diagnostic rate (6/9), which could be a coincidence owing to the mutations detected in various genes. Taken together, because the number of case of demyelinating CMT with onset age older than 40 years was limited, more samples should be collected for validating these unexpected results in the future.

We performed a geographic distribution analysis to elucidate the effect of geography on the genetic spectrum of our cases. *GJB1*, *MFN2*, and *MPZ* were the top three causative genes associated with CMT throughout the most regions in Japan.

Although our case numbers were limited, we observed an unexpectedly high frequency of *NEFL* variants in Shikoku region, which was indicated in a previous study reporting that *NEFL* variants accounted for a much higher proportion of CMT cases (2.7%) [8] Unfortunately, geographic distribution data were not available in the previous report. This unusually high frequency of *NEFL* variants was unlikely to be caused by a founder effect, because all these variants were completely different. High frequency of *MFN2* variants and the absence of the *MPZ* variant were identified in Hokkaido. Our findings suggest that geographic regions could give rise to the variable genetic spectrum and diagnostic rate of CMT, and future well-powered analyses will be helpful to clarify these findings.

Within our CMT-related gene panels, the role of several genes still require further validation. Therein, six variants were detected in *MARS*, consisting of a previously reported P800T mutation (4 cases) and two novel likely pathogenic variants. In two of the four pedigrees with P800T, cosegregation of genotype and phenotype was identified. For the two novel variants, further study is required to validate their pathogenicity. Besides, we also found two heterozygous variants, G585S and W426G in

GALC gene (NM_000153.3) from one patient. *GALC* was known as the responsible gene for Krabbe disease; however, peripheral neuropathy was the original and predominant symptom of our patient, which is comparable with a former Japanese patient with isolated peripheral neuropathy. [20]

Recently, a number of new causative genes, such as those encoding MORC family CW-type zinc finger 2 (*MORC2*), minichromosome maintenance complex component 3 associated protein, neurofilament protein heavy polypeptide, diacylglycerol O-acyltransferase 2, dystrophin-related protein 2, cytochrome c oxidase subunit VIa polypeptide 1 and peripheral myelin protein 2 (*PMP2*) were associated with CMT phenotypes. [21 22 23 24 25 26] These genes were not involved in any of our gene panels, but a high frequency (2.7%) of *MORC2* variants in axonal CMT was revealed by whole-exome sequencing in our laboratory. [27] These genes should be included in the upcoming version of our gene panel, and the diagnostic rate would be increased.

In conclusion, using targeted gene panel sequencing, we demonstrated the genetic features and geographical differences in a nationwide group of cases with CMT in Japan. Together with results of onset age analysis, our findings advanced the

understanding of this intractable disease. Our sequencing strategy was proved effective, exempting for complicated and undefined subtyping in clinic. A limitation of this study is that our library could not yield either the non-coding region or the structural variant of these genes.

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Competing interests None declared.

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Figure legends

Figure 1 Genetic spectrum of 301 cases with pathogenic or likely pathogenic variants. The following genes are indicated: *GJB1* (21.9%), *MFN2* (21.9%), *MPZ* (16.9%), *HSPB1* (4.6%), *PMP22* point mutation (4.3%), *GDAP1* (3.0%), *NEFL* (3.0%), *MME* (2.7%), *BSCL2* (2.0%), *MARS* (2.0%), *DNM2* (1.7%), *SETX* (1.7%), *SH3TC2* (1.7%), *PRX* (1.3%), *GARS* (1.0%), *IGHMBP2* (1.0%), *LRSAM1* (1.0%), *AARS* (0.7%), *ARHGEF10* (0.7%), *FGD4* (0.7%), *SACS* (0.7%), *SBF2* (0.7%), *TRPV4* (0.7%), *TTR* (0.7%), *COA7* (0.3%), *DCTN1* (0.3%), *DHTKD1* (0.3%), *EGR2* (0.3%), *FBLN5* (0.3%), *GALC* (0.3%), *GAN* (0.3%), *HARS* (0.3%), *HSPB3* (0.3%), *HSPB8* (0.3%), *INF2* (0.3%), *KARS* (0.3%), *MTMR2* (0.3%), *PRPS1* (0.3%), *RAB7A* (0.3%), and *SOX10* (0.3%).

