Short Communication

Clinico-Electrophysiological and Genetic Overlaps and Magnetic Resonance Imaging Findings in Charcot–Marie– Tooth Disease: A Pilot Study from Western India

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Abstract

Background: Charcot–Marie–Tooth (CMT) disease is clinically and genetically heterogeneous. There are no published series describing clinical, electrophysiological, and genetic information on CMT from the Indian subcontinent. Magnetic resonance imaging (MRI) neurography technique provides useful information about the plexus and roots and can be employed in patients with CMT. **Settings and Design:** A prospective, observational study carried out at a tertiary care hospital in Western India. **Subjects and Methods:** CMT patients fulfilling the UK Genetic Testing Network criteria were included. They underwent clinical, electrophysiological, radiological, and multigene panel testing. **Results:** Totally 22 patients (19 males, 3 females; 18 sporadic and 4 familial cases) were studied. Pes cavus (19), hammer toes (16), and scoliosis was seen in 1 patient. Electrophysiology revealed motor predominant neuropathy with 15 demyelinating (10 uniform and 5 multifocal) and 7 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. **Conclusions:** This small series documents the pattern of CMT neuropathies as seen in Western India. 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.

Keywords: Charcot–Marie–Tooth disease, magnetic resonance imaging neurography, multigene panel testing, PMP22

Introduction

Charcot–Marie–Tooth disease (CMT) is the most common inherited peripheral neuropathy, affecting 1 in 2500.[1] At present, the diagnosis of CMT rests mainly on phenotype, inheritance pattern, and electrophysiological differentiation into demyelinating and axonal types (CMT 1 and 2).[2-4] Large numbers of genetic abnormalities are associated with CMT phenotype and are known to vary with the studied populations. Magnetic resonance imaging (MRI) studies are being increasingly used in the diagnostic process of neuropathies, particularly for visualization of the proximal roots and plexuses. Currently, limited data exist on MRI neurography studies in patients having CMT. Genetic analysis has recently become available in India, and present, there is no systematic information available on Indian patients having CMT.

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Hence, the present study was undertaken to perform the clinical, electrophysiological, radiological, and genetic evaluation of CMT patients presenting to a tertiary care hospital in Western India.

Subjects and Methods

This is a prospective, observational study carried out from July 2016 to December 2016 at the neurology department of a tertiary care hospital in Western India. Informed consents

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were obtained, and the study was approved by the Institutional Ethics Committee. All patients with clinical presentation of neuropathy attending the neuromuscular clinic were subjected to detailed neurologic examination, electrophysiology testing, radiological evaluation, and genetic testing in whom it was feasible. The electrophysiology of family members was performed when possible.

Inclusion and exclusion criteria

Only index cases were included in this study. The UK Genetic Testing Network criteria were used to include and exclude the patients.[5]

The criteria include as follows:

- "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
- Supportive nerve conduction test result (defining Type I or II according to nerve conduction velocity)
- Absence of other nongenetic causes (alcohol, B12 deficiency, diabetes, and trauma)
- No associated CNS involvement.

Electrophysiological criteria used by our laboratory for the diagnosis of inherited neuropathies for CMT were as per Harding and Thomas guidelines (or criteria).[6] Thus, median nerve: Conduction velocity <38 m/s was considered as demyelinating. 38–45 intermediate and >45 m/s with low amplitude were considered as axonal.

Method of genetic testing

Selective capture and sequencing of the protein-coding regions of the genome/genes is performed. Most commonly encountered gene mutations such as in PMP22 gene were the part of targeted gene sequencing method. DNA extracted from blood was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean >80–100X coverage on illumina-sequencing platform. The sequences obtained are aligned to human reference genome (GRCh37/hg19) using BWA program[7,8] and analyzed using Picard and GATK-Lite toolkit[9,10] to identify variants relevant to the clinical indication. We follow the GATK best practices framework for the identification of variants in the sample. Gene annotation of the variants was performed using Victim Empowerment programme[11] against the Ensembl release 84 human gene model.[12] Silent variations that do not result in any change in amino acid in the coding region were not reported.

Results

Demographic variables

Twenty-two patients were included (19 males and 3 females). The median age of presentation was 23.5 years (3–69 years). The median duration of disease from onset to presentation was variable among different genetic cohorts [Table 1]. Family members were affected in 4 index cases, and 18 were sporadic (82%).

