RESEARCH ARTICLE

Mutation spectrum of Charcot-Marie-Tooth disease among the Han Chinese in Taiwan

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Abstract

Objective: Charcot-Marie-Tooth disease (CMT) is a clinically and genetically heterogeneous group of inherited neuropathies. Mutations in more than 90 genes have been implicated in CMT; however, the mutational spectrum of CMT in Chinese population remains obscure. This study aims to provide a comprehensive overview of the frequency of mutations in Taiwanese patients with CMT and look for genotype-phenotype correlations. **Methods**: Mutational analyses were performed on 427 unrelated Taiwanese patients with CMT by polymorphic microsatellite markers analysis or real-time fluorescent PCR for *PMP22* duplication, Sanger sequencing for *GJB1* mutations, and targeted sequencing covering 124 genes causing or relevant to inherited neuropathies. We also correlated the genotypes with the phenotypic features, such as age at disease onset and ulnar motor nerve conduction velocity. **Results**: Pathogenic mutations were identified in 312 patients (73.1%; 312/427), including 208 patients with a *PMP22* duplication, 40 patients with a *GJB1* mutation, and 64 patients with a mutation in one of other 18 CMT genes. A confirmed molecular diagnosis was achieved in 84.4% (266/315) of the patients with demyelinating CMT and 41.1% (46/112) of the patients with axonal CMT. Mutations in *MPZ*, *MFN2*, or *NEFL* are the most frequent disease causes in patients with infantile-onset CMT (≤2 years), while *PMP22* duplications and mutations in *GJB1*, *MFN2*, or *MPZ* are the frequent causes among patients with childhood- or adolescence-onset CMT (3–9 years). **Interpretation**: This study provides a genotype-phenotype landscape of CMT in Taiwan and highlights the unique spectrum of CMT genes frequencies among patients of Chinese origin.

Introduction

Charcot-Marie-Tooth disease (CMT), also known as hereditary motor and sensory neuropathy, is a clinically and genetically heterogeneous group of inherited neuropathies characterized by progressive distal muscle atrophy and weakness, distal sensory loss, foot deformities, and depressed tendon reflexes.[1,2] It is one of the most common inherited neurological disorders with a prevalence of approximately one in 2500 individuals.[3,4] There were several kinds of CMT classification from historical perspectives.[5] In clinical practice, one widely used classification is dichotomizing CMT into demyelinating and axonal forms using the median or ulnar motor nerve conduction velocity (MNCV) with a cut-off value of 38 m/sec.[6] Actually, most patients categorized into demyelinating CMT group have dysmyelination due to the genetic defect.[7,8] Sometimes, patients with CMT having an upper limb MNCV between 25 and 45 m/sec are classified as intermediate CMT.[9] CMT can also be categorized by the inheritance pattern and electrophysiological features.[1] CMT patients mostly are inherited in autosomal dominant manner (CMT1 for the demyelinating forms and CMT2 for the axonal forms), in autosomal recessive manner (CMT4) or in X-linked manner (CMTX).

To date, mutations in more than 90 genes have been implicated in CMT,[10,11] and only a few of the genes, such as *PMP22, GJB1, MFN2,* and *MPZ,* account for a significant percentage of CMT cases when mutated.[12–15] It is still not fully clear about the contributions of other CMT-related genes, especially those in which mutations have just identified in one or two families or sporadic cases in past few years. Although a number of studies have investigated the frequencies and spectrum of mutations in one or some particular genes in CMT patients of different ethnicities, only a few provided a comprehensive landscape of the mutation spectrum and frequencies of multiple genes. Most of the studies were performed in Caucasian cohorts with CMT[12–16] and two studies were conducted in the Japanese population.[17,18] Except for a small-scale study investigating 82 Chinese patients with CMT,[19] no extensive mutational analysis has been performed in CMT patients of Han Chinese populations yet.

To fill this knowledge gap and increase the understanding of rare CMT subtypes, we assessed 427 unrelated Taiwanese CMT patients of Han Chinese origin for known CMT-related genes using traditional methods and next-generation sequencing (NGS) techniques. Clinical and neurophysiological features of these CMT patients were also analyzed. The aim of this study is to provide a comprehensive overview of the spectrum and frequencies of mutations in Taiwanese patients with CMT and look for genotype-phenotype correlations.

Subjects and Methods

Patient cohort

Between 1996 and 2018, 427 index patients with CMT were consecutively recruited from the Neurology Clinics of Taipei Veterans General Hospital. The diagnosis and classification of CMT were based on the clinical manifestations, family history and the electrophysiological features.[1] During this period, there were 81 unrelated patients with clinical presentations and genetic mutations compatible with the diagnostic guideline of hereditary neuropathy with liability to pressure palsies (HNPP).[20] Eighty of them carried a *PMP22* deletion and one patient harbored the *PMP22* p.C42R mutation that had been reported previously.[21] Although HNPP is traditionally categorized as a subtype of CMT, we did not include the 81 patients in the present study because of the discrepant phenotypes between HNPP and other CMT subtypes.

Nerve conduction studies were performed by standard techniques utilizing a Medelec MS25 electromyograph (Mistro, Surrey, U.K.) with surface electrode stimulations and recordings. Distal and proximal motor latencies and compound muscle action potential amplitudes were recorded from the median, ulnar, peroneal, and tibial nerves. Sensory nerve action potential amplitudes and distal latencies were recorded from median, ulnar, and sural nerves. MNCV was calculated by standard techniques. Forearm ulnar MNCV with a cutoff value of 38 m/sec was used to distinguish between demyelinating and axonal CMT.[6]

We further categorized the patients into four groups according to the age at disease onset: infancy (≤2 years), childhood/adolescence (3–19 years), early adulthood (20–39 years) and late adulthood (≥40 years). All the patients were of Han Chinese descent. To investigate the relationship between genotype and electrophysiological features, the patients were also divided into four groups according to their forearm ulnar MNCV: (1) MNCV ≤15 m/sec, (2) MNCV between 15 and 25 m/sec, (3) MNCV between 25 and 38 m/sec, and (4) MNCV >38 m/sec.