Figure 2 Detection rate and proportional detection of variants in cases with demyelinating and axonal CMT. * *PMP22* point mutation.

Figure 3 Onset age analyses of mutation-positive cases. (A and B) Curve graph and column diagram of varied onset age and diagnostic rate of axonal or demyelinating CMT. (C) Diagram of disease onset features in cases with *GJB1*, *MFN2*, *MPZ*,

HSPB1, *PMP22*, and *NEFL* variants. (D) Diagram of disease onset features of cases with monoallelic or biallelic variants of *GDAP1* and *MME* genes. A, axonal type; D, demyelinating type; N, number; Y, year. * *PMP22* point mutation.

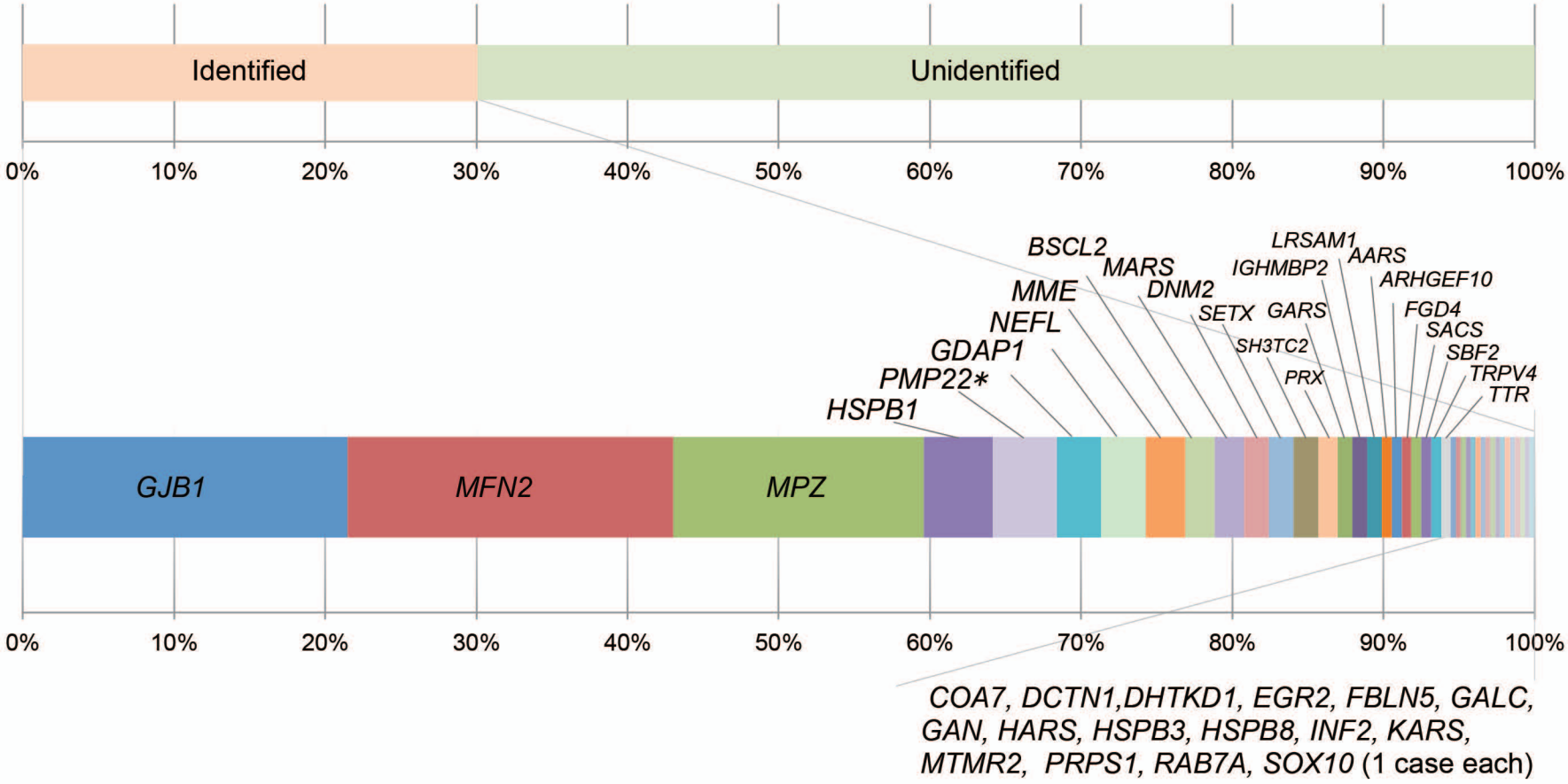
Figure 4 Geographic analysis of genetic spectrum of CMT in Japan. Japan is divided into eight regions (A–H), and axonal/demyelinating type and the causative genes are indicated in different colors. Mutation-positive and total numbers of each region are indicated around the pie chart. NE, not examined.

