Original Article

Genetic Spectrum of Inherited Neuropathies in India

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Abstract

Background and Objectives: Charcot-Marie-Tooth (CMT) disease is the commonest inherited neuromuscular disorder and has heterogeneous manifestations. Data regarding genetic basis of CMT from India is limited. This study aims to report the variations by using high throughput sequencing in Indian CMT cohort. **Methods:** Fifty-five probands (M:F 29:26) with suspected inherited neuropathy underwent genetic testing (whole exome: 31, clinical exome: 17 and targeted panel: 7). Their clinical and genetic data were analysed. **Results:** Age at onset ranged from infancy to 54 years. Clinical features included early-onset neuropathy (*n*=23), skeletal deformities (*n*=45), impaired vision (*n*=8), impaired hearing (*n*=6), facial palsy (*n*=8), thickened nerves (*n*=4), impaired cognition (*n*=5), seizures (*n*=5), pyramidal signs (*n*=7), ataxia (*n*=8) and vocal cord palsy, slow tongue movements and psychosis in one patient each. Twenty-eight patients had demyelinating electrophysiology. Abnormal visual and auditory evoked potentials were noted in 60.60% and 37.5% respectively. Sixty two variants were identified in 37 genes including variants of uncertain significance (*n*=34) and novel variants (*n*=45). Eleven patients had additional variations in genes implicated in CMTs/ other neurological disorders. Ten patients did not have variations in neuropathy associated genes, but had variations in genes implicated in other neurological disorders. In seven patients, no variations were detected. **Conclusion:** In this single centre cohort study from India, genetic diagnosis could be established in 87% of patients with inherited neuropathy. The identified spectrum of genetic variations adds to the pool of existing data and provides a platform for validation studies in cell culture or animal model systems.

Keywords: Charcot-Marie-Tooth Disease, genetic modifiers, *MFN2*, next-generation sequencing, novel variations, *SH3TC2*

Introduction

Inherited neuropathies are a heterogeneous group of diseases that predominantly affect the peripheral nerves but may be associated with other features such as spasticity, ataxia, global developmental delay, etc., resulting in complex syndromes.[1] These are slow-progressive disorders characterized by distal symmetrical weakness of the limbs, hypo/areflexia, and skeletal deformities that are more pronounced in the lower limbs.[2,3] They commonly begin in the first two decades of life, cause progressive disability, and impair quality of life.[4,5] They are broadly categorized on the basis of electrophysiological studies as: (a) demyelinating with predominant involvement of peripheral myelin wherein nerve conduction velocities are reduced, and (ii) axonal with predominant involvement of peripheral nerve axons wherein amplitudes of compound muscle action potentials are reduced with normal or slightly reduced nerve conduction velocities.

Among the inherited neuropathies, the hereditary motor and sensory neuropathies (HMSNs) or Charcot-Marie-Tooth diseases (CMTs) constitute the most frequent genetically determined neuromuscular disorders.[6] They are caused by mutations in genes encoding proteins involved in different peripheral nerve functions such as maintenance and compaction of myelin in Schwann cells, axonal transport, as well as mitochondrial metabolism and dynamics.[7] Progress in the field of genomics with the advent of the next generation sequencing (NGS) technology has led to the identification of a number of genes involved in various subtypes of CMTs in different ethnic groups across the globe.[3,8-10] Identifying the molecular genetic abnormality establishes the diagnosis as well as aids in the treatment and reproductive planning.

India is a home to about one-sixth of the world population which is ethnically diverse, and has a distinct genetic landscape. Hospital based audits suggest that hereditary neuropathies account for 4.8% of all neuropathies.[11] Data on genetics of CMTs from India are rather limited.[12,13] This study is aimed to identify variants in disease-associated genes using high throughput sequencing in a cohort of CMT from India.

Patients and Methods

This study was carried out at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India.

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Probands were recruited from a single neurology unit between March 2017 and February 2020, after obtaining written informed consent. They included subjects with chronic progressive sensorimotor neuropathy of suspected genetic etiology who did not have evidence of alternative etiologies such as acquired, autoimmune, or metabolic causes. Patients with duplication or point mutations in the *PMP22* gene were published earlier and have not been included in the present study.[13] The clinical data included age, gender, symptom–duration, type of neuropathy, and sites of neuraxis affected. The functional/ambulatory status of the patients was objectively quantified using the CMT neuropathy score (CMTNS) and the modified Rankin Score (mRS).[14,15] Nerve conduction studies were carried out using standard protocols at a laboratory temperature of 32–34°C (Neuropack S1 MEB-9400K, Nihon Kohden Corporation, Tokyo, Japan). Three motor nerves (median, ulnar, and common peroneal nerves) and three sensory nerves (median, ulnar, and sural nerves) were examined. Conduction blocks were considered if the ratio of the amplitudes of compound muscle action potentials following proximal and distal stimulation was <0.5, provided the distal amplitude was at least 20% of the lower limit of normal.[16] Recordings of evoked potentials to visual and auditory stimuli were carried out wherever possible. The study was approved by the Institute Ethics Committee of NIMHANS.

Genomic DNA was extracted using standard phenol–chloroform method from about 6 mL of peripheral blood collected in an ethylenediaminetetraacetic acid (EDTA)-coated vacutainer. Genetic analysis was carried out using NGS (whole exome: 31, clinical exome: 17, and targeted panel: 7). The libraries were prepared, followed by enrichment as per manufacturer's instruction for sequencing with 80-100X denotes the coverage of the exonic regions. The sequences obtained were aligned to the human reference genome (GRCh37/hg19) and analyzed using Sentieon for removing duplicates, recalibration, and re-alignment of indels. Sentieon haplotype caller was used to identify variants relevant to the clinical phenotype.[17] Common variants were filtered based on allele frequency in 1000Genome Phase 3, ExAC (v1.0), gnomAD (bv2.1), EVS, dbSNP (v151), and 1000 Japanese Genome.[18-21] The identified variants were interpreted based on the recommendations of American College of Medical Genetics and Genomics (ACMG).[22] The pathogenicity of the identified variants was predicted using multiple tools namely PolyPhen-2, sorting intolerant from tolerant (SIFT), and mutation taster. The data were entered in a predesigned proforma and incorporated into a Microsoft Excel Spreadsheet for analysis.