Written informed consent was obtained from all of the participants or their parents on behalf of those who were younger than 18 years. This study was approved by the Institutional Review Board of Taipei Veterans General Hospital.

Molecular genetic analysis

In patients with demyelinating CMT, we first investigated *PMP22* duplication by polymorphic microsatellite markers analysis or real-time fluorescent PCR,[22,23] and then screened for mutations in *GJB1* (RefSeq NM_000109764)

by direct nucleotide sequencing. In patients with axonal CMT, we first sequenced *GJB1* because of its small size and a proportion of patients with *GJB1* mutations having a forearm ulnar NCV above 38 m/sec. For most of the patients without *PMP22* duplication and *GJB1* mutations, further mutational analysis was performed by utilizing a high-throughput targeted NGS panel for detecting mutations in other CMT-related genes. For a minor group of the patients recruited before 2014, the mutation analyses were conducted by direct nucleotide sequencing of *PMP22* (RefSeq NM_000304.3), *MPZ* (RefSeq NM_000530.5), *MFN2* (RefSeq NM_014874.3), *NEFL* (RefSeq NM_006158.4), *AARS* (RefSeq NM_001605.2), *HSPB1* (RefSeq NM_001540.5), and *GDAP1* (RefSrq NM_018972.3) as employed in our previous studies.[21,24–26]

The targeted NGS panel covering the coding exons of 124 genes associated with inherited neuropathies (Table S1) was designed by using NimbleGen Design website (http://www.nimblegen.com/products/nimbledesign/). The NimbleGen SeqCap EZ Choice Library system (Roche NimbleGen, Madison, WI) was used to enrich the targeted regions. The enriched samples were sequenced using the HiSeq2000 platform (Illumina, San Diego, CA) with a paired-end 100bp protocol. All sequenced reads were mapped to the Human Genome version 19 (hg19/GRCh37). The BaseSpace pipeline (https://basespace.illumina.com/) and the Illumina VariantStudio software (http://variantstudio.software.illumina.com/) were used for variant calling and annotation. After annotation, only rare nonsynonymous variants with minor allele frequencies lesser than 0.1% for dominant CMT and 1% for recessive CMT disease genes in the Taiwan Biobank (https://taiwanview.twbiobank.org.tw/index), which contains whole genome sequences of 1517 Taiwanese healthy controls, were taken for further analysis. Sanger sequencing was performed to confirm the potentially pathogenic variants.

Bioinformatics analyses

Previously reported pathogenic mutations were confirmed by literature reviews and querying the Inherited Neuropathy Variant Browser (http://hihg.med.miami.edu/code/http/cmt/public_html/index.html#/) and the Human Gene Mutation Database Professional (https://portal.biobase-international.com/hgmd/pro). To evaluate the pathogenicity of the novel variants, we surveyed the variants in the genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org).[27] The novel and potential pathogenic variants were further checked among another 500 neurologically healthy individuals of Han Chinese origin. Segregation analysis was also done in those patients whose family members were available.

The pathogenicity of these variants were predicted in silico by Mutation Taster (http://www.mutationtaster.org),[28] PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/),[29] combined annotation dependent depletion (CADD) (http://cadd.gs.washington.edu),[30] and SIFT (http://sift.jcvi.org/).[31] The UniProt website (http://www.uniprot.org) was used to evaluate evolutionary conservation of the mutated amino acid by aligning amino-acid sequences of the orthologues from several species.[32]

Statistical analyses

Descriptive statistics were performed, and the data were represented as mean ± standard deviation (SD) for age of disease onset and ulnar MNCV. The undetectable nerve conduction parameters were excluded from mean and SD analyses. Statistical analysis was performed with the statistical software package SPSS for Windows (version 19.0; SPSS, Inc., Chicago, IL). A $P < 0.05$ was defined as statistically significant.

Results

Characteristics of study participants

Among the 427 unrelated CMT patients, 248 are male and 179 are female. The average age of disease onset was 23.8 ± 17.4 (range 1–72) years. Three hundred fifteen patients (73.8%) had demyelinating CMT and 112 patients (26.2%) had an axonal polyneuropathy. Family history of CMT was present in 58.6% of patients, in whom 48.6%, 7.3%, and 2.7% of cases were inherited in an autosomal dominant, X-linked, and autosomal recessive manner, respectively.

Distribution of CMT subtypes

We identified the pathogenic mutations in 312 patients (73.1%; 312/427), including 208 patients with a *PMP22* duplication, 40 patients with a *GJB1* mutation, and 64 patients with a mutation in one of other 18 CMT-related genes (Table 1). Up to 84.4% (266/315) of the patients with demyelinating CMT and 41.1% (46/112) of the patients with axonal CMT were confirmed to harbor a mutation responsible for their diseases. Among the 427 unrelated CMT patients, the most common genetic causes for the CMT patients were *PMP22* duplication (48.7%), *GJB1* mutations (9.4%), *MPZ* mutations (3.3%), *MFN2* mutations (3.3%), and *NEFL* mutations (1.9%) (Fig. 1A and Table 1). These five groups of mutations accounted for 91.0% of all the CMT patients who had achieved a molecular diagnosis and 66.5% of all the CMT patients in the present cohort. Each of the remaining CMT-related

Table 1. Distribution of CMT subtypes and associated clinical/neurophysiological features.

