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Wide phenotypic variations in Charcot-Marie-Tooth 1A neuropathy with rare copy number variations on 17p12

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Charcot-Marie-Tooth disease (CMT) is clinically heterogeneous hereditary motor and sensory neuropathies with genetic heterogeneity, age-dependent penetrance, and variable expressivity. Rare copy number variations by nonrecurrent rearrangements have recently been suggested to be associated with Charcot-Marie-Tooth 1A (CMT1A) neuropathy. In our previous study, we found three Korean CMT1A families with rare copy number variations (CNVs) on 17p12 by nonrecurrent rearrangement. Careful clinical examinations were performed in all the affected individuals with rare CNVs (n = 19), which may be the first full study of a subject from a large CMT1A family with nonrecurrent rearrangement. The clinical phenotype showed no significant difference compared with common CMT1A patients, but with variable phenotypes. In particular, a broad intrafamilial phenotypic spectrum was observed within the same family, which may suggest the existence of a genetic modifier. This study may broaden the understanding of the role of CNVs in the pathogenesis of CMT.

Keywords: Charcot-Marie-Tooth disease type 1A (CMT1A); copy number variation (CNV); nonrecurrent rearrangements; phenotype; PMP22

Introduction

Charc ot-Marie-Tooth disease (CMT) is genetically and clinically heterogeneous hereditary motor and sensory neuropathies with an estimated prevalence of 1/2500. Frequent clinical symptoms of CMT patients are distal muscle weakness, sensory loss, and foot deformities. Based on the median motor nerve conduction velocities (NCVs), CMT is usually divided into two forms, the demyelinating form (CMT1) and the axonal form (CMT2) (Harding and Thomas 1980). Genetically more than 50 genes or loci have been reported as the underlying cause of CMT at the Inherited Peripheral Neuropathies Mutation Database (http://www.molgen.ua.ac.be/CMTMutations/Mutations/).

The most frequent autosomal dominant demyelinating type is CMT1A genomic disorder (MIM# 118220), which is usually relevant with the tandem duplication between proximal and distal repeat sequences (d-REP and p-REPs) on chromosome 17p12 (Lupski et al. 1991; Pentao et al. 1992). The two flanking CMT1A–REPs share 98.7% sequence identity, and the duplication comprises approximately a 1.4-Mb genomic region that includes the dosage-sensitive *PMP22* gene (Reiter et al. 1997). Duplication is found in up to approximately 70% of patients with CMT1 (Szigeti et al. 2006). Deletion of the same region by the unequal crossover is associated with hereditary neuropathy with liability to pressure palsies (HNPP, MIM# 162500) (Chance et al. 1993; Mariman et al. 1994). It is estimated that about 5–24% of the duplication occur *de novo* (Hoogendijk et al. 1992; Nelis et al. 1996). Recently, copy number variations (CNVs) in the vicinity of the *PMP22* gene have been also reported to lead CMT (Weterman et al. 2010; Zhang et al. 2010). No other CMT form has been reported to be associated with CNV.

Rearrangements of the human genome can occur by two types of events, recurrent rearrangements and nonrecurrent rearrangements. The major mechanism of recurrent rearrangements is non-allelelic homologous recombination (NAHR), which is associated with several genomic repeated sequences, like long interspersed nuclear elements (LINEs). A putative recombination-based mechanism of the nonrecurrent rearrangements is nonhomologous end joining (NHEJ). Recently, the DNA replication-based models of fork stalling template switching (FoSTeS) and microhomology-mediated break-induced replication (MMBIR) have been proposed to explain the nonrecurrent

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rearrangements (Lee et al. 2007; Zhang et al. 2009). According to the FoSTeS/MMBIR model, the active replication fork could stall and switch templates via complementary template microhomology to anneal and prime DNA replication. Although the common NAHR mechanism is a prevalent underlying cause of CMT1A, rare CNVs by nonrecurrent rearrangements have been also reported to be associated with the CMT1A and HNPP (Zhang et al. 2009; Zhang et al. 2010; Huang et al. 2010; Choi et al. 2011).