Results

The current cohort comprised of 55 patients. The age at evaluation ranged from 2 to 72 years. The clinical and electrophysiological characteristics of patients are summarized in Table 1. In the present study, 62 variants were identified in 37 genes in these 55 probands. They included pathogenic/likely pathogenic variants (n = 28) and variants

Table 1: Clinical and electrophysiological characteristics of the current cohort of inherited neuropathy (N=55)

Parameter	Observed value
Males:Females	29:26
Age at evaluation	2-72 years.
Children (≤18 years)	25
Age at onset	Infancy to 54 years
Early onset neuropathy (onset ≤10 years)	23
Consanguineous parents	16
Positive family history	22
Developmental delay	9
Global delay	3
Motor delay	6
Skeletal deformities	
Pes cavus	38
Hammer toes	30
Clawed fingers	19
Kyphoscoliosis	5
Pes planus	2
Dyschromatosis universalis hereditaria	1
Impaired vision	8
Ocular abnormalities	7
Optic atrophy	6
Retinitis pigmentosa	1
Cataract	1
Sensorineural hearing impairment	8
Facial palsy	8
Vocal cord palsy	1
Slow tongue movements	1
Thickened nerves	4
Intellectual disability or cognitive decline	5
Psychosis	1
Seizures	4
Pyramidal involvement	7
Ataxia	8
Cerebellar	4
Sensory	2
Mixed	2
Functional rating scales	
CMT neuropathy score (mean±SD)	15.48±6.9
Modified Rankin score (mean±SD)	2.66±1.0
Electrophysiological tests	
Demyelinating neuropathy (conduction velocity of ulnar nerve <38 m/sec)	26
Conduction blocks	7
Abnormal visual evoked potentials*	20**
Prolonged P100 latency	18
Absent waveforms	2
Abnormal brainstem auditory evoked responses*	11***
All waveforms absent	5
Only waves I and III present	1
Only wave V present	5

*Testing for visual evoked potentials and brainstem auditory evoked responses was carried out in 33 patients. **Seven of these patients with abnormal visual evoked potentials were symptomatic for impaired vision. ***Six of these patients with abnormal brainstem auditory evoked responses were symptomatic for impaired hearing

of uncertain significance (n = 34). There were 17 reported and 45 novel variants. Of these, eight patients had variants in more than one gene (*MFN2+SBF, SH3TC2+AARS, SH3TC2+JPH 1, SH3TC2+UBQLN2, FGD4+WNK1, MARS+SBF2, MPZ+DNMT1,* and *GARS+GAN*) implicated in the neuropathy phenotype. Three patients had additional variants in genes that so far have not been associated with neuropathy (*DCTN1+BLK, IGHMBP2+SLC12A6,* and *MFN2+VPS13D*). Ten patients did not have variants in neuropathy associated genes, but had variants in genes such as *ATM*, *SETX*, *COX15*, *MPV17*, *OPA1*, *SACS*, *C10ORF2*, *APOB*, and *CDH23* which are implicated in other neurological disorders. In seven patients, no variants were detected [Tables 2 and 3]. *In silico* analysis showed that the detected variants were damaging [Supplementary Table 1].

Discussion

This study led to the identification of pathogenic/likely pathogenic variants in 87.3% cases, in addition to a number of novel variants as well as variants of uncertain significance (VUS). However, in other cohorts the detection rates ranged from 24% to 87% based on high throughput sequencing.[38-40] The clinical, demographic, and genetic features in various cohorts of CMT are compared with the present study [Supplementary Table 2].[87] Previous studies have shown that variants in *PMP22*, *GJB1*, *MPZ* and *MFN2* genes account for vast majority of the CMTs.[9,41] Variants in other genes though individually rare, constitute a large number, with nearly 100 genes being implicated in the pathogenesis of CMT.[42] Given the large number of genes implicated in CMT, it may be difficult to precisely pinpoint the genetic abnormality based on the phenotype, because of significant overlapping clinical features. For instance, vocal cord palsies have been described in both axonal and demyelinating neuropathies due to *MFN2*, *GDAP1*, *TRPV4*, *SH3TC2*, and *MTMR2* mutations.[43] Sensorineural hearing loss (SNHL) has also been reported in demyelinating and axonal CMT due to *PMP22*, *GJB1*, *MPZ*, *PRPS1*, and *SH3TC2* mutations, among others.[44-47] We used NGS to identify the genetic basis in patients of Indian origin with suspected inherited neuropathies in whom the *PMP22* variants had been excluded. The most frequent abnormality in the present study was in the *MFN2* gene (all pathogenic/likely pathogenic), which is similar to that noted in the previous studies. *MFN2* is reported to be the commonest cause of axonal CMT followed by *MORC2*.[48] In the present study, no variants in *MORC2* were identified. Variants in *SH3TC2* were the second most frequent abnormality in the present cohort (pathogenic/likely pathogenic = 3, VUS = 4), which is reported to be the commonest cause of recessively inherited demyelinating CMT.[49,50] Variants in *GJB1* and *MPZ* were identified in four and two patients, respectively. An interesting finding in the present cohort is that a proportion of patients had conduction blocks on electrophysiological testing. Classically, demyelination in CMT is considered to be uniform and conduction block is generally not expected in electrophysiological testing. However, while in the "pre-genetic" era it was believed that inherited neuropathies have uniform reduction in conduction parameters, there is growing evidence that some of the CMTs may exhibit non-uniform conduction abnormalities as well as conduction

Table 2: Summary of genetic analysis of patients with inherited neuropathy included in the present cohort

Parameter	Number
Total number of genes identified with variations	37
Total number of variants	62
Missense	46
Intronic	1
Insertion	0
Deletions	1
Frameshift	8
Nonsense	4
Splice site	2
Zygosity	
Homozygous (pathogenic/likely pathogenic/VUS)	23 (6/6/11)
Heterozygous (pathogenic/likely pathogenic/VUS)	36 (4/9/23)
Hemizygous (pathogenic/likely pathogenic/VUS)	3 (1/2/0)
Genes involved in patients with early onset neuropathy	*APOB, C10ORF2, CDH23, COX6A1, FGD4, GDAP1, HK, HSPB8, IGHMBP2, JPH1, MTMR2, OPA1, SBF1, SLC12A6, UBQLN2, WNK1*
Genes involved in patients with late onset neuropathy	*AARS, ATM, BLK, COX15, DCTN1, DNMT1, GARS, LRSAM1, MARS, MME, MPV17, MPZ, NAGLU, SACS, SETX, VPS13D*
Genes involved in patients with early and late onset neuropathy	*GAN, GJB1, MFN2, SBF2, SH3TC2*
Genes involved in patients with axonal neuropathy	*APOB, BLK, C10ORF2, CDH23, COX15, COX6A1, DCTN1, GDAP1, LRSAM1, MME, MPV17, MTMR2, SBF1, VPS13D*
Genes involved in patients with demyelinating neuropathy	*AARS, ATM, DNMT1, FGD4, GAN, GARS, GJB1, HK, HSPB8, IGHMBP2, JPH1, MPZ, NAGLU, OPA1, SACS, SBF2, SH3TC2, SLC12A6, UBQLN2*
Genes involved in patients with axonal and demyelinating neuropathy	*MARS, MFN2, SETX, WNK1*