Genetic subtype	N	% in patients with demyelinating or axonal CMT	% in all patients with CMT	Age of disease onset, year (mean ± SD)	Ulnar MNCV, m/sec (mean ± SD)
Demyelinating CMT	315	100	73.8		
PMP22 duplication	208	66.0	48.7	26.5 ± 17.3 (3–64)	19.1 ± 5.4 (5.7–36.8)
GJB1	32	10.2	7.5	19.8 ± 8.5 (7–40)	32.8 ± 2.9 (25.0–38.0)
Male	29			19.6 ± 8.8 (7–40)	32.5 ± 2.9 (25.0–38.0)
Female	3			21.7 ± 5.7 (17–28)	35.5 ± 0.4 (35.0–35.8)
MPZ	12	3.8	2.8	15.5 ± 16.5 (1–42)	15.0 ± 6.9 (5.1–33.3)
PMP22[1]	4	1.3	0.9	13.5 ± 8.7 (1–20)	11.3 ± 8.0 (5.9–20.5)
SH3TC2	3	1.0	0.7	26.3 ± 20.6 (5–46)	26.9 ± 1.8 (25.5–28.9)
NEFL	2	0.6	0.5	1.5 ± 0.7 (1–2)	21.1 ± 1.5 (20.0–22.1)
EGR2	2	0.6	0.5	1.0	4.7
GNB4	2	0.6	0.5	25.0 ± 28.3 (5–45)	25.6 ± 9.5 (18.8–32.3)
LITAF	1	0.3	0.2	40.0	31.0
Unknown	49	15.6	11.5	23.2 ± 16.7 (1–64)	25.9 ± 8.8 (8.1–43.0)
Axonal CMT	112	100	26.2		
MFN2	14	12.5	3.3	13.7 ± 17.6 (1–72)	47.6 ± 9.2 (30.0–61.4)
GJB1	8	7.1	1.9	25.9 ± 15.1 (7–45)	45.3 ± 6.4 (38.3–57.0)
Male	4			26.8 ± 18.6 (7–45)	44.4 ± 5.6 (38.3–51.4)
Female	4			24.7 ± 12.9 (10–34)	46.2 ± 7.8 (38.9–57.0)
NEFL	6	5.4	1.4	17.2 ± 14.7 (1–40)	41.9 ± 4.3 (36.8–48.7)
MPZ	2	1.8	0.5	53.0 ± 11.3 (45–61)	51.0 ± 1.4 (50.0–52.0)
GARS	2	1.8	0.5	1.5 ± 0.7 (1–2)	43.5
HSPB1	2	1.8	0.5	33.5 ± 19.1 (20–47)	39.5 ± 7.1 (34.5–44.5)
GDAP1	2	1.8	0.5	1.5 ± 0.7 (1–2)	48.1
IGHMBP2	2	1.8	0.5	11.5 ± 14.8 (1–22)	38.8 ± 16.8 (26.9–50.6)
BSCL2	2	1.8	0.5	14.5 ± 13.4 (5–24)	48.5 ± 4.9 (45.0–52.0)
KIF5A	2	1.8	0.5	15.0 ± 7.1 (10–20)	48.1 ± 1.6 (47.0–49.2)
LRSAM1	1	0.9	0.2	65.0	63.2
AARS	1	0.9	0.2	30.0	42.1
TFG	1	0.9	0.2	32.0	63.5
MORC2	1	0.9	0.2	1.0	33.0
Unknown	66	58.9	15.5	26.5 ± 19.4 (1–70)	47.7 ± 7.6 (32.0–62.8)

CMT, Charcot-Marie-Tooth disease; SD, standard deviation; MNCV, motor nerve conduction velocities.
[1]*PMP22* point mutation.

genes (i.e. *PMP22, SH3TC2, EGR2, GNB4, LITAF, GARS, HSPB1, GDAP1, IGHMBP2, BSCL2, KIF5A, LRSAM1, AARS, TFG*, and *MORC2*) accounted for <1% of total CMT patients each (Fig. 1B and Table 1).

Among the demyelinating CMT patients, the most common genetic causes are *PMP22* duplication (66%), *GJB1* mutations (10.2%), and *MPZ* mutations (3.8%) (Table 1). These three groups of mutations accounted for 94.7% (252/266) of the demyelinating CMT patients whose mutations had been identified in the present study. Among the axonal CMT patients, the most common genetic causes are *MFN2* mutations (12.5%), *GJB1* mutations (7.1%), and *NEFL* mutations (5.4%). These three groups of mutations accounted for 60.9% (28/46) of the axonal CMT patients with an identified mutation.