There is a broad spectrum of phenotypic manifestation depending on the causative mutations within the same gene in CMT. As an extreme case, Kleopa et al. (2004) reported a Cypriot family with *PMP22* mutation in which some family members presented HNPP, while other family members had a slowly progressive chronic polyneuropathy typical of the CMT1 phenotype. Mutations in the MPZ gene, which encodes the major integral membrane protein of the peripheral nerve system, also exhibit a broad spectrum of phenotypes from severe early-onset neuropathies, such as Dejerine-Sottas syndrome (DSS), to the mild CMT phenotype (Shy et al. 2004; Lee et al. 2010).

We previously identified three CMT1A families with partial duplication by nonrecurrent rearrangements in Korean CMT families (Choi et al. 2011). The detailed clinical phenotypes have still not been reported in CMT1A with rare CNVs on 17p12. In the present study, exact clinical phenotypes were investigated in subjects from three CMT1A families with rare CNVs. We revealed wide phenotypic heterogeneity within a large CMT1A partial duplicated family.

Materials and methods

Subjects

A total of 19 CMT1A patients were included in this study. They were from three Korean CMT1A families (FC85, FC116 and FC388) with rare CNV on 17p12 by nonrecurrent rearrangement (Choi et al. 2011). All participants in this study provided written informed consent.

Molecular analysis

DNA was isolated from peripheral whole blood using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) (Ahn et al. 2011). The common 1.4-Mb duplication or deletion on 17p12 was determined by genotyping six microsatellites (D17S921, D17S9A, D17S918, D17S4A, D17S122, and D17S2230) (Choi et al. 2007). The high resolution of the rearrangement map was obtained by genotyping three more microsatellites (D17S1296, D17S1357, and D17S9B) and by the subsequent determination of CNVs with a customer-designed high-density array comparative genomic hybridization (aCGH) (HG18 CGH 2X 135K, Roche-NimbleGen, Madison, WI, USA). A long-range template PCR was attempted to amplify the breakpoint junctions using a long template PCR kit (Roche, Mannheim, Germany). PCR products were sequenced on the automatic genetic analyzer ABI 3100 using the BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).

Clinical assessments

Clinical information was obtained in a standard manner and included motor and sensory impairments, deep tendon reflexes, and muscle atrophy. Muscle strengths of flexor and extensor muscles were assessed manually using the standard medical research council (MRC) scale. In order to determine physical disability, we used two scales, a functional disability scale (FDS) (Birouk et al. 1998) and a CMT neuropathy score (CMTNS) (Shy et al. 2005). Disease severity was determined for each patient using a nine-point FDS that was based on the following criteria: 0, normal; 1, normal but with cramps and fatigability; 2, an inability to run; 3, walking difficulty but unaided; 4, walking with a cane; 5, walking with crutches; 6, walking with a walker; 7, wheelchair bound; and 8, bedridden. In addition, we determined the CMTNS based on motor and sensory symptoms and on pain and vibration, muscle strength, and neurophysiological tests. Sensory impairments were assessed in terms of the level and severity of pain, temperature, vibration, and position.

Table 1. Summarization of CNVs identified in CMT1A families with rare partial duplication.

	Duplication (kb)[a]					
Family	Start	End	Size (kb)	Gain/loss[b]	Cytoband	Genes in the duplication region
FC85	13,997	14,462	465	Gain: +1	17p12	*COX10*
	14,984	15,434	450	Gain: +1	17p12	*PMP22, TEKT3*
FC116	14,839	15,443	607	Gain: +1	17p12	*PMP22, TEKT3*
FC388	14,585	15,310	725	Gain: +1	17p12	*PMP22, TEKT3*

[a]The distance was from the short arm end of chromosome 17 (hg18 at the UCSC Genome Browser: http://genome.ucsc.edu/); [b]+1 means one additional copy gain for the rearrangement region.

Table 2. Clinical features in 19 CMT1A patients with rare rearrangements on 17p12.

