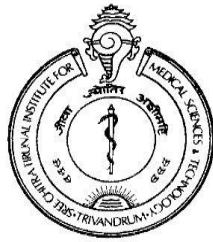


**SREE CHITRA TIRUNAL INSTITUTE FOR
MEDICAL SCIENCES AND TECHNOLOGY**

THIRUVANANTHAPURAM, KERALA



**ELECTROCLINICAL PROFILE OF CHARCOT
MARIE TOOTH DISEASE AND GENOTYPE
PHENOTYPE CORRELATION**

Dissertation submitted in partial fulfilment of the rules and regulations for

Post Doctoral Fellowship in Neuromuscular disorders

Sree Chitra Tirunal Institute for Medical Sciences and Technology

By

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01/01/2022 to 31/12/2022

DECLARATION

I, Dr. Aravinda Mandalapu, hereby declare that this project was undertaken by me under the supervision of Dr Sruthi S Nair, Associate Professor, Program supervisor of Neuromuscular disorders, Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology.

Thiruvananthapuram,
Date: 30-11-2022.

Dr. Aravinda Mandalapu

Forwarded:

The candidate, Dr. Aravinda Mandalapu, has completed the project under my guidance.

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INDEX

i.	Introduction	1
ii.	Review of Literature	5
iii.	Aims and objectives of the study	44
iv.	Materials and methods	46
v.	Results	50
vi.	Discussion	74
vii.	Conclusions	85
viii.	References	88
ix.	Appendix	97
	a. Abbreviations	98
	b. Participant proforma	101
	c. Ethics Committee approval letter	108
	d. Plagiarism Check Report	111

INTRODUCTION

"Inherited peripheral neuropathies" are a group of disorders that comprise Hereditary motor and sensory neuropathies (HMSN), Hereditary motor neuropathies (HMN), and Hereditary sensory neuropathies (HSN) or hereditary sensory and autonomic neuropathies (HSAN). Hereditary motor and sensory neuropathies are the commonest, also known as Charcot-Marie-Tooth disease (CMT).^{1, 2}

Jean Marie Charcot and Pierre Marie Howard Henry Tooth, in 1886, first described CMT.¹ Dyck and Lambert classified CMT types based on inheritance patterns, natural history, biochemical features, nerve conduction velocity, and pathologic characteristics.³ They introduced the term "hereditary motor and sensory neuropathy (HMSN)". They classified the autosomal-dominant form with decreased conduction velocities as HMSN type I and the autosomal-dominant form with normal conduction velocities as HMSN type II.

CMT has a crude global prevalence estimate of 1 in 2500.^{4, 5} Based on the literature from adult neuropathy clinics, it has been reported that CMTs are more frequent in comparison with inflammatory or paraneoplastic neuropathies.^{1, 6} No systematic data is available on Indian patients having CMT. CMT disease has been scarcely reported in the Indian literature.

CMT is a disease with genetic heterogeneity. The mutations causing CMT may occur in several different genes.⁷ mutations in the same gene can lead to clinically distinct entities. CMTs are categorized into several types and subtypes based on the affected gene. It can be classified according to the mode of transmission (autosomal dominant, autosomal recessive, or X-linked), electrophysiological findings (demyelinating or axonal), or the causative genetic variation. The

classification of CMT is complex and undergoing constant revision as newer genes and mutations are being discovered. As more genes causing CMT were identified, and the overlap of neuropathy phenotypes and modes of inheritance became apparent, the alphanumeric classification system proved unwieldy and inadequate. Magy L et al.⁸, in 2018, proposed a gene-based classification of inherited neuropathies, including a comprehensive list of CMT-associated genes and their correlation with the alphanumeric classification. Based on this classification system, a patient's findings can be described based on the mode of inheritance, neuropathy type, and gene.

Normal structure and functions of peripheral nerve depend upon the close anatomical and physiological interaction between Schwann cells and axons. Axons determine the survival, proliferation, and differentiation of Schwann cells which, in turn, play an important role in ion channel regulation and the maintenance, survival, and regeneration of axons. Abnormalities in genes that regulate myelin assembly and axonal transport lead to primary demyelination and axonopathy, respectively.

CMT is the most common inherited neuromuscular disorder with clinical heterogeneity. It affects the peripheral nerves and leads to progressive weakness of extremities. Occasionally it involves cranial nerves and other sites of the neuraxis resulting in complex syndromes and other organ systems, though these are more commonly seen in secondary inherited neuropathies.

CMT is a complex molecular disorder with over 1000 putative mutations in 80 disease-associated genes.⁹ CMT is characterized by a clinically and genetically heterogeneous group of neuropathies with all types of Mendelian patterns of inheritance. Genetic research of CMT has evolved in discovering genomic disorders and helped in understanding the effects of copy

number variation and the mechanism of genomic rearrangements. Approximately 30 genes involved in axonal transport, the structure of myelin, and membrane metabolism have been found in multiple unrelated families or confirmed by functional studies.^{10, 11} The wide spectrum of genetically identifiable disease-causing alleles complicates the molecular diagnosis, but a few genes account for over 90% of known genetic causes.¹² Over 100 genes have been associated with CMT¹³. It is reported that over 90% of all genetically diagnosed cases are due to mutations in four genes: *PMP22*, *GJB1*, *MFN2*, and *MPZ*.^{13, 14} The 1.4 Mb duplication on chromosome 17 (17p) accounts for over 60% of all genetically diagnosed cases of CMT in Europe and America.¹³ This region contains nine genes, including the peripheral myelin protein 22 gene (*PMP22*), which is amenable to therapeutic manipulation of CMT1A, mainly aiming at reducing *PMP22* transcription. Global data suggests that the predominant inheritance pattern is autosomal dominant, with autosomal recessive and X-linked forms being less commonly identified.

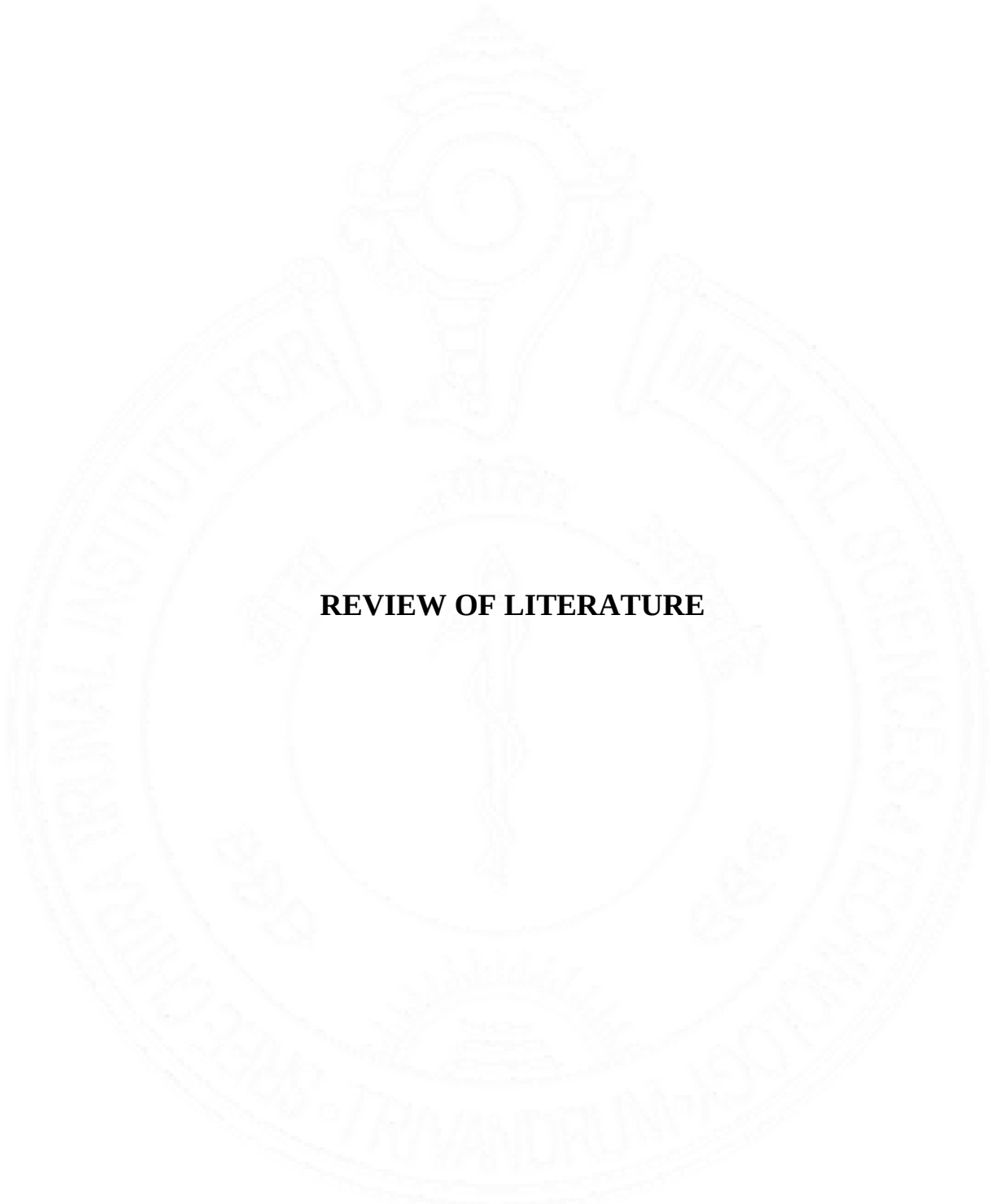
The clinical presentation includes an average onset at the age of 12 years, progressive weakness of distal musculature, impaired deep tendon reflexes and abnormalities of the peripheral nerve axon or its adjacent myelin sheath.^{15, 16} The majority of these patients manifest in the first and second decade with insidious onset and slowly progressive weakness, which starts in the lower limbs and later involves the upper limbs.^{17, 18}

Diagnosis of CMT is important for patient education and counselling and aids in the treatment and reproductive planning.

There is scarce data on the distribution of subtypes and natural history from India. A lack of financial resources and the vast heterogeneity of the mutations limit genetic studies.

In a retrospective study in south India on a small series of patients on CMT, where the clinico-electrophysiological data and genetic diagnosis were performed, ¹⁹ showed general concordance and some overlaps and reiterated the advantages of gene panel testing in this heterogeneous group of neuropathies. MRI neurography was useful as an additional investigation to detect nerve enlargement in patients with inherited neuropathies.

Hence the present study is carried out to analyze the clinical and electrophysiological profile of patients with CMT attending a single tertiary care center in South India over 24 years. The present study also aims to correlate the electroclinical profile with nerve biopsy and genotype.



REVIEW OF LITERATURE

REVIEW OF LITERATURE

Charcot Marie Tooth disease

Charcot Marie Tooth disease (CMT) is a clinically and genetically heterogeneous disease affecting the peripheral nerves and leading to progressive weakness, especially in the extremities. It is the most common inherited neuromuscular disorder.¹ It may also involve cranial nerves and other organs occasionally. 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, and mitochondrial metabolism and dynamics. The majority of these patients manifest in the first or second decade with insidious onset and slowly progressive weakness that starts in the lower extremities and later involves the upper extremities.^{17, 18}

"Inherited peripheral neuropathies" are a group of disorders that comprise of

1. Hereditary motor and sensory neuropathies (HMSN),
2. Hereditary motor neuropathies (HMN), and
3. Hereditary sensory neuropathies (HSN) or hereditary sensory and autonomic neuropathies (HSAN).

Hereditary motor and sensory neuropathies are the most common inherited neuropathies. It is also known as Charcot-Marie-Tooth disease (CMT).^{1, 2}

The U.K. Genetic Testing Network criteria for defining a CMT is as follows

1. "Idiopathic" peripheral neuropathy diagnosed by clinical presentation with progressive weakness in hands/wrists and/or feet/ankles and/or associated pes cavus or finger flexion contractures and/or peripheral sensory loss.
2. Supportive nerve conduction test result (defining Type I or II according to nerve conduction velocity)
3. Absence of other nongenetic causes (alcohol, B12 deficiency, diabetes, and trauma)
4. No associated CNS involvement.

Electrophysiological criteria for the diagnosis of inherited neuropathies for CMT according to Harding and Thomas guidelines ²⁰:

Conduction velocity (median nerve)	Type
< 38 m/s	demyelinating
38–45 m/s	intermediate
>45 m/s with low amplitude	axonal

History:

Jean Marie Charcot, Pierre Marie Howard Henry Tooth first described the entity in 1886.¹ In February 1886, Charcot and Marie published their original description of five patients with Progressive Muscular Atrophy. They construed that the lesion could be in the spinal cord. Three months later, Tooth presented his thesis entitled "Peroneal Type of Progressive Muscular Atrophy" to the University of Cambridge, UK. Tooth localized the pathology to the peripheral nerves.³ Then, Hoffman described thickened nerves in a patient with 'peroneal muscular atrophy'.

^{1,3} Dyck and Lambert classified several CMT kinships based on differences in modes of inheritance, natural history, biochemical features, nerve conduction velocity, and pathologic characteristics. ³ introduced the terminology of "hereditary motor and sensory neuropathy" and further classified the autosomal-dominant form with reduced conduction velocities as HMSN type I and the autosomal-dominant form with normal conduction velocities as HMSN type II. Harding and Thomas observed that the motor nerve conduction velocities (NCV) showed a bimodal distribution and set a median NCV of 38 m/s as an arbitrary but rational cut-off between the demyelinating HMSN type I (motor NCV <38 m/s) and the axonal HMSN type II (motor NCV >38 m/s). ²¹

Epidemiology:

The crude global prevalence of CMT has been estimated at around one per 2500. ^{4,5} CMTs occur more frequently, based on literature from neuropathy clinics comparing with neuropathies of inflammatory or paraneoplastic aetiology. ^{1,6}

CMTs are broadly categorized as demyelinating (CMT1) and axonal (CMT2) subtypes.

CMT1 - demyelinating with autosomal-dominant transmission and

CMT2 - axonal and usually dominant

The frequency ranges from 37.5% to 84% for CMT1 and 12% to 35.9% for CMT2.²² But there needs to be more information on its global prevalence and genetic epidemiology because of the challenges in diagnosis in countries with limited resources. Population-based surveys have revealed a varied frequency of CMT in different countries.

Its prevalence ranges from 9.7/per 100,000 in Serbia to 82.3 per 100,000 in Norway. (22) Libya: 8 per 100,000, Nigeria: 10 per 100,000, South Wales: 17 per 100,000, Northern Sweden: 20 per 100,000, and Northern Spain: 28 per 100,000.²³ The estimated prevalence in western Norway for autosomal dominant, X-linked, and autosomal recessive subtypes was 36 per 100,000, 3.6 per 100,000, and 1.4 per 100,000, respectively.²²

It has been reported from western countries that the most common cause of CMT1 is CMT1A, which results from the duplication of a genomic fragment that encompasses the *PMP22* gene on chromosome 17.²¹ CMT1B is more common in Japan than in Western countries.^{24, 25} CMT2A has been the most common cause of CMT2.²⁶

India: India is one of the most populous countries with a wide range of ethnic and genetic diversity. Hospital-based studies suggest that hereditary neuropathies constitute 4.8% of all neuropathies.¹ Data on the genetics of CMTs from India are rather limited.¹

There is no systematic information available on Indian patients having CMT. CMT disease has been scarcely reported in the Indian literature. Kalane UD et al.²⁷ reported a case of the rare but relatively more severe autosomal recessive CMT type 4C disease with a few features that are distinct from its regular presentation. It was one of the common mutations in the *SH3TC2* gene. Chakravarty A et al.²⁸ reported on a family with clinical and electrophysiological profiles with CMTX. Umesh et al. reported a case of CMT4C from India with *SH3TC4* mutation. Ghodasara

MK et al.²⁹ reported a patient with an adult-onset CMT caused by heterozygous *HSPB1* mutation encoding small heat shock protein 27 (HSP27), which is seen in the CMT2F subtype. A prospective, observational study was carried out by S.V Khadilkar et al. at a tertiary care hospital in Western India in 22 CMT patients fulfilling the U.K. Genetic Testing Network criteria.¹⁹ They underwent clinical, electrophysiological, radiological, and multigene panel testing. Twenty-two patients (19 males, three females, 18 sporadic and four familial cases) were studied. Pes cavus (19), hammer toes (16), and scoliosis were seen in 1 patient. Electrophysiology revealed predominant motor neuropathy with 15 demyelinating (10 uniform and five multifocal) and seven axonal patterns of neuropathy. Thickened lumbosacral plexuses on MRI neurography were noted in 6/10 studied patients, all 6 having demyelinating neuropathy. Genetic analysis identified PMP22, GJB1, SH3TC2, HSPB1, SPTLC2, MPZ, AARS, and NEFH gene mutations.

Classification:

CMT is a heterogeneous group of diseases, and the mutations linked to it may occur in a number of different genes.⁷ Based on the genes affection, CMT is categorized into several types and subtypes. It can be classified according to the pattern of transmission (autosomal dominant, autosomal recessive, or X-linked), according to electrophysiological findings (demyelinating or axonal), or according to the causative mutant gene. The classification of CMT is complex and undergoes constant revision as new genes and mutations are discovered.

Classically, CMT is divided into two main types:

1. Type 1 (CMT1) when the disease is primarily demyelinating with the median motor nerve conduction velocities (MNCVs) <38 m/s, and

2. Type 2 (CMT2) when the disease is axonal with MNCVs >38 m/s.¹³

An intermediate type is suggested when the MNCVs are between 25 and 45 m/s. Other sub-classifications are based on the inheritance pattern, which can be autosomal dominant (A.D.), autosomal recessive (A.R.), or X-linked (CMTX).^{13, 14}

CMTs have

1. Autosomal dominant,
2. Autosomal recessive or
3. X-linked inheritance.

The autosomal dominant pattern is the most common type of inheritance.³⁰

The most commonly used classification combines clinical findings, the inheritance pattern (autosomal dominant, autosomal recessive, or X-linked), and electrophysiological or pathological findings (axonal or demyelinating).²¹ The demyelinating forms predominantly affect the myelin sheath surrounding the axon of the nerve. The function of myelin is to increase conduction velocity. Therefore, this disorder's most notable electrophysiological finding is a low NCV. Specific effects on the axon cause the axonal forms of the disease; electrophysiological studies reveal preserved conduction velocity, while the amplitude of the motor and sensory potentials is greatly reduced. Recently, disease variants have been described that show combined effects on the myelin and the axon and hence have intermediate conduction velocities. The transmission of these new variants is X linked.³¹

The current classification of CMT is driven by electrophysiological profile and inheritance pattern. These are characterized by slowly progressive distal muscle weakness, wasting, and sensory disturbances. CMT is often characterized by motor deficits in the form of peroneal nerve paralysis or palsy, pes cavus and hammer toes with sensory disturbances in the form of impaired proprioception. Neuropathic pain, scoliosis, skeletal deformities, deafness, cognitive deficits, tremor, impaired speech and swallowing difficulty, breathing difficulties, or structural changes of the central nervous system may also accompany the disorder. The long nerves in the lower limbs are often affected earlier, more severely than those in the upper limbs. Especially in severe autosomal-recessive forms of CMT, the disorder usually starts in childhood.

CMTs have varied ages of onset of symptoms: neonatal or 'congenital', infantile, or late-onset.³²

They are categorized into early infantile (before 24 months), childhood (2 -10 years), juvenile (10- 20 years), adult (20 - 50 years), and late adult (after 50 years) onset.³³

Based on the clinical, electrophysiological, and pathological findings, CMTs were initially termed hereditary motor and sensory neuropathies (HMSN) and classified as¹

1. HMSN I: demyelinating subtype
2. HMSN II: axonal subtype
3. HMSN III: Dejerine Sottas disease
4. HMSN IV: Refsum disease
5. HMSN V: with pyramidal signs
6. HMSN VI: with optic atrophy

7. HMSN VII: with retinitis pigmentosa

The classification was changed later based on the mode of inheritance and clinical features as CMT1 (A, B, C...), CMT2 (A, B, C..), CMT3, CMTX (1,2, 3..) and CMT4, etc. However, due to overlapping phenotypic and genotypic presentations and advances in molecular testing, the classification has changed over many years, e.g., CMT1 as autosomal dominantly inherited demyelinating CMT, CMT4 as recessively inherited demyelinating CMT, while AR-CMT2 as recessively inherited axonal CMTs. There exists a need for more consensus in the nosological terms provided by different experts.³⁴

There is significant phenotypic diversity with certain mutations, e.g., variations in *MPZ*, *GDAP1*, and *NEFL* can cause both the axonal demyelinating types of CMTs. Likewise, mutations in *MFN2*, *MPZ*, *GDAP1*, and *EGR2* may have autosomal dominant or recessive modes of inheritance. Hence, with expanding knowledge, it is appropriate to classify CMTs based on genetic abnormality and not by electrophysiological or pathological observations. A revised classification incorporating phenotype, electrophysiology, type of inheritance, and causal genetic variation is also proposed.³²

As more genes causing CMT were identified, and the overlap of neuropathy phenotypes and modes of inheritance became apparent, the alphanumeric classification system proved unwieldy and inadequate. Magy L et al. 8, in 2018, proposed a gene-based classification of inherited neuropathies, including a comprehensive list of CMT-associated genes and their correlation with the alphanumeric classification. Based on this classification system, a patient's findings can be described based on the mode of inheritance, neuropathy type, and gene.