Table 3: Genetic abnormalities identified in the present cohort of patients with inherited neuropathies ($n=55$)

Patient No	Clinical phenotype in addition to neuropathy	Demyelinating electrophysiology	Gene	Disease associated with genetic variant	Nucleotide change/Amino acid change/Zygosity	Inheritance	Classification	Reference	rsID
1	Facial weakness, pyramidal signs	No	*MFN2*	CMT2A	c. 281G>A/p.Arg94Gln/2Het	AD	Pathogenic	Reported[23]	rs28940291
2	Thickened nerves	No	*MFN2*	CMT2A	c. 605G>A/p.Gly202Asp/Het	AD	Likely pathogenic	Novel	Not available
			VPS13D	Spinocerebellar ataxia 4	c. 3005G>A/p.Gly1002Asp/Het	AR	VUS	Novel	Not available
3	Mild SNHL	No	*MFN2*	CMT2A	c. 281G>A/p.Arg94Gln/Het	AD	Pathogenic	Reported[23]	rs28940291
4	Seizures, intellectual disability, pyramidal signs	No	*MFN2*	CMT2A	c. 833T>C/p.Met278Thr/Het	AD	Likely pathogenic	Novel	Not available
5	-	No	*MFN2*	CMT2A	c. 371C>T/p.Ser124Phe/Homo	AR	Likely pathogenic	Novel	Not available
6	-	No	*MFN2*	CMT2A	c. 334G>A/p.Val112Met/Homo	AR	Likely pathogenic	Novel	rs757937208
7	Pyramidal signs	No	*MFN2*	CMT2A	c. 334G>A/p.Val112Met/Homo	AR	Likely pathogenic	Novel	rs757937208
8	-	No	*MFN2*	CMT2A	c. 310C>T/p.Arg104Trp/Het	AD	Pathogenic	Reported[24]	rs119103268
9	-	No	*MFN2*	CMT2A	c. 752C>G/p.Pro251Arg/Het	AD	Likely pathogenic	Reported[23]	rs1557525153
			SBF1	CMT 4B3	c. 2335C>G/p.Leu779Val/Het	AR	VUS	Novel	Not available
10	-	Yes	*SH3TC2*	CMT4C, mild mononeuropathy of median nerve	c. 1105C>T/p.Arg369Cys/Het	AR/AD	VUS	Novel	rs569974719
			AARS	CMT2	c. 2053G>A/p.Val685Met/Het	AD	VUS	Novel	Not available
11	-	Yes	*SH3TC2*	CMT4C, mild mononeuropathy of median nerve	c. 1412del/p.Leu471TrpfsTer53/Homo	AR	Pathogenic	Novel	Not available
			JPH1	CMT 2K	c. 803C>T/p.Pro268Leu/Het	AR/AD	VUS	Novel	rs756049890
12	Moderate SNHL, cerebellar and sensory ataxia	Yes	*SH3TC2*	CMT4C, mild mononeuropathy of median nerve	c. 3152G>A/p.Gly1051Glu/Homo	AR	VUS	Novel	Not available
			UBQLN2	ALS-15 with or without frontotemporal dementia	c. 1573C>T/p.Pro525Ser/Het	X-linked	VUS	Reported[25]	rs369947678
13	Facial weakness	Yes	*SH3TC2*	CMT4C, mild mononeuropathy of median nerve	c. 69del/p.Lys24ArgfsTer10/Homo	AR	Pathogenic	Novel	Not available
14	Thickened nerves	Yes	*SH3TC2*	CMT4C, mild mononeuropathy of median nerve	c. 3511C>T/p.Arg1171Cys/Het	AR/AD	Likely pathogenic	Reported[26]	rs759785462
			SH3TC2	CMT4C, mild mononeuropathy of median nerve	c. 2028G>C/p.Leu676Phe/Het	AR/AD	VUS	Novel	Not available
			SH3TC2	CMT4C, mild mononeuropathy of median nerve	c. 254A>T/p.Asp85Val/Het	AR/AD	VUS	Novel	Not available
15	-	Yes	*GJB1*	CMT1, HNPP	c. 548G>A/p.Arg183His/Het	X-linked	Pathogenic	Reported[27]	rs1555937233
16	-	Yes	*GJB1*	CMT1	c. 65G>A/p.Arg22Gln/Hemi	X-linked	Likely pathogenic	Reported[28]	rs1060501002

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Table 3: Contd...

Patient No	Clinical phenotype in addition to neuropathy	Demyelinating electrophysiology	Gene	Disease associated with genetic variant	Nucleotide change/Amino acid change/Zygosity	Inheritance	Classification	Reference	rsID
17	-	Yes	*GJB1*	CMT1	c. 217del/p.His73MetfsTer11/Hemi	X-linked	Pathogenic	Reported[29]	Not available
18	-	Yes	*GJB1*	CMT1	c. 77C>T/p.Ser26Leu/Hemi	X-linked	Likely pathogenic	Reported[30]	rs587777876
19	Facial weakness	Yes	*FGD4*	CMT4H	c. 1062_1063insT/p.Tyr355LeufsTer2/Homo	AR	Likely pathogenic	Novel	Not available
			WNK1	HSAN2, pseudohypoaldosteronism type 2	c. 7526C>A/p.Ser2509Tyr/Homo	AD/AR	VUS	Novel	Not available
20	-	Yes	*WNK1*	HSAN2, pseudohypoaldosteronism type 2	c. 2500G>A/p.Gly834Arg/Het	AD/AR	VUS	Novel	Not available
			WNK1	HSAN2, pseudohypoaldosteronism type 2	c. 4501+96C>A/Nil/Het	AD/AR	VUS	Novel	Not available
21	-	No	*GDAP1*	CMT 2K	c. 197C>G/p.Pro66Arg/Het	AR/AD	VUS	Novel	Not available
22	-	No	*GDAP1*	CMT 2K	c. 431C>T/p.Pro144Leu/Het	AR/AD	Likely pathogenic	Novel	rs786205591
23	Slow tongue movements, cerebellar ataxia	No	*SETX*	Spinocerebellar ataxia-1, spinocerebellar ataxia with axonal neuropathy 2, ataxia with oculomotor apraxia, Juvenile AlS-4	c. 3127_3128insA/p.Arg1043fs/Homo	AR	Likely pathogenic	Novel	Not available
24	Facial weakness	Yes	*SETX*	Spinocerebellar ataxia-1, spinocerebellar ataxia with axonal neuropathy 2, ataxia with oculomotor apraxia, juvenile AlS-4	c. 7195A>T/p.Ile2399Phe/Homo	AR	VUS	Novel	Not available
25	-	Yes	*ATM*	Ataxia telangiectasia	c. 4852C>T/p.Arg1618Ter/Het	AR	VUS	Novel	Not available
			ATM	Ataxia telangiectasia	c. 6899G>T/p.Trp2300Leu/Het	AR	VUS	Novel	Not available
26	OA, moderate SNHL, cerebellar ataxia, psychosis	No	*MARS*	CMT2U	c. 918_919del/p.Tyr307SerfsTer6/Het	AD	VUS	Novel	Not available
27	-	Yes	*MARS*	CMT2U	c. 2209C>T/p.Arg737Trp/Het	AD	Likely pathogenic	Reported[31]	Not available
			SBF2	CMT4B	c. 3110G>A/p.Arg1037His/Homo	AR	VUS	Novel	Not available
28	Cognitive decline, thickened nerves	Yes	*SBF2*	CMT4B	c. 5345_5354del/p.Asp1782ValfsTer10/Homo	AR	Pathogenic	Novel	Not available
29	-	No	*MME*	CMT2T	c. 1270G>A/p.Gly424Arg/Homo	AR	VUS	Novel	Not available
30	-	Yes	*MPZ*	CMT1B, CMT2J, CMT 2I, CMT with hearing loss and pupillary abnormalities	c. 223G>T/p.Asp75Tyr/Het	AD	VUS	Novel	Not available