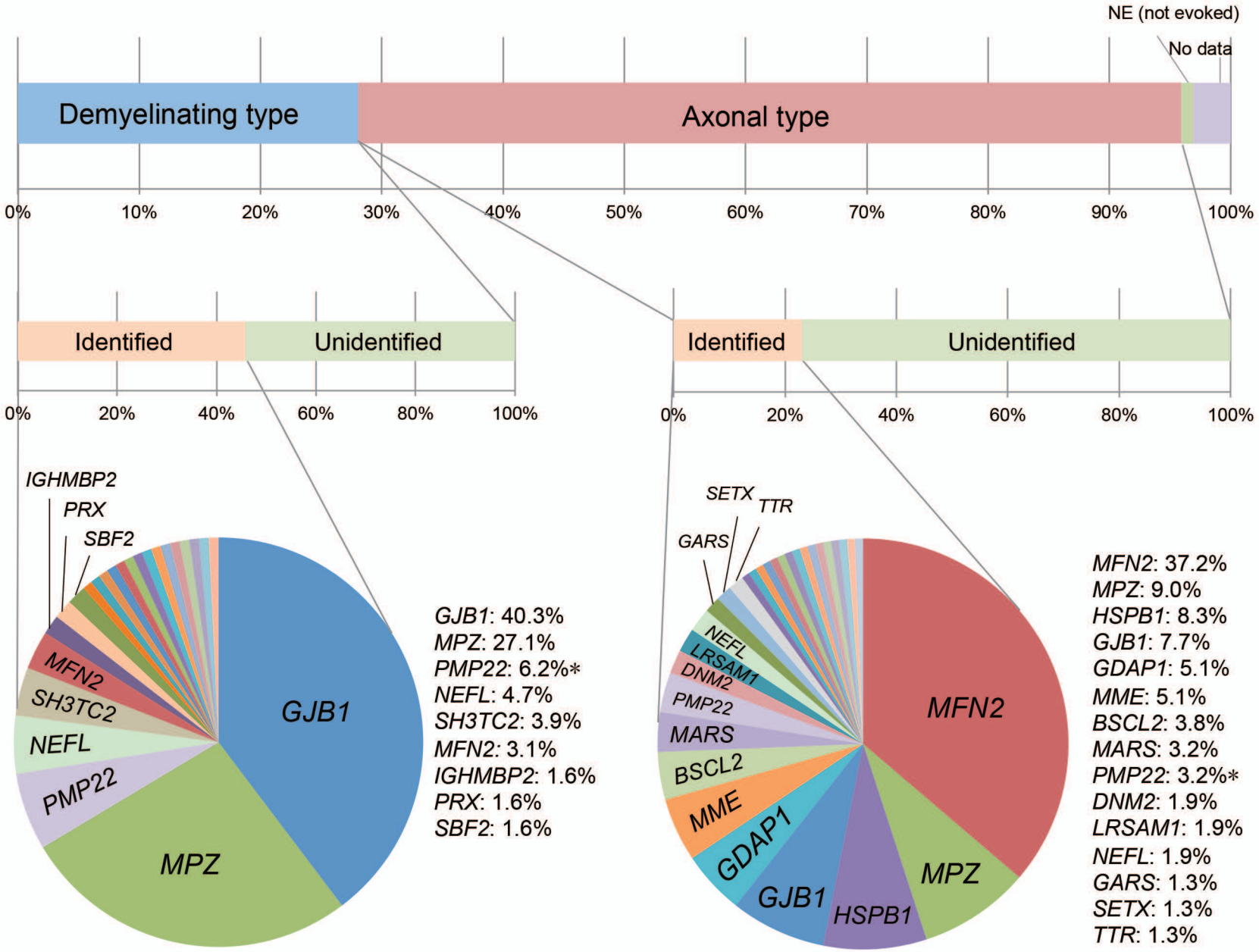
Table 1 Mutation detection rates of CMT patients in various populations