Table 1: Genes, Mode of Inheritance, Neuropathy Phenotype in CMT (adapted from Bird TD.²

Gene	MOI	Neuropathy Type			Other Phenotypic Features / Comments
		Ax	De	In	
Most commonly involved genes					
GDAP1	AR	●			Vocal cord paresis
	AR	●	●	●	
	AD, AR	●			
GJB1	XL	●	●	●	Family history may appear to be AD as females can be as severely affected as males; CNS myelin may be affected.
HINT1	AR	●			Neuromyotonia
MFN2	AD, AR	●			Optic atrophy
MPZ	AD	●	●	●	
PMP22	AD		●		
SH3TC2	AR	●			
SORD	AR	●		●	Distal muscle atrophy & weakness
Other genes					
AARS1	AD	●			
ABHD12	AR		●		Deafness, cataract, retinitis pigmentosa
AIFM1	XL	●			Deafness, intellectual disability
ARHGEF10	AD		●		
ATP1A1	AD	●			
ATP7A *	XL	●			Distal lower extremities
BAG3	AD	●			Myofibrillar myopathy, cardiomyopathy
BSCL2	AD	●			Distal lower extremities: UMN involvement can cause spastic paraplegia
CADM3	AD	●			Forearm & hand atrophy as well as lower limb
CNTNAP1	AR	●	●		Arthrogryposis, leukodystrophy
COA7	AR	●			
DCTN1	AD				Distal lower extremities
DCTN2	AD	●			Vocal cord paresis
DGAT2	AD	●			
DHTKD1	AD	●			

Gene	MOI	Neuropathy Type			Other Phenotypic Features / Comments
		Ax	De	In	
<i>DNAJB2</i>	AR	•			Distal motor neuropathy
<i>DNMT1</i>	AD	•			Hearing loss, dementia
<i>DNM2</i>	AD			•	
<i>DRP2</i>	XL			•	Autism
<i>DYNC1H1</i>	AD	•			SMA
<i>EGR2</i>	AD		•		
	AR		•		
<i>FGD4</i>	AR		•		
<i>FIG4</i>	AR		•		
<i>GARS1</i>	AD	•			Onset in hands
<i>GNB4</i>	AD			•	
<i>HARS1</i>	AD	•	•		
<i>HSPB1</i>	AD	•			
<i>HSPB3</i>	AD				
<i>HSPB8</i>	AD	•			Adult onset
<i>IGHMBP2</i>	AR	•			
<i>INF2</i>	AD			•	Glomerulosclerosis
<i>KIF1B</i>	AD	•			
<i>KIF5A</i>	AD	•			Spasticity
<i>LITAF</i>	AD		•		
<i>LMNA</i>	AR	•			
<i>LRSAM1</i>	AD, AR	•			
<i>MARS1</i>	AD	•			
<i>MCM3AP</i>	AR	•	•		Childhood onset, severe
<i>MME</i>	AR, AD	•			
<i>MORC2</i>	AD	•			
<i>MPV17</i>	AR	•			Navaho neurohepatopathy
<i>MPZ</i>	AD	•	•	•	
<i>MTMR2</i>	AR		•		Vocal cord paresis
<i>NAGLU</i>	AD	•			
<i>NDRG1</i>	AR		•		
<i>NEFH</i>	AD	•			
<i>NEFL</i>	AD, AR	•	•		

Gene	MOI	Neuropathy Type			Other Phenotypic Features / Comments
		Ax	De	In	
<i>PKD3</i>	XL	•			
<i>PLEKHG5</i>	AR			•	Distal predominant
<i>PMP2</i>	AD		•		
<i>PNKP</i>	AR	•			
<i>PRPS1</i>	XL				Retinopathy, deafness
<i>PRX</i>	AR	•			
<i>PTRH2</i>	AR				Hearing loss
<i>RAB7A</i>	AD	•			Prominent sensory loss
<i>SBF1</i>	AR	•			
<i>SBF2</i>	AR		•		
<i>SCO2</i>	AR	•			Motor neuropathy
<i>SETX</i>	AD				Distal lower extremities
<i>SIGMAR1</i>	AR	•			Motor neuropathy
<i>SGPL1</i>	AR	•			Recurrent mononeuropathy
<i>SPG11</i>	AR	•			Spasticity, cognitive decline
<i>SPTLC1</i>	AD	•			May be Assoc w/a juvenile ALS syndrome ³
<i>TRIM2</i>	AR	•			Vocal cord paresis
<i>TRPV4</i>	AD	•			Vocal cord paresis, skeletal dysplasia
<i>VCP</i>	AD	•			Inclusion body myopathy, dementia
<i>VWA1</i>	AR	•			Motor neuropathy, <i>pes cavus</i> , & proximal muscle weakness
<i>WARS</i>	AD	•			Motor neuropathy
<i>YARS1</i>	AD			•	
Unknown #	XL		•		Rapid progression, severe hand weakness

AD = [autosomal dominant](#); ALS = amyotrophic lateral sclerosis; AR = [autosomal recessive](#); Ax = axonal; De = demyelinating; dHMN = distal hereditary motor neuropathy; DI-CMT = dominant intermediate CMT; DSMA = distal spinal muscular atrophy; HSAN = hereditary sensory and autonomic neuropathy; In = intermediate; UMN = upper motor neuron; XL = [X-linked](#)

Pathophysiology:

The close anatomical and physiological interactions between Schwann cells and axons are responsible for the normal functioning of the peripheral nerves. The axons determine the survival, proliferation, and differentiation of Schwann cells. Regulation of ion channels and maintenance, axon survival and regeneration is regulated by these Schwann cells. Primary demyelination occurs due to abnormalities in genes that regulate myelin assembly. Axonopathy occurs due to abnormalities in genes that regulate axonal transport.

CMTs may occur due to any of the following molecular and cellular mechanisms ¹:

1. Myelin assembly: genes involved in myelin compaction (*MPZ*), gap junctions formation (*GJB1*), the interaction of Schwann cells with the extracellular matrix, as well as in regulating cell spreading, cell migration and apoptosis (*PMP22*)
2. Cytoskeletal structure: genes involved in the polymerization of actin (*INF2*), membrane-protein interactions for stabilizing the myelin (*PRX*), intermediate filaments (*NEFL*), and axonal transport (*DYNC1H1*)
3. Endosomal sorting and cell signalling: genes that regulate vesicular transport, membrane trafficking, transport of intracellular organelles and cell signalling like *LITAF*, *MTMR2*, *SBF1*, *SBF2*, *SH3TC2*, *NDRG1*, *FIG4*, *RAB7A*, *TFG*, *DNM2*, *FGD4*, *SIMPLE*

4. Proteasome and protein aggregation: genes regulating microtubules (*HSPB1*, *HSPB8*), cell adhesion (*LRSAM1*), ubiquitin ligase (*TRIM2*)
5. Mitochondria: genes which regulate mitochondrial structure, function and dynamics of the respiratory chain (*MFN2*, *GDAP1*, *MT-ATP6*, *PDK3*)
6. Others: genes regulating cell fusion-fission apparatus (*DNM2*), calcium homeostasis (*TRPV4*) glucose metabolism (*HK1*), transcription factors (*EGR2*, *HINT1*, *PRPS1*, *AARS*, *GARS*, *MARS*, *KARS*, *YARS*)

Because of the functional interaction, demyelinating neuropathies may eventually lead to functional axonopathies and manifest as secondary axonal degeneration.^{35, 36} Thus, common secondary phenomena in CMTs include loss of axon, secondary Schwann cell proliferation, and immune-mediated acceleration of pathology³⁷

Genotype profile:

More than 1000 different putative mutations in 80 disease-associated genes have been reported.⁹ Around 30 genes involved in functions such as axonal transport, myelin structure, and membrane metabolism have been reported in several unrelated families or confirmed by functional studies.^{10, 11} More than 100 genes have been defined in association with CMT.¹³ Mutations in the four genes - *PMP22*, *GJB1*, *MFN2*, and *MPZ*- account for over 90% of all genetically diagnosed cases.^{13, 14} In Europe and America, a chromosome 17 (17p) duplication is responsible for more than 60% of all genetically diagnosed CMT cases.¹³ It contains nine genes, including the peripheral myelin protein 22 gene (*PMP22*). The global data suggests that the predominant

pattern of inheritance is autosomal dominant, with autosomal recessive and X-linked forms being less commonly identified.

Table 2: Summary of CMT classification, including clinical data (such as electrophysiological results) and molecular findings: (adapted from Banchs I et al. ²¹)

Type	Gene	Inheritance	Locus
CMT 1 (demyelinating)			
CMT1A:	PMP-22	Dominant/sporadic	17p11
CMT1B:	P ₀ protein	Dominant	1q22
CMT1C:	LITAF	Dominant	16p13
CMT1D:	EGR2	Dominant	10q21
CMT1E:	P ₀ protein	Dominant	1q22
CMT1F:	Neurofilament	Dominant/sporadic	8p21
	Light chain		
CMT 2 (axonal)			
CMT 2A1:	KIF1B	Dominant	1p36
CMT 2A2:	MFN2	Dominant	1p36
CMT 2B:	RAB7	Dominant	3q13-q22
CMT 2C:		Dominant	12q23-q24
CMT 2D:	GARS	Dominant	7p15
CMT 2E:	Neurofilament light chain	Dominant	8p21
CMT 2F:	HSPB1	Dominant/recessive	7q11-q21
CMT 2G:		Dominant	12q12
CMT 2I:	P ₀	Dominant	1q22
CMT 2J:	P ₀	Dominant	1q22
CMT 2L:	HSPB8	Dominant	12q24
AR-CMT2A	Lamin A/C	Recessive	1q21.2
AR-CMT2B	Med25	Recessive	19q13.3
CMT 2K:	GDAP1	Recessive/dominant	8q21
Intermediate NCV			
Connexin-32	GJB1	X-linked	Xq13
CMT DIA		Dominant	10q24

Type	Gene	Inheritance	Locus
CMT DIB	DNM2	Dominant	19p12
CMT DIC	Tyrosyl-tRNA synthetase	Dominant	1p34
CMT DI3	P ₀	Dominant	1q22
CMT 2E:	Neurofilament light chain	Dominant	8p21
CMT 4			
CMT 4A:	GDAP1	Recessive	8q21
CMT 4B1:	MTMR2	Recessive	11q23
CMT 4B2:	SBF2	Recessive	11p15
CMT 4C:	SH3TC2 (KIAA1985)	Recessive	5q32
CMT 4D (Lom):	NDRG1	Recessive	8q24
CMT 4E:	EGR2	Recessive/dominant	10q21
CMT 4F:	Periaxin	Recessive	19q13
HMSN-Russe	(4G)	Recessive	10q23
CMT 4H:	FGD4	Recessive	12q12

Clinical presentation, Diagnosis and Phenotype:

CMT presents at a relatively younger age, around 12 years, with progressive weakness in the extremities.^{15, 16} Diagnosis of CMT is important for educating the patients and genetic counselling, appropriate rehabilitation measures, and consideration for therapeutic trials.

The age of onset ranges from infancy to the elderly. These have an insidious onset and a slowly progressive course.

Congenital hypo-myelinating neuropathy and Dejerine Sottas disease may have onset in the neonatal period or infancy. Manifests include deformities of the foot, delayed motor milestones, difficulty walking, and impaired sensation.³⁸ Severe phenotypes may present with neonatal hypotonia, feeding difficulty, and respiratory compromise.³⁹

The majority of these individuals have an insidious onset and slowly progressive weakness that starts in the lower extremities and later involves the upper extremities.^{17, 18} The common clinical

features of most CMTs are symmetrical distal weakness, wasting, hypo/areflexia, and skeletal deformities.¹ These are more pronounced in the lower limbs than the upper limbs. Other commonly reported symptoms include difficulty walking fast or running, tripping, falls, and twisting ankles. Patients may have delayed motor milestones. During childhood, these patients are clumsy with poor athletic performance. As the weakness worsens, patients develop foot drop and high-stepping gait. The weakness of the hands manifests as difficulty in buttoning, holding, zipping, and writing. The Proximal onset of weakness and upper limb onset of symptoms are infrequently seen. Sensory symptoms and signs are relatively less frequent. About a quarter of patients complain of musculoskeletal or neuropathic pain. Thus, positive sensory symptoms like paresthesia are rather infrequent.

Clinical examination reveals deformities like pes cavus, hammertoes, and clawing of hands in patients due to the long-standing weakness of intrinsic muscles. Thinning of distal thighs and legs may seem like an inverted champagne bottle appearance. Spinal deformities like scoliosis may also occur.

Early and severe scoliosis is suggestive of, but not an exclusive feature of, *SH3TC2* mutations.⁴⁰ gait abnormalities and imbalance may result from proprioceptive loss and foot deformities³⁰. These clinical manifestations result from axonal loss, even in demyelinating CMTs, since these patients have slowed velocities even before the onset of clinical manifestations.⁴¹⁻⁴³

Demyelinating subtypes may have hypertrophied nerves. Upper motor signs in spasticity may coexist, designated as HMSN type V and commonly associated with *MFN2* mutations.⁴⁴

Patients may have various forms of disability, ranging from minimally symptomatic to severe.⁴⁵

Patients have phenotypic heterogeneity in the age at onset, motor deficits, and sensory loss. This variability is observed even in families sharing the same genetic abnormalities.⁴⁶

For example, mutations in *PMP22* may manifest as only minor abnormalities on electrophysiology, classical CMT1A, hereditary neuropathy with liability to pressure palsies, Dejerine-Sottas syndrome, or congenital hypo-myelinating neuropathy⁴⁷, Coexisting illness like hypothyroidism, diabetes, obesity, or exposure to toxins increases impairment in these patients.

⁴⁸ Early or infantile onset usually correlates with worse disability.

In addition to the classical presentation of distal wasting, weakness, and deformities, patients may have other features. In a study by Werheid et al., out of 49 patients with genetically established CMT, 88% had at least one additional component, with 65% having two or more other manifestations.⁴⁹ This may give a clue for underlying genetic variations. Patients with mutations in *MPZ*, *GJB1* and copy number variations in *PMP22* may have abnormal pupillary findings like tonic pupils, miosis, mydriasis, anisocoria, and impaired pupillary reaction.

Other ocular manifestations include ptosis, optic atrophy, cataract, glaucoma, and macular degeneration. Cranial neuropathies like hearing impairment, vocal cord palsy, facial/ bulbar weakness, and tongue atrophy may also occur. Patients with *PMP22* and *MPZ-associated* neuropathies may report positive sensory symptoms like pain and paresthesias, as seen in acquired neuropathies. Individuals with mutations in *MPZ* viz Thr124Met and Gly123Ala may present with late-onset phenotype with pupillary abnormalities, shooting pains, paresthesias, and sometimes have rapid progression.⁴⁹ Milley et al. reported *PMP22* duplication has been associated with that deafness and autoimmune disorders.⁵⁰

Features of autonomic disturbances, including sweat abnormalities, urinary urgency and incontinence and orthostatic hypotension, have also been reported.⁴⁹ Focal segmental glomerulosclerosis is seen in patients with mutations in the inverted formin gene (INF2).⁵¹ Restless leg syndrome (RLS) is more common in patients with CMT than in the general population.⁴⁹ Axonal loss increases axonal excitability in the sensory nerve endings and leads to abnormal sensations.⁵² Other rare clinical features include hyperkeratosis, skin hyperlaxity, arthrogryposis, impaired cognition, learning disability, lip or chin myokymia, respiratory insufficiency, tremor, nystagmus, ataxia, fasciculations, cold-induced cramps, hip dysplasia, and Ehlers-Danlos syndrome among others.^{49, 53} A few individuals with genetically diagnosed CMT may have acute worsening resembling inflammatory polyradiculoneuropathies.⁵⁴

Complications: Deformities of the foot are a major cause of disability in CMTs, which might develop over time and may progress despite physiotherapy and orthosis use, which might require surgical correction plantigrade foot and correct muscle imbalance.⁵⁵ calluses may develop over the extremities due to increased local pressure. Respiratory insufficiency may rarely occur due to severe neuropathy, diaphragmatic palsy, or spinal deformities.⁴⁹ Worsening of CMT-related symptoms may occur during pregnancy with an increased risk of an abnormal fetal presentation, post-partum haemorrhage, and the need for emergency intervention during delivery. Therefore, a regular ante-natal checkup is recommended. Neurotoxic drugs like chemotherapeutic agents and co-morbid neuropathic diseases also worsen neuropathy in CMT.⁵⁶

Prognosis

CMTs are slowly progressive disorders. Patients develop progressive weakness and wasting of extremities that interfere with mobility and daily living activities, and the progression rate varies among various CMTs. Periodic evaluation and interventions by a multidisciplinary rehabilitation team are essential for maintaining functional independence and ambulation. Life expectancy is usually not affected unless in severe disease with a very early presentation.

Electroclinical profile of CMT disease

Electrophysiology: Nerve conduction studies help confirm the diagnosis of neuropathy and broadly categorize patients into demyelinating and axonal types. They are also useful in screening asymptomatic family members of the affected individual. The parameters measured are distal latencies, amplitudes, and conduction velocities of motor and peak latency and amplitudes of sensory nerves. A decrease in conduction velocities indicates myelin dysfunction, while the reduced compound muscle action potential amplitudes with preserved conduction velocity imply axonal loss. Some individuals may have features of both demyelinating and axonal neuropathy. Evidence of denervation on needle electromyography is useful in establishing axonal pathology.

The median motor nerve conduction velocity of 38 m/sec is the commonly used cut-off for differentiating demyelinating and axonal subtypes of CMT. In addition, there is an intermediate form of CMT where the nerve conduction velocity ranges between 25 to 45 m/sec.³⁰

Based on nerve conduction velocities, CMTs are categorized into (11)

Type of CMT	Nerve conduction velocity
very slow	<15 m/s
slow	15 to 35 m/s
intermediate	35 to 45 m/s
normal	>45 m/s

The usefulness of this classification has been reported by Saporta et al., patients with inherited neuropathies selected for genetic testing.¹¹

Uniform and diffuse slowing in Conduction velocity are seen in inherited neuropathies, whereas in acquired neuropathies, the changes may be segmental, non-homogeneous, and asymmetrical. However, in *GJB1-associated* neuropathy, conduction velocity slowing is non-uniform and variable within a single nerve and between multiple nerves, with the conduction slowing more seen in the median than in the ulnar nerve. This inter-nerve variability is prominent in females.⁵⁷ However, severe and uniform demyelination is seen in males, which is reflected by a marked reduction in conduction velocities.