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Table 3: Contd...

Patient No	Clinical phenotype in addition to neuropathy	Demyelinating electrophysiology	Gene	Disease associated with genetic variant	Nucleotide change/Amino acid change/Zygosity	Inheritance	Classification	Reference	rsID
31	Facial weakness, moderate SNHL	Yes	*MPZ*	CMT 1B	c. 207_212delGCCCGA/p. Pro70_Glu71del/Het	AD	VUS	Novel	Not available
			DNMT1	HSAN 1E, AD cerebellar ataxia, CMT	c. 1018G>A/p.Ala340Thr/Het	AD	VUS	Novel	rs529074384
32	Cognitive decline, thickened nerves	No	*LRSAM1*	CMT 2P	c. 2120C>T/p.Pro707Leu/Het	AD	Likely pathogenic	Novel	rs797044913
			LRSAM1	CMT 2P	c. 49C>T/p.Arg17Cys/Het	AD	VUS	Novel	rs368646898
33	-	No	*DCTN1*	dHMN VIIB, Perry syndrome, ALS	c. 3746C>T/p.Thr1249Ile/Het	AD	Likely pathogenic	Reported[32]	rs72466496
			BLK	Maturity onset diabetes of young type 11, systemic scleroderma, rheumatoid arthritis	c. 211G>A/p.Ala71Thr/Het	AD	VUS	Reported[33]	rs55758736
34	-	No	*COX15*	Leigh syndrome due to cytochrome c oxidase deficiency	c. 520G>A/p.Gly174Ser/Homo	AR	Likely pathogenic	Novel	rs763842058
35	Pyramidal signs	No	*COX6A1*	Intermediate CMTD	c. 247-7_247-3del (3' proximal splice site)/Homo	AR	Pathogenic	Reported[34]	rs587777783
36	Facial weakness	Yes	*GARS*	CMT 2D, dHMN VA	c. 1172G>A/p.Arg391His/Het	AD	VUS	Novel	rs370057212
			GAN	Giant axonal neuropathy 1	c. 944C>G/p.Pro315Arg/Het	AR	VUS	Reported[35]	rs144486241
37	-	Yes	*GAN*	Giant axonal neuropathy 1	c. 444C>G/p.His148Gln/Homo	AR	VUS	Novel	Not available
38	Intellectual disability, facial weakness, seizures	Yes	*HK*	Russe type of HMSN	c. 19C>T/p.Arg7Ter/Homo	AR	Pathogenic	Novel	rs779250530
39	Sensory ataxia, pyramidal signs	No	*IGHMBP2*	CMT2S, AR distal SMA1, dHMN	c. 1523C>T/p.Ser508Leu/ Homo	AR	Likely pathogenic	Reported[36]	rs754465226
			SLC12A6	Hartnup disease, Andermann syndrome	c. 1625T>C/p.Ile542Thr/Homo	AR	VUS	Novel	Not available
40	-	No	*MPV17*	Mitochondrial DNA depletion syndrome-6	c. 280G>T/p.Gly94Trp/Homo	AR	VUS	Novel	Not available
41	Facial weakness, vocal cord palsy	No	*MTMR2*	CMT4B	c. 484C>T/p.Arg162Ter/Homo	AR	Pathogenic	Novel	rs756723587
42	-	Yes	*NAGLU*	CMT2V	c. 325C>T/p.Arg109Cys/Het	AD	VUS	Novel	Not available
43	OA, RP, cataract, cerebellar ataxia	No	*OPA1*	Optic atrophy plus syndrome	c. 1045C>T/p.Arg349ter/Het	AD	Pathogenic	Novel	Not available
44	Mild SNHL	Yes	*SACS*	Spastic ataxia of Charlevoix-Saguenay type	c. 8980C>T/p.Pro2994Ser/ Homo	AR	VUS	Novel	Not available
45	Severe SNHL, sensory ataxia	No	*C10ORF2*	Mitochondrial DNA depletion syndrome-7	c. 876delT/p. Ala293ProfsTer33/Het	AR	Likely pathogenic	Novel	rs772683219
46	Pyramidal signs	Yes	*HSPB8*	CMT 2L, dHMN	c. 71C>T/p.Ser24Phe/Het	AD	VUS	Novel	rs781475312
47	OA, seizures, cerebellar ataxia	No	*APOB*	Familial hypercholesterolemia, familial hypobetalipoproteinemia	c. 13441G>A/p.Ala4481Thr/ Homo	AR	VUS	Reported[37]	rs1801695

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Table 3: Contd...