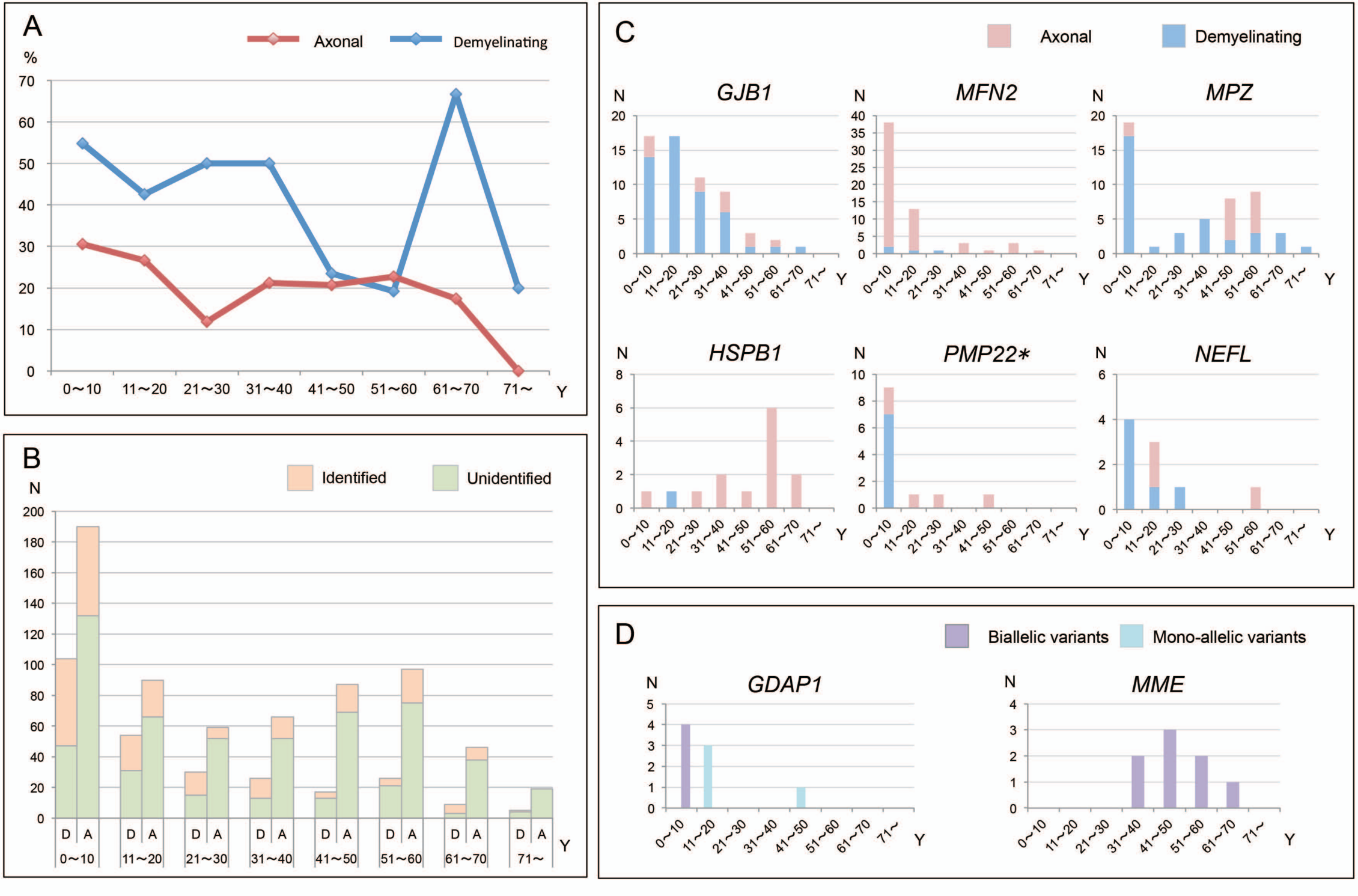
	GJB1	***MFN2***	***MPZ***	***HSPB1***	***PMP22****	***GDAP1***	***NEFL***	***MME***	***BSCL2***	***MARS***	***DNM2***	***SETX***	***SH3TC2***	***PRX***	***GARS***	***IGHMBP2***	***LRSAM1***	**Total**
Our study	6.6%	6.6%	5.1%	1.4%	1.3%	0.9%	0.9%	0.8%	0.6%	0.6%	0.5%	0.5%	0.5%	0.4%	0.3%	0.3%	0.3%	30.0%
	(66)	(66)	(51)	(14)	(13)	(9)	(9)	(8)	(6)	(6)	(5)	(5)	(5)	(4)	(3)	(3)	(3)	(301/1005)
Japan, 2011[8]	8.3%	4.7%	1.7%	0	3.3%	0.3%	2.7%	/	/	/	0	/	/	1.7%	0.3%	/	/	29.9%
	(25)	(14)	(5)	(0)	(10)	(1)	(8)	/	/	/	(0)	/	/	(5)	(1)	/	/	(90/301)
UK, 2012[11]	12.3%	5.0%	2.6%	0.3%	0.9%	1.0%	0.3%	/	0.2%	/	/	/	0.8%	/	/	/	/	24.9%