Clinical profile

All patients presented with motor weakness and had sensory symptoms or signs [Table 1]. Motor weakness was most prominent in distal part of lower limbs in all 22 patients. Upper limb was affected in 19 patients, but weakness was less severe as compared to lower limbs. The weakness was symmetrical in 17 patients (82%) and 5 patients showed mild asymmetry of weakness. Pes cavus was evident in 19 patients (86%), hammer toes in 16 (73%), and scoliosis was seen in one patient [Figure 1a-c]. Thickening of nerves on clinical examination was detected in 9 patients. Sensory deficit was documented in 7 patients which was predominantly of the large fiber type. A single patient had membranous glomerulopathy.

Electro diagnosis

Motor nerve conduction studies were abnormal in all 22 patients. Lower limb motor nerves were affected more severely as compared to the upper limb. Tibial (abductor hallucis) compound motor action potentials (CMAP) were absent in 19/22 patients. In the upper limbs, median motor nerve was affected more in terms of CMAP amplitude, conduction velocity, distal latencies, and temporal dispersion. Multifocal demyelinating electrophysiology with conduction blocks were observed in 5 patients. Uniform conduction slowing was observed in 10 patients. Sensory nerve action potentials were affected in both extremity but more in lower limbs particularly sural nerves in 12 patients. Axonal electrophysiology was observed in 7 patients. Nerve conduction results in different genetic cohorts are described in Tables 1 and 2.

Based on the clinical and electrophysiological features, 10 patients were classified as CMT 1, 7 as CMT 2, and HNPP in four patients. One patient was diagnosed to have CMT X.

Magnetic resonance imaging

MRI of the lumbar plexus with contrast was done in 10 out of 22 patients (45%), which showed thickened plexus in 6 patients [Figure 1d]. All the six patients had demyelinating neuropathies. The plexus studies were normal in 4 patients who had axonal neuropathy.

Molecular diagnosis

Genetic testing was available in 13 patients (63.07%). The distribution of genetic mutations was as follows. Three patients

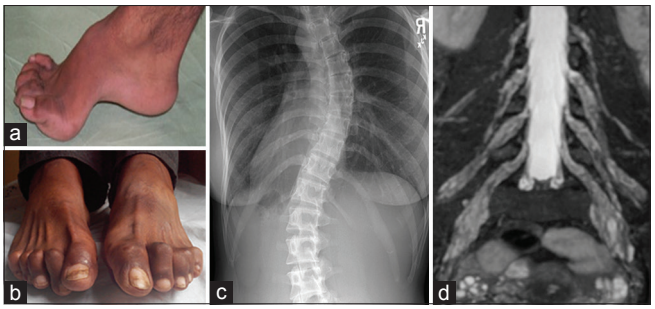

Figure 1: (a) Pes cavus; (b) Hammer toes; (c) X-ray anteroposterior of dorsolumbar spine of patient with Charcot–Marie–Tooth 4C; (d) magnetic resonance neurography with contrast (volumetric interpolated breath-hold examination sequence) showing thickened lumbosacral plexus

Table 1: Clinical, radiological, and genetic features

Clinical features	PMP 22 (HNPP) (*n*=3)	GJB1 (CMT X) (*n*=2)	SH3TC2 (CMT 4c) (*n*=2)	HSPB1 (CMT 2F) (*n*=2)	SPTLC2 (CMT 1) (*n*=1)	MPZ (CMT 2D) (*n*=1)	AARS (CMT 2N) (*n*=1)	NEFH (CMT 1) (*n*=1)	Cohort without genetic confirmation (*n*=9)
Clinical parameters									
Age (years)	31±20	19.5±4	9.5±6	57±2	26	40	31	20	32.5±17.5
Age of onset (years)	17±13	10±5	4.5±2	27.5±2	10	20	9	6	21±14
Disease duration (years)	13.5±8.5	9±2	5±4	30±2	16	20	22	14	20.5±13.5
Sex (male/female)	3/0	2/0	1/1	2/0	1/0	0/1	0/1	1/0	7/2
Family history	2	1	0	0	1	0	0	0	0
Muscle weakness	3	2	2	2	1	1	1	1	8
Muscular atrophy	0	2	2	2	1	1	1	1	8
Thickened peripheral nerves	0	1	2	0	1	0	0	0	5
Motor asymmetry	3	1	0	0	0	0	0	0	0
Sensory impairment	0	1	1	0	1	1	1	1	1
Areflexia	0	2	0	0	1	1	1	1	8
MRI lumbar roots									
Thickening of roots	0	0	1	1	1	0	0	0	3
Electrophysiology studies*									
A-axonal	0	0	0	2	0	1	1	0	3
D-demyelinating									
Uniform, symmetric	0	1	2	0	1	0	0	1	5
Multifocal, asymmetric	3	1	0	0	0	0	0	0	1

MRI = Magnetic resonance imaging, CMT = Charcot–Marie–tooth, HNPP = Hereditary neuropathy with pressure palsies

had mutation in PMP22 gene. Two patients had mutation in GJB. Two patients had mutation in SH3TC2 gene, 2 in HSPB1 gene. One mutation in each of SPTLC2, MPZ, AARS and NEFH was documented.

