Novel homozygous mutations in Pakistani families with recessive Charcot-Marie-Tooth disease

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Abstract

Background

Charcot-Marie-Tooth disease (CMT) is a group of genetically and clinically heterogeneous peripheral nervous disorders. Few studies have identified genetic causes in the Pakistani CMT patients.

Methods

This study was performed to identify pathogenic mutations in five consanguineous Pakistani CMT families negative for *PMP22* duplication. Genomic screening was performed by application of whole exome sequencing

Results

We identified five pathogenic or likely pathogenic homozygous mutations in four genes: c.2599C > T (p.Gln867*) and c.3650G > A (p.Gly1217Asp) in *SH3TC2*, c.19C > T (p.Arg7*) in *HK1*, c.247delG (p.Gly83Alafs*44) in *REEP1*, and c.334G > A (p.Val112Met) in *MFN2*. All the mutations were not reported in the CMT patients. Mutations in the *SH3TC2*, *HK1*, *REEP1*, and *MFN2* have been reported to be implicated to CMT4C, CMT4G, dHMN5B (DSMA5B), and CMT2A, respectively. The genotype-phenotype correlations were confirmed in all the examined families. We also confirmed that both alleles from the homozygous variants were originated from a single founder using homozygosity mapping.

Conclusions

This study found five novel mutations as the underlying causes of CMT. Pathogenic mutations in *SH3TC2, HK1*, and *REEP1* have been reported rarely in other populations, suggesting ethnic-specific distribution. This study will be useful for the exact molecular diagnosis and treatment in the Pakistani CMT patients.

Background

Charcot-Marie-Tooth disease (CMT) and related neuropathies are a group of genetically and clinically quite heterogeneous rare peripheral neuropathies with the prevalence of approximately 1 in 2,500 people. CMT, also called hereditary motor and sensory neuropathy (HMSN), is impaired in both sensory and motor nerves, whereas, both distal hereditary motor neuropathy (dHMN) and hereditary sensory and autonomic neuropathy (HSAN) only affect motor and sensory nerves, respectively. Their common clinical phenotypes include progressive distal muscle weakness and atrophy, loss of sensation, and anti-reflection symptom of the upper and lower limbs [1]. CMT is commonly divided into demyelinating type of CMT1 with the reduced motor nerve conduction velocity (NCV) of less than 38 m/s, axonal type of CMT2 with normal or slightly reduced NCV of 38 m/s or more, and intermediate CMT type [2, 3]. CMT is often viewed as a monogenic Mendelian disease; however, mutations in more than 130 genes are associated with the development of peripheral neuropathies in an autosomal or X-linked dominant or recessive manner [4].

Several studies have attempted to determine the underlying causes of CMT. In particular, recent application of next generation sequencing (such as whole exome or targeted sequencing) has enhanced unveiling of the genetic pathogenicity. However, limited studies have been performed to determine the genetic causes of CMT and related peripheral neuropathies in Pakistan [5–8]. Pakistani patients whose genetic causes were identified showed unusually high rate of recessive homozygous mutations. Pedurupillay et al. reported three patients with CMT2S or spinal muscular atrophy with respiratory distress type 1 (SMARD1) with *IGHMBP2* mutations [6]. Two of them presented with homozygous mutations. Wright et al. reported a homozygous *Fig. 4* variant in four independent patients with combined phenotypes of CMT4J and Yunis-Varón syndrome [8]. Houlden et al. reported several patients with *HSPB1* mutations which contained a homozygous mutation in addition to heterozygous mutations [5]. Zambon et al. reported a patient with CMT4B1 with homozygous *MTMR2* mutation [7]. The high rates of homozygous mutations in Pakistani patients can be attributed to the relatively frequent consanguineous marriages.

This study aimed to determine the genetic causes in Pakistani patients with CMT or related neuropathies using whole exome sequencing (WES) and subsequent filtering process of called variants. We identified five pathogenic or likely pathogenic homozygous mutations in the CMT-related genes. Additionally, we determined that all the observed homozygous mutations originated from a single founder through homozygosity mapping.

Methods

Subjects

This study examined five CMT patients and 15 of their unaffected familial members that originated from consanguineous Pakistani pedigrees, negative for the duplication or deletion of the 17p12 chromosomal region including *PMP22* gene (Fig. 1). All participants were recruited from Care Hospital Sahiwal, Pakistan, and provided written informed consent. For the minors involved in the study, consent was provided by their parents. This study was approved by the Institutional Review Boards for Kongju National University (KNU_IRB_2018-06) and Sungkyunkwan University, Samsung Medical Center (2014-08-057-002).