Patient No	Clinical phenotype in addition to neuropathy	Demyelinating electrophysiology	Gene	Disease associated with genetic variant	Nucleotide change/Amino acid change/Zygosity	Inheritance	Classification	Reference	rsID
48	OA, severe SNHL	No	*CDH23*	Usher syndrome, non-syndromic hearing loss, age-related hearing loss	c. 1589-7C>T/Homo	AR		Novel	Not available
49	-	Yes	Nil						
50	-	No	Nil						
51	Pyramidal signs	No	Nil						
52	-	Yes	Nil						
53	Mild SNHL, seizures	Yes	Nil						
54	OA	No	Nil						
55	Cognitive decline, OA, cerebellar and sensory ataxia	No	Nil						

AD: Autosomal dominant, ALS: Amyotrophic lateral sclerosis, AR: Autosomal recessive, dHMN: distal hereditary motor neuronopathy, Hemi: hemizygous, Het: heterozygous, HNPP: hereditary europathy with liability to pressure Palsy, Homo: Homozygous, HSAN: Hereditary sensory autonomic neuropathy

blocks. In fact, conduction block and non-uniform slowing can form the basis for targeted genetic testing (e.g., X-linked CMT).[51]

In the present study, in addition to the common genes (*GJB1*, *MPZ*, and *MFN2*), variants were identified in a number of genes implicated in various cellular functions such as growth and differentiation (*SBF1*, *DCTN1*), endocytosis (*SH3TC2*, *LRSAM1*), tRNA synthetases (*AARS*, *MARS*, *GARS*), intracellular calcium homeostasis (*JPH 1*), ubiquitin-proteasome system (*UBQLN2*, *GAN*), actin cytoskeleton regulation (*FGD4*), DNA repair (*SETX*, *ATM*), transcriptional regulation (*IGHMBP2*, *DNMT1*), protein homeostasis (*MME*, *HSPB8*), mitochondrial function including dynamics and maintenance (*MPV17*, *COX6A1*, *COX15*, *VPS13*, *OPA1*, *C10ORF2*), and ion transport (*SLC12A6*). Besides these, alterations in endoplasmic reticulum structure (*TFG*, *ATL1*), membrane or vesicle trafficking (*LITAF*, *SBF1*, *DNM2*, *FIG4*), myelin structural organization (*PRX*), axonal cytoskeleton maintenance (*NEFL*, *NEFH*), and axonal transport (*KIF1*, *DCTN1*, *SPG11*) have been reported from other cohorts of HMSN.[66,67]

The CMTs may follow autosomal or X-linked, dominant or recessive pattern of inheritance. Homozygous or compound heterozygous variants in the "dominantly" inherited genes resulting in recessive CMTs with early onset and more severe disability have been reported. For example, *MFN2* is usually linked to autosomal dominant (AD) inheritance but autosomal recessive (AR, homozygous or compound heterozygous variants) pattern is also reported where the proband inherits one mutation from each parent. The heterozygous parents can be asymptomatic or may manifest with late-onset milder phenotype, in contrast to early-onset severe phenotype in the proband bearing two mutations.[68,69] We also report two homozygous variants in *MFN2* in three subjects with early-onset neuropathy [Patients 5–7, Table 3]. In contrast to compound heterozygous variants that occur *in trans*, distantly spaced double variants *in cis* have also been reported uncommonly in *MFN2*.[70,71] Likewise, *LRSAM1* variants are dominantly inherited and are associated with CMT2 phenotype with onset in the second decade of life and moderate disability.[72] In the present study, patient 32 had early-onset neuropathy with severe disability and two heterozygous variants in *LRSAM1*. The presence of two variants might have contributed to the increased disease severity. This phenomenon may be comparable to severe phenotypes associated with homozygous/compound heterozygous mutations in *MFN2*.

The present study identified variants in multiple genes in 11 patients. There are a few reports highlighting co-occurrence of variants of multiple genes in the same individual with CMT.[73-78] Such variants are often inherited not only from heterozygous carrier parents, but can also occur *de novo*. High throughput sequencing permits unbiased analysis of several genes and helps in identifying all the variants which could have been missed in sequential analysis. Traditional

sequencing of genes in tandem does not extend testing for other genes once a genetic variant that explains the phenotype is identified. Using NGS one can identify multiple genetic variants in different combinations: (a) more than one CMT-associated genes, or (b) CMT-associated gene(s) implicated in other neurodegenerative disorders. Two variants identified in different neuropathy-related genes may cause mild phenotype when they occur in isolation, but when present together may have additive effect on severity of symptoms by causing pathology at different sites. The non-neuropathy related genetic variants may act as modifier when present together with another gene known to cause neuropathy.[77] The most common cause of demyelinating CMT, that is, *PMP22* duplication has been reported with additional dose of *PMP22* (triplication) resulting in more severe clinical phenotype.[79,80] There are reports on *PMP22* mutation along with other related genes like *LITAF*, *SMN2*, *DCTN1*, *GJB1*, *FSHD*, and *ABCD1* [Supplementary Table 3].[73-76] Coexistence of variants in *MFN2* and *GDAP1* in axonal CMT has also been reported. The two variants act in a synergistic manner resulting in major mitochondrial defects as each gene is involved in mitochondrial bioenergetics either for adenosine triphosphate (ATP) production or respiratory chain complex I activity.[81-84] Apart from *PMP22* and *MFN2*, there are selected reports on other gene combinations such as *JPH 1*/*GDAP1* and *EGR2*/*GJB1*.[55,77,85] Increased genetic "burden" arising from this combination of genetic mutations may contribute to phenotypic variability including age at onset and disease severity.[78] Based on the available literature, we hypothesize that the co-occurence of multiple genetic variants may have impacted the clinical phenotype including the severity in the present cohort. However, we did not establish the synergy between the multiple variants and their impact on the phenotypes by using *in vitro* studies or animal models. This is a limitation of the present study.

The present study identified a number of novel variants and VUS which require to be validated for confirming their pathogenicity. Previous studies have also reported a large number of VUS, ranging from 10 to as high as 215 in a single cohort.[56,86] Reporting of VUS is dependent on the ACMG guidelines. Validating individual variants in various genes may not be an efficient approach given the low frequency of individual genetic variants other than *PMP22*, *MPZ*, *GJB*, and *MFN2* genes. Due to the prevailing high levels of genetic heterogeneity, narrowing down to common cellular pathways through network biology approach and forming "disease modules" may prove to be more useful in understanding the pathobiology even in patients who are "negative" for genetic abnormalities by whole exome sequencing (WES). Various genes reported in the context of hereditary neuropathies act on interconnected pathways and share common proteins to carry out the overlapping biological functions. The peripheral nervous tissue being highly metabolically active needs constant maintenance of a pool of proteins and other molecular interactors. Mutations in any one of the associated genes resulting in abnormal protein can have a cascading effect on the protein interactome and may fail to maintain the cellular homeostasis. This effect is propagated along the nerve function adding to disease pathology. These networks of proteins and their molecular partners can be exploited further to understand the disease pathogenesis and further translated for drug development and therapeutics.[52]

In conclusion, we report the NGS findings in a fairly large cohort of patients with inherited neuropathies from India and highlight the spectrum of genetic abnormalities. This study brings out a number of novel variants and VUS. Establishing an accurate genetic diagnosis is important not only for genetic counseling but also in the perspective of including patients for upcoming therapeutic trials. NGS identified variants in several genes, including those that have pathobiological significance in neuropathy and other non-neuropathic disorders. The functional validation of novel variants and the impact of their interactions with other molecular partners remain to be established in future studies.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: *In silico* prediction of the pathogenicity of the identified variants