Among the 81 pathogenic mutations in CMT-related genes identified in the present study, 12 mutations were novel (Table 2). These novel mutations are p.F51C, p.Y135D, p.R183F and p.T185Pfs*11 in *GJB1*, p.[L139del];[L1048P] in *SH3TC2*, p.N98Y in *NEFL*, p.E356K in *EGR2*, p.R280P in *MFN2*, p.[L40R];[R595W] and p.[A786Pfs*45];[711+1G>C] in *IGHMBP2*, p.Q764* in *KIF5A*, and p.E680* in *LRSAM1*. The following points support the pathogenicity of these mutations (Table 3). First, all the 12 novel mutations are absent in the 500 healthy Taiwanese controls. Among these mutations, the nine dominant CMT mutations were also absent from both gnomAD and Taiwan Biobank database containing genome sequence of 1517 healthy Taiwanese. The three recessively inherited CMT with compound heterozygous
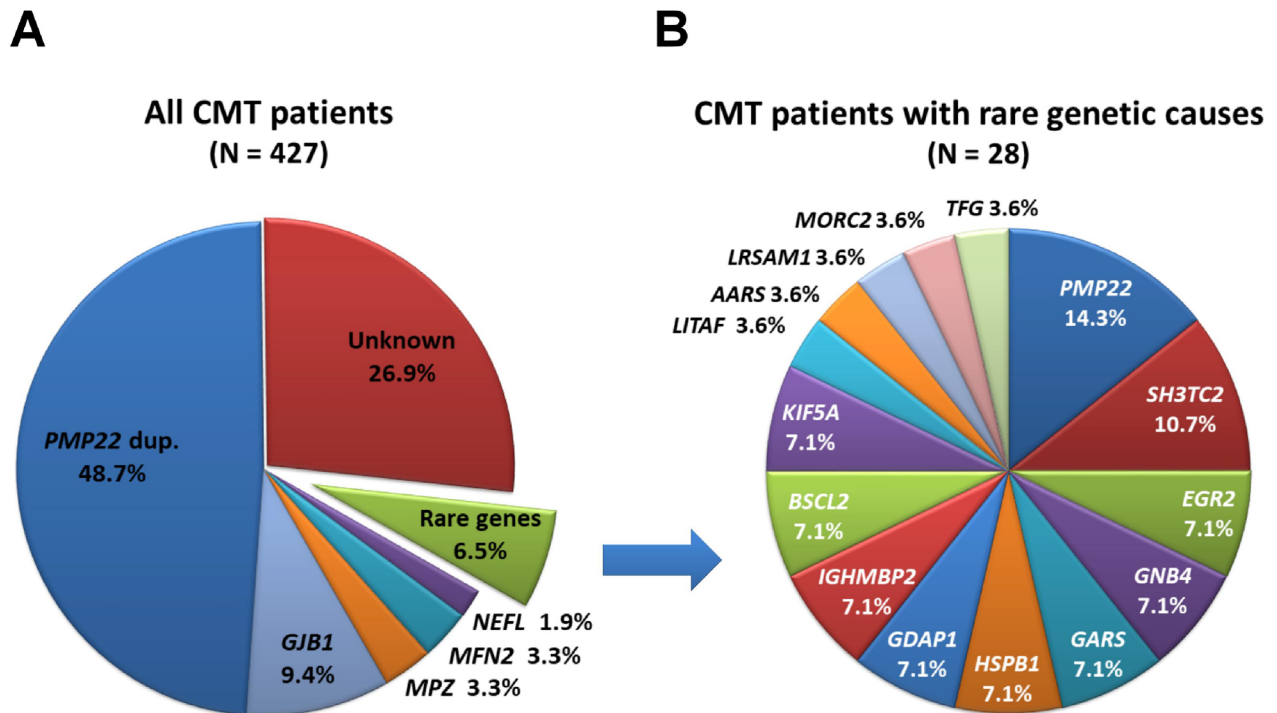
Figure 1. Genetic spectrum of CMT among the Han Chinese population in Taiwan. (A) The common genetic causes, and (B) rare genetic causes in our CMT cohort. CMT, Charcot-Marie-Tooth disease; dup., duplication.

mutations were absent or present in a very low allele frequency in the both databases. Second, these mutations alter the evolutionarily conserved amino acid residues of the mutated proteins and their pathogenicity was supported by in silico prediction, using Mutation Taster, PolyPhen-2, CADD score, and SIFT programs. Third, six of the novel missense mutations change an amino acid residue where a different missense change determined to be pathogenic has been seen before, including *GJB1* p.F51C,[33] *GJB1* p.Y135D,[34] *GJB1* p.R183F,[35,36] *NEFL* p.N98Y,[37,38] *MFN2* p.R280P,[39] and *IGHMBP2* p.R595W.[40] Five of these novel mutations lead to protein length changes, such as *GJB1* p.T185Pfs*11, *SH3TC2* L139del, *LRSAM1* p.E680*, *IGHMBP2* p.A786Pfs*45, and *KIF5A* p.Q764*.

Genotype-phenotype correlations

To investigate the genotype-phenotype relationship within this CMT cohort, we analyzed the mutation spectrum in patients with different age of disease onset and in patients with different scopes of ulnar MNCV.

The age of disease onset was ascertained in 352 patients by self-reporting the time when the parents noticed any motor abnormalities in their children, or when the patients themselves began to be aware of their motor or

sensory dysfunctions. Most of the CMT patients (43.8%) have a childhood- or adolescence-onset disease (3–19 years), around one-fourth (26.4%) with an early adulthood-onset disease (20–39 years), another one-fourth (23.3%) with a late adulthood-onset disease (≥40 years), and only 6.5% of them have disease onset at infancy (≤2 years). In the patients with infantile-onset CMT, mutations in *MPZ*, *MFN2*, or *NEFL* are the most frequent disease causes and mutations in each gene account for 13.0% of the infantile-onset patients (Fig. 2). Among the patients with childhood- or adolescence-onset CMT, *PMP22* duplications (42.2%) and mutations in *GJB1* (12.3%), *MFN2* (5.8%), or *MPZ* (3.2%) are the frequent causes of diseases. In the early adulthood-onset CMT group, *PMP22* duplications (40.9%) and *GJB1* mutations (18.3%) are the major etiologies. For CMT patients whose first symptom occurs during late adulthood, *PMP22* duplication was the most common cause (51.2%), followed by *MPZ* mutations (6.1%), and then *GJB1* mutations (3.7%).

When the CMT patients were grouped based on their ulnar MNCV, 14.6% of the CMT patients have MNCV ≤15 m/sec, 36.2% have MNCV between 15 and 25 m/sec, 24.6% have MNCV between 25 and 38 m/sec, and 24.6% have MNCV faster than 38 m/sec. In the CMT patients with ulnar MNCV ≤15 m/sec, *PMP22* duplications and

Table 2. Pathogenic mutations identified in Taiwanese CMT patients.