Clinical and electrophysiological assessments

Motor and sensory impairments, deep tendon reflexes, and muscle atrophy were measured as the clinical information. Onset age was determined through patient interviews about when symptoms such as distal muscle weakness, foot deformity, or sensory change first appeared. Disease severity was determined

using the functional disability scale (FDS). Motor and sensory conduction velocities of median, ulnar, peroneal, tibial, and sural nerves were determined by surface stimulation and recording electrodes. Motor nerve conduction velocities (MNCVs) of the median and ulnar nerves were determined by stimulating at the elbow and wrist while recording compound muscle action potentials (CMAPs) over the abductor pollicis brevis and adductor digiti quinti, respectively. In the same way, the NCVs of peroneal and tibial nerves were determined by stimulating at the knee and ankle, while recording CMAPs over the extensor digitorum brevis and adductor hallucis, respectively. Sensory nerve conduction velocities (SNCVs) and sensory nerve action potential (SNAP) amplitudes were obtained over a finger-wrist segment from the median and ulnar nerves by orthodromic scoring, and were also recorded for sural nerves. Electromyography was tested for investigation of neuromuscular disease. MRIs of the brain and spinal cord were obtained using a 1.5-T system (Siemens Healthineers, Erlangen, Germany).

DNA purification and paternity testing

Genomic DNA was purified from whole blood by using the HiGene Genomic DNA Prep Kit (Biofact, Daejeon, Korea). Paternity was confirmed for all the examined families by PCR amplification of STR markers using the PowerPlex Fusion System (Promega, Wisconsin-Madison, USA) and resolution of the PCR products by the SeqStudio genetic analyzers (Life Technologies-Thermo Fisher Scientific, Foster City, CA, USA).

Exome sequencing and filtering

WES was performed for patients in the five examined families. Exome was captured using the SureSelect Human All Exon 50M kit (Agilent Technologies, Santa Clara, CA, USA), and sequencing was performed using the HiSeq 2000 Genome Analyzer (Illumina, San Diego, CA, USA). The UCSC assembly hg19 (GRCh37) was used as the reference sequence (http://genome.ucsc.edu). Small nucleotide variants (SNVs) were called using the programs of GATK (https://software.broad institute.org/gatk/) and SAMtools (http://samto ols.sourceforge.net/). Rare alleles with minor allele frequencies (MAFs) of < 0.1 were obtained from the 1000 Genomes Project (1000G, http://www.1000genomes.org/), and the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/). Candidate variants for the genetic causes were also checked in the dbSNP (http://www.ncbi.nlm.nih.gov/snp) and the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Pathogenicity of the variants was evaluated into five grades (pathogenic, likely pathogenic, uncertain significance, likely benign, and benign) based on the guidelines of the American College of Medical Genetics and Genomics (ACMG) [9]. Pathogenic candidate variants were confirmed by Sanger sequencing using the SeqStudio genetic analyzers (Life Technologies-Thermo Fisher Scientific).

In silico prediction and conservation analysis

In silico analyses to predict the mutation effect were performed using the programs of MUpro (http://www.ics.uci.edu/~baldig/mutation), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and PROVEAN (http://provean.jcvi.org/). Conservation analysis of the mutation sites were performed by the MEGA-X software, ver. 5.05 (http://www.megasoftware.net/). Genomic evolutionary rate profiling score (GERP) were determined by the GERP++ program (http://mendel.stanford.edu/SidowLab/downloads/gerp/).

Homozygosity mapping

For the putative pathogenic homozygous variants, homozygosity mapping was performed to determine whether two same alleles originated from a single founder. Homozygosity mapping was achieved through haplotyping of SNPs distributed around the corresponding mutations from the WES data of the affected persons by using the method outlined by Park et al. [10].

Results

Clinical manifestations

This study examined five consanguineous families with CMT. CMT types and clinical phenotypes are provided in Table 1.