Patient No	Gene	Nucleotide change/Amino acid change	Zygosity	ACMG Classification	SIFT	Polyphen2	LRT	Mutation taster
1	*MFN2*	c. 281G>A/p.Arg94Gln	Het	Pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
2	*MFN2*	c. 605G>A/p.Gly202Asp	Het	Likely pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
	VPS13D	c. 3005G>A/p.Gly1002Asp	Het	VUS	Affect protein function	-	-	Damaging
3	*MFN2*	c. 281G>A/p.Arg94Gln	Het	Pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
4	*MFN2*	c. 833T>C/p.Met278Thr	Het	Likely pathogenic	Tolerated	Benign	Damaging	Damaging
5	*MFN2*	c. 371C>T/p.Ser124Phe	Homo	Likely pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
6	*MFN2*	c. 334G>A/p.Val112Met	Homo	Likely pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
7	*MFN2*	c. 334G>A/p.Val112Met	Homo	Likely pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
8	*MFN2*	c. 310C>T/p.Arg104Trp	Het	Pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
9	*MFN2*	c. 752C>G/p.Pro251Arg	Het	Likely pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
	SBF1	c. 2335C>G/p.Leu779Val	Het	VUS	Affect protein function	Possibly damaging	Damaging	Damaging
10	*SH3TC2*	c. 1105C>T/p.Arg369Cys	Het	VUS	Tolerated	Possibly damaging	-	Damaging
	AARS	c. 2053G>A/p.Val685Met	Het	VUS	Tolerated	-	Damaging	Damaging
11	*SH3TC2*	c. 1412del/p.Leu471TrpfsTer53	Homo	Pathogenic	-	-	-	Damaging
	JPH 1	c. 803C>T/p.Pro268Leu	Het	VUS	Tolerated	Benign	Damaging	Damaging
12	*SH3TC2*	c. 3152G>A/p.Gly1051Glu	Homo	VUS	Affect protein function	Probably damaging	Damaging	Damaging
	UBQLN2	c. 1573C>T/p.Pro525Ser	Het	VUS	Tolerated	Benign	-	Damaging
13	*SH3TC2*	c. 69del/p.Lys24ArgfsTer10	Homo	Pathogenic	-	-	-	Damaging
14	*SH3TC2*	c. 3511C>T/p.Arg1171Cys	Het	Likely pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
	SH3TC2	c. 2028G>C/p.Leu676Phe	Het	VUS	Affect protein function	Probably damaging	Damaging	Damaging
	SH3TC2	c. 254A>T/p.Asp85Val	Het	VUS	Tolerated	Possibly damaging	-	Damaging
15	*GJB1*	c. 548G>A/p.Arg183His	Het	Pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
16	*GJB1*	c. 65G>A/p.Arg22Gln	Hemi	Likely pathogenic	Affect protein function	Possibly damaging	Damaging	Damaging
17	*GJB1*	c. 217del/p.His73MetfsTer11	Hemi	Pathogenic	-	-	-	Damaging
18	*GJB1*	c. 77C>T/p.Ser26Leu	Hemi	Likely pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
19	*FGD4*	c. 1062_1063insT/p.Tyr355LeufsTer2	Homo	Likely pathogenic	-	-	-	-
	WNK1	c. 7526C>A/p.Ser2509Tyr	Homo	VUS	-	-	-	-
20	*WNK1*	c. 2500G>A/p.Gly834Arg	Het	VUS	-	Damaging	-	-
	WNK1	c. 4501+96C>A/Nil	Het	VUS	-	-	-	-
21	*GDAP1*	c. 197C>G/p.Pro66Arg	Het	VUS	Affect protein function	-	-	-
22	*GDAP1*	c. 431C>T/p.Pro144Leu	Het	Likely pathogenic	Affect protein function	-	-	-
23	*SETX*	c. 3127_3128insA/p.Arg1043fs	Homo	Likely pathogenic	-	-	-	-
24	*SETX*	c. 7195A>T/p.Ile2399Phe	Homo	VUS	Affect protein function	Possibly damaging	Damaging	Damaging
25	*ATM*	c. 4852C>T/p.Arg1618Ter	Het	VUS	-	-	-	-
	ATM	c. 6899G>T/p.Trp2300Leu	Het	VUS	Tolerated	-	-	-
26	*MARS*	c. 918_919del/p.Tyr307SerfsTer6	Het	VUS	-	-	-	Damaging
27	*MARS*	c. 2209C>T/p.Arg737Trp	Het	Likely pathogenic	Affect protein function	Possibly damaging	Damaging	Damaging
	SBF2	c. 3110G>A/p.Arg1037His	Homo	VUS	Affect protein function	Possibly damaging	Damaging	Damaging
28	*SBF2*	c. 5345_5354del/p.Asp1782ValfsTer10	Homo	Pathogenic	-	-	-	Damaging

Contd...

Supplementary Table 1: Contd...

Patient No	Gene	Nucleotide change/Amino acid change	Zygosity	ACMG Classification	SIFT	Polyphen2	LRT	Mutation taster
29	*MME*	c. 1270G>A/p.Gly424Arg	Homo	VUS	Affect protein function	Probably damaging	Damaging	Damaging
30	*MPZ*	c. 223G>T/p.Asp75Tyr	Het	VUS	Affect protein function	Probably damaging	Damaging	Damaging
31	*MPZ*	c. 207_212delGCCCGA/p.Pro70_Glu71del	Het	VUS	-	-	-	-
	DNMT1	c. 1018G>A/p.Ala340Thr	Het	VUS	Tolerated	Benign	Benign	Benign
32	*LRSAM1*	c. 2120C>T/p.Pro707Leu	Het	Likely pathogenic	Affect protein function	Probably damaging	Damaging	Damaging
	LRSAM1	c. 49C>T/p.Arg17Cys	Het	VUS	Affect protein function	Probably damaging	Damaging	Damaging
33	*DCTN1*	c. 3746C>T/p.Thr1249Ile	Het	Likely pathogenic	Affect protein function	-	-	Damaging
	BLK	c. 211G>A/p.Ala71Thr	Het	VUS	-	Possibly damaging	-	-
34	*COX15*	c. 520G>A/p.Gly174Ser	Homo	Likely pathogenic	Tolerated	Probably damaging	Damaging	Damaging
35	*COX6A1*	c. 247-7_247-3del (3' proximal splice site)	Homo	Pathogenic	-	-	-	Damaging
36	*GARS*	c. 1172G>A/p.Arg391His	Het	VUS	Tolerated	Possibly damaging	Damaging	Damaging
	GAN	c. 944C>G/p.Pro315Arg	Het	VUS	Tolerated	Benign	-	Damaging
37	*GAN*	c. 444C>G/p.His148Gln	Homo	VUS	Tolerated	Probably damaging	Damaging	Damaging
38	*HK*	c. 19C>T/p.Arg7Ter	Homo	Pathogenic	-	-	-	Damaging
39	*IGHMBP2*	c. 1523C>T/p.Ser508Leu	Homo	Likely pathogenic	Affect protein function	Damaging	Damaging	Damaging
	SLC12A6	c. 1625T>C/p.Ile542Thr	Homo	VUS	Tolerated	Possibly damaging	-	-
40	*MPV17*	c. 280G>T/p.Gly94Trp	Homo	VUS	Tolerated	Possibly damaging	-	Damaging
41	*MTMR2*	c. 484C>T/p.Arg162Ter	Homo	Pathogenic	-	-	-	Damaging
42	*NAGLU*	c. 325C>T/p.Arg109Cys	Het	VUS	-	Possibly damaging	-	Damaging
43	*OPA1*	c. 1045C>T/p.Arg349ter	Het	Pathogenic	-	-	-	Damaging
44	*SACS*	c. 8980C>T/p.Pro2994Ser	Homo	VUS	-	Possibly damaging	Damaging	Damaging
45	*C10ORF2*	c. 876delT/p.Ala293ProfsTer33	Het	Likely pathogenic	-	-	-	-
46	*HSPB8*	c. 71C>T/p.Ser24Phe	Het	VUS	Tolerated	Possibly damaging	Damaging	Damaging
47	*APOB*	c. 13441G>A/p.Ala4481Thr	Homo	VUS	-	Benign	-	-
48	*CDH23*	c. 1589-7C>T	Homo	-	-	-	-	-