Gene	Ever reported in literatures or public database[1]	Novel
Demyelinating CMT		
GJB1	p.M1I, p.L6S, p.I20F, p.S26L, p.S49Y, p.V91M, p.I101Rfs*8, p.L144del, p.F153L, p.Y160C, p.R164Q, p.C173Y, p.R183C, p.R183H, p.E186K p.A197V, p.R215P,	p.F51C, p.Y135D p.R183F, p.T185Pfs*11
MPZ	p.V58D, p.S63F, p.T65I, p.R98C, p.R98H, p. G123S, p.D128G, p.I135M, p.Q187Pfs*63, p.S233fs	–
PMP22	p.Q86*, p.I104Ffs*7, c.319+G>A	–
SH3TC2	p.W245*, p.E657K	p.[L139del];[L1048P]
NEFL	p.N98S	p.N98Y
EGR2	p.E412K	p.E356K
GNB4	p.G53D, p.K89E	–
LITAF	p.G112S	–
Axonal CMT		
MFN2	p.R94Q, p.T159_Q162del, p.L218P, p.L233V, p.R280H, p.R364W, p.R400P, p.L724P, p.W740C, p.E744M	p.R280P
GJB1	p.S138G, p.M162L, p.R220*, p.D278V, c.-459C>T, c.-529T>C	
NEFL	p.P8R, p.P22S, p.E396K	–
MPZ	p.T124M	–
GARS	p.D146Y, p.M238R	–
HSPB1	p.R127L, p.T164A	–
GDAP1	p.[H256R];[R282H], p.[H256R];[118-1G>C]	–
IGHMBP2	–	p.[L40R];[R595W], p.[A786Pfs*45];[711+1G>C],
BSCL2	p.N88S, p.S90L	–
KIF5A	p.R280C	p.Q764*
LRSAM1	–	p.E680*
AARS	p.N71Y	–
TFG	p.G269V	–
MORC2	p.S25L	–

CMT, Charcot-Marie-Tooth disease.
[1]Previously reported pathogenic mutations were confirmed by literature reviews and querying the Inherited Neuropathy Variant Browser (http://hihg.med.miami.edu/code/http/cmt/public_html/index.html#/) and the Human Gene Mutation Database Professional (https://portal.biobase-international.com/hgmd/pro).

MPZ mutations are the two major causes and account for 67.9% and 15.1% of the patients, respectively (Fig. 3). Among the patients with ulnar MNCV between 15 and 25 m/sec, *PMP22* duplication is the sole major etiology, accounting for 84% of the cases. In the patients with ulnar MNCV between 25 and 38 m/sec, the major etiologies are *GJB1* mutations (32.6%) and *PMP22* duplications (23.6%). In patients with ulnar MNCV faster than 38 m/sec, the major disease causes are mutations in *MFN2* (12.4%), *GJB1* (9.0%), and *NEFL* (4.5%).

Discussion

This study demonstrated the genotypic and phenotypic profiles of CMT in a Han Chinese population by investigating 427 unrelated CMT patients in Taiwan. Eighty-one different pathogenic mutations were identified in 312 patients (73.1%; 312/427), including 208 patients with a *PMP22* duplication, 40 patients with a *GJB1* mutation, and 64 patients with a mutation in one of other 18 CMT genes. Among the pathogenic mutations identified in the present study, 12 were novel. This is the first large-scale genetic research on CMT in the Chinese population. In comparison with similar studies in Caucasian or Japanese populations (Table 4), the present study revealed that CMT in Taiwanese population had a higher proportion of *PMP22* duplication and relatively more common *NEFL* mutations. The present study also showed the prioritization of genetic testing for CMT patients of Han Chinese descent by using the age of disease onset and ulnar MNCV to separate the patients into specific subgroups.

In our CMT cohort, the most frequent genetic causes are *PMP22* duplication and mutations in *GJB1*, *MPZ*, and *MFN2*. These genetic alterations comprised 88.5% of cases with positive molecular diagnosis in our study, which is in accordance with the findings in the U.S., U.K., and Germany studies.[12–14] The *PMP22* duplication accounted for 66.7% of the genetically confirmed CMT cases and was responsible for 48.7% of all CMT patients in our cohort. The proportion of *PMP22* duplication among

Table 3. Bioinformatics prediction and population analyses of the novel pathogenic mutations.

Gene	Nucleotide changes	Amino acid changes	Bioinformatics prediction				Population genetics[1]		Conservation
			Mutation taster	PolyPhen2	CADD score	SIFT	gnomAD	Taiwan Biobank	
GJB1	c.152T>G	p.F51C	Disease causing	Probably damaging	23.3	Damaging	Not found	Not found	Zebrafish
GJB1	c.403T>G	p.Y135D	Disease causing	Probably damaging	26.0	Damaging	Not found	Not found	Zebrafish
GJB1	c.547_548delinsTT	p.R183F	N/A	Probably damaging	27.6	Damaging	Not found	Not found	Zebrafish
GJB1	c.552del	p.T185Pfs*11	Disease causing	Probably damaging	23.6	N/A	Not found	Not found	Zebrafish
NEFL	c.292A>T	p.N98Y	N/A	Probably damaging	27.1	Damaging	Not found	Not found	Zebrafish
EGR2	c.1066G>A	p.E356K	Disease causing	Probably damaging	27.5	Damaging	Not found	Not found	C. elegans
MFN2	c.839G>C	p.R280P	Disease causing	Probably damaging	33	Damaging	Not found	Not found	C. elegans
KIF5A	c.2290C>T	p.Q764*	Disease causing	N/A	43	N/A	Not found	Not found	Drosophila
LRSAM1	c.2038G>T	p.E680*	Disease causing	N/A	48	N/A	Not found	Not found	Zebrafish
SH3TC2	c.[415_417del]; [3143T>C]	p.[L139del]; [L1048P]	Disease causing	N/A	22.2	N/A	1/246236	Not found	Zebrafish
IGHMBP2	c.[119T>G]; [1783C>T]	p.[L40R]; [R595W]	Disease causing	Probably damaging	28.3	Damaging	14/246082	1/1517	Zebrafish
			Disease causing	Probably damaging	28.1	Damaging	Not found	Not found	Zebrafish
IGHMBP2	c.[2354del]; [711+1G>C]	p.[A786Pfs*45]; [711+1G>C]	Disease causing	Probably damaging	35	Damaging	4/245652	Not found	Zebrafish
			Disease causing	Benign	23.9	N/A	5/276040	Not found	N/A
			N/A	N/A	N/A	N/A	N/A	N/A	N/A