In the PaC2 family, a 6-year-old boy was born with a full term pregnancy to healthy parents. He showed delayed development and frequent fall during walking since early onset of 3 years old. He did not complain of sensory symptoms, however, his vibration sense were reduced. Deep tendon reflex at the knee was absent. Scoliosis, and foot deformities were observed. No family history of such complaints was recorded. Motor nerves conduction studies showed prolonged distal latencies and low distal CMAP amplitudes with no reproducible f-wave latencies and wave forms. He showed absent SNAP amplitudes in upper and lower extremities. These findings are compatible with demyelinating CMT neuropathy.

In the PaC3 family, a 10-year-old boy born full term from healthy normal parents had demyelinating CMT. At the age of 3, he was noticed frequent fall and difficulty in standing from sitting position. In addition to CMT phenotype, he showed the scoliosis and short stature. He had walking difficulty but still possible unaided. Motor nerves conduction studies of median and peroneal nerves showed prolonged distal latencies and low distal CMAP amplitudes of median and peroneal nerves were absent, and those of ulnar and tibial nerves were decreased. Motor and sensory NCVs were decreased on upper and lower extremities. Brain MRI showed no area of abnormal signal intensity.

In the PaC4 family, an 11-year-old boy was the product of a normal full-term pregnancy from healthy parents. At the age of 15 months, he was unable to walk without support. His parents first noticed gait disturbance at the age of 2.5 years. No family history of such complaints was recorded. Neurologic examination revealed decreased vibration and pain sense, which were consistent with the results of sensory nerve conduction study. Deep tendon reflex at the knee was absent, and foot deformities were observed. Lumbo-sacral spine MRI showed no noticeable abnormal signal.

In the PaC6 family, a 2.5-year-old girl born full term from unaffected parents had congenital motor neuropathy. She showed delayed development. The affected girl showed foot deformity and contractures of the distal phalanges before 6 months old, and her parents noticed the neuromuscular defect before 1

years. She had frequent fall during walking, and mild respiratory distress. Deep tendon reflex at the knee was decreased, and foot deformities were observed. No family history of such complaints was recorded. CMAPs of median, ulnar, peroneal and tibial nerves were not evoked, at all. But, normal SNAPs and SNCVs were observed in the sensory median, ulnar and sural nerves.

In the PaC14 family, a 7-year-old boy showed axonal CMT with onset of 5 years old. He had vocal cord hoarseness as the additional symptom. He showed delayed development. At 5 years, he showed gait disturbances, and frequent fall during walking. He did not complain of sensory symptoms, however, his vibration and position sense were reduced. Deep tendon reflex at the knee was absent, and foot deformities were observed. No family history of such complaints was recorded.

Identification of novel homozygous pathogenic mutations

This study identified five pathogenic or likely pathogenic homozygous mutations from *SH3TC2, HK1, REEP1*, and *MFN2* genes in the examined families by the WES and subsequent annotation and filtering processes (Table 2). All the candidate pathogenic mutations were confirmed by Sanger sequencing (Fig. 2a).

Mutations in the *SH3TC2* (MIM 608206) are implicated to the recessive CMT4C (MIM 601596) [11] and the relatively mild dominant mononeuropathy of the median nerve (MNMN, MIM 613353) [12]. We identified two novel or rare homozygous variants of *SH3TC2* in two families. As the first mutation, a novel homozygous c.2599C>T mutation which results in a stop-gain mutation (p.Gln867*) was identified in a 6-year-old boy (family ID: PaC2). The *SH3TC2* mutation was heterozygous in both unaffected parents and a brother (Fig. 1a). This mutation has not been reported as pathogenic, nor has it been registered in the public databases of dbSNP, 1000G, and ExAC. The p.Gln867* mutation is expected to produce a truncated protein of which many tetratricopeptide repeat (TPR) domains are deleted. Although the p.Gln867* was not reported yet, several stop-gain mutations, such as p.Gln892*, p.Arg904*, and p.Tyr943*, have been reported to the underlying causes of the patients with CMT4C [11, 13, 14]. As the second *SH3TC2* mutation, a c.3650G>A resulting p.Gly1217Asp was identified in a 10 years old boy (family ID: PaC3). This mutation was heterozygous in the unaffected parents and two siblings (Fig. 1b). The homozygous p.Gly1217Asp was still not reported as pathogenic, although the same heterozygous variant was recently registered as “uncertain significance” in the ClinVar database. It was registered in the dbSNP (rs758669363) and ExAC with a very low allele frequency (1.6E-5). The p.Gly1217Asp mutation was located at a highly conserved TPR domain which has a putative function for protein-protein interactions (Fig. 2b, 2c), and was suggested to be pathogenic by the PolyPhen-2 and PROVEAN *in silico* prediction programs.