Temporal dispersion and conduction blocks help to distinguish acquired demyelinating neuropathies from inherited ones.⁵⁸ Lack of temporal dispersion, conduction blocks, and reduction in conduction velocities in a range of 20 to 30 m/sec gives a clue for hereditary neuropathy. Exceptionally, temporal dispersion can also occur in *GJB1* mutations.^{57, 59}

Reduction in the amplitude of motor and sensory potential reflects the degree of axonal loss, which correlates with the clinical disability, which might be seen in demyelinating neuropathies. Sometimes, patients present late during their disease⁵⁸ In this situation, it might be difficult to

distinguish primary axonal and demyelinating forms of CMT due to the in elicitable motor and sensory potentials, where studies of proximal nerves, such as musculocutaneous, radial and facial nerves, are helpful.⁵⁹

Nerve Imaging

Advances in neuroimaging have enabled visualization of peripheral nerves along their entire course with great spatial resolution. In evaluating neuropathies, Nerve ultrasound and Magnetic Resonance Neurography have a promising role. Diffuse enlargement of roots, plexuses, and peripheral nerves, with no variation between entrapment and non-entrapment sites, is seen in CMTs.⁶⁰ Cranial nerve enlargement is also described. The enlargement is more obviously visualized in the upper limbs and CMT1A subtype compared to other CMTs, but in CMT2, there is no significant increase in peripheral nerve cross-sectional area (CSA). An increase in CSA indirectly predicts disability and disease progression.⁶¹ Vascularity, altered signal characteristics within the nerve, fascicular architecture, and abnormal contrast enhancement differentiate CMTs from other neuropathies, such as chronic inflammatory demyelinating polyneuropathy (CIDP) and leprosy.⁶² Besides, muscle volume and intramuscular fat accumulation (IMFA) in the legs also correlate with disability.⁶³

Correlation of Electroclinical profile with nerve biopsy and genotypes

Genetic testing is useful for establishing a conclusive diagnosis (particularly in a sporadic patient), medical counselling, reproductive planning, and selecting patients for therapeutic trials and research.⁵⁶ All patients should have a phenotypic characterization, detailed pedigree analysis, and proper pre-test counselling by an expert.

Nerve Biopsy has a role in identifying underlying genetic aetiology in sporadic cases and helps to distinguish CMT from acquired disorders like CIDP. Nerve biopsy also supports a functional association when genetic tests detect a novel variant or "variants of uncertain significance". Pathological changes in the axon, myelin, nodes of Ranvier, and mitochondria help understand the functions of the mutated genes in identifying the disease pathogenesis.

Non-specific changes may also be seen in axonal loss, demyelination/remyelination, and occasionally features suggestive of inflammation. Formation of onion bulbs due to concentric proliferation of Schwann cell cytoplasm or basal lamina will lead to Chronic demyelination and remyelination. In CMT1, demyelination remains stable while axonal loss progresses. The nerve biopsy in CMT1A may show 'tomaculae', multifocal hyper-myelinating processes appearing in longitudinal sections like a chain of sausages, which are the characteristic feature of HNPP.^{64, 65} The presence of inflammatory cell infiltration with lymphocytes and macrophages may lead to a misdiagnosis of CIDP, particularly in *PMP22*, *MPZ*, *GJB1*, and *GDAP1*-associated neuropathies. Increased susceptibility to inflammation in CMT1A, involvement of immune-mediated cells in genetically mediated demyelinating neuropathies, and superimposed CIDP are some possible hypotheses explaining inflammatory infiltrates.^{66, 67}

MPZ-associated neuropathy shows loss of compaction of myelin sheath layers, loss of the paired intra-period lines, the separation between major dense lines, and irregularly un-compacted myelin sheaths.⁶⁵

Thickened, folded myelin with redundant myelin loops is seen in mutations caused by *MPZ*, *MTMR2*, *MTMR12*, *MTMR5*, *SBF2*, *FGD4*, *SH3TC2*, *PRX*, and *NEFL*^{65, 68, 69} These foldings differ from typical tomaculae, with focal hypermyelination and 'smooth' external

contours.^{70, 71} *SH3TC2*-associated neuropathy shows abnormal extensions of the cytoplasm of Schwann cells and 'onion bulbs' formation.⁷² *INF2*-neuropathy shows whorl-like proliferation, and supernumerary elongated extensions of Schwann cell cytoplasm, prominent axoplasmic reticulum, and nodal widening.^{64, 73} Disruption of Cajal bands with focal hypermyelination is noted in *PRX* mutations.^{74, 75}

MFN2 and *GDAP1* mutations cause abnormally swollen and rounded mitochondria, vacuolated with the accumulation of amorphous material with loss of cristae⁶⁵ occasionally. In *CNTNAP1* mutations, ultrastructural observations show an absence of transverse bands and the widening of the paranodal junctional gap between myelin loops and axolemma.⁷⁶ The pathological hallmark of *NDRG1*-associated neuropathy is the pleomorphic granular, sometimes with filaments or glycogen-filled vesicular depositions, in the adaxonal space of myelinated fibres. In *NEFL*-associated neuropathies, unmyelinated fibres are abnormally condensed and show aggregates containing glycogen granules and dense microtubules. *SH3TC2* and *NEFL* mutations may show "giant axons."⁶⁵ In congenital demyelinating neuropathy due to *EGR2* mutations, there is a total absence of myelin with normal axons.⁷⁷

MOST RELEVANT STUDIES

Yalcouye A et al. ⁷⁸ (2022), in their systematic review, aimed to summarize available data on CMT in Africa, with emphasis on the epidemiological, clinical, and genetic features. They searched PubMed, Scopus, Web of Sciences, and the African Journal Online for articles published from the database inception until April 2021 using specific keywords. Three hundred ninety-eight articles were screened, and 28 fulfilled our selection criteria. A total of 107 families totalling 185 patients, were reported. Most studies were reported from North Africa (n = 22). The demyelinating form of CMT was the commonest subtype, and the phenotype varied greatly between families, and one family (1%) of CMT associated with hearing impairment was reported. The inheritance pattern was autosomal recessive in 91.2% (n = 97/107) of families. CMT-associated variants were reported in 11 genes: LMNA, GDAP1, GJB1, MPZ, MTMR13, MTMR2, PRX, FGD4/FRABIN, PMP22, SH3TC2, and GARS. The most common genes reported are LMNA, GDAP1, and SH3TC2 which have been found mostly in Northern African populations. They concluded that CMT is not rare in Africa and described the current clinical and genetic profile. They emphasized the need to invest in genetic research to inform counselling, prevention, and care for CMT in numerous settings on the continent.

Wang H et al. ⁷⁹ (2019) reviewed published cases with MTMR2 mutations and described four novel cases identified through whole-exome sequencing (WES). The four unrelated families were harbouring novel homozygous mutations in MTMR2 (NM_016156, Family 1: c.1490dupC, p.Phe498IlefsTer2, Family 2: c.1479+1G>, Family 3: c.1090C>T, p.Arg364Ter, Family 4: c.883C>T, p.Arg295Ter) present with CMT4B1-related severe early-onset motor and sensory neuropathy, generalized muscle atrophy, facial and bulbar weakness, and pes cavus deformity. The clinical description of the new mutations reported here overlaps with previously reported

CMT4B1 phenotypes caused by mutations in the phosphatase domain of MTMR2, suggesting nonsense MTMR2 mutations are expected to cause loss or disruption of the phosphatase domain, which are associated with a severe phenotype and walking without support by the early twenties. The few reported missense mutations and those truncating mutations occurring at the C-terminus after the phosphatase domain cause a mild phenotype. Patients were still ambulatory above the age of 30 years. Charcot-Marie-Tooth neuropathy and Centronuclear Myopathy causing mutations to have been shown to occur in proteins involved in membrane remodelling and trafficking pathways mediated by phosphoinositides. They proved that the regulation of expression of these proteins involved in the membrane remodelling pathway might compensate for each other's loss- or gain-of-function mutations by restoring the phosphoinositides equilibrium. This provides a potential therapeutic strategy for neuromuscular diseases resulting from mutations in the membrane remodelling pathway.

Hoebeker C et al. ¹⁸ (2018) did a retrospective single-centre study of 75 children with peripheral inherited neuropathy and correlated their genotype and phenotype. They described a cohort of 75 patients with CMT onset in childhood to explore genotype-phenotype correlations. Between 1992 and 2016, patients with CMT diagnosed in childhood and a molecular diagnosis were included, and symptoms were retrieved over time in the patients' files, as well as from molecular data and an electrodiagnostic exam. They distinguished three groups: PMP22 compared CMT (CMT1A), MFN2 compared CMT (CMT2A2) and "all genes except PMP22". Seventy-five patients were included with 11 genes involved, PMP22 being the most frequent (61.3%), then MFN2 (14.7%) and other sporadic mutations in various genes. Limitations in walking tended to occur earlier and more often, and distal strength impairment tended to progress further in CMT2A2 and "other genes than PMP22". The mean age at diagnosis was 8.4 years, with a mean

age when parents first expressed concern of 4.1 years. Only three patients lost their ability to walk. They described two cases of digenism and one case of GAN mutation with a CMT-like presentation. An electromyogram was not systemically performed. They concluded that there is a wide genetic and clinical heterogeneity in CMT. We tend to describe more severe patterns in CMT2A2 and less progressive presentations in CMT1A, considering distal strength impairment and limitations in walking. Prospective studies with more objective principal judgement criteria would be necessary to confirm these observations.

Wen Q et al.⁸⁰ (2018), in their review, analyzed published reports to determine the electrophysiological features of CMTX1 patients with transient central nervous system deficits. 21 CMTX1 patients with transient central nervous system deficits were found in 17 published case reports/series. The onset age ranged from 0.5 to 18 years (mean 12.02 ± 0.78 years). All patients were male. Recurrent central nervous system deficits were reported in all 21 cases and resolved in periods ranging from several minutes to 3 days. All 20 patients who had MRIs at presentation had bilaterally symmetrical abnormal T2/Diffusion signals in the white matter without enhancement of gadolinium. All subsequent MRIs showed improvement or were within normal limits. The median motor nerve conduction velocity (MNCV), motor latencies, and compound muscle action potential (CMAP) amplitude were the most commonly measurable electrophysiological parameters (85.7%). All cases with MNCV at presentation had slower and significantly decreased MNCV compared with the normal value (34.1 ± 1.10 m/s vs 46.8 ± 2.05 m/s, $P < 0.0001$; 95% CI, -17.4 to -7.92). The average variations of MNCV in the median nerve, ulnar nerve, peroneal nerve, and tibial nerve were $22.0 \pm 5.96\%$, $27.6 \pm 11.9\%$, $25.9 \pm 4.36\%$, and $27.3 \pm 4.30\%$, respectively. All cases with measured sensory nerve conduction velocity (SNCV) at presentation had slower and significantly decreased SNCV compared with

the normal value (35.3 ± 1.33 m/s vs 47.7 ± 2.40 m/s, $P < 0.001$; 95% CI -18.2 to -6.46). The average variations of SNCV in the median, ulnar, and sural nerve were $19.9 \pm 8.24\%$, $25.2 \pm 7.75\%$, and $23.2 \pm 3.95\%$, respectively. They concluded that this case series serves as a reminder that electrophysiological examination should be included in diagnosing recurrent and episodic neurological deficits with white matter lesions. Median MNCV is a sensitive and valuable parameter to support the diagnosis of CMTX1 with transient central nervous system deficits.

Khadilkar SV et al.¹⁹ (2017) did a pilot study on clinico-electrophysiological and genetic overlaps and Magnetic Resonance Imaging Findings in CMT disease. A prospective, observational study has been conducted at a tertiary care hospital in Western India. CMT patients fulfilling the U.K. Genetic Testing Network criteria were included. Twenty-two patients (19 males, three females, 18 sporadic and four familial cases) were studied. They underwent clinical, electrophysiological, radiological, and multigene panel testing. Clinical examination revealed Pes cavus (19), hammer toes (16), and scoliosis were seen in 1 patient. Electrophysiology revealed predominant motor neuropathy with 15 demyelinating (10 uniform and five multifocal) and seven axonal patterns. Thickened lumbosacral plexuses on MRI neurography were evident in 6/10 studied patients, all 6 having demyelinating neuropathy. Genetic analysis identified PMP22, GJB1, SH3TC2, HSPB1, SPTLC2, MPZ, AARS, and NEFH gene mutations. They concluded that clinico-electrophysiological and genetic diagnosis showed general concordance, some overlaps and reiterated advantages of gene panel testing in this heterogeneous group of neuropathies. MRI neurography was useful as an additional investigation to detect nerve enlargement in patients with demyelinating neuropathies.

Werheid F et al.⁴⁹ (2016) studied a wide range of additional features in a cohort of 49 genetically confirmed CMT patients and performed a systematic literature revision. Patients

harbored a PMP22 gene alteration (n = 28) or a mutation in MPZ (n = 11), GJB1 (n = 4), LITAF (n = 2), MFN2 (n = 2), INF2 (n = 1), NEFL (n = 1). They identified four novel mutations (3 MPZ, 1 GJB1). A total of 88% presented at least one additional feature. In MPZ patients, we detected hypertrophic nerve roots in 3/4 of cases that underwent spinal MRI and pupillary abnormalities in 27%. In our cohort, restless legs syndrome (RLS) was present in 18%. They described for the first time, "RLS is associated with LITAF or MFN2 and predominant upper limb involvement with LITAF". Cold-induced hand cramps occurred in 10% (PMP22, MPZ, MFN2), and autonomous nervous system involvement in 18% (PMP22, MPZ, LITAF, MFN2). RLS and respiratory insufficiency were mostly associated with severe neuropathy and pupillary abnormalities with mild to moderate neuropathy. They concluded that in CMT patients, additional features occur frequently. Some of them might help orient genetic diagnosis. Our data broaden the clinical spectrum and genotype-phenotype associations with CMT.

Fridman V et al. ¹⁷ (2015) described CMT subtypes and disease burden in patients enrolled in the Inherited Neuropathies Consortium natural history study. They analyzed 1652 patients evaluated at 13 INC centres. The distribution of CMT subtypes and pathogenic genetic mutations were determined. The CMT Neuropathy Score (CMTNS) and CMT Examination Score (CMTES) assessed the disease burden of all the mutations. 997 of the 1652 patients (60.4%) received a genetic diagnosis. The most common CMT subtypes were CMT1A/PMP22 duplication followed by CMT1X/GJB1 mutation, CMT2A/MFN2 mutation, CMT1B/MPZ mutation, and hereditary neuropathy with liability to pressure palsy/PMP22 deletion, which accounted for 89.2% of all genetically confirmed mutations. Mean CMTNS for some subtypes were similar to those previously reported. Their findings demonstrate that large numbers of patients with a specific type of CMT subtypes have been enrolled, and the various subtypes of

CMT and the frequency of achieving a molecular diagnosis reflect similar to those previously reported. This study demonstrates that it is possible to assess patients uniformly between international centres, which is critical to study the natural course of the disease in planning future clinical trials, further providing a representative baseline for longitudinal studies of CMT.

DiVincenzo C et al. ¹⁶ (2014) described the allelic spectrum of Charcot-Marie-Tooth disease in individuals with neuropathy. They reported the frequency, positive rate, and type of mutations in 14 genes (*PMP22*, *GJB1*, *MPZ*, *MFN2*, *SH3TC2*, *GDAP1*, *NEFL*, *LITAF*, *GARS*, *HSPB1*, *FIG4*, *EGR2*, *PRX*, and *RAB7A*) associated with CMT in a cohort of 17,880 individuals referred to a commercial genetic testing laboratory. Deidentified results from sequencing assays, and multiplex ligation-dependent probe amplification (MLPA) were analyzed, including 100,102 Sanger sequencing, 2338 next-generation sequencing (NGS), and 21,990 MLPA assays. Genetic abnormalities were identified in 18.5% ($n = 3312$) of all individuals. Testing by Sanger and MLPA ($n = 3216$) showed that duplications (dup) (56.7%) or deletions (del) (21.9%) in the *PMP22* gene accounted for the majority of positive findings, followed by mutations in the *GJB1* (6.7%), *MPZ* (5.3%), and *MFN2* (4.3%) genes. *GJB1* del and mutations in the remaining genes explained 5.3% of the abnormalities. Pathogenic mutations were distributed as follows: missense (70.6%), nonsense (14.3%), frameshift (8.7%), splicing (3.3%), in-frame deletions/insertions (1.8%), initiator methionine mutations (0.8%), and nonstop changes (0.5%). Mutation frequencies, positive rates, and the types of mutations were similar between tests performed by either Sanger ($n = 17,377$) or NGS ($n = 503$). Among patients with a positive genetic finding in a CMT-related gene, 94.9% were positive in one of four genes (*PMP22*, *GJB1*, *MPZ*, or *MFN2*).

Kang JH et al. ⁸¹ (2013) in their study analyzed and compared the electrophysiological characteristics observed in nerve conduction studies (NCS) of chronic inflammatory demyelinating polyneuropathy (CIDP) and Charcot-Marie-Tooth disease type 1 (CMT 1). A differential diagnosis of acquired and congenital demyelinating neuropathies was based on a study of 35 patients with NCS-confirmed CIDP and 30 patients with CMT 1 genetically proven by peripheral myelin protein-22 (PMP-22) gene analysis, pulsed-field gel electrophoresis (PFGE), and Southern blot analysis. They analyzed values collected in motor nerve conduction studies. They conducted a dispersion analysis of the amplitudes of various nerve types' of compound muscle action potential (CMAP) and a correlation coefficient analysis of the motor nerve conduction velocity (MNCV). They found that CIDP and CMT 1 were attributable to severe polyneuropathy. In dispersion analysis, CIDP showed greater differences in proximal-to-distal amplitude ratios. Moreover, CMT 1 showed relatively high correlations compared to CIDP based on the correlation coefficient analysis of MNCV. Their study suggested that CIDP showed greater asymmetry than CMT 1 in MNCV and CMAP amplitudes.

Calvo J et al. ⁸² (2009) described MFN2 mutations and associated phenotypes in patients with hereditary motor and sensory neuropathy (HMSN). They did direct genetic sequencing for the MFN2 gene and clinical parameters of patients with MFN2 mutations in the Molecular genetics' laboratory. One hundred fifty index patients with HMSN and a median motor nerve conduction velocity of 25 m/s or greater without mutations in the genes encoding connexin 32 and myelin protein zero. Genetic analyses and phenotypic observations were the main outcome measures. Twenty different missense mutations were identified in 20 index patients. The mutation frequency was 19 of 107 (17.8%) in patients with CMT2 and 1 of 43 (2.3%) in patients with a median motor nerve conduction velocity of less than 38 m/s. Four patients had proven de novo

mutations, eight families had an autosomal dominant inheritance, and 3 had an autosomal recessive inheritance. The remaining five patients were sporadic cases with heterozygous mutations. Phenotypes varied from mild forms to early-onset severe forms. Additional features were encountered in 8 patients (32%). Six patients underwent sural nerve biopsy, and electronic microscopy showed prominent mitochondrial abnormalities on longitudinal sections. They concluded that MFN2 mutations are a frequent cause of CMT2, with variable severity and either dominant or recessive inheritance. MFN2 gene testing must be a first-line analysis in axonal HMSN irrespective of the mode of inheritance or the severity of the peripheral neuropathy.

Pareyson D et al. ⁵⁸ (2006), in their review, observed that Charcot-Marie-Tooth disease (CMT) is a genetically heterogeneous group of disorders sharing the same clinical phenotype, characterized by distal limb muscle wasting and weakness, usually with skeletal deformities, distal sensory loss, and abnormalities of deep tendon reflexes. Mutations of genes involved in different functions eventually lead to a length-dependent axonal degeneration, which is the likely basis of the distal predominance of the CMT phenotype.

Ishpekova BA et al. ⁸³ (2005) described electrophysiological findings in hereditary motor and sensory neuropathy-Lom (HMSN-L) containing 27 individuals. Their disease onset was in early childhood, with gait difficulty secondary to progressive lower limb weakness. Upper limb weakness developed later. Bulbar involvement was noted in one-third, and deafness appeared during the second or third decades. Electromyographic evidence of denervation was more severe distally and greater in the legs, totaling distal lower limb muscles being the most affected. Sensory action potentials were in elicitable, motor nerve conduction velocities were severely reduced, including the proximal upper limb (musculocutaneous and axillary), facial nerves, and hypoglossal nerves, and the severity of slowing worsens in childhood. Multiple M-waves were

recorded in all affected individuals. The blink reflex showed unusual three-component responses, and latencies of all three components were prolonged. They concluded that HMSN-L is shown to be a demyelinating neuropathy involving severe and early axonopathy changes. The progressive reduction in conduction velocities during childhood differs from the static reduction in type I HMSN.