CADD, combined annotation dependent depletion; gnomAD, genome aggregation database; N/A, not applicable.
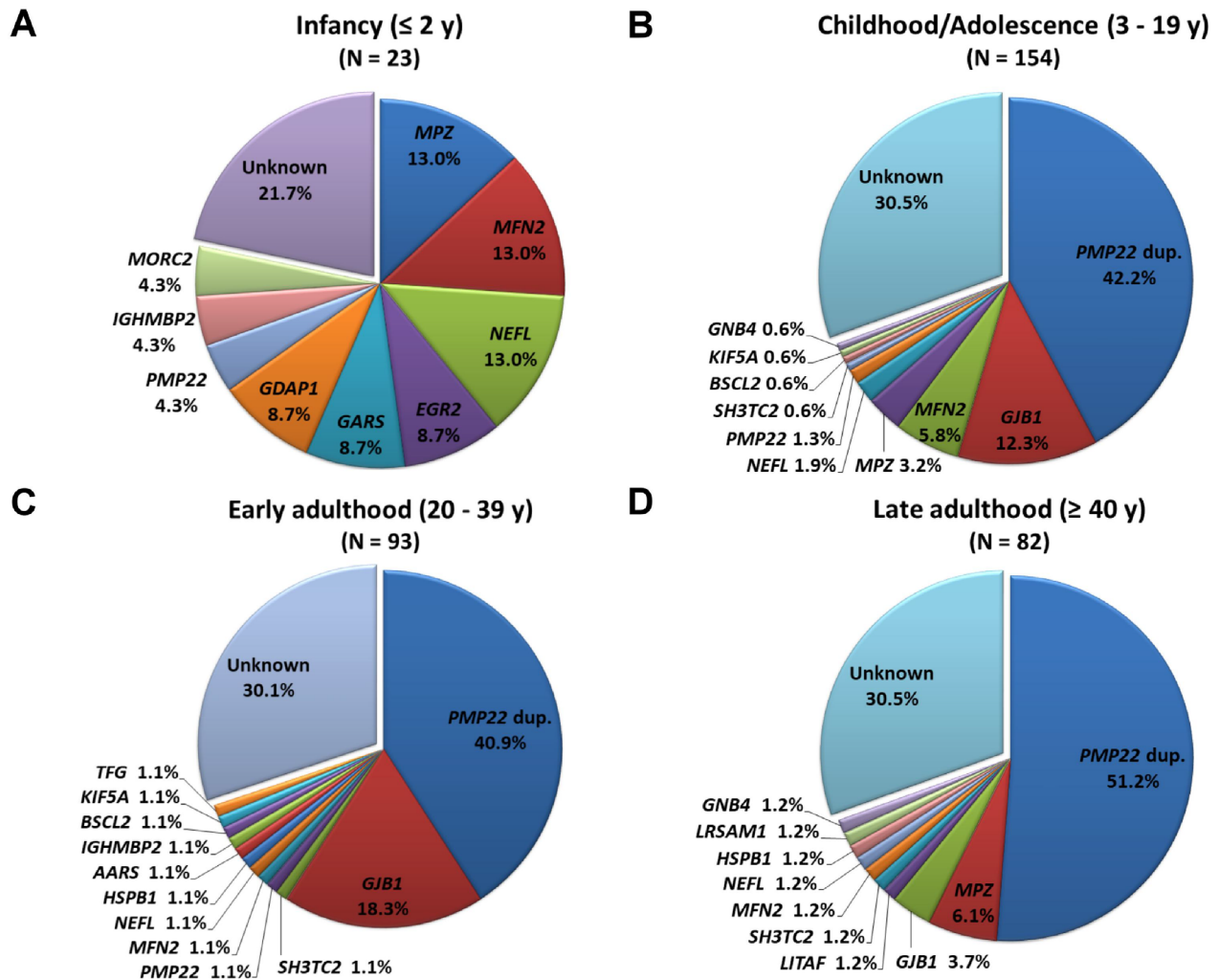[1]Minor allele count/total allele count.

Figure 2. The mutational distribution of CMT-related genes based on age at disease onset. The frequencies of genetic diagnoses in patients with (A) infantile-onset CMT (≤2 years), (B) childhood- or adolescence-onset CMT (3–19 years), (C) early adulthood-onset CMT (20–39 years), and (D) late adulthood-onset CMT (≥40 years). CMT, Charcot-Marie-Tooth disease; dup., duplication.