Few cases with homozygous mutations in *HK1* have been reported to recessive CMT4G (MIM 605285), also called HMSN Russe type [15, 16]. An 11 years old boy with demyelinating CMT (family ID: PaC4) showed a stop-gain mutation of c.19C>T (p.Arg7*) in *HK1* (MIM 142600), which putatively resulted in a very short premature peptide. The mutation was homozygous in the affected boy and heterozygous in the unaffected father and brother (Fig. 1c). This *HK1* mutation was not reported as pathogenic, although registered in the dbSNP (rs779250530) and the ExAC database with a very low allele frequency (1.7E-5).

A small number of mutations in *REEP1* (MIM 609139) have been reported to cause several neuromuscular disorders, such as the dominant dHMN5B (MIM 614751), also called distal spinal muscular atrophy type 5B (dSMA5B) [17], and the dominant spastic paraplegia-31 (SPG31, MIM 610250) [18]. A homozygous splicing site mutation was also recently reported in a patient having similar symptoms of the spinal muscular atrophy with respiratory distress (SMARD), of which phenotype is similar to the SMA but with additional symptom of diaphragmatic palsy [19]. This study identified a homozygous frameshift *REEP1* mutation of c.247delG in a 2.5-year-old girl with dHMN (family ID: PaC6). This deletion was expected to produce a truncated premature peptide (p.Gly83Alafs*44). The mutation was heterozygous in the unaffected parents and sister (Fig. 1d). It has not been registered in any databases, nor has it been reported as a pathogenic mutation.

Most mutations in *MFN2* (MIM 608507) are relevant with autosomal dominant CMT2A2A (MIM 609260) [20] and CMT6A (MIM 601152) [21], whereas, recessive *MFN2* mutations have been rarely reported with more severe and earlier onset CMT2A2B (MIM 617087) [22]. The affected 7-year-old boy in the PaC14 family revealed a homozygous c.334G>A (p.Val112Met) in *MFN2*. The mutation was heterozygous in the unaffected parents and a sister (Fig. 1e). The mutation was reported in the ExAC with a very low frequency (1.6E-5), and was registered as likely pathogenic in the ClinVar database. It was predicted to be pathogenic by the *in silico* analysis using the PolyPhen-2 and PROVEAN programs, and was located at the highly conserved GTPase domain among vertebrate species (Fig. 2b, 2c).

From the filtering of the WES data for the affected individuals of five families, several rare functionally significant variants (MAF < 0.1) were observed in the CMT-related genes, in addition to the above mentioned five pathogenic or likely pathogenic mutations (Table 3). A homozygous c.1933A>G (p.Ile645Val) variant in *DST* was observed in the PaC4 patient. The *DST* mutation was cosegregated with the affected individual. However, *in silico* analyses with PolyPhen-2 and PROVEAN programs predicted it to be nonpathogenic. *DST* mutations have been reported to be implicated in HSAN6 (MIM 614653) [23], thus, we classified this homozygous variant as ‘variant of uncertain significance (VUS)”. All other rare variants were considered as nonpathogenic because they were either nonsegregated with affected individuals or did not fit the inheritance modes.

Homozygosity mapping

Homozygous blocks (HBs) were found at the chromosomal regions including pathogenic or likely pathogenic mutations in all the five affected individuals by the SNP haplotype analysis using WES data (Fig. 3). The lengths of HBs were from approximately 12 Mbp to 53 Mbp: 16 Mbp HB from *FGF1* to *THG1L* in the PaC2 family with *SH3TC2* mutation, 12 Mbp HB from *PKD2L2* to *SLC6A7* in the PaC3 family with *SH3TC2* mutation, 38 Mbp HB from *PPYR1* to *NRG3* in the PaC4 family with *HK1* mutation, 53 Mbp HB from *CTNNA2* to *MZT2A* in the PaC6 family with *REEP1* mutation, and 14 Mbp HB from *NADK* to *CLCNKB* in the PaC14 family with *MFN2* mutation. This homozygosity mapping suggests that both homozygous alleles in each family originated from a single founder.

Discussion

From the genetic screening of the consanguineous Pakistani CMT families, we identified five homozygous mutations in *SH3TC2, HK1, REEP1*, and *MFN2* as the underlying causes. All the identified homozygous mutations were not reported in the CMT patients.