Sevilla T et al.⁸⁴ (2003) described three Spanish families with an autosomal recessive severe hereditary motor and sensory neuropathy, showing mutations in the ganglioside-induced-differentiation-associated protein 1 (GDAP1) gene in the Charcot-Marie-Tooth (CMT) type 4A locus. The disorder started in the neonatal period or early infancy with weakness and wasting of the feet and, subsequently, involvement of the hands, causing severe disability. By the late teens, some patients developed a hoarse voice and vocal cord paresis. Peripheral motor nerve conduction velocity (MNCV) could not be measured in many cases because of the absence of muscle response due to distal atrophy. However, latencies to proximal muscles were in the normal range; median MNCV was >40 m/s in those cases where it could be measured. Sural nerve biopsy from two patients showed a pronounced depletion of myelinated fibres, regenerative clusters, and signs of axonal atrophy. Additionally, a small proportion of thin myelinated fibres and proliferation of Schwann cells forming onion bulb structures were also found. The unmyelinated fibre population markedly increased. These findings are indicative of a predominant axonal degeneration with some demyelinating features. These Spanish families share the severe CMT clinical phenotype with some Tunisian families who also presented mutations in the GDAP1 gene, to which the CMT4A locus was originally assigned. However, the families differed with laryngeal involvement and values of MNCV and pathological features, which were more in line with the CMT2 type.

Feng SY et al. ⁹⁰ (2019) identified a novel homozygous *VRK1*p.W375X mutation causing recessive dHMN. The proband presented with juvenile-onset weakness in the distal lower extremities, slowly progressing to the distal upper limbs, with bilateral pes cavus and no upper motor or sensory neuron involvement. Nerve conduction studies showed pure motor axonal neuropathy. Our findings extend the ethnic distribution of *VRK1* mutations, indicating that these mutations should be included in genetic diagnostic testing for dHMN. Vaccinia-related kinase 1 (*VRK1*) mutations can cause motor phenotypes, including axonal sensorimotor neuropathy, distal hereditary motor neuropathy (dHMN), spinal muscular atrophy, and amyotrophic lateral sclerosis.

Gonzaga-Jauregui C et al. ⁸⁹ (2013) elucidated the molecular cause of a complex neuropathy phenotype in 3 patients by applying genomic sequencing strategies. Three affected individuals from 2 unrelated families presented with a complex neuropathy phenotype characterized by axonal sensorimotor neuropathy and microcephaly. They were recruited into the Centers for Mendelian Genomics research program to identify the molecular cause of their phenotype. Whole-genome targeted whole-exome sequencing and high-resolution single-nucleotide polymorphism arrays were performed in genetics clinics of tertiary care pediatric hospitals and biomedical research institutions. Whole-genome and whole-exome sequencing identified the variants responsible for the patient's clinical phenotype. They identified compound heterozygous alleles in 2 affected siblings from 1 family and a homozygous nonsense variant in the third unrelated patient in the vaccinia-related kinase 1 gene (*VRK1*). In the latter subject, we found a common haplotype on which the nonsense mutation occurred and that segregates in the Ashkenazi Jewish population. They reported the identification of disease-causing alleles in 3

children from 2 unrelated families with a previously uncharacterized complex axonal motor and sensory neuropathy accompanied by severe nonprogressive microcephaly and cerebral dysgenesis, which has raised the question of whether *VRK1* mutations disturb cell cycle progression and may result in apoptosis of cells in the nervous system. The application of unbiased genomic approaches allows the identification of potentially pathogenic mutations in unsuspected genes in highly genetically heterogeneous and uncharacterized neurological diseases.

Wei N et al. ⁹¹ (2019) reported that genetic defects in six aminoacyl-tRNA synthetases (aaRSs) – GARS (Antonellis et al., 2003), YARS (Jordanova et al., 2006), AARS (Latour et al., 2010), KARS (McLaughlin et al., 2010), HARS (Vester et al., 2013) and MARS (Gonzalez et al., 2013) lead to CMT, highlighting the importance of this protein family in PNS homeostasis. They reported that aminoacyl-tRNA synthetases are ubiquitously expressed proteins that charge tRNAs with their cognate amino acids. Paradoxically, every cell requires the aaRSs function, as they catalyze the aminoacylation of cognate tRNAs during protein biosynthesis, yet mutations specifically affect the maintenance of peripheral neurons.

Santos-Cortez RL et al. ⁹² (2013) reported about DFNB89, a locus associated with autosomal-recessive nonsyndromic hearing impairment (ARASHI), was mapped to chromosomal region 16q21–q23.2 in three unrelated, consanguineous Pakistani families. Through whole-exome sequencing of a hearing-impaired individual from each family, missense mutations were identified at highly conserved residues of lysyl-tRNA synthetase (KARS): the c.1129G>A (p. Asp377Asn) variant was found in one family, and the c.517T>C (p. Tyr173His) variant was found in the other two families. Both variants were predicted to be damaging by multiple bioinformatics tools. The two variants were segregated with the nonsyndromic hearing

impairment phenotype within the three families, and neither mutation was identified in ethnically matched controls or within variant databases. Individuals homozygous for KARS mutations had symmetric, severe hearing impairment across all frequencies but did not show evidence of auditory or limb neuropathy. It has been demonstrated that KARS is expressed in the hair cells of zebrafish, chickens, and mice. Moreover, KARS has strong localization to the spiral ligament region of the cochlea, as well as to Deiters' cells, the sulcus epithelium, the basilar membrane, and the surface of the spiral limbus. It is hypothesized that KARS variants affect aminoacylation in inner-ear cells by interfering with binding activity to tRNA or p38 and tetramer formation. The identification of rare KARS variants in ARNSHI-affected families defines a gene that is associated with ARASHI.

The ERCC2 gene plays an important role in forming a protein called XPD, an essential subunit of a group of proteins constituting the general transcription factor IIH (TFIIH) complex. This complex has two major functions: it is involved in gene transcription and helps repair damaged DNA. The role of hereditary neuropathy has not been reported in previous studies.

The NTRK1 gene plays its role in developing and surviving neurons, which helps transmit sensations such as pain, temperature, and touch (sensory neurons). **NTRK1** mutation results in congenital insensitivity to pain with anhidrosis (*NTRK1-CIPA*) is characterized by pain insensitivity, anhidrosis, and intellectual disability.⁸⁵ The ability to sense pain modality (including visceral pain) is absent, resulting in repeated injuries, including oral self-mutilation (biting of the tongue, lips, and buccal mucosa), biting of fingertips, bruising, scarring, and cutaneous infections, multiple bone fractures (many of which fail to heal properly) and recurrent joint dislocations resulting in deformities of joints. Touch, vibration, and joint position sense are normal. Anhidrosis predisposes to recurrent febrile episodes, often the initial manifestation

of *NTRK1*-CIPA. Hypothermia in cold environments also occurs. Intellectual disability of varying degrees is observed in most individuals; hyperactivity and emotional lability are common.

SCN-9A gene belongs to a family of genes which has its role in the alpha subunit of sodium channels called NaV 1.7, which are found in the nociceptors that transmit the pain signals, which transport positively charged sodium ions intracellularly, play a key role in a cell's ability to generate and transmit electrical signals. Mutations in SCN-9A have been reported to cause insensitivity to pain, generalized epilepsy with febrile seizures, primary erythromelalgia, and small fibre neuropathy.

SLC-25A46 gene encodes a mitochondrial solute carrier protein family member. It promotes mitochondrial fission and prevents the formation of hyperfilamentous mitochondria. Mutation of this gene results in neuropathy and optic atrophy. It encodes a mitochondrial solute carrier protein family member. It promotes mitochondrial fission and prevents the formation of hyperfilamentous mitochondria. Mutation of this gene results in neuropathy and optic atrophy. Biallelic mutations in SLC25A46 have been identified as novel causes of a wide spectrum of neurological diseases with recessive inheritance, including optic atrophy, Charcot-Marie-Tooth neuropathy (CMT) type 2A neuropathy, Leigh syndrome, progressive myoclonic ataxia, and lethal congenital pontocerebellar hypoplasia.⁸⁶

MTMR-2

Myotubularin-Related 2 (*MTMR2*) gene is a member of the myotubularin family of phosphoinositide lipid phosphatases. The encoded protein possesses phosphatase activity towards phosphatidylinositol-3-phosphate and phosphatidylinositol-3,5-bisphosphate. Mutations in this gene cause Charcot-Marie-Tooth disease type 4B, an autosomal recessive demyelinating neuropathy.⁷⁹ Alternatively, spliced transcript variants encoding multiple isoforms have been found for this gene. MTMR2 protein is crucial for the modulation of membrane trafficking.

HARS

HARS variants are associated with axonal CMT type 2W (MIM#616625) with an autosomal dominant inheritance pattern. Axonal CMT type 2W is a neurological disease characterized by peripheral neuropathy typically involving the lower limbs and causing gait impairment and distal sensory loss.⁸⁷ Mutations in genes encoding aminoacyl-tRNA synthetases have been implicated in peripheral neuropathies, suggesting that these tRNA charging enzymes are uniquely important for the peripheral nerve. Recently, a mutation in histidyl-tRNA synthetase (*HARS*) was identified in a single patient with a late-onset, sensory-predominant peripheral neuropathy; however, the genetic evidence was lacking, making the significance of the finding unclear. Alonso RL et al.⁸⁸ described a variant of the gene *HARS* detected in the clinical exome - aetiology of a peripheral neuropathy undiagnosed for 20 years.

VRK-1

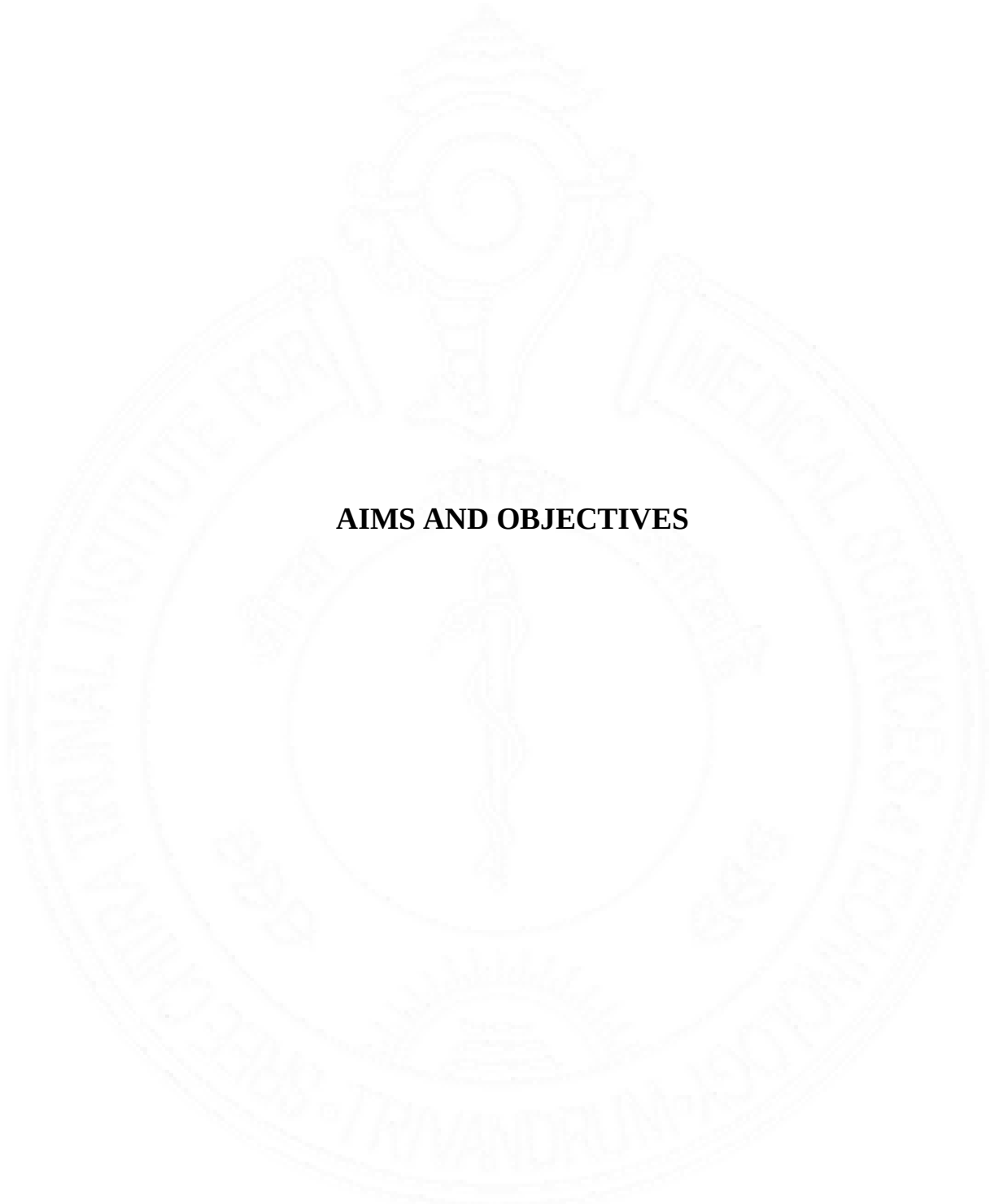
The vaccinia-related kinase-1, *VRK1* gene plays an important role in forming a protein called VRK serine/threonine kinase 1. which is present in all cells. It plays a critical role in directing cell growth and division. This protein regulates several transcription factors, which control the activity of genes by binding to specific regions of DNA.⁸⁹ Mutations in VRK 1 gene have been associated with the axonal type of sensorimotor neuropathy, recessive distal hereditary motor neuropathy, spinal muscular atrophy, and amyotrophic lateral sclerosis.

KARS

KARS is a dual localized lysyl-tRNA synthetase, and its cytosolic isoform belongs to the multiple aminoacyl-tRNA synthetase complex (MSC). Biallelic mutations in the KARS gene were described in a wide phenotypic spectrum ranging from nonsyndromic deafness to complex impairments. Mutations in KARS have been reported to cause autosomal recessive intermediate CMT with nonsyndromic hearing impairment.

WNK-1

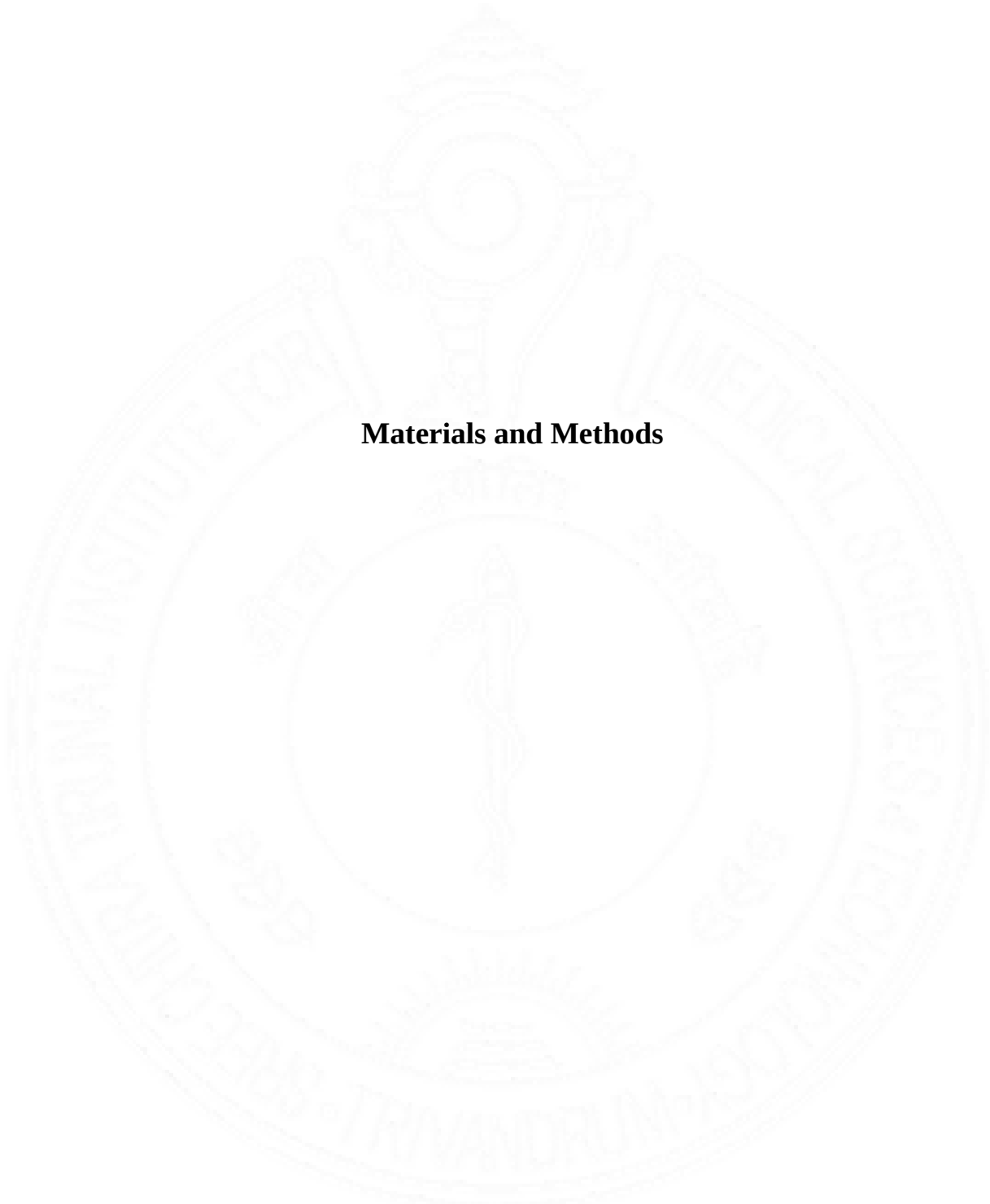
WNK (With-No-Lysine (K)) kinases are serine-threonine kinases characterized by an atypical placement of a catalytic lysine within the kinase domain. Mutations in human WNK1 or WNK4 cause an autosomal dominant syndrome of hypertension and hyperkalemia, reflecting that WNK kinases are critical regulators of renal ion transport processes.⁹³ Mutations in WNK 1 have been associated with hereditary sensory autonomic neuropathy type 2.



AIMS AND OBJECTIVES

Aims of the study

1. To study the clinical, electrophysiological, and genetic profiles of patients with CMT attending a single tertiary care center in South India.
2. To correlate the genotype with phenotypic presentations in CMT.



Materials and Methods

Methodology:

This study was carried out at Sree Chitra Tirunal institute for science and technology, Trivandrum, Kerala, India.

We included patients with a diagnosis of Hereditary sensorimotor neuropathy evaluated in the Neuromuscular Clinic and neurology wards in our centre between January 1998 to December 2021 by reviewing electronic medical records.

Study type: Retrospective observational and descriptive study.

Study duration: January 1998 to December 2021.

Inclusion criteria:

We included patients with sensorimotor or motor-dominant peripheral polyneuropathy if they had a positive family history or clinical and nerve conduction test data supportive of inherited neuropathy.

Exclusion criteria:

Immune-mediated, nutritional, drug-induced, infectious, toxic, metabolic neuropathy.

No evidence of co-existent neurodegenerative conditions like leukodystrophies suggesting a secondary inherited neuropathy. (Saporta 2011 Ann Neurology).

All patients with a negative family history with inadequate primary evaluation to rule out an alternate etiology were excluded from the analysis.

The Institute Ethics Committee of SCTIMST approved the study, as the present study is a retrospective study, consent waiver has been obtained.

The clinical data included age, gender, symptom–duration, clinical features, type of neuropathy, and sites of neuraxis affected, and electrophysiological and pathological data were extracted from the patient records using a structured proforma. The nerve biopsy and genetic reports were reviewed in detail if available.

Nerve conduction study records were analyzed, and the standard protocols for recording the Conduction studies at our laboratory were a temperature of 32–34°C (Natus Viking EDX machine AT2+6). Recordings from the four motor nerves (median, ulnar, tibial, and common peroneal nerves) and four sensory nerves (median, ulnar, superficial peroneal and sural nerves) were noted. Conduction velocity from the upper limb motor nerves was noted and classified into axonal, demyelinating intermediate and unclassifiable types of CMTs. If the median and ulnar CMAP potentials are inelicitable, they have been categorized as unclassifiable forms.

Conduction blocks were considered if the ratio of the amplitudes of compound muscle action potentials following proximal and distal stimulation was <0.6 in the tibial nerve and 0.5 in the rest of the nerves and provided the distal amplitude was at least 20% of the lower limit of normal.[16] Nerve biopsy findings were reviewed and classified based on the biopsy findings into axonal, demyelinating, mixed neuropathies, or superadded inflammation, mild neuropathy. Furthermore, inconclusive, insufficient reports were noted. Magnetic resonance imaging and recordings of evoked potentials to visual and auditory stimuli, pure tone audiometry was carried out wherever possible.