Discussion

Our neuromuscular clinic is a major referral center for patients residing in Western India, and hence, the present study can be considered as representative of the region, up to a point. The population of Western India is heterogeneous with multitudes of castes and subcastes, some of whom practice intracommunal exogamy. In the present study, CMT 1 (59%) was more common than CMT 2 (32%), which is comparable with large studies available on the subject.[13,14]

Clinico-electrophysiological and genetic correlation

Clinical and electrophysiological features have conventionally formed the mainstay of differential diagnosis of the CMT group. In the present study, based on the electrophysiology, 10 patients had uniform demyelination, 5 had multifocal demyelination, and 7 had axonal features.[Table 1]

As expected, the multifocal demyelination group fitted in two genetic diagnoses, HNPP and CMT X. Out of the 5 patients who had multifocal demyelination and history of acute mononeuropathies, 3 tested positive for PMP22 proving HNPP as the diagnosis. The fourth patient had a strong X-linked inheritance pattern, and GJB1 mutation was detected in him, confirming the diagnosis of CMT X. Genetic data were not available in the fifth patient. The initial diagnoses in all these 5 patients were of various acquired neuropathies. This restates the need of considering inherited disease processes in the differential diagnosis of acute mononeuropathies, along with the acquired ones.[15]

Ten patients showed uniform demyelination on electrophysiology and were grouped as CMT 1. Out of these, clinically, 2 patients had severe disease and prominent scoliosis [Figure 1c] which raised the possibility of CMT 4c. Genetic testing confirmed and correlated well with this combination of clinical and electrophysiological features, in both the patients [Table 3]. A single patient with sporadic disease [Tables 1 and 2] who showed uniform demyelination on electrophysiology and clinical features fitting with CMT1 was found to have GJB 1 mutation. This mutation, while mostly associated with CMT X, has been uncommonly shown to present with CMT 1 phenotype and electrophysiology.[13] This patient exemplifies the superiority of panel testing over targeted testing, as the later would have missed the genetic abnormality in him.[16] In the remaining seven patients, who had clinical and electrophysiological features of CMT1, genetic testing was available in 2 showing NEFH and SPTLC2 gene mutations, confirming the diagnosis of CMT 1.[14,17]

Seven patients exhibited axonopathic electrophysiology. In this cohort of CMT 2, a high rate of concordance for clinic-electrophysiological and genetic correlation was seen. All the 4 patients in whom the genotype was available

showed type specific mutations consistent with diagnosis of CMT 2.[18-20] Myelin protein zero (MPZ) mutations have been described with multiple CMT subtypes, mainly CMT 1B.[14,20] In our single patient with this mutation, electrophysiology showed axonal type of neuropathy and MRI did not show thickening of roots. Hence, we classified our patient as CMT 2. Interestingly, this patient with MPZ mutation had membranous glomerulopathy. MPZ knockout mouse models have showed increased glomerular permeability to albumin, suggesting a role for MPZ in the control of glomerular permeability and its possible implication in CMT-associated renal disease.[21]

Table 2: Clinico-electrophysiological and genetic correlation

Electrophysiology	Clinico-electrophysiological diagnosis	Genetic diagnosis
Demyelinating (*n*=15)		
Multifocal, asymmetric demyelination (*n*=4)	HNPP	HNPP
Multifocal demyelination (*n*=1)	CMT X	CMT X
Symmetric, homogenous demyelination (*n*=10)	CMT 1	CMT 1 (*n*=2), CMT X (*n*=1), CMT 4C (*n*=2)
Axonal (*n*=7)		
Symmetric, axonal neuropathy (*n*=7)	CMT 2	CMT 2

CMT = Charcot–Marie–tooth, HNPP = Hereditary neuropathy with pressure palsies

Thus, in the present investigation, clinico-electrophysiological and genetic correlation were achieved in 84% patients in whom complete data were available. The electrophysiological differentiation into uniform demyelination, multifocal demyelination, or axonopathy helped the basic grouping. The clinical features which helped to predict the genetic diagnosis in this cohort of patients are as shown in Table 4.