	(147)	(60)	(31)	(3)	(11)	(12)	(4)	/	(2)	/	/	/	(9)	/	/	/	/	(297/1192)
German, 2013[9]	13.1%	3.3%	5.8%	/	2.2%	0	0	/	/	/	/	/	0	0	0.6%	/	/	30.6%
	(47)	(12)	(21)	/	(8)	(0)	(0)	/	/	/	/	/	(0)	(0)	(2)	/	/	(110/360)
Norway, 2013[12]	4.0%	3.6%	3.3%	/	0	/	0.7%	/	/	/	/	/	/	/	/	/	/	11.6%
	(12)	(11)	(10)	/	(0)	/	(2)	/	/	/	/	/	/	/	/	/	/	(35/302)
Spain, 2013[14]	22.0%	1.6%	7.5%	2.8%	0.8%	16.5%	1.6%	/	/	/	/	/	11.0%	1.6%	0.4%	/	/	71.3%
	(56)	(4)	(19)	(7)	(2)	(42)	(4)	/	/	/	/	/	(28)	(4)	(1)	/	/	(181/254)
USA, 2014 [10]	1.4%	0.9%	1.1%	0.1%	0.2%	0.1%	0.1%	/	/	/	/	/	0.2%	0.01%	0.1%	/	/	4.6%
	(215)	(138)	(170)	(10)	(30)	(22)	(22)	/	/	/	/	/	(26)	(1)	(13)	/	/	(679/14840