Supplementary Table 2: Epidemiological, demographic, clinical and genetic features in various cohorts of Charcot Marie Tooth disease

Author/Year	Country	Cohort Number	M: F ratio	Age at onset (years)	Sporadic/ Familial	Genetic Test	Genetic Diagnosis	Reported/Novel	VUS
Mostacciuolo *et al*., 2001[53]	Italian	172 cases	-	-	35/104	Targeted gene sequencing	*PMP22* duplication=98/170 cases *MPZ* point mutations: 4 variants *PMP22* missense mutations: 2 variants C×32: 12 variants	Reported=14 Novel=4	-
Sivera *et al*., 2013[54]	Spanish	438 cases	-	-	-	Targeted gene sequencing	Total yield=365/438 (83.3%) Most common: PMP22 duplication=184 cases Point mutations- GJB1=56 cases *GDAP1*=42 cases *SH3TC2*=27 cases *MPZ*=19 cases *NDRG1*, *HSPB1*=7 cases each *MFN2*=6 cases *HK1*=5 cases	Novel=17	-
Manganelli *et al*., 2014[57]	Italian	197 cases	-	-	47/101	Targeted gene sequencing	Total yield=148/197 (75.1%) *PMP22*=107 *GJB1*=14 *GDAP1*=8 *MPZ*=7 *SH3TC2*=3 *MFN2*=2	Novel=12	-
Hoyer *et al*., 2015[56]	Norway	103 cases	48:55	-	-	MLPA, Targeted gene sequencing	Total yield=35/103 (33.9%) Point mutations=28 cases Copy number variations=7 cases	-	10
Antoniadi *et al*., 2015[86]	-	448 cases	-	-	-	Targeted gene sequencing	Total yield=137/448 (30.5%) 195 variants in 31 genes for 137 patients AD inheritance=93/137 AR inheritance=32/137 X-linked inheritance=9/137	Reported=107 Novel=88	215
Drew *et al*., 2015[58]	-	110 cases	-	-	2/108	Whole exome sequencing	Total yield=21/110 (19.09%) HMN=2 cases HMNP=4 cases CMT2=10 cases CMT1=1 case CMTX=4 cases	Reported=9 Novel=12	-

Contd...

Supplementary Table 2: Contd...

Author/Year	Country	Cohort Number	M: F ratio	Age at onset (years)	Sporadic/ Familial	Genetic Test	Genetic Diagnosis	Reported/Novel	VUS
Rudnik-Schöneborn *et al*., 2016[59]	German	1330 cases	-	Varied from early infantile (<2 years), to late adult (>50 years)	894/436	MLPA, Targeted gene sequencing	AD/X-linked inheritance (axonal) = 108/340 (31.8%) AD/X-linked inheritance (demyelinating) = 275/674 (40.8%) Autosomal recessive inheritance (axonal) = 15/340 (4.4%) Autosomal recessive inheritance (demyelinating) = 38/674 (5.6%)	-	
Nam *et al*., 2016[60]	Korean	78 families	-	1-49 years	-	Hexaplex microsatellite PCR, Targeted panel sequencing	Total yield=17/78 (21.7%) *GJB1*=6 variants *MPZ*=2 variants *SH3TC2*=1 pair of compound heterozygous *PMP22, MARS, MFN2, SPTLC2, DCTN1*=1 variant each	Reported=7 Novel=8	-
Li *et al*., 2016[61]	Chinese	22 cases	17:5	Childhood to 46 years	-	MLPA, Targeted gene sequencing	*PMP22* duplication=8/22 Possible pathogenic variants: 11/22	Reported=7 Novel=3	-
Sun *et al*., 2017[10]	Chinese Han	106 patients, NGS done on 82	57:25	Mean 30±15 years	86/20	NGS	*PMP22* duplication=10 patients *GJB1* mutation=9 patients *PMP22* deletion=2 patients *MFN2* mutation=2 patients *NEFL, SH3TC2, HSPB1, PRX*=1 patient each	Reported=15(single base exchange) Reported Copy number variation=2(PMP22 duplication, PMP22 deletion) Novel=6(single base exchange)	-
Dohrn *et al*., 2017[62]	German	612 cases	294:318	-	289/217	MLPA and NGS	Total yield=121/612 (19.7%) *PMP22*=16.4% *GJB1*=10.7% *MPZ & SH3TC2*=9.9% *MFN2*=8.3%	Reported=121 cases Novel=34 variants	201
Bacquet *et al*., 2018[38]	French	179 cases (123 prospective and 56 retrospective)	-	-	-	Targeted panel of genes causing inherited disorders	Total yield=49/123 (39.8%) CMT1=19/28 CMT2=27/64 dHMN=5/11 HSAN=5/9 Internediate CMT=4/8	Reported=26 Novel=52	17
Milley *et al*., 2018[9]	Hunagrian and Roma	531 cases	289:242	First decade to seventh decade of life	142/148	MLPA, qPCR, targeted gene sequencing	Total yield=59.9% CMT1=276 CMT2=42	Reported=30 Novel=6	-

Contd...

Supplementary Table 2: Contd...