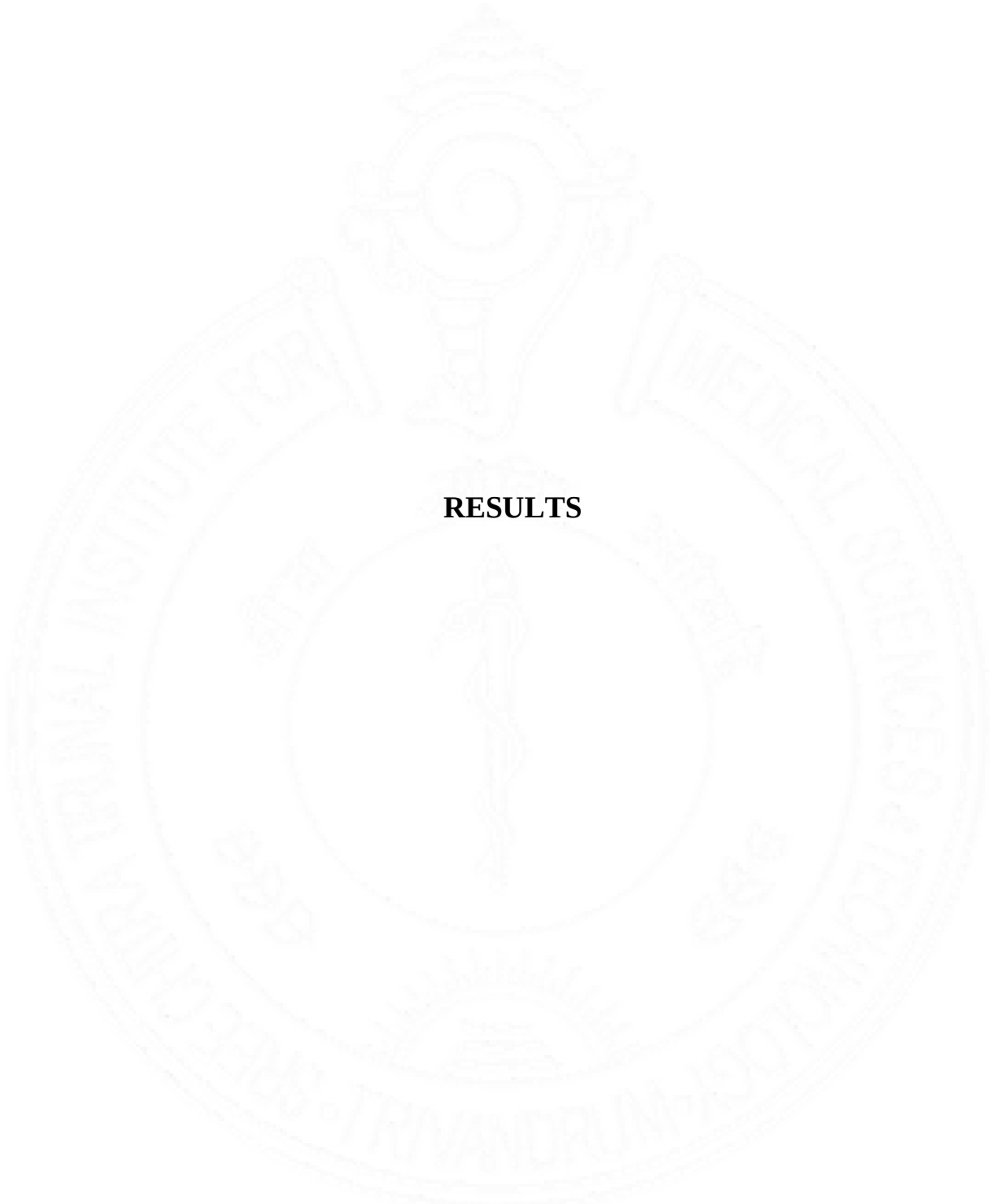
The study group, individuals who underwent genetic analysis (MLPA or NGS), were recruited for genotype and phenotype correlation. The identified variants were interpreted based on the recommendations of the American College of Medical Genetics and Genomics (ACMG),

Clinically relevant mutations were annotated using published variants in literature and a set of diseases database- Clinvar, OMIM (updated on 11th May 2020), GWAS, HGMD and SwissVar. Common variants are filtered on allele frequency in the 1000 genome phase 3, GenomeAD (v.2.1), EVS dbSNP (v151), 1000 Japanese genome and the internal Indian population database. The nonsynonymous variant's effect is calculated using multiple algorithms such as PolyPhen-2, sorting intolerant from tolerant SIFT, Mutation Taster 2, and LRT. Only nonsynonymous and splice site variants found in the clinical exome panel consisting of 6670 genes were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not considered. The data were entered in a predesigned proforma and incorporated into a Microsoft Excel Spreadsheet for analysis. However, genetic studies were not used to confirm the diagnosis, as very few had studies performed.

STATISTICAL METHODS

The data was entered into an EXCEL spreadsheet and analyzed with SPSS version 25 software (SPSS Inc, Illinois, Chicago).

Descriptive analysis was carried out by mean and standard deviation for quantitative variables and frequency and proportion for categorical variables. Data was also represented using appropriate diagrams like bar diagrams and pie diagrams and the patients with genetic reports were studied for genotype and phenotype correlation.



RESULTS

RESULTS:

A total of 191 subjects were included in the final analysis.

Table 1: Descriptive analysis of the age of presentation, age of onset, and duration of symptoms in the study population (N=191)

Parameter	Mean $\pm$ SD (years)	Median (years)	Range
Age of presentation	24.59 $\pm$ 14.78	20.00	3-67 years
Age of onset	15.5 $\pm$ 13.44	13.00	Birth-67 years
Duration of symptoms	9.06 $\pm$ 10.01	5.00	0.08-50years

The mean age of presentation score was 24.59 ± 14.78 years in the study population, with ages ranging between 3 to 67 years. The mean Age of onset was 15.5 ± 13.44 years (range birth to 67 years). The mean Duration of symptoms was 9.06 ± 10.01 years (range 0.08 to 50 years). (Table 1)

Table 2: Descriptive analysis of gender in the study population (N=191)

Gender	Frequency	Percentages
Female	71	37.17%
Male	120	62.83%

Among the study population, 71 (37.17%) were female, and the remaining 120 (62.83%) were male. (Table 2 & Figure 1)

Figure 1: Pie chart of gender in the study population (N=191)

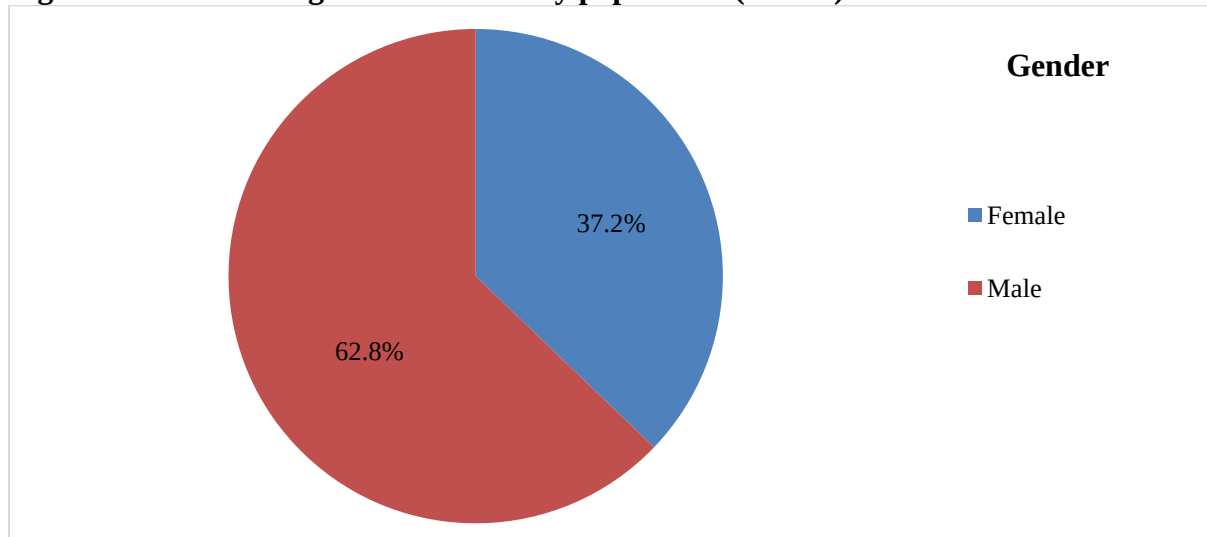


Table 3: Baseline characteristics of symptoms in the study population (N=191)

Symptomatology	Frequency	Percentages
Developmental delay	26	13.61%
Poor athletic performance	35	18.32%
Asymmetry at onset	46	24.08%
Mild	42	21.99%
Significant	4	2.09%
Symptom onset		
Sensory symptoms	53	27.74%
Ataxia	23	12.04%
Paresthesia	17	8.9%
Numbness	17	8.9%
Cramps	6	3.14%
Pain insensitivity	2	1.05%
Pain	20	10.47%
Weakness	112	58.63%
Lower limb weakness	75	39.26%
Upper limb weakness	14	7.33%
Both upper and lower limb weakness	23	12.04%
Distal weakness	30	15.7%
Distal lower limb weakness	23	12.04%
Distal upper limb weakness	12	6.28%
Distal upper and lower limb weakness	5	2.61%

Thinning of the limbs	11	5.76%
Deformities	68	35.60%
Upper limb onset of symptoms	60	31.41%
Gait difficulty	103	53.93%
Falls at onset	53	27.75%
Respiratory disturbances	3	1.57%
Tremors in limbs	15	7.85%
Cranial nerve involvement	6	3.14%

Among the study population, 26 (13.61%) had Developmental delay, and 35 (18.32%) had Poor athletic performance. Forty-six individuals (24.08%) had asymmetric onset of symptoms.

Among them, 42 (91.3%) had mild asymmetry, and 4 (8.69%) had Significant asymmetry at the onset. 11 (5.76%) individuals had thinning of the limbs, and 68 (35.60%) had deformities of the limbs at the onset of symptoms. Among the study population, 60 (31.41%) individuals had upper limb symptoms at the onset of the illness. 103 (53.93%) individuals had Gait difficulty, and 53 (27.75%) had falls at the onset of symptoms. 3 (1.57%) had respiratory disturbances, 15 (7.85%) had tremulousness in limbs, 6 (3.14%) individuals had Cranial nerve disturbances, and 112 (58.63%) individuals had a weakness as the initial presentation. 53 (27.74%) had Sensory symptoms at the onset of symptoms, and the most common sensory symptoms were Swaying while walking, numbness, and paresthesia. (Table 3)

Table 4: Baseline characteristics of symptoms in the study population (N=191)

Other Symptoms	Frequency	Percentages
Urge incontinence	4	2.10%
Recent acute worsening	3	1.57%
Recurrent symptoms	1	0.52%
Cataract	2	1.05%
Visual impairment	2	1.05%
Stiffness in limbs	2	1.05%
Non-healing ulcers	2	1.05%
Neck pain	2	1.05%
Headache	1	0.52%
Low back ache	1	0.52%

Blepharospasm	1	0.52%
Autonomic disturbances	5	2.61%
Erectile dysfunction	2	1.05%
Early satiety, resting tachycardia	1	0.52%
Increased sweating	1	0.52%
Poikiloderma-fluctuations in skin temperature	1	0.52%
Fasciculations	1	0.52%
Hearing impairment, slurred speech	1	0.52%
Myotonia	1	0.52%
Respiratory disturbances	1	0.52%
Poor academic performance	1	0.52%
Weight loss	1	0.52%

Among the study population, 5 (2.61%) had autonomic disturbances, 3(1.57%) had bladder symptoms, 2(1.05%) had visual impairment and 1(0.52%) had hearing impairment ad slurring of speech (Table: 4)

Table 5: Descriptive analysis of family history of similar complaints in the study population (N=191)

Family history	Frequency	Percentages
Consanguineous parentage	46	24.08%
Positive Family History	92	48.16%

Among the study population, 46 (24.08%) individuals were born out of consanguineous parentage, 92 individuals had a positive family history. (Table 5) out of which 83 had ≤ 3 family members affection, for nine individuals >3 family members were affected.

Examination/ clinical signs:

Table 6: Baseline characteristics of general examination findings in the study population (N=191)

Gen Exam	Frequency	Percentages
Skin and appendages changes	12	6.28%
Dysmorphic facies	6	3.14%
Microcephaly	4	2.10%
Calf hypertrophy	3	1.57%
Ulcers	3	1.57%

Marfanoid habitus	3	1.57%
Alopecia	2	1.05%
Curly hair	1	0.52%
Dental caries	1	0.52%
No facial and axillary hair, tanner 2 to 3	1	0.52%
Prognathism	1	0.52%
Microglossia	1	0.52%
Open angle glaucoma	1	0.52%
Pallor	1	0.52%
Microorchidism, hypospadias	1	0.52%

Among the study population, the majority had skin and appendages changes in 12 (6.28%)

followed by dysmorphic facies in 6 (3.14%), microcephaly in 4(2.10%) (Table 6)

Table 7: Descriptive analysis of deformities in the study population (N=191)

Deformities	Frequency	Percentages
Foot Deformities	165	86.39%
Pes cavus	123	64.40%
Hammer toes	69	36.13%
Claw hands	38	19.90%
Scoliosis	32	16.75%
Talipes equinovarus	17	8.90%
Inverted champagne bottle appearance of lower limbs	14	7.33%
Exaggerated lumbar lordosis	9	4.71%
High arched palate	6	3.14%
Pes planus	5	2.61%
Dystrophic changes	5	2.61%
Hallux valgus	4	2.10%
Short stature	4	2.10%
Ape thumb deformity	4	2.10%
Barrel chest	1	0.52%
Low set ears	1	0.52%
Hyper extensibility of joints	1	0.52%
Foot eversion	1	0.52%
Swan neck deformity of hands	1	0.52%
Simian crease in hand	1	0.52%

Among the study population, 165 (86.39%) had Deformities, 123 (64.40%) had Pes Cavus, 69 (36.13%) had Hammer Toes, 32 (16.75%) had Scoliosis, 38 (19.90%) had Claw Hands, 6 (3.14%) had High Arch Palate, 17,14 and 9 had talipes equino varus, inverted champagne bottle appearance of lower limbs exaggerated lumbar lordosis respectively. (Table 7)

Table 8: Descriptive analysis of clinical signs in the study population (N=191)

Examination/ clinical signs	Frequency	Percentages
Facial Weakness	19	9.95%
Contractures	57	29.84%
Tibialis anterior	55	28.79%
Gastrocnemius, biceps, pronators	1	0.52%
Elbow, wrist	1	0.52%
Hamstring	1	0.52%
Wasting	166	86.91%
Asymmetric signs	16	8.37%
Power charting		
Lower limb: predominant distal weakness	164	85.86%
Predominant proximal weakness	1	0.52%
Equal weakness in proximal and distal muscles	26	13.6%
Upper limbs: predominant distal weakness	139	72.77%
Predominant proximal weakness	4	2.10%
Equal weakness in proximal and distal weakness	48	25.13%
Trunk weakness	17	8.90%
Neck	22	11.51%
Generalized areflexia	53	27.74%
Plantar		
Extensor	15	7.85%
Spasticity	20	10.47%
Brisk deep tendon reflexes	34	17.80%
Pyramidal signs	42	21.98%
Abnormal sensory Examination	110	57.59%
Abnormal pain sensation	56	29.32%
Abnormal Touch sensation	53	27.75%
Impaired Joint position sense	74	38.74%
Impaired vibration sense	92	48.17%
Tremors	26	13.61%

Among the study population, 19 (9.95%) individuals had Facial Weakness. 166 (86.91%) had wasted, and 16 had asymmetric signs. 15 (7.85%) individuals had Extensor. Among the study population, 20 (10.47%) had Spasticity, 34 (17.80%) had Brisk DTR, 110 (57.59%) had Sensory abnormalities, 56 (29.32%) had abnormal pain sensation, 53 (27.75%) had abnormal Touch, 74 (38.74%) had impaired joint position sense, 92 (48.17%) had Vibration abnormalities, 26 (13.61%) had tremulousness in limbs. (Table 8)

Table 9: Descriptive analysis of gait patterns in the study population (N=191)

Gait	Frequency	Percentages
Affected	155	81.15%
Normal	36	18.85%
Specify (Gait)		
High stepping gait	109	57.06%
waddling	15	7.85%
ataxic	13	15.7%
Could not be tested due to severe disability	12	6.28%
spastic	9	4.71%
Inverted foot	7	3.66%
Impaired heel walking	4	2.10%
cautious	2	1.04%
Impaired toe walking	1	0.52%
Nonspecific limping gait	1	0.52%

Among the study population, 155 (81.15%) individuals had gait abnormalities, and most common gait pattern identified was high stepping gait in 109 (57.06%) (Table 9)

Table 10: Descriptive analysis of nerve thickening in the study population (N=191)

Nerve thickening	Frequency	Percentages
Present	27	14.14%
Specify (nerve thickening)		
Common peroneal nerve	17	8.90%
Ulnar nerve	15	7.85%
Greater auricular	6	3.14%
Diffuse	2	1.05%

superficial radial	2	1.05%
Sural	2	1.05%

Among the study population, 27 (14.14%) had Nerve thickening, among them common peroneal nerve thickening was seen in majority of individuals, followed by ulnar nerve in 15(7.85%), Diffuse nerve thickening was noted in 2 (1.05%) individuals (Table 10)

Table 11: Descriptive analysis of neurological findings in the study population (N=191)

Neurological Findings	Frequency	Percentages
Hearing loss	5	2.61%
Preserved Extensor digitorum brevis	3	1.57%
Calf hypertrophy	3	1.57%
Polyminimyoclonus	3	1.57%
Gaze restriction	3	1.57%
Tremor	3	1.57%
Scapular winging	3	1.57%
Autonomic tests abnormal	2	1.05%
Intellectual disability	2	1.05%
Limb incoordination	2	1.05%
Mild ptosis plus	2	1.05%
Myotonia	1	2.86%
Optic disc pallor	1	2.86%
Myokymia	1	2.86%
Gower's phenomenon	1	2.86%
Teres minor, infraspinatus weakness	1	2.86%
Visual field impairment	1	2.86%
Gaze evoked nystagmus	1	2.86%
Attention deficit	1	0.52%
Blepharospasm	1	0.52%

Among the study population the majority of 5 (2.61%) and 3 (1.57%) had hearing loss and calf hypertrophy, preserved extensor digitorum brevis, calf hypertrophy, polyminimyoclonus, tremor, gaze restriction, scapular winging (Table 11)

Table 12: Descriptive analysis of EP Findings in the study population (N=191)

Parameter	Mean $\pm$ SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Conduction velocity in upper limbs m/s	37.12 $\pm$ 17.45	41.0	0.0	66.0	34.6	39.6
Conduction velocity in lower limbs m/s	26.83 $\pm$ 18.25	34.0	0.0	58.0	24.2	29.4
Peroneal CMAP	1.79 $\pm$ 2.38	0.8	0.0	14.0	1.5	2.1
Tibial CMAP	3.49 $\pm$ 5.08	1.2	0.0	29.0	2.8	4.2
Median CMAP	6.6 $\pm$ 6.27	4.8	0.0	29.5	5.7	7.5
Ulnar CMAP	6.57 $\pm$ 5.26	5.3	0.0	22.0	5.8	7.3
Sural SNAP	7.53 $\pm$ 9.08	5.0	0.0	51.0	6.2	8.8
Median SNAP	27.57 $\pm$ 32.53	13.6	0.0	205.0	22.9	32.2
Ulnar SNAP	21.31 $\pm$ 23.53	11.6	0.0	125.0	18.0	24.7

The mean of Conduction velocity in upper limbs, lower limbs, Peroneal CMAP, Tibial CMAP, Median CMAP, Ulnar CMAP, Sural SNAP, Median SNAP and Ulnar SNAP were 37.12 $\pm$ 17.45, 26.83 $\pm$ 18.25, 1.79 $\pm$ 2.38, 3.49 $\pm$ 5.08, 6.6 $\pm$ 6.27, 6.57 $\pm$ 5.26, 7.53 $\pm$ 9.08, 27.57 $\pm$ 32.53 and 21.31 $\pm$ 23.53. (Table 12)