CMT patients in Taiwan was higher than that in U.S., U.K., Germany, Spain and Japan (15–42%).[12–18] This phenomenon may come from ethnic differences. All patients that carried *PMP22* duplications had ulnar MNCV <38 m/sec (Fig. 3E). The age of clinical onset varied from 3 to 64 years, and sporadic cases accounted for 24.2% of them. Therefore, onset at late adulthood and lack of family history did not exclude the possibility of CMT1A.

Mutations in *GJB1* were the second most frequent cause of CMT and accounted for 12.8% of CMT patients with a confirmed pathogenic mutation and 9.4% of overall CMT patients in the study. The majority of the patients carrying a *GJB1* mutation were male. The ulnar MNCV of the patients with *GJB1* mutations varied widely, ranging from 25 to 57 m/sec and crossing the

usually used cutoff value 38 m/sec for distinguishing between demyelinating and axonal CMT. Several mutations in *GJB1* affected more than two index patients, including four cases with p.S26L, four with p.R164Q, and three with p.V91M, suggesting the possibility of multiple founders of *GJB1* mutations in our cohort. Because *GJB1* mutations are not rare in CMT and the coding region of *GJB1* is not large, in our lab, we do traditional *GJB1* sequencing for CMT patients without *PMP22* duplication before considering the NGS targeted sequencing panel.

Among the patients with axonal CMT, we identified 37 distinct mutations in 14 genes. In Caucasian populations, *GJB1*, *MFN2*, and *MPZ* were the most common mutated genes in axonal CMT.[12–14] However, our data suggested that *MFN2*, *GJB1*, and *NEFL* genes are the three main disease genes for axonal CMT in our population. The
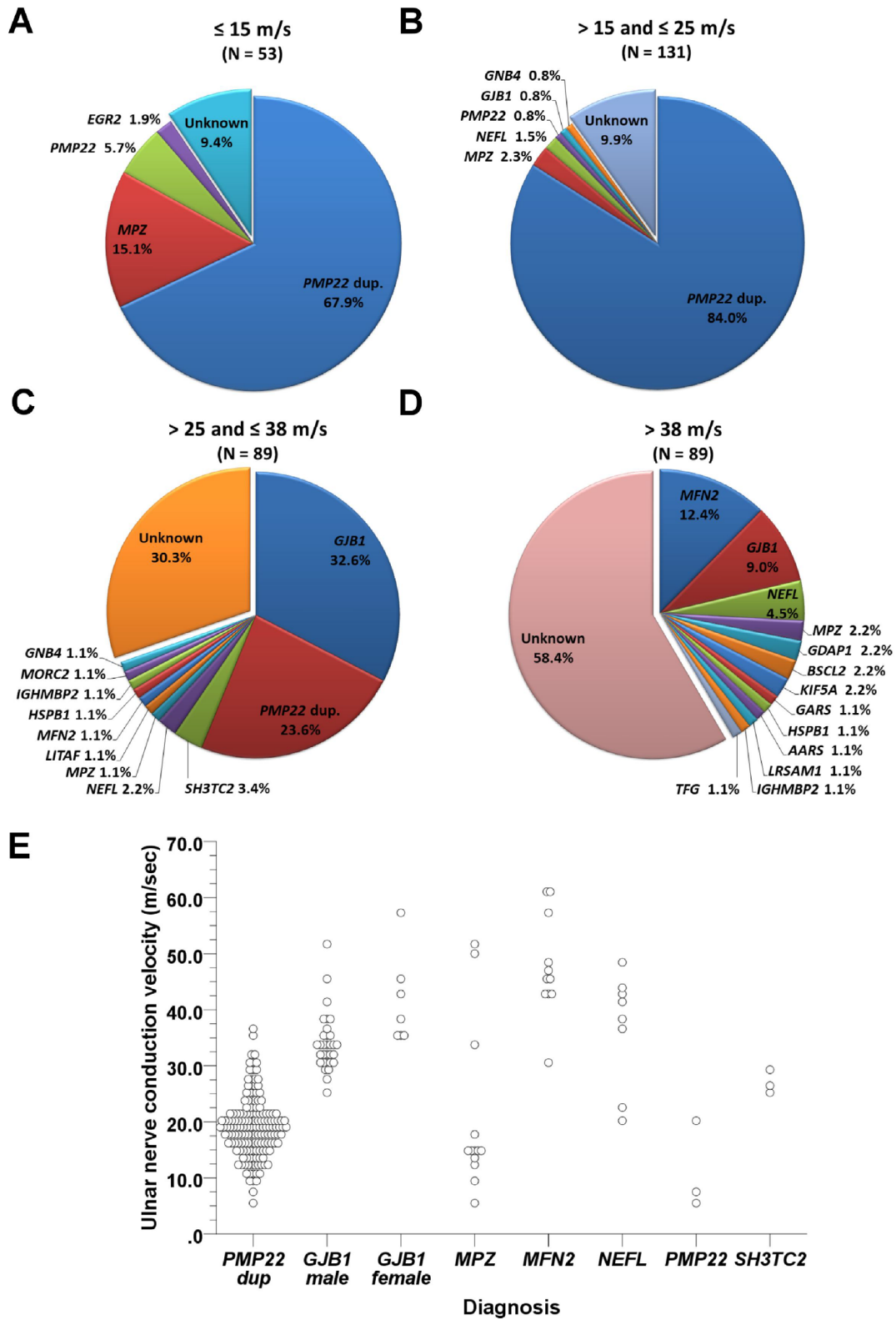
Figure 3. The mutational distribution of CMT-related genes based on ulnar MNCV. The frequencies of genetic diagnoses in the CMT patients with (A) ulnar MNCV ≤15 m/sec, (B) ulnar MNCV between 15 and 25 m/sec, (C) ulnar MNCV between 25 and 38 m/sec, and (D) ulnar MNCV faster than 38 m/sec. (E) The distribution of ulnar MNCV in relation to each CMT-related genes. CMT, Charcot-Marie-Tooth disease; dup., duplication.