SH3TC2 which encodes a protein of SH3 domain and tetratricopeptide repeats containing protein 2, expressed in Schwann cells of peripheral nerves, suggesting a possible role in myelination [24]. Mutations in *SH3TC2* cause recessive CMT4C usually concurrent with scoliosis, with the onset ranging from infancy to early teens [11], however, cases with late onset (≤ 30 years) were also reported [25].

Mutations in *HK1* cause recessive CMT4G (HMSNR), mostly found in the Spanish Gypsy patients [15]. Hexokinase 1 encoded by *HK1* catalyzes the phosphorylation of glucose. HK1 localizes at the outer membrane of mitochondria (OMM) through a porin-binding domain, and it was suggested that the involvement of the non-OMM-binding HK1 protein in the CMT4G pathogenesis [15]. Several *HK1* mutations are also associated with autosomal dominant retinitis pigmentosa-79 (RP79, MIM 617460), which exhibits variable phenotype with ages of onset ranging from childhood to 70 years [26]. Here, the affected 11-year-old boy with the *HK1* mutation did not show retinitis pigmentosa symptom until his examined age.

REEP1 encodes a receptor accessory protein 1 that suggested to have a role in facilitating endoplasmic reticulum (ER)-mitochondrial interactions [27]. It is known that the *REEP1* mutations exhibited considerable phenotypic heterogeneity [28]. The dominant *REEP1* mutations have been reported to cause dHMN5B (DSMA5B) and SPG21 with the onset ages falling in either the first or second decades [17, 18]. Recently, a recessive *REEP1* mutation was reported in a Lebanese 5-year-old boy with a SMARD-similar phenotype [19]. The affected boy presented foot deformity and contractures of the distal phalanges at birth. This case was similar to our PaC6 case having a p.Gly83Alafs*44 mutation in view of premature termination, onset age, and some clinical symptoms. In the nerve conduction studies, all motor nerves were not evoked at all, but all sensory nerves showed normal SNAPs and SNCVs. From the clinical and NCV findings, this patient's symptoms are apparently similar with SMARD.

MFN2 encodes mitofusin 2 which plays an important role maintaining equilibrium between mitochondrial fusion and fission [29]. Most *MFN2* mutations have been reported to cause dominant CMT2A2A [20]. However, some homozygous or compound heterozygous mutations cause recessive CMT2A2B (MIM 617087) with more severe and earlier onset phenotypes. Nicholson et al. suggested that CMT2A2B may semidominant and carriers with a single mutant allele may show weak phenotype with incomplete penetrance [22]. Our case with the homozygous *MFN2* mutations showed relatively early onset (5 years old) and severe phenotypes, which are matched with the characteristics of CMT2A2B. Additionally, the vocal cord paralysis seen in the affected boy has been occasionally reported in CMT2A patients with *MFN2* mutations [30, 31]. The patient showed no symptom of optic atrophy which is a characteristic of CMT6A until his examined age (7 years old). His parents were apparently seemed to be unaffected; however, exact clinical and electrophysiological tests were not done.

Although a small number of CMT cases were investigated in this study, the incidence of the recessive patients with homozygous mutations was certainly frequent compared to other populations. Homozygosity mapping showed that both alleles of the homozygous mutations identified in each family originated from a single founder. Mutations in *MFN2* are well known as the cause of dominant CMT2, however, recessive homozygous mutations have been rarely reported. This suggests an increased risk of consanguinity prone to develop rare recessive genetic diseases.

Conclusions

In conclusion, we identified five pathogenic or likely pathogenic mutations in the consanguineous Pakistani families with early onset CMT. All the mutations were novel, and the genotype-phenotype correlations were confirmed. We believe that our findings will contribute to expanding understanding of the genetic basis of peripheral neuropathy, improving molecular diagnostics and treatment options.