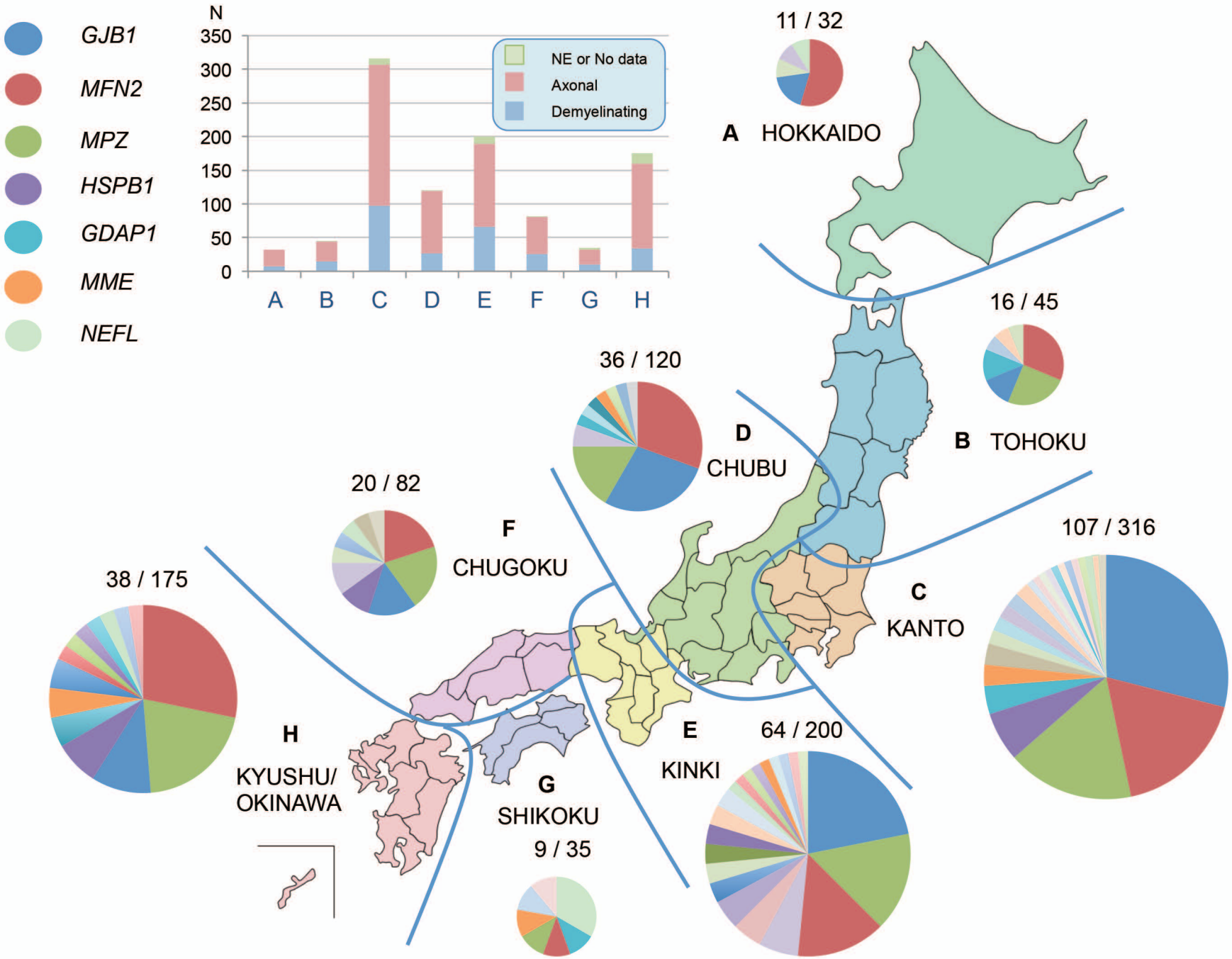
Italy, 2014[15]	14.4%	2.1%	7.2%	1.0%	7.2%	8.2%	1.0%	/	/	/	/	/	3.1%	/	/	/	/	49.5%
	(14)	(2)	(7)	(1)	(7)	(8)	(1)	/	/	/	/	/	(3)	/	/	/	/	(48/97)
CC, 2015[17]	11.2%	7.3%	7.0%	0.7%	1.8%	0.9%	1.2%	/	0.5%	/	/	/	1.5%	0.3%	0.2%	/	/	34.4%
	(107)	(70)	(67)	(7)	(17)	(9)	(11)	/	(5)	/	/	/	(14)	(3)	(2)	/	/	(328/954)
Korea, 2016[16]	14.8%	1.6%	3.3%	0	1.6%	0	0	/	0	1.6%	0	0	1.6%	0	0	0	0	26.2%
	(9)	(1)	(2)	(0)	(1)	(0)	(0)	/	(0)	(1)	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(16/61)
Denmark, 2018[13]	2.7%	2.0%	2.3%	0	0.4%	0	0.1%	/	0	/	0.1%	0	0.1%	0	0.1%	/	0	8.3%
	(32)	(24)	(27)	(0)	(5)	(0)	(1)	/	(0)	/	(1)	(0)	(1)	(0)	(1)	/	(0)	(98/1177)