Table 4. Comparison of genetic distribution of CMT subtypes in diverse populations.

Gene	This study N = 427	U.S.A.[12] N = 787 (48 HNPP)	U.K.[13] N = 425	Germany[14] N = 589 (83 HNPP)	Spain[16] N = 438	Japan[17] N = 354	International (INC)[15] N = 1652 (36 HNPP)
PMP22 dup	48.7%	36.9%	39.5%	35.6%	42.0%	15.0%	37.2%
GJB1	9.4%	10.2%	10.8%	9.3%	12.8%	7.1%	6.5%
MPZ	3.3%	5.7%	3.1%	4.2%	4.3%	7.1%	4.1%
MFN2	3.3%	2.7%	2.8%	2.4%	1.4%	4.0%	4.2%
NEFL	1.9%	0.5%	0.5%	0.0%	0.9%	2.3%	0.66%
PMP22[1]	0.9%	0.6%	1.4%	0.8%	0.5%	2.8%	1.0%
SH3TC2	0.7%	0.4%	1.2%	0.0%	6.2%	n.t.	0.85%
EGR2	0.5%	0.1%	0.0%	0.0%	0.0%	0.3%	0.06%
GARS	0.5%	0.4%	n.t.	0.4%	0.9%	0.3%	0.12%
HSPB1	0.5%	n.t.	0.5%	0.0%	1.6%	n.t.	0.42%
GDAP1	0.5%	0.6%	0.5%	0.0%	9.6%	0.3%	0.54%
IGHMBP2	0.5%	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
BSCL2	0.5%	n.t.	0.2%	n.t.	0.0%	n.t.	0.30%
KIF5A	0.5%	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
GNB4	0.5%	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
LITAF	0.2%	0.6%	0.9%	0.0%	0.0%	0.0%	0.12%
LRSAM1	0.2%	n.t.	n.t.	n.t.	0.0%	n.t.	n.t.
AARS	0.2%	n.t.	n.t.	n.t.	0.0%	n.t.	n.t.
TFG	0.2%	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
MORC2	0.2%	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Unknown	26.9%	33.0%	37.4%	42.4%	16.7%	59.6%	39.6%

CMT, Charcot-Marie-Tooth disease; HNPP, Hereditary neuropathy with liability to pressure palsy; dup, duplication; n.t., not tested.
[1]PMP22 point mutation.

relatively high percentage of NEFL mutations in our CMT cohort (1.9%) with recurrent mutations of p.P8R and p.E396K (two and three pedigrees, respectively) suggested population-specific founder effects of the NEFL mutations in Chinese population. NEFL mutations were also relatively prevalent in Japan, accounting for 0.9% and 2.3% of total CMT patients in two Japanese cohorts.[17,18]

After a series of mutational analyses, including PMP22 dosage assay, Sanger sequencing, and targeted sequencing with NGS technique, a causal mutation could be identified in 73.1% of the patients in our CMT cohort. The diagnostic yield rate of demyelinating CMT was higher than that of axonal CMT (84.4% vs. 41.1%). Moreover, we identified 81 different mutations in 19 CMT-related genes in our CMT cohort, and point mutations in 15 genes accounted for <1% of the patients each. The number of the mutated genes identified in this study was higher than similar studies. Possible explanations include the ethnic factor, different inclusion criteria, and different mutation-detecting strategies.

Forearm MNCV is a popular parameter to categorize CMT into demyelinating or axonal subtype. Moreover, Saporta et al.[12] analyzed data from 787 CMT patients and proposed a genetic testing strategy for CMT based on different ranges of ulnar MNCV. In this study, we utilized ulnar

MNCV and age of disease onset to separate the patients with identified mutations into 16 subgroups (Table S2). This information can help predict the genotype according to the MNCV and age of onset and provide a guide for the prioritization of genetic testing for CMT patients of Han Chinese origin. We acknowledged that clinical assessment scales like Charcot-Marie-Tooth neuropathy score[41] and Charcot-Marie-Tooth disease Pediatric Scale[42] could provide precious information toward phenotype-genotype correlations; unfortunately, we have just started to use them to evaluate our CMT patients and only a limited number of our patients had such data. We also acknowledged that additional functional experiments are needed to confirm the causal roles of the 12 novel mutations observed in the present CMT cohort. Although their pathogenicity can be partially supported by in silico analysis and population data, there are still some degrees of uncertainty.

In conclusion, this study presents the mutational spectrum and genotype-phenotype correlations of 427 patients of Han Chinese descent in Taiwan. There is substantial difference in the frequencies of PMP22 duplication and NEFL mutations between Taiwanese population and Caucasian populations. These findings broaden the spectrum of mutations causing CMT and are useful for optimal strategies of mutational analysis and genetic counseling of CMT for patients of Han Chinese origin.

Author Contributions

Yun-Hsin Hsu: collecting and analyses of the data, drafting the manuscript, Kon-Ping Lin: patient enrollment, collecting the clinical data. Yuh-Cherng Guo: patient enrollment, colleting the clinical data. Yu-Shuen Tsai: bioinformatics support, analyses of the next generation sequencing data. Yi-Chu Liao: analyses and interpretation of the data, revising the manuscript for intellectual content. Yi-Chung Lee: conceptualizing and designing the study, interpretation of the data, revising the manuscript for intellectual content.

Acknowledgments

Conflict of Interest

All authors read and approved the final manuscript. They declared no conflicts of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Genes in the targeted next-generation sequencing panel.
Table S2. Genetic distribution in accordance with age of onset and ulnar MNCV (n = 227).