Table 13: Descriptive analysis of EP Findings in the study population (N=191)

Parameters	Frequency	Percentages
Distal latency prolongation (102)		
Severe	41	21.47%
Mild	61	31.94%
Conduction block present	27	14.14%
Peak latency prolongation (25)		
Mild	20	10.47%
Severe	5	2.62%

Among the study population, 41 (21.47%) individuals had reported severe, and 61 (31.94%) had reported mild distal latency prolongation. 27 (14.14%) had reported conduction blocks. 20 (10.47%) people had reported mild, and 5 (2.62%) had reported severe peak latency prolongation. (Table 13)

Table 14: Clinical classification of CMT in study population (N=191)

Clinical classification of CMT	Frequency	Percentages
Sensorimotor	108	56.54%
Pure motor	76	39.79%
Pure sensory	6	3.14%
Probable autonomic	1	0.52%

The majority of 108 (56.54%) had Sensorimotor findings clinically, followed by 76 (39.69%) had a pure motor, 6 (3.14%) had pure sensory, and 1 (0.52%) had probable autonomic involvement. (Table 14)

Table 15: Electrophysiological classification of CMT in study population

Electrophysiological classification of CMT	Frequency	Percentages
Pure motor	65	34.03%
Sensorimotor	122	63.87%
Pure sensory	3	1.57%
normal	1	0.52%

Electrophysiologically Sensorimotor affection was noted in 122 (63.87%), followed by pure motor in 65 (34.03%), pure sensory involvement in 3(1.57%) in the present study.(Table 15)

Table 16: Electrophysiological classification of CMT based on the conduction velocity in upper limb (N=191)

3 group classification based on conduction velocity in upper limbs	Frequency	Percentages
Axonal	99	51.83%
Intermediate	55	28.79%
Demyelinating	24	12.56%
Unclassifiable	13	6.80%
<38m/s	69	36.1%

>38m/s	109	57.06%
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Based on the conduction velocity from upper limbs, CMT in present study have been classified into Axonal type in 99 (51.83%), Intermediate type in 55 (28.79%), Demyelinating type in 24 (12.56%), unclassifiable in 13 (6.8%). Conduction velocity less than 38m/s was noted in 69 (36.1%) individuals. (Table 16)

Table 17: Classification of CMT based on the nerve biopsy findings (N=65)

Parameters	Frequency	Percentages
BIOPSY PERFORMED	65	
Demyelinating	15	23.1%
Axonal	26	40%
Mixed	5	7.69%
Superadded inflammation	3	4.61%
Inconclusive	5	7.69%
Very mild changes	3	4.61%
Normal	2	3.07%
Unavailable report	6	9.23%

Biopsy was performed in 65 individuals in our study out of which 26 (40%) showed axonal loss, 15 (23.1%) showed myelin loss, followed by mixed pathology, very mild changes in 3 (4.61%) respectively. (Table 17)

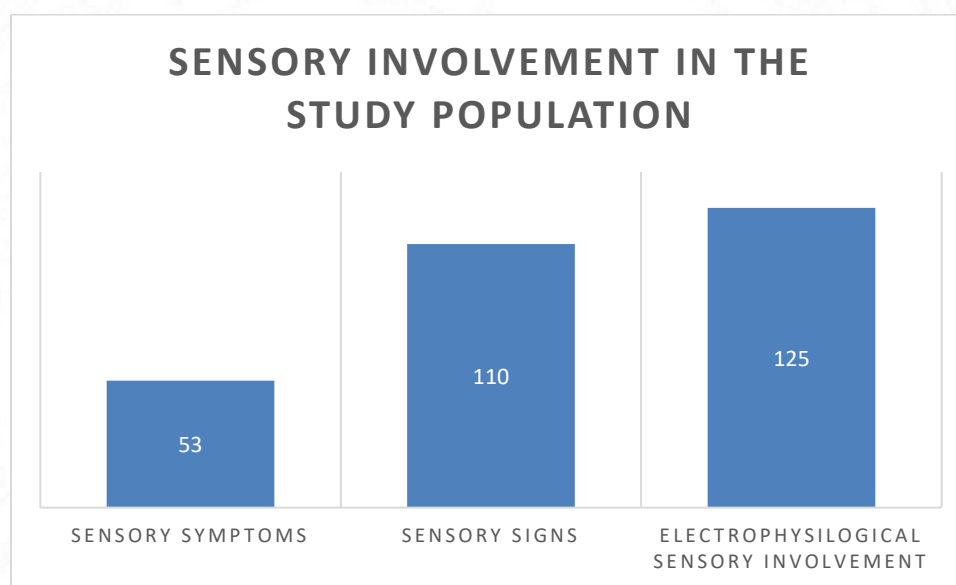
Table 18: Final diagnosis of CMT after clinical, electrophysiological, and pathological workup (N=191)

Final Diagnosis	Frequency	Percentages
Axonal	76	39.79%
Demyelinating	60	31.41%
DMN	28	14.66%
Pyramidal	13	6.81%
Mixed	4	2.09%
Indeterminate	3	1.57%

Superadded inflammation	3	1.57%
HSAN	4	2.09%

After the Clinical, electrophysiological, and pathological work up, the majority of individuals in study group had axonal 76 (39.79%), followed by demyelinating 60 (31.41%) and DMN in 28 (14.66%), associated pyramidal findings in 13 (6.815), mixed pathology in 4(2.09%), indeterminate type in 3(1.57%)

Figure:2 Bar diagram showing sensory involvement in study population:



Among the study population, 53 individuals had sensory symptoms, 110 individuals had sensory involvement on examination and 125 had electrophysiological sensory involvement

Table 19: Descriptive analysis of supportive tests in the study population

Other Tests	Frequency
CSF protein elevation (14)	7
ABNORMAL BRAIN IMAGING (7)	4
CT-BRAIN: Diffuse calcification and Atrophy of bilateral caudate, putamen	1
MRI BRAIN: symmetrical white matter hyperintensities with diffusion restriction	1
MRI-BRAIN: Bilateral basal ganglia calcification with inferior vermis, cerebellar hypoplasia, cerebellar dysplastic cortex, mild atrophy of pons	1
MRI- Peri trigonal hyperintensities, posterior limb of internal capsule without volume loss	1
MRI lumbosacral and cervical root enhancement	1
Hypertrophied nerve with uniform onion bulbs	1
Elevated lead levels	1
Arachnoid cyst spinal cord	1
CPK elevation	2
VEP Prolongation (8)	3
Puretone audiometry abnormality (12)	4
Neurogenic muscle biopsy (5)	3
Skin biopsy- hyperkeratosis (2)	2
Skin biopsy: hyperkeratosis, spongiosis, orthokeratosis, Elongated rete ridges with suprapapillary thinning, exostosis, moderate lymphocytic infiltration	1
Skin biopsy: thick laminated ichthyosiform Orthokeratosis stratum corneum	1
Vocal cord palsy on laryngoscopy (3)	1
Pulmonary function test (3)	1
POSITIVE AUTONOMIC TESTS	3
Abnormal sympathetic skin response	2
Polysomnography-mild nocturnal hypoxemia, no OSA (1)	1

Summary of genetic analysis with inherited neuropathy included in the present study:

Table 20: Descriptive analysis of genetic defect in the study population (N=38)

Total number of genes identified- 26

Genetic Defect	Frequency	Percentages
PMP22	7	18.42%
PMP22 DUPLICATION	6	16.22%
PMP22 DELETION	1	2.63%
IGHBP2	3	7.89%
ERCC-2	2	5.26%
GDAP1	2	5.26%
BSCL-2	1	2.63%
COL-Q7	1	2.63%
DHTKD1	1	2.63%
DNM2	1	2.63%
HINT-1	1	2.63%
INF2	1	2.63%
KARS	1	2.63%
MFN-2	1	2.63%
MTMR2, HARS	1	2.63%
NAGLU	1	2.63%
NTRK 1C	1	2.63%
PLEKHG5	1	2.63%
PRX	1	2.63%
SCN-9A	1	2.63%
SH3TC2, LITAF	1	2.63%
SIGMAR-1	1	2.63%
SLC25A46	1	2.63%
TRPV4	1	2.63%
VRK1	1	2.63%
WNK1	1	2.63%
Negative test	4	10.5%

Among the study population, genetic testing was performed in 38 individuals, among them 6 (16.22%), 3 (8.11%), 2(5.42%) and 2 (5.41%) had PMP22 duplication, IGHBP2, ERCC-2 and GDAP-1 genetic defects. Negative genetic test was noted in 4 individuals.

FIGURE: 3 Pie diagram showing the distribution of genetic defects in the study population

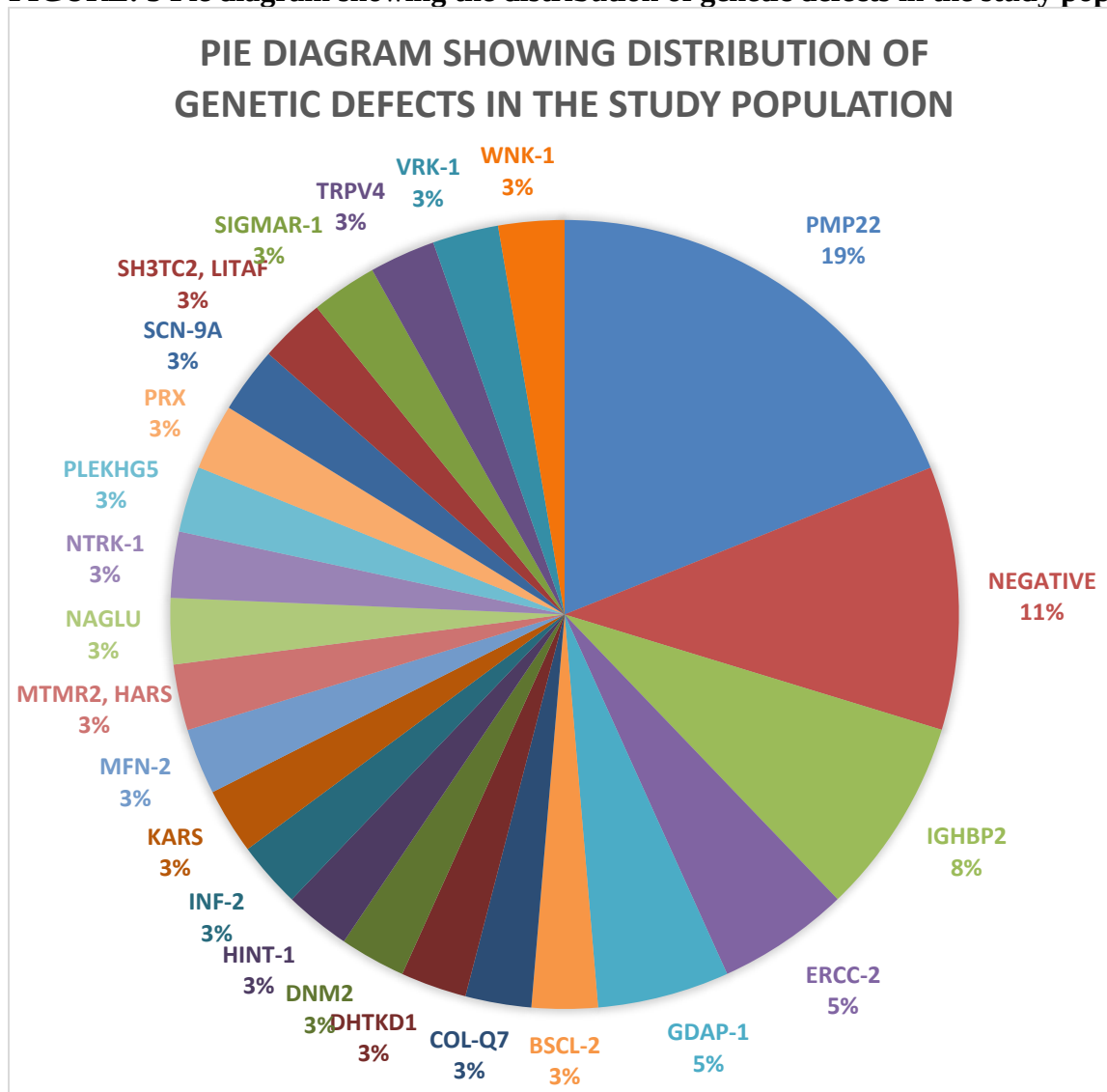


Table 21: Observational analysis of genetically positive group in present study

Total number of genes identifies with variations	26
Genes in patients with early onset neuropathy	PRX, NTRK 1C, IGHBP2, PMP22, WNK1, SCN9A, SLC25A6, GDAP1, MFN 2 BSCL-2, MTMR2, HARS
Male	ERCC-2, HINT-1 TRPV4, VRK1, DHTKD1, PLEKHG5, PMP22 WNK1, SCN-9A, PMP22, GDAP1 SIGMAR-1, MFN-2, MTMR2, HARS, SH3TC2, LITAF
Developmental delay	PRX, NTRK 1C IGHBP2, PMP22, WNK1, GDAP1

Poor athletic performance	PRX, IGHBP2, PMP22, WNK1 SLC25A46, GDAP1, MTMR2, HARS
Asymmetrical onset	TRPV4, DHTKD1, PMP22, SLC25A46, GDAP1, BSCL2, INF2
Distal weakness	ERCC-2, TRPV4, VRK1 DNM2, GDAP1 MFN-2, PMP22
deformities	ERCC-2, TRPV4 VRK1, KARS, NTRK 1C DHTKD1, IGHBP2, PMP22, WNK1, NAGLU DNM2, SLC25A46, GDAP1, SIGMAR-1 BSCL-2, MTMR2, HARS, INF2, SH3TC2, LITAF
History of consanguinity	ERCC-2, NTRK 1C, IGHBP2, SLC25A46 GDAP1, MTMR2, HARS, SH3TC2, LITAF
pyramidal	ERCC-2, HINT-1, COL-Q7, TRPV4, KARS WNK1, PMP22, SLC25A46, GDAP1
Family history present	ERCC-2, IGHBP2 PLEKHG5, PMP22, WNK1 PMP22, SLC25A46, MFN-2 MTMR2, HARS, SH3TC2, LITAF
Axonal neuropathy	HINT-1, COL-Q7, VRK1, KARS NTRK 1C, DHTKD1, NAGLU DNM2, SLC25A46, GDAP1 MFN-2, BSCL-2, INF2
Demyelinating neuropathy	PRX, TRPV4, IGHBP2, PMP22 GDAP1, MTMR2, HARS
Conduction blocks	PLEKHG5, PMP22, NAGLU
Intermediate	ERCC-2, IGHBP2, PLEKHG5 WNK1, PMP22, SIGMAR-1, SH3TC2, LITAF
PURE MOTOR	TRPV4, DHTKD1, PLEKHG5 DNM2, SIGMAR-1, BSCL-2, INF2
HSAN	SCN-9A, NTRK-1C

Table 22: Genetic analysis in the study group

6/F	PRX	NONSENSE EXON-7	c.1174c>t chr 19: g.40903085g>a	HOMOZYGOUS	A. R	Pathogenic
15/M	ERCC-2	MISSENSE EXON 21	c.1906c>t chr 19	ERCC-2 homozygous	A.R	VUS
	GAN	MISSENSE EXON10	c.1523a>g chr 16	GAN- homozygous	A. R	VUS
18/M	ERCC-2	MISSENSE EXON 21	c.1906c>t chr 19	ERCC-2 homozygous	A. R	VUS
	GAN	MISSENSE EXON10	c.1523a>g chr 16	GAN- homozygous		VUS
11/M	HINT1	MISSENSE EXON3	c.253g>c chr.5	HINT-1 homozygous	A. R	VUS
12/F	COLQ7	SINGLE NUCLEOTIDE VARIATION EXON4	chr 16	COL-Q7 Heterozygous Biallelic mutation		
		SINGLE NUCLEOTIDE DELETION EXON5				

19/M	TRPV4	MISSEN SE EXON4	c.656g>a chr12	TRPV4 Heterozygous	A. D	VUS
25/M	VRK1+			VRK-1		VUS
20/F	KARS	MISSEN SE EXON10 MISSEN SE EXON 11	c.1262g>a c.1343g>a	KARS Heterozygous HETRO ZYGOUS	A. R	VUS
3/F	NTRK 1	Intronic 13	homozygous c.1787+1 g>t	NTRK 1 Homozygous intronic		Pathogenic
17/M	DHTKD1	MISSEN SE EXON 5	c.981c>g	DHTKD1 heterozygous	A. D	VUS
7/F	IGHBP2	EMISSE NSE XON8	c.1198g>a	IGHBP2 homozygous	A. R	VUS
5/F	IGHBP2	MISSEN SE EXON8	c.1198g>a	IGHBP2 homozygous	A. R	VUS
9/F	IGHBP2	MISSEN SE EXON8		IGHBP2 homozygous		VUS
17/M	PLEKHG5	MISSEN SE	c.2347- g>a	PLEKG5 Homozygous		Pathogenic
29/M	PMP-22 DUPLICATION in exon 1-5	COPY NUMBER VARIATION IN PMP22 EXON1-5	copy number variation	PMP22 Heterozygous duplication		Pathogenic
17/M	Wnk1	MISSEN SE Chr 12	c.6664g>t exon 27	WNK-1 Heterozygous	A. R	VUS

35/F	PMP-22 DUPLICATION	Heterozygous duplication on entire PMP-22	copy number variation	PMP-22 Heterozygous duplication	A. D	pathogenic
3/M	SCN-9A	EXON 26	c.a660a>t --	SCN-9A Homozygous		Pathogenic
29/F	NAGLU- EXON6	Missense Exon6	c.2146c>g	NAGLU Heterozygous	A. D	VUS
17/F	DNM2-			DNM-2 Heterozygous	A. D	VUS
35/F	PMP22, COX10	Duplication - Chromosome 17	copy number variation	PMP22, - copy number variation COX10 Heterozygous duplication	A. D	Likely pathogenic
13/M	PMP22	Deletion of exonic regions chromosome 17	copy number variation	PMP-22 Copy number variation Heterozygous	A. D	LIKELY PATHOGENIC
13/F	SLC25A4 6	MISSENSE EXON7	c.670a>g	SLC25A4 6 Homozygous	A. R	VUS
25/M	GDAP-1, (BOTH PARENTS HETEROZYGOUS FOR SAME MUTATION)	DELETION EXON7	c.1018 del	GDAP-1 Homozygous	A. R	.