Abbreviations

ACMG: American College of Medical Genetics and Genomics; CMAP: Compound muscle action potential; CMT: Charcot-Marie-Tooth disease; dHMN: Distal hereditary motor neuropathy; dSMA5B: Distal spinal muscular atrophy type 5B; ER: Endoplasmic reticulum; FDS: Functional disability scale; GERP: Genomic evolutionary rate profiling score; HB: Homozygous block; HMSN: Hereditary motor and sensory neuropathy; HSAN: Hereditary sensory and autonomic neuropathy; MAF: Minor allele frequency; MNCV: Motor nerve conduction velocity: NCV: Nerve conduction velocity; OMM: Outer membrane of mitochondria; SMARD: Spinal muscular atrophy with respiratory distress; SNAP: Sensory nerve action potential; SNCV: Sensory nerve conduction velocity; TPR: tetratricopeptide repeat; WES: Whole exome sequencing

Declarations

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Author contributions

Conceptualization: Chung KW, Kanwal S; Sampling: Kanwal S, Nuzhat R, Khan A, Perveen S; Genetic investigation: Choi YJ, Lim SO, Choi HJ, Park JH, Son WS; Clinical data analysis: Choi BO; Funding acquisition: Chung KW, Choi BO; Writing original draft preparation: Son WS, Choi BO, Chung KW; Review and editing: all authors.

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Availability of data and materials

All pathogenic variants and data from this study are available upon reasonable request. Raw data on the exome sequencing are available upon request.

Ethics approval and consent to participate

This study was approved by the Institutional Review Boards for Kongju National University (KNU_IRB_2018-06) and Sungkyunkwan University, Samsung Medical Center (2014-08-057-002). All participants were recruited from Care Hospital Sahiwal, Pakistan, and provided written informed consent. For the minors involved in the study, the consent was provided by their parents.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1. Clinical characterization of the five Pakistani CMT patients

Item Patient (sex)	PaC2:IV-1 (male)	PaC3:IV-1 (male)	PaC4:IV-1 (male)	PaC6:IV-2 (female)	PaC14:IV-2 (male)
Gene: mutation	*SH3TC2*: p.Q867*	*SH3TC2*: p.G1217D	*HK1*: p.R7*	*REEP1*: p.G83Afs*44	*MFN2*: p.V112M
Type	CMT4C	CMT4C	CMT4G	dHMN5B/SMARD1	CMT2A2B
Ages at exam/onset (yr)	6/3	10/3	11/1	2.5/< 1	7/5
Muscle atrophy	Yes	Yes	Yes	Yes	Yes
FDS	3	3	3	3	3
Sensory loss	Yes	Yes	Yes	No	Yes
DTR, ankle	Absent	Absent	Absent	Decreased	Absent
Foot deformities	Yes	Yes	Yes	Yes	Yes
Brain/Spine MRI	ND	Normal brain	Normal spine	Normal brain	ND
Other symptoms	Scoliosis	Scoliosis, short stature	-	Mild respiratory distress	Vocal cord hoarseness
Motor nerve conduction studies					
Median CMAP (mV)	1.3	Absent	ND	Absent	ND
Median MNCV (m/s)	14.1	Absent	ND	Absent	ND
Ulnar CMAP (mV)	0.8	4.1	ND	Absent	ND
Ulnar MNCV (m/s)	12.8	25.0	ND	Absent	ND
Peroneal CMAP (mV)	0.8	Absent	ND	Absent	ND
Peroneal MNCV (m/s)	14.9	Absent	ND	Absent	ND
Sensory nerve conduction studies					
Median SNAP (μV)	Absent	10.6	ND	22.0	ND
Median SNCV (m/s)	Absent	26.0	ND	51.3	ND
Ulnar SNAP (μV)	Absent	ND	ND	22.2	ND
Ulnar SNCV (m/s)	Absent	ND	ND	39.5	ND
Sural SNAP (μV)	Absent	8.8	ND	16.3	ND
Sural SNCV (m/s)	Absent	32.0	ND	39.8	ND

CMAP: compound muscle action potential, DTR: deep tendon reflexes, FDS: functional disability scale, MNCV: motor nerve conduction velocity, ND: not done, SNAP: sensory nerve action potential, SNCV: sensory nerve conduction velocity.