Genetic heterogeneity

In the present study, a significant spread of genetic mutations was seen [Table 1]. These genetic results reflect the heterogeneity of studied population. While our results are largely comparable with the reported studies outside India, some differences were seen in the mutation patterns. PMP22, MPZ, GJB1, and MFN 2 gene mutations are documented to account for most of the known abnormalities. This has been consistently seen in three prominent studies studying different populations.[13,14,22] In the present study, these four genes accounted for only 27%. While the mutation set seen in our patient needs attention, a larger study will be necessary to substantiate this fact. At present, there are no documented Indian series available on inherited neuropathies for comparison.

Magnetic resonance imaging studies

MRI imaging is being increasingly utilized in the study of nerves, roots, and plexuses[23-25] but has not been systematically applied to cohorts of CMT patients. In the present study, MRI neurography was available on 10 patients. Hypertrophy of nerve roots was found in 6 of them, all of whom had demyelinating neuropathy and genetic diagnoses of CMT 1, CMT 4, or HNPP [Figure 1d]. Thus, MRI demonstration of

Table 3: Clinical clues

Clinical clue	Clinical type	Electrophysiology type	Genetic mutation	Genotype
Scoliosis	CMT 1	Demyelinating neuropathy	SH3TC2	CMT 4C
Early severe disease	CMT 1	Demyelinating neuropathy	SH3TC2	CMT 4C
Membranous glomerulopathy	CMT 2	Axonal neuropathy	MPZ	CMT 2J or 2I
Focal mononeuropathies	HNPP	Multifocal demyelinating neuropathy	PMP 22	HNPP

CMT = Charcot–Marie–tooth, HNPP = Hereditary neuropathy with pressure palsies

Table 4: Details of genetic mutations identified in the study

Gene (transcript)	Location	Variant	Zygosity	Classification
MPZ (−) (ENST00000533357)	Exon 2	c.205C > C/T (p.Gln69Ter)	Heterozygous	Pathogenic
HSPB1 (+) (ENST00000248553)	Exon 2	c.418C > C/G (p.R140G)	Heterozygous	Pathogenic
GJB1 (+) (ENST00000374022)	Exon 2	c.536G > C (p.Cys179Ser)	Heterozygous	Likely pathogenic
SH3TC2 (−) (ENST00000515425)	Exon 11	c.2775G > A (p.Trp925Ter)	Homozygous	Likely pathogenic
NEFH (+) (ENST00000310624)	Exon 4	c.1349C > C/G (p.Ser450Cys)	Heterozygous	Uncertain significance
SPTLC2 (−) (ENST00000216484)	Exon 8	c.1151C > C/T (p.Ser384Phe)	Heterozygous	Uncertain significance
SH3TC2 (−) (ENST00000515425)	Exon 14	c.3325C > T (p.Arg1109Ter)	Homozygous	Pathogenic
AARS (−) (ENST00000261772)	Exon 7	c.904G > G/A (p.Ala302Thr)	Heterozygous	Uncertain significance
HSPB1 (+) (ENST00000248553)	Exon 2	c.418C > C/G (p.Arg140Gly)	Heterozygous	Likely pathogenic
GJB1 (+) (ENST00000374022)	Exon 2	c.514G > C (p.pro172Ser)	Hemizygous	Pathogenic
PMP 22	Entire gene deletion	-	Heterozygous	Pathogenic
PMP 22	Entire gene deletion	-	Heterozygous	Pathogenic
PMP 22	Entire gene deletion	-	Heterozygous	Pathogenic

enlargement of the nerves and roots correlated completely with the demyelinating pathophysiology in CMT patients. MRI neurography can be considered as an additional tool to evaluate proximal nerve, plexus, and roots in CMT patients. This investigation may be particularly relevant in advanced cases in whom the axonal changes dominate the electrophysiology.

Conclusions

This small study documents the clinical, electrophysiological, radiological, and mutation spectrum in patients with CMT from Western India. Electrophysiological and clinical information formed the mainstay of the broad divisions in CMT 1, 2 and CMT X. The mutation spectrum was somewhat different than the well-known pattern. The study also reaffirms the benefits of gene panel by highlighting those cases of phenotype-genotype discordance. MRI features of this cohort demonstrate that the root thickening is specific to the demyelinating electrophysiology. This expands the role of imaging in CMT patients as a complementary investigation.

Limitations of the study

This is a single-center investigation and has limited numbers of study individuals.

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Conflicts of interest

There are no conflicts of interest.

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