Author/Year	Country	Cohort Number	M: F ratio	Age at onset (years)	Sporadic/ Familial	Genetic Test	Genetic Diagnosis	Reported/Novel	VUS
Hartley *et al.*, 2018[24]	Canadian	50 index patients and 23 affected/ unaffected family members	-	Adult onset: 34 cases Pediatric onset: 16 cases	11/39	NGS	Total yield=12/50 (24%) HMSN=8/34 HMN=4/11 HSN or HSAN=0/5	-	11
Hoebeke *et al.*, 2018[39]	French	75 cases from 59 different families	1.8:1	Mean: 4.1 years	21/54	MLPA, Targeted gene sequencing, Targeted inherited disease panel for NGS	*PMP22* duplication=46/75 *MFN2* mutations=11/75 Other genes=18/75	Reported=12 Novel=10	-
Yoshimura *et al.*, 2018[63]	Japanese	1005 patients	-	-	570/413	NGS (CMT panel)	Total yield=301/1005 (30%)	-	-
Khadilkar *et al.*, 2017[12]	Indian	22 patients	19:3	-	18/4	NGS	Total yield=13/22 (63.07%)	-	3
Hsu *et al.*, 2019[64]	Taiwanese	427 patients	248:179	Mean=23.8±17.4 years Range=1-72 years	177250	Real time fluorescent PCR for *PMP22*, direct sequencing of *PMP22, GJB1, MPZ, MFN2, NEFL, AARS, HSPB1, GDAP1*. NGS	Total yield=312/427 (73.1%) Demyelinating CMT=266/315 (84.4%) Axonal CMT=46/112 (41.1%)	Reported=69 Novel=12	-
Cortese *et al.*, 2020[8]	UK and US	220 cases	136:84	-	111/109-	NGS	Demyelinating CMT=30/41 axonal or intermediate=32/143 dHMN =/21 HSN=2/15	Novel=30	98
Taghizadeh *et al.*, 2020[65]	Iran	58 patients	-	Mean=13 years Range=4 months to 63 years	-	NGS (WES)	Total yield=27/58 (46.6%)	Reported=16 Novel=11	-
Xie *et al.*, 2021[87]	Chinese	435 families	268:167	1-60 years	221/214	MLPA for *PMP22*, NGS (CMT panel)	Total yield=304/435 (70%)	Reported=140 Novel=20	66
Current cohort	Indian	55 patients	29:26	Infancy to 54 years	Sporadic=33 Familial=22	NGS (WES=31, CES=17, Neurology/CMT panel=7)	28/55 (50.9%)	Reported=17 Novel=45	33

dHMN: distal Hereditary motor neuronopathy, HSAN: Hereditary sensory and autonomic neuropathy, HSN: Hereditary sensory neuropathy, MLPA: Multiplex ligation-dependent probe amplification

Supplementary Table 3: Impact of mutations in multiple genes on neuropathy phenotype

Author/year	Gene combination	Number of subjects	Impact on phenotype
Kim *et al*., 2015[70]	*PMP22* triplication	1 case	Proband: severely affected: triplication, mildly affected family members: duplication
Liu *et al*., 2014[71]	*PMP22* triplication		Triplication is caused due to Lenovo mutation from maternal origin and results in severe phenotype as compared to usual duplication
Meggouh *et al*., 2005[50]	*PMP22* and *LITAF*	1 case	PMPP22 causes inefficient protein folding and variations in *LITAF* may hamper protein degradation pathway, altogether affecting the clearance of misfolded protein. Modifier genes can play role for pathogenesis of disease
Fernández *et al*., 2016[51]	*SMN2* and *PMP22*	1 case	Clinical phenotype suggestive of SMN, Elder brother was diagnosed with CMT
Hodapp *et al*., 2006[66]	*PMP22* and *GJB1*, *PMP22* and *DCTN1*, *PMP22* and *ABCD1*	3 families	Presence of two gene variants resulted in cumulative effect on severity of symptoms, and individual variant itself was correlated with respective function in peripheral nerve
Chung *et al*., 2005[76]	*EGR2* (R359W) and *GJB1* (V136A)	Screening: 125 CMT families, described: 1 family; 5 members	Proband carrying both the mutations had severe phenotype while father having mutation only in *EGR2* gene presented with mild phenotype. The difference in clinical presentation might be either due to genetic modifier in *EGR2* (mild phenotype) or cumulative effect of both the mutations (severe phenotype)
Kim *et al*., 2010[77]	*DMPK* (CTG repeats) and *GJB1* (R149Q)	1 family	*DMPK* inherited from father (80 fold as compared to 220 fold in proband), mother was found normal for the repeats, *GJB1*: mother carried heterozygous mutation but this variant was absent in father
Vital *et al*., 2012[75]	*MFN2* (V160fs) and *GDAP1* (R120W)	1 family	Mother and father both heterozygous carriers of one mutation each. Proband and her daughter have both the variants. Synergistic effect of two mild variants resulted in severe phenotype observed in the second generation (proband) and her daughter as well
Kostera-Pruszczyk *et al*., 2014[74]	*MFN2* (T236M) and *GDAP1* (H123R)	1 case	*MFN2* variant: inherited from maternal line (mother and paternal grandfather carriers); results in impaired mitochondrial energy coupling and *GDAP1* variant: denovo; impairs mitochondrial transmembrane potential. Individual variants presents with mild phenotypes as reported earlier in literature, but this combination resulted in severe phenotype
Anghelescu *et al*., 2017[72]	*MFN2* (P201L) and *GDAP1* (E222K)	1 case	Proband: both *GDAP1* and *MFN2* mutations. Father and paternal grandmother carriers of *GDAP1* variant, *MFN2* variant was not found in any of the family members examined. Father and paternal grandmother had mild phenotypic presentation, de novo *MFN2* mutation or co-existence of *MFN2* and *GDAP1* may explain the severity of disease in the proband
Cassereau *et al*., 2011[73]	*MFN2* (R468H) and *GDAP1* (Q163X)	1 family	*MFN2* variant results in mild phenotype, as ATP production remains normal in this case even when there is defect in energy coupling, but *GDAP1* variant results in decreased ATP production because of impairment in Complex I activity. Simultaneous *MFN2* and *GDAP1* mutations cause major mitochondrial defects in a patient with CMT. The synergistic effect of these two mutations prove to be deleterious and hence explaining the severity of phenotype
Pla-Martín *et al*., 2015[68]	*JPH 1* and *GDAP1*	-	*GDAP1* variant (R120W) and JPH 1 variant (R213P) collectively mimics the phenotype of *GDAP1* knock-down cells as they both are involved in calcium homeostasis
Schreiber *et al*., 2013[67]	FSHD and *PMP22*	1 case	Overlap of two phenotypes