34/M	SIGMAR-1			SIGMAR-1 HOMOZYGOUS	A. R	
9/M	GDAP1 MFN2	MISSENSE EXON 7	c.922c>t	GDAP-1 Heterozygous	A.D/ A. R	VUS
		MISSENSE EXON9	c.907t>c	MFN2 Heterozygous	A.D/ A. R	VUS
7/F	MFN2	MISSENSE EXON3	c.281g>a	MFN-2 Heterozygous	A. D	Pathogenic
37/M	PMP22 DUPLICATION OF ENTIRE GENE			PMP 22 Heterozygous duplication of entire gene	A. D	Pathogenic
10/F	BSCL 2	MISSENSE Exon4		BSCL-2 missense heterozygous		Pathogenic
6/M	MTMR2 HARS	NONSENSE EXON4	c.304c>t	MTMR-2 Homozygous	A.R	Pathogenic
		MISSENSE EXON7	c.710c>t	HARS HETEROZYGOUS	A. D	VUS
24/F	INF2	MISSENSE EXON8	c.1317a>t	INF-2 Heterozygous	A. D	VUS
60/M	PMP22 HETEROZYGOUS DUPLICATION OF	HETEROZYGOUS DUPLICATION OF ENTIRE	copy number variation	PMP-22 Heterozygous duplication of entire gene	A. D	Pathogenic

	ENTIRE GENE	PMP-22 GENE				
50/M	SH3TC2	Nonsense Exon11	c.2710c>t	SH3TC2 Homozygous	A.R/ A. D	Pathogenic
	LITAF	MISSENSE EXON2	c.134c>t	LITAF Heterozygous	A. D	VUS
13/F	PMP22 duplication	Copy number variation	chr 17	duplication	A. D	Pathogenic

Table: 23 Genotype phenotype correlation in the study group

PMP22 (7)	Asymmetric onset, Pes cavus, Clawing of hands, hammer toes, Tibialis anterior contracture, pseudo athetosis, thickened nerves, microorchidism NCS: Demyelinating sensorimotor neuropathy
IGHBP2 (3)	Ape thumb deformity, everted foot, inverted champagne bottle appearance, Tibialis anterior contracture, pes cavus, callosities, delayed social development NCS: Mixed sensorimotor neuropathy with conduction blocks
ERCC2, GAN (2)	Cataract, talipes equinovarus Prolonged P100 Latency NCS: sensorimotor axonal neuropathy (1), mixed neuropathy (1)
GDAP-1 (2)	Vocal cord palsy, hyperpigmented knuckles, hammer toes NCS: Axonal sensorimotor neuropathy
HINT-1 (1)	Less interactive, walking difficulty, lordotic waddling, Intellectual disability, Exaggerated lumbar lordosis, mild scoliosis, subtle dynamic spasticity in lower limbs, MRI-BRAIN: Symmetric FLAIR HI in bilateral periventricular regions NCS: Pure motor neuropathy Giant SSEP
PRX (1)	Developmental delay, poor athletic performance, Diffuse nerve thickening, thoracic scoliosis, hyper extensibility of joints, pes cavus, talipes equinovarus, scoliosis, lordosis, respirator insufficiency, hyper extensible joints, Tibialis anterior contracture, NCS: Severe demyelinating sensorimotor neuropathy

COL-Q7 (1)	Recurrent abdominal pain, oral ulcers, Slow vertical saccades, Tibialis anterior contractures, pyramidal signs NCS: Pure motor axonal neuropathy
TRPV4 (1)	Asymmetric onset, bilateral pes cavus, thinning of calves, Pyramidal signs NCS: Pure motor axonal neuropathy
VRK-1 (1)	Pes cavus, increased sweating in palms, wasting in distal upper and lower limbs, mild neck flexion weakness, Pyramidal signs MRI-thinning of dorsal spine NCS: Pure motor axonal neuropathy
KARS (1)	Proximal weakness, truncal weakness, pyramidal symptoms NCS: Pure motor axonopathy
NTRK-1 (1)	Congenital insensitivity to pain, non-healing ulcers Low set ears, microcephaly, upturned philtrum, retrognathia, dystrophic teeth, poikilothermic, non-healing ulcers, amputated right great toe, pseudo joint effusion of elbow NCS- normal, SSR- in elicitable,
DHTKD1 (1)	Asymmetric onset, proximo-distal lower limb weakness, Inverted champagne bottle, pes cavus, hammer toes, right greater auricular nerve hypertrophied, Pyramidal signs NCS: Pure motor axonal neuropathy
PLEKHG5 (1)	Shortness of breath on exertion, Marfanoid habitus, arachnodactyly, high arched palate, thumb sign, wrist sign, pes cavus, hammer toes, lax joint, mild neck weakness NCS: Motor demyelinating neuropathy with conduction blocks
WNK-1 (1)	Developmental delay, intellectual sub normality, short stature, wide space teeth, pes cavus NCS: Demyelinating sensorimotor neuropathy VEP: P100 prolongation
SCN-9A (1)	Congenital insensitivity to pain, non-healing ulcers over foot, ulcers over knees, pain, temperature absent perception, reflexes- intact NCS, SSR: NORMAL
NAGLU (1)	Retention of urine, proximal f/b distal weakness, hammer toes NCS: Demyelinating sensorimotor neuropathy with conduction blocks
DNM2 (1)	Asymmetric motor distal weakness NCS: Pure motor axonopathy
SLC25A46 (1)	Visual complaints, distal asymmetric weakness, Optic atrophy, pes cavus, hammer toes, TA contracture, bilateral foot drop NCS; Sensorimotor axonal neuropathy VEP: prolonged

SIGMAR-1 (1)	Pure motor asymmetric, pes cavus, clawing of hands, intrinsic foot wasting NCS: Mixed motor neuropathy
MFN2 (1)	Bilateral tibialis contractures, pes cavus, thenar wasting NCS: Sensorimotor axonal neuropathy
BSCL2 (1)	Asymmetric onset, motor claw hand, pes cavus, hammer toes, NCS: Motor axonal neuropathy
MTMR2, HARS (1)	Proximo-distal weakness, pes cavus, tibialis anterior contracture, bifacial weakness, partial ptosis neck flexion mild weakness, knee flexion mild weakness, NCS: Demyelinating sensorimotor neuropathy
INF-2 (1)	Distal pure motor asymmetric weakness, wasting, hypotonia NCS: Motor axonal neuropathy
SH3TC2, LITAF (1)	Hearing difficulty, neck, and truncal weakness, Pes cavus, clawing in hands, hyperpigmentation in lower limbs, eye: restricted abduction and elevation, bilateral sensorineural hearing loss, NCS: Demyelinating sensorimotor polyneuropathy



DISCUSSION

CMT is the commonest inherited neuromuscular disorder affecting the peripheral nerves and leads to progressive lower limb followed by upper limb weakness. The present study was conducted in a single tertiary care center in south India to study the clinical and electrophysiological profile of patients with CMT over 24 years. The present study also aims to correlate the electroclinical profile with nerve biopsy and genotype. The objective of the present study is similar to that of the study by Gemelli C et al.⁹⁴ and Khadilkar SV et al.¹⁹, with respect to the description of the genetic profile, clinic-electrophysiological profile and phenotype.

Baseline characteristics of the study group:

A total of 191 subjects were included in the final analysis of the present study. 585 patients were evaluated in the study by Gemelli C et al.⁹⁴ 22 patients were included in the study by Khadilkar SV et al.¹⁹ the median duration of disease presentation was variable in their studies. The mean age at presentation was 24.59 ± 14.78 years in the present study, with a range of 3 to 67 years, and then the mean duration of symptoms was 9.06 ± 10.01 years. The mean age at onset was 15.5 ± 13.44 years, Their findings were similar to the present study, as the socio-demographic settings are similar in both studies. Their study was among the pioneer series with a genetic evaluation of CMT patients in India. CMT presents at a relatively younger age, around 12 years, with progressive weakness in the extremities.^{15, 16} Age at onset ranges from infancy to the elderly. It has an insidious onset and slow progression. However, in the study by Gemelli C et al.⁹⁴, the overall mean age was 53 years $\pm$ (16), with an age range of 13–94 years. This could be due to the single-site retrospective experience of their study covering patients enrolled in the past 15 years which might led to an overestimation of their mean age.

In the present study, the majority (62.83%) were males. Gemelli C et al.⁹⁴ observed an equal sex distribution (47% female, 53% male). However, in the study by Khadilkar SV et al.¹⁹, 19 of the 22 individuals were males. In their study, out of 22 subjects, Family members were affected in 4 cases, and 18 were sporadic (82%). Gemelli C et al.⁹⁴ observed that index cases were 170, out of which 20 were family positivity and 150 isolated cases in their study, and they were more frequently presented with axonal neuropathy (69%). More than three members of the same family were affected in only 2.11% of the study individuals in the present study,. 24.08% of the subjects had a history of consanguinity, and 49.74% had a similar family history.

Genetic defects in the study group:

Genetic diagnosis in hereditary neuropathy has rapidly evolved over the last few years with the introduction of next-generation sequencing (NGS) into routine practice, which involves sequencing of individual genes addressed by the mode of inheritance, clinical and electrophysiological phenotypes, and data about the prevalence of different genetic subtypes, as well as peculiar genotype-phenotype associations. In NGS, several disease-associated genes can be tested in parallel. The most observed genetic mutations in the present study were PMP22 (18.42%) and IGHBP2 (7.89%), followed by ERCC-2 (5.26%), GDAP-1 (5.26%) 4 (10.5%) had a negative report. Other genetic defects found in the present study were GDAP1, BSCL-2, COL-Q7, DHTKD1, DNM2, HNT-1, INF2, KARS, MFN-2, MTMR2, HARS, NAGLU, NTRK 1C, PLEKHG5, PRX, SCN-9A, SH3TC2, LITAF, SIGMAR-1, SLC25A46, TRPV4, VRK1 and WNK1.

By genetic diagnosis in 391 patients, Gemelli C et al.⁹⁴ observed that most patients were affected by demyelinating neuropathy (86%), whereas axonal forms accounted for 14% of genetically identified cases. They observed that demyelinating cases achieved a positive genetic

diagnosis more frequently than axonal and intermediate ones. Gemelli C et al.⁹⁴ observed that the most common genetic diagnoses were CMT1A, caused by PMP22 duplication, accounting for one-half of all patients (48%); HNPP, caused by PMP22 deletion (14%); CMT1X, caused by pathogenic variants in GJB1 (13%); P0-related neuropathies caused by MPZ pathogenic variants (7%); CMT2A due to MFN2 pathogenic variants (5%). Altogether, these accounted for 87% of all molecularly defined neuropathies in their study. Pathogenic variants in rarely mutated genes (SH3TC2, LITAF, RAB7A, NEFL, AARS, MTMR2, NDRG1, PRPS1, INF2, PMP2, DNMT2, FBLN5, HINT1, IGHMBP2, PMP22) each accounted for less than 1% of the total, except for HSPB1 pathogenic variants that were found in 3% of all index cases and GDAP1, which accounted for 2% of all index cases in their study.

DiVincenzo C et al.¹⁶, in their study, observed that genetic abnormalities were identified in 18.5% ($n = 3312$) of all individuals. Testing by Sanger and MLPA ($n = 3216$) showed that duplications (dup) (56.7%) or deletions (del) (21.9%) in the *PMP22* gene accounted for the majority of positive findings, followed by mutations in the *GJB1* (6.7%), *MPZ* (5.3%), and *MFN2* (4.3%) genes. *GJB1* del and mutations in the remaining genes explained 5.3% of the abnormalities. Pathogenic mutations were distributed as follows in their study: missense (70.6%), nonsense (14.3%), frameshift (8.7%), splicing (3.3%), in-frame deletions/insertions (1.8%), initiator methionine mutations (0.8%), and nonstop changes (0.5%).

Clinical presentation:

Regarding clinical presentation, 13.61% had developmental delays, and 18.32% had poor athletic abilities. 24.08% had asymmetrical symptoms at onset. Also, in the present study, 86.91% had lower limb onset, while 31.41% had upper limb onset. Symptoms are more pronounced in lower limbs as compared to upper limbs. With regards to symptoms at onset, 12.04% had ataxia, 8.9%

had paraesthesia, and with regards to weakness, 39.26% had lower limb weakness, 12.04% had distal lower limb weakness, and 12.04% had both upper limb and lower limb weakness in the present study. 26.7% of the study population had sensory defects. Commonly reported symptoms are difficulty in running, tripping, falls, and twisting or spraining of ankles. Patients may have delayed motor milestones. During childhood, these patients are 'slow' in sports, with reduced athletic performance. As the weakness progresses, patients eventually develop foot drop and the hands manifests as difficulty in holding objects. with few sensory symptoms and signs. About 10.47% of patients complain of musculoskeletal or neuropathic pain.

Khadilkar SV et al.¹⁹ observed that all the patients presented with motor weakness and had sensory complaints in their study. Motor weakness was most prominent in the distal part of the lower limbs in all 22 patients. The upper limb was affected in 19 patients, but the weakness was less severe than in the lower limbs. The weakness was symmetrical in 17 (82%), and five patients showed mild asymmetry of weakness. 86% had Pes cavus, 73% had hammer toes and Scoliosis was seen in one patient. Nerve thickening on clinical examination was detected in 9 patients. The sensory deficit was documented in 7 patients, predominantly of the large fibre type. A single patient had membranous glomerulopathy.

Abnormal gait and imbalance usually occur because of proprioceptive loss and foot deformities.

30 These clinical manifestations result from the axonal loss, even in demyelinating CMTs.⁴¹⁻⁴³

In the present study, 56.54% had sensorimotor neuropathy, 39.79% had pure motor neuropathy and 3.14% had pure sensory neuropathy. 64.4% had Pes cavus, 36.13% had hammer Toe, 29.84% had contracture, and many had tibialis anterior contracture. Spasticity was present in 10.47% of individuals. The most common deformities were Scoliosis (16.75%) and talipes

equinovarus (8.9%), followed by inverted Champagne Bottle appearance of lower limbs (7.33%).

81.15% had an abnormal gait, and 57.06% had a high stepping gait. 14.14% had nerve thickening. Diffuse nerve thickness was noted in 1.05% of individuals. CPN was thickened in the majority of the individuals.

On general examination, 12 (6.28%) subjects had involvement of skin and appendages involvement, 6 (3.14%) subjects had dysmorphic facies, microcephaly in 2.10%, and calf hypertrophy in 1.57%.

Electrophysiological findings in the study population:

Electrophysiological and neuropathological findings differentiate CMT into demyelinating and axonal types based on motor conduction velocities from median and ulnar nerves. The mean of Conduction velocity in upper and lower limbs, Peroneal CMAP, Tibial CMAP, Median CMAP, Ulnar CMAP, Sural SNAP, Median SNAP and Ulnar SNAP, respectively, were 37.12 ± 17.45 , 26.83 ± 18.25 , 1.79 ± 2.38 , 3.49 ± 5.08 , 6.6 ± 6.27 , 6.57 ± 5.26 , 7.53 ± 9.08 , 27.57 ± 32.53 and 21.31 ± 23.53 . In the present study, 21.47% had severe prolonged distal latency in motor conduction. Based on the conduction velocity from upper limb nerves, 51.83% were axonal, followed by intermediate (28.79%) and demyelinating (12.56%). 6.8% were classified as unclassifiable type as the in elicitable CMAP from the tested motor nerves. 12.57% of subjects had inflammatory suspicion. Gemelli C et al.⁹⁴ observed that in neurophysiology, the demyelinating phenotype was found in 64.9% and an axonal phenotype in 35.1% of their study. Khadilkar SV et al.¹⁹ observed that motor nerve conduction studies were abnormal in all the 22 patients. Lower limb motor nerves were severely affected than the upper limb. Tibial nerve (abductor hallucis) compound motor action potentials were absent in 19/22 patients in their

study. In the upper limbs, the median motor nerve was more affected in terms of CMAP amplitude, conduction velocity, distal latencies, and temporal dispersion. Conduction blocks were noted in 5 patients. Uniform conduction slowing was observed in 10 patients. Sensory nerve action potentials were affected in both extremities but more in lower limbs, particularly sural nerves, in 12 patients. Axonal electrophysiology was observed in 7 patients in their study.

Biopsy findings in the study population:

Nerve biopsy was done in 65 individuals, of which 40% had axonal changes, followed by 23.1% had demyelinating changes on biopsy.

Electroclinical diagnosis in the study population:

Nerve conduction studies help confirm the diagnosis of neuropathy and broadly categorize patients into demyelinating and axonal subtypes. They help in screening asymptomatic relatives of the index patient. Nerve Biopsy has a role in identifying underlying genetic etiology in sporadic cases, which helps to distinguish CMT from acquired disorders like CIDP. Nerve biopsy may also support a functional association when the genetic tests detect "variants of uncertain significance" or a novel variant.

In the present study, the majority had Axonal type at 39.79%, followed by demyelinating at 31.41%, DHMN at 14.66%, association with pyramidal signs at 6.81%, mixed type at 2.09%, indeterminate type at 1.57%, 1.57% had superadded inflammation, HSAN in 2.09% after detailed electrophysiological and pathological evaluation.

Khadilkar SV et al.¹⁹ observed that based on the clinical and electrophysiological features, ten patients were classified as CMT 1, 7 as CMT 2, and HNPP in four patients. One patient was diagnosed with CMT X. Some patients may have demyelinating and axonal neuropathy features.

Evidence of denervation on concentric needle electromyography is useful in establishing axonal pathology. By genetic diagnosis in 391 patients, Gemelli C et al.⁹⁴ observed that demyelinating neuropathy accounted for 86% and axonal form in 14% of genetically identified cases. They observed that a positive genetic diagnosis was achieved in demyelinating cases more frequently than axonal and intermediate types.

The final diagnosis in the study population:

The majority (35.6%) had axonal, followed by demyelinating (30.37%) and DMN (17.28%) types among the study group.

Genotype-phenotype correlation:

Yalcouye A et al.⁷⁸ observed that demyelinating form of CMT was the commonest subtype, and the phenotype varied greatly between families. Gemelli C et al.⁹⁴ observed that their population had the most common genetic abnormality detected in the MPZ and HSPB1 genes. They identified fifteen patients affected by axonal CMT associated with the MPZ pathogenic variant (CMT2I/2J) and nine patients affected by CMT (CMT2F) or distal hereditary motor neuropathies (dHMN). A length-dependent phenotype with exclusive involvement of the lower limbs in the earlier stage was found in 60% of cases with a pathogenic variant in HSPB1 and 80% of patients with the late-onset MPZ pathogenic variant (CMT2) (12 out of 15 patients).

They described a new phenotype with

1. the onset of the disease during adulthood (a fourth decade on average),
2. the early exclusive or prevalent involvement of the lower limbs, and
3. the mild to moderate severity of the disease.

Genetic advances have led to a continuous increase in genes related to neuromuscular diseases, and in clinical practice, gene panels must be periodically updated.

Strengths of the study:

It is a large retrospective study performed with a meticulous screening of all the CMT cases from the available electronic medical records and electromyographic records.

NGS findings in a large cohort of patients with inherited neuropathies from India highlight genetic abnormalities.

This study brings out a number of novel variants and VUS. Establishing an accurate genetic diagnosis is important for genetic counselling and the perspective of including patients in upcoming therapeutic trials.

Limitations:

It is a retrospective study; hence the possibility of recall bias is likely.

It is a single-centre study, and the generalizability of the present study findings may be questionable.

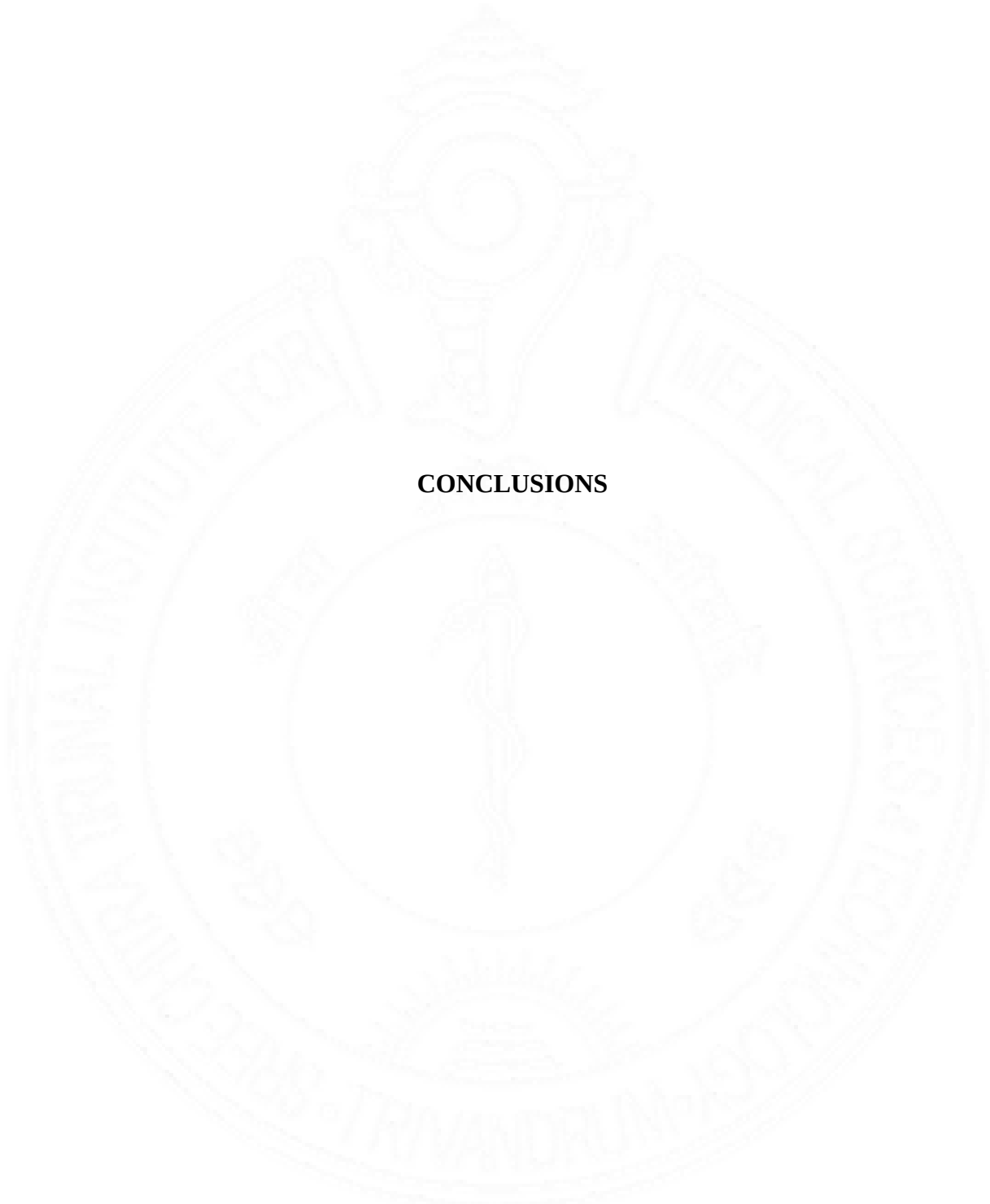
NGS identified variants in several genes, including those that have pathobiological significance in neuropathy and other non-neuropathic disorders. The functional validation of novel variants and the impact of their interactions with other molecular partners remain to be established in future studies.