Table 2. Homozygous mutations and clinical phenotypes in the Pakistani CMT patients

Family ID	Gene	Mutation[a]	Type	Onset age (yr)	Other symptom	Allele frequency		GERP	*In silico* prediction[b]			Note
						1000G	ExAC		PP2	MU	PRO	
PaC2	*SH3TC2*	c.2599C>T;p.Q867*	CMT4C	3	Scoliosis	UR	UR	2.17	-	-	-	P
PaC3	*SH3TC2*	c.3650G>A;p.G1217D	CMT4C	3	Scoliosis, short stature	UR	1.6E-5	6.17	1.00*	0.10	-5.79*	P
PaC4	*HK1*	c.19C>T;p.R7*	CMT4G	1		UR	1.7E-5	1.13	-	-	-	P
PaC6	*REEP1*	c.247delG;p.G83Afs*44	SMARD/dHMN5B	< 1		UR	UR	5.33	-	-	-	LP
PaC14	*MFN2*	c.334G>A;p.V112M	CMT2A2B	5	Vocal cord hoarseness	UR	1.6E-5	4.70	1.00*	0.32	-2.76*	P

1000G: 1000 Genomes Project, CMT: Charcot-Marie-Tooth disease, ExAC: Exome Aggregation Consortium, GERP: genomic evolutionary rate profiling score, P: pathogenic, LP: likely pathogenic, UR: unreported.

[a]Reference DNA and protein sequences: *SH3TC2*: NM_024577.4 and NP_078853.2, *HK1*: NM_033498.3 and NP_277033.1, *REEP1*: NM_022912.3 and NP_075063.1, *MFN2*: NM_014874.3 and NP_055689.1.

[b]Scores of PolyPhen-2 (PP2) ~1, MUpro (MU) <0, and PROVEAN (PRO) <-2.5 indicate pathogenic prediction (* denotes a pathogenic prediction).

Table 3. Rare variants observed in the CMT-related genes from patients of five Pakistani CMT families

Family	Gene	Variant		ClinVar	dbSNP151	Allele frequency		GERP	*In silico* analysis		Note
		Nucleotide	Amino acid			1000G	ExAC		PP2	PRO	
PaC2	*KIF1B*	c.3209C>T	p.A1070V	UR	rs768176241	UR	1.7E-5	4.82	0.02	-0.01	Nonseg LB
	DST	[c.7252G>A + c.7765A>G]	[p.V2418I + p.I2589V]	UR	rs62621210	0.0400	0.0356	4.80	0.00	0.76	*Cis*, nonseg LB
				B,LB	rs150191284	0.0102	0.0239	4.45	0.02	0.07	
	MYH14	c.3748G>T	p.V1250L	LB	rs202242879	0.0006	0.0009	3.78	0.11	-0.39	Nonseg LB
	SCN11A	c.1732T>A	p.F578I	UR	rs772393665	UR	0.0001	5.58	0.98*	-4.61*	Nonseg LB
PaC3	*KIF1B*	[c.2107T>C] + [c.2455A>C]	[p.W703R] + [p.S819R]	B,LB	rs551543997	0.0054	0.0033	5.32	0.99*	-10.0*	*Trans*, nonseg LB
				LB	rs140015591	0.0002	0.0005	-2.96	0.02	-2.19	
	NTRK1	[c.2339G>A + c.2360C>T]	[p.R780Q + p.A787V]	B,LB	rs35669708	0.0038	0.0064	4.10	0.87*	-1.25	*Cis*, nonseg LB
				UR	rs761207548	UR	7.E-05	4.07	0.36	-3.00*	
	NAGLU	c.2209C>A	p.R737S	B	rs86312	0.0116	0.0192	4.01	0.47*	-0.19	Nonseg LB
	SCN10A	c.3887G>T	p.S1296I	LB	rs779527264	UR	0.0002	4.88	1.00*	-5.79*	Nonseg LB
PaC4	*DST*	[c.1933A>G] + [c.1933A>G]	[p.I645V] + [p.I645V]	UR	rs754692637	UR	8.E-06	2.51	0.00	-0.20	Homoz cosegre VUS
	ARHGEF10	c.2566G>A	p.V856I	UR	rs773521162	UR	0.0003	4.25	0.87*	-0.62	Nonseg LB
PaC6	*TFG*	c.175A>G	p.K59E	UR	rs1232918261	UR	UR	5.90	1.00*	-3.02*	Nonseg LB
PaC14	*SETX*	c.2385_2387delTTT	p.I795_K796delinsM	UR	rs755971927	UR	6.E-05	-	-	-	Nonseg LB

1000G: 1000 Genomes Project, ExAC: Exome Aggregation Consortium, B: benign, LB: likely benign, VUS: variant of uncertain significance, *trans*: *trans* arrangement of variants in homologous chromosomes (bi-alleles), *cis*: *cis* arrangement of variants in a chromosome, UR: unreported.