Numbers in () indicate case numbers; /, no data. * *PMP22* point mutation; CC, cross-country.


Identified
Unidentified
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
BSCL2
MARS
MME
DNM2
NEFL
SETX
GDAP1
SH3TC2
PMP22*
PRX
HSPB1
LRSAM1
AARS
IGHMBP2
ARHGEF10
GARS
FGD4
SACS
SBF2
TRPV4
TTR
GJB1
MFN2
MPZ
COA7, DCTN1,DHTKD1, EGR2, FBLN5, GALC,
GAN, HARS, HSPB3, HSPB8, INF2, KARS,
MTMR2, PRPS1, RAB7A, SOX10 (1 case each)


NE (not evoked)
No data
Demyelinating type
Axonal type
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Identified
Unidentified
0%
20%
40%
60%
80%
100%
Identified
Unidentified
0%
20%
40%
60%
80%
100%
IGHMBP2
PRX
SBF2
MFN2
SH3TC2
NEFL
PMP22
GJB1
MPZ
GJB1: 40.3%
MPZ: 27.1%
PMP22: 6.2%*
NEFL: 4.7%
SH3TC2: 3.9%
MFN2: 3.1%
IGHMBP2: 1.6%
PRX: 1.6%
SBF2: 1.6%
SETX
TTR
GARS
NEFL
LRSAM1
DNM2
PMP22
MARS
BSCL2
MME
GDAP1
GJB1
HSPB1
MPZ
MFN2
MFN2: 37.2%
MPZ: 9.0%
HSPB1: 8.3%
GJB1: 7.7%
GDAP1: 5.1%
MME: 5.1%
BSCL2: 3.8%
MARS: 3.2%
PMP22: 3.2%*
DNM2: 1.9%
LRSAM1: 1.9%
NEFL: 1.9%
GARS: 1.3%
SETX: 1.3%
TTR: 1.3%


A
Axonal
Demyelinating
%
70
60
50
40
30
20
10
0
0~10
11~20
21~30
31~40
41~50
51~60
61~70
71~
Y
B
Identified
Unidentified
N
200
180
160
140
120
100
80
60
40
20
0
D
A
0~10
11~20
21~30
31~40
41~50
51~60
61~70
71~
Y
C
Axonal
Demyelinating
GJB1
MFN2
MPZ
HSPB1
PMP22*
NEFL
N
Y
D
Biallelic variants
Mono-allelic variants
GDAP1
MME


GJB1
MFN2
MPZ
HSPB1
GDAP1
MME
NEFL
N
350
300
250
200
150
100
50
0
A B C D E F G H
NE or No data
Axonal
Demyelinating
11 / 32
A HOKKAIDO
16 / 45
B TOHOKU
107 / 316
C KANTO
36 / 120
D CHUBU
64 / 200
E KINKI
20 / 82
F CHUGOKU
9 / 35
G SHIKOKU
38 / 175
H KYUSHU/ OKINAWA

Supplementary table 1 Gene panel list of Illumina MiSeq and Ion Proton

Gene panel for Miseq (60genes)	*AARS*	*APTX*	*ARHGEF10*	*DHH*	*DNM2*	*EGR2*
	FGD4	*FIG4*	*GAN*	*GARS*	*GDAP1*	*GJB1*
	HARS	*HK1*	*HOXD10*	*HSPB1*	*HSPB8*	*KARS*
	LITAF	*LMNA*	*MARS*	*MED25*	*MFN2*	*MPZ*
	MTMR2	*NDRG1*	*NEFL*	*PMP22*	*PRPS1*	*PRX*
	RAB7A	*SBF2*	*SETX*	*SH3TC2*	*SLC12A6*	*SOX10*
	TDP1	*TRPV4*	*TTR*	*YARS*	20 candidate genes	
Gene panel for Ion Proton (72genes)	*AARS*	*APTX*	*ARHGEF10*	*BSCL2*	*DCAF8*	*DCTN1*
	DHH	*DHTKD1*	*DNM2*	*DYNC1H1*	*EGR2*	*FBLN5*
	FBXO38	*FGD4*	*FIG4*	*GALC*	*GAN*	*GARS*
	GDAP1	*GJB1*	*GJB3*	*GNB4*	*HARS*	*HK1*
	HOXD10	*HSPB1*	*HSPB3*	*HSPB8*	*IGHMBP2*	*INF2*
	KARS	*KIF1A*	*LITAF*	*LMNA*	*LRSAM1*	*MARS*
	MED25	*MFN2*	*MME*	*MPZ*	*MTMR2*	*NDRG1*
	NEFL	*PDK3*	*PLEKHG5*	*PMP22*	*PRPS1*	*PRX*
	RAB7A	*REEP1*	*SACS*	*SBF1*	*SBF2*	*SETX*
	SH3TC2	*SLC12A6*	*SLC5A7*	*SOX10*	*SURF1*	*TDP1*
	TFG	*TRIM2*	*TRPV4*	*TTR*	*YARS*	*COA7*
	6 candidate genes					