Parental testing for segregation analysis in the case of VUS was not performed in all the cases.

Table 24: Comparison of the previous studies with the present study

	Type of study	Study period	Genetic study	Demographic data	Genes identified	Most common type of CMT
Present study*	Retrospective, India	January 1998 to December 2021	38 out of 191	M: 120 F: 71 Age: 3-67Years	PMP22 IGHBP2, ERCC-2, GDAP1, BSCL-2, COL-Q7, DHTKD1, DNMT2, HNT-1, INF2, KARS, MFN-2, MTMR2, HARS, NAGLU, NTRK 1C, PLEKHG5, PRX, SCN-9A, SH3TC2, LITAF, SIGMAR-1, SLC25A46, TRPV4, VRK1 and WNK1.	Axonal and type of CMT
Shivani Sharma et al ¹ 2022	Prospective, India	2017-2020	55	M: 29 F: 26 Age: infancy-54y	<i>MFN2+SBF, SH3TC2+AARS, SH3TC2+JPH 1, SH3TC2+UBQLN2, FGD4+WNK1, MARS+SBF2, MPZ+DNMT1, and GARS+GAN, DCTN1+BLK, IGHMBP2+SLC12A6, and MFN2+VPS13D). ATM, SETX, COX15, MPV17, OPA1, SACS, C10ORF2, APOB, and CDH23</i>	Demyelinating
Gentile, L. et al 2021⁹⁵	Prospective, Italy		17	6-74Y	BSCL2, MORC2, HINT1, LITAF, GARS, and autosomal dominant GDAP1	Axonal

NGUYEN-LE ET AL. ⁹⁶	Prospective, Vietnam,		31	1.6:1 0-60years	PMP22 DUPLICATION, PMP22 DELETION, MFN2, NEFL, GJB1	Demyelinati ng
Gamelli Et al ⁹⁴	Retrospecti ve. Genova Italy	2004- 2020	391 out of 585	53:47 13-94 years	PMP22 duplication, PMP22 deletion, GJB1 MFN2 , MPZ, HSPB1, SH3TC2, LITAF, RAB7A, NEFL, AARS, MTMR2, NDRG1, PRPS1, INF2, PMP2, DNM2, FBLN5, HINT1, IGHMBP2, PMP22	Demyelinati ng
S V Khadilkar ¹⁹	Prospective, India	2016	22	19:3 23.5 years (3-69 years).	PMP22, GJB1, SH3TC2, HSPB1, SPTLC2, MPZ, AARS, and NEFH gene	Demyelinati ng



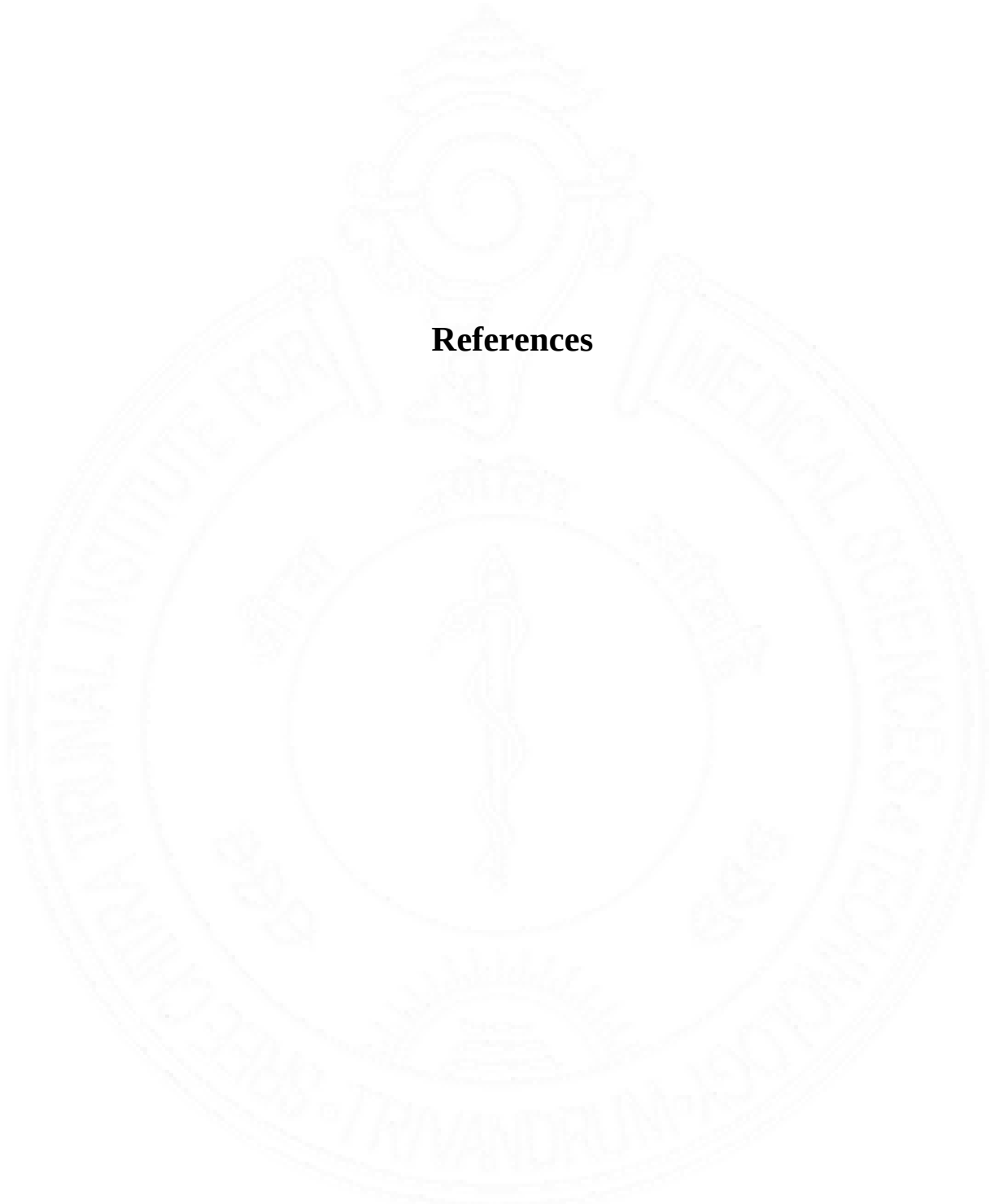
CONCLUSIONS

Conclusions:

1. CMT is the commonest inherited neuromuscular disorder, affecting the peripheral nerves and leading to progressive weakness of extremities.
2. It is a retrospective, descriptive study conducted to analyze the clinical and electrophysiological profile of patients with CMT attending a single tertiary care centre in South India over 24 years. A total of 191 subjects with the diagnosis of CMT were included for the study out of which nearly 2/3rds were males with mean age at presentation was 24.59 ± 14.78 years with a mean age at onset of symptoms was 15.5 ± 13.44 years. Family history positivity was present in a quarter of individuals
3. Among the study group, 18.32% had poor athletic abilities, 13.61% had developmental delays, 31.41% had upper limb onset of symptoms and 24.08% had asymmetrical symptoms at onset.
4. Regarding symptoms at onset, motor symptoms were present in 58.63%, and, 27.74% had sensory symptoms. 31.41% had upper limb symptoms at the onset of illness
5. 86.39% had deformities in the examination, and the most common deformity identified were Pes cavus in 64.4%, followed by hammertoes in 36.13%. Spasticity was present in 10.47% of individuals.
6. Based on the clinical, electrophysiology and biopsy findings, the Axonal type of CMT was more commonly seen in 39.79%, followed by demyelinating in 31.41%, Distal motor neuropathy in 14.6%, associated with pyramidal tract involvement in 6.81%, mixed type in 2.09%, indeterminate type in 1.57%, HSAN in 2.09%. 1.57% had superadded inflammation in the background of hereditary neuropathy.

7. The most commonly observed genetic mutations in the present study were PMP22 (18.42%), Negative results (10.5%), IGHBP2 (7.89%), followed by ERCC-2 (5.26%) and GDAP-1 (5.26%) Other genetic defects found in the present study were BSCL-2, COL-Q7, DHTKD1, DNM2, HNT-1, INF2, KARS, MFN-2, MTMR2, HARS, NAGLU, NTRK1C, PLEKHG5, PRX, SCN-9A, SH3TC2, LITAF, SIGMAR-1, SLC25A46, TRPV4, VRK1 and WNK1.





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APPENDIX

Abbreviations

CMT	Charcot Marie Tooth disease
AD-CMT	Autosomal dominantly inherited CMT
ALS	Amyotrophic lateral sclerosis
ARASHI	Autosomal recessive non- syndromic hearing impairment
AR-CMT	Autosomal recessively inherited CMT
BAEP	Brainstem auditory evoked potential
CB	Conduction block
CIDP	Chronic inflammatory demyelinating polyradiculoneuropathy
CMAP	Compound muscle action potential
CMTES	CMT examination score
CMTNS	CMT neuropathy score
CMT-X	X-linked Charcot Marie Tooth disease
CPK	Creatinine phosphokinase
CSA	Cross sectional area

DHMN/DMN	Distal hereditary motor neuropathy
EDTA	Ethylene diaminetetraacetic acid
GDAP-1	Ganglioside induced differentiation associated protein
GJB1	Gap junction formation
Gly	Glycine
HMN	Hereditary motor neuropathy
HMSN	Hereditary motor and sensory neuropathy
HMSN	Hereditary motor and sensory neuropathy-Lom
HSAN	Hereditary sensory and autonomic neuropathy
HSN	Hereditary sensory neuropathy
HSP27	Heat shock protein 27
IMFA	Intramuscular fat accumulation
m/s	Meter/second
Met	Methionine
MLPA	Multiplex ligation dependent probe amplification
MNCV	Motor nerve conduction velocity
MPZ	Myelin protein zero

MRI	Magnetic resonance imaging
NCV	Nerve conduction velocity
NGS	Next generation sequencing
OSA	Obstructive sleep apnoea
PFGE	Pulsed field gel electrophoresis
PFT	Pulmonary function test
PMP22	Peripheral myelin protein 22
PSG	Polysomnography
RLS	Restless leg syndrome
SNAP	Sensory nerve action potential
UL, LL	Upper limb, lower limb
VEP	Visual evoked potential
VUS	Variants of unknown significance

PARTICIPANT PROFORMA
PROFORMA

1. Identification information

- 1.1 Serial number -----
- 1.2 Unique identification number -----

2. Demographic data

- 2.1 Age-----years
- 2.2 Sex ----- 1. Male 2. Female
- 2.3 Occupation -----
- 2.4 Year of registration -----

3. History (1 = Yes, 0 = No)

- 3.1 Age of onset -----
- 3.2 Duration of illness -----
- 3.3 Developmental delay -----
- 3.3.1 If yes, specify -----
- 3.4 Poor athletic performance -----
- 3.5 Distal lower limb weakness ----- Duration -----
- 3.6 Proximal lower limb weakness ----- Duration -----
- 3.7 Distal upper limb weakness ----- Duration -----
- 3.8 Proximal lower limb weakness ----- Duration -----
- 3.9 Gait difficulty ----- Duration -----
- 3.10 Falls ----- Duration -----
- 3.11 Neck weakness -----
- 3.12 Trunk weakness -----
- 3.13 Respiratory difficulty -----
- 3.14 Cranial nerve involvement -----
- 3.14.1 I yes, specify -----

3.15 Sensory symptoms ----- Duration -----

3.15.1 If yes, specify -----

3.16 Pain -----

3.17 Cramps -----

3.18 Deformities -----

3.19 Functional status -----

(0 = normal, 1 = mild difficulty, independent, 2 = moderate difficulty, independent, 3 = partly dependent, 4 = bed/ wheel chair bound)

4. Other history (1= Yes, 0 = No)

4.1 Other illnesses -----

4.1.1 If yes, details -----

4.2 Family history -----

4.2.1 If yes, details -----

4.3 Consanguinity -----

4.9.1 If yes, details -----

5. Physical signs (1 = Yes, 0 = No)

5.1 Systemic signs ----- Specify -----

5.2 Cranial neuropathy ----- Specify -----

5.3 Deformities -----

5.3.1 Pes cavus -----

5.3.2 Hammer toes -----

5.3.3 Equinovarus -----

5.3.4 Claw hands -----

5.3.5 Kyphoscoliosis -----

5.3.6 Others -----

5.4 Contractures ----- Specify -----

5.5 Wasting ----- Specify -----

5.6 Power of lower limbs (Right/ Left)

5.6.1 Toe flexors -----

5.6.2 Toe extensors -----

5.6.3 Ankle dorsiflexion -----

5.6.4 Ankle plantar flexion -----

5.6.5 Knee flexion -----

5.6.6 Knee extension -----

5.6.7 Hip abduction -----

5.6.8 Hip adduction -----

5.6.9 Hip flexion -----

5.6.10 Hip extension -----

5.7 Power of upper limbs (Right/ Left)

5.7.1 Intrinsic muscles -----

5.7.2 Hand grip -----

5.7.3 Wrist flexion -----

5.7.4 Wrist extension -----

5.7.5 Elbow flexion -----

5.7.6 Elbow extension -----

5.7.7 Shoulder abduction -----

5.7.8 Shoulder adduction -----

5.7.9 Shoulder flexion -----

5.7.10 Shoulder extension -----

5.8 Axial muscles

5.8.1 Neck flexion -----

5.8.2 Neck extension -----

5.8.3 Trunk -----

5.9 Reflexes

5.9.1 Biceps jerk -----

5.9.2 Supinator jerk -----

5.9.3 Triceps jerk -----

5.9.4 Knee jerk -----

5.9.5 Ankle jerk -----

5.9.6 Abdominal reflex -----

5.9.7 Plantar response -----

5.10 Sensory involvement -----

5.10.1 Pain impairment -----

5.10.2 Touch impairment -----

5.10.3 Vibration impairment -----

5.10.4 Joint position sense impairment -----

5.10.5 Pattern -----

(1= stocking-glove, 2=stocking, 3= glove, 4=asymmetric)

5.11 Gait (describe) -----

5.12 Tremors of upper limbs -----

5.13 Other signs (specify) -----

6. Electrophysiology and nerve biopsy

6.1 Conduction velocity (right/ left)

6.1.1 Median -----

6.1.2 Ulnar -----

6.1.3 Peroneal -----

6.1.4 Tibial -----

6.2 CMAP amplitude

6.2.1 Median -----

6.2.2 Ulnar -----

6.2.3 Peroneal -----

6.2.4 Tibial -----

6.3 Sensory peak latency

6.3.1 Median -----

6.3.2 Ulnar -----

6.3.3 Sural -----

6.4 Sensory amplitudes

6.4.1 Median -----

6.4.2 Ulnar -----

6.4.3 Sural -----

6.5 EMG -----

(0=normal, 1= denervation, 2= reinnervation only, 3= not done)

6.6 Final impression -----

(1 = Axonal, 2 = demyelinating, 3= intermediate, 4 =
indeterminate)

6.7 Other etiology workup -----

(0 = not done, 1= negative, 2 = incomplete)

6.8 Nerve biopsy done -----

6.8.1 Report (details) -----

6.9 Genetic study -----(1= Done, 2= Not done)

6.9.1 If done, details of tests done -----

1. MLPA for PMP22 duplication/deletion
2. Targeted CMT panel
3. Clinical exome sequencing
4. Whole exome sequencing
5. Sanger sequencing for a single gene----- Specify -----
6. Others_Specify _____

6.10 Final clinical diagnosis _____

7.Follow up

7.1Date of last follow up -----

7.2Total follow up duration -----

Status at last follow up -----



Technical Advisory Committee (Clinical Studies)
SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES & TECHNOLOGY
THIRUVANANTHAPURAM – 695011, INDIA

TAC Registration No: SCT-/S/2020/1180

Date: 13.10.2020

Project title: PHENOTYPIC CHARACTERIZATION OF CHARCOT MARIE TOOTH DISEASE AND CORRELATION WITH NERVE HISTOPATHOLOGY AND GENOTYPE

Dr Sruthi S. Nair, Associate Professor, Department of Neurology , SCTIMST	Degree: MBBS, MD, DM (Neurology)
Co-Principal Investigator(s):	
Dr Soumya Sundaram, Associate Professor, Department of Neurology, SCTIMST	Degree: MBBS, MD, DM (Neurology)
Co-Investigator	
Dr Rajalakshmi P, Assistant Professor, Department of Pathology , SCTIMST	Degree: MBBS, MD

Members who participated in the TAC meeting on 25/09/2020

Dr Harikrishnan S (Chairman)
Dr Manikandan S
Dr Sylaja P N
Dr Narayanan Namboodiri
Dr Sanjay G
Dr Jayadevan E R
Dr Unnikrishnan K P
Dr Syam K
Dr Ramshekhar N Menon
Dr Jayanand Sudhir B
Dr Sabarinath Menon
Dr Madhusoodanan U K
Dr Srinivas G (Member Secretary)

Dr Sylaja P N, Dr Ramshekhar N Menon, Dr Sanjay G, Dr Narayanan Namboodiri, Dr Jayadevan E R and Dr Harikrishnan S stayed away from the proceedings when the projects in which they are involved as investigator were discussed (#1173, 1175, 1178, 1186, 1169. 1183, 1184, 1185, 1191, 1193, 1171, 1176, 1177).

Risk Classification of the project (Minimum/ Moderate/ High): Minimum

Requirement of DSMB: No

Recommended members of DSMB: Not applicable

Recommendations of TAC:

Recommended for consideration of IEC in the light of the responses received from the investigator

The PI may note that there can be no additions / alterations in the documents approved by TAC when they are submitted to the IEC.

MEMBER SECRETARY
TAC (Clinical Studies)
SCTIMST

Dr Srinivas G

Note for IEC

Copy of the investigator's responses to questions/suggestions from TAC is attached (Appendix-1).

Appendix-1

1. The plan is to include patients from 1996 to 2020 in the study. The aim is to correlate the clinical and electrophysiological pattern with genetic and nerve biopsy data. But do we have the genetic data from 1996? Since when did we start regularly doing genetic analysis for CMT?

Answer: Genetic studies were not done prior to 2015 for Charcot Marie Tooth disease (to the investigator's knowledge). We had started sending genetic studies for complex diagnostic situations from 2015 and regularly from 2018. A few of the patients registered prior to 2015 and on long-term follow up (approximately 4 - 5 patients) had opted to do genetic testing once the facility became freely available. We believe that even this small cohort will be helpful in providing some insight into the genotypic patterns as this data is quite scarce from India. We have detailed nerve histopathology reports from 1996 onwards which can be correlated with the phenotype. A duration of 25 years was taken to ensure a meaningful sample for analysis.

Action taken: No modifications were made in the project protocol.

2. There is a need of a pathologist in the study

Answer: We appreciate this suggestion. Dr Rajalakshmi P, Assistant Professor of Pathology, is added as a co-investigator

Action taken: The changes are reflected in the project proforma, IEC form, declaration and relevant section of the TAC application.

MEMBER SECRETARY
TAC (Clinical Studies)
SCIENTIST



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