Family/ patients	Sex	Age at exam (yrs)	Age at onset (yrs)[a]	Disease duration (yrs)	FDS	CMTNS[b]	Muscle weakness[c]		Muscle atrophy[d]	Sensory loss[e]		DTR[f]		Heel gait defect	Toe gait defect	Foot deformity
							U/E	L/E		U/E	L/E	U/E	L/E			
FC116																
III-7	M	67	10	57	4	21	++	+++	Severe (U < L)	P < V	P < V	A	A	Yes	Yes	Yes
III-11	F	82	60	22	2	10	+	+	Mild (U = L)	Nl	P < V	D	A	Yes	No	Yes
III-22	M	75	8	67	5	27	++	+++	Severe (U < L)	P < V	P < V	A	A	Yes	Yes	Yes
III-29	F	58	35	23	2	9	−	+	Mild (L)	Nl	P < V	Nl	A	Yes	No	Yes
IV-2	F	41	15	26	3	16	+	++	Moderate (U < L)	P < V	P < V	A	A	Yes	No	Yes
IV-9	F	34	8	26	3	16	+	++	Moderate (U < L)	P < V	P < V	A	A	Yes	Yes	Yes
IV-14	M	29	14	15	2	8	+	++	Mild (U < L)	Nl	P < V	Nl	D	Yes	No	Yes
IV-17	M	66	43	23	3	15	+	++	Moderate (U < L)	P = V	P < V	A	A	Yes	Yes	Yes
IV-25	F	52	35	17	3	15	+	++	Moderate (U < L)	P < V	P < V	A	A	Yes	Yes	Yes
IV-38	F	45	8	37	1	7	−	+	Mild (L)	Nl	P = V	Nl	D	No	No	Yes
IV-39	F	42	14	28	1	5	−	+	No	Nl	P = V	D	A	No	No	Yes
V-1	M	11	4	7	1	6	+	+	Mild (U < L)	Nl	P = V	D	D	Yes	No	Yes
V-18	M	25	As	0	0	ND	−	−	No	Nl	Nl	Nl	Nl	No	No	No
V-19	F	23	20	3	0	2	−	−	No	Nl	Nl	Nl	Nl	Yes	No	Yes
V-23	F	19	17	2	0	2	−	−	No	Nl	Nl	Nl	D	No	No	No
FC388																
II-2	F	36	8	28	3	17	+	++	Moderate (U < L)	P < V	P < V	A	A	Yes	Yes	Yes
III-1	F	6	4	2	1	7	+	+	Mild (U = L)	Nl	P = V	Nl	D	No	No	Yes
III-2	M	3	As	0	0	ND	−	−	No	Nl	Nl	Nl	Nl	No	No	No
FC85																
II-2	M	23	8	15	1	7	−	+	Mild (U < L)	Nl	P = V	D	A	Yes	No	Yes

[a]As = asymptomatic; [b]ND = nerve conduction study not done; [c]muscle weakness in upper limbs (U/E): + = intrinsic hand weakness 4/5 on medical research council (MRC) scale, ++ = intrinsic hand weakness <4/5 on MRC scale, − = no symptom; muscle weakness in lower limbs (L/E): + = ankle dorsiflexion 4/5 on MRC scale, ++ = ankle dorsiflexion <4/5 on MRC scale, +++ = proximal weakness and wheelchair dependent; [d]muscle atrophy: U < L = lower limb predominant muscle atrophy, L = only lower limb muscle atrophy; [e]sensory loss: P = pain sense, V = vibration sense, Nl = normal sense; [f]deep tendon reflexes: Nl = normal, D = diminished, A = absent.

Age at onset was determined by asking patients their ages when the symptoms first appeared.

Electrophysiological study

Neurophysiologic studies were carried out on 17 affected individuals (six males and 11 females). Nerve conduction studies were performed with surface electrodes in median, ulnar, peroneal, tibial, and sural nerves. Recordings were obtained by standard methods using surface stimulation and recording electrodes. The motor conduction velocities (MCVs) of the median and ulnar nerves were determined by stimulation at the elbow and wrist while recording compound muscle action potentials (CMAPs) over the abductor pollicis brevis and adductor digiti quinti, respectively. In the same way, the MCVs of peroneal and tibial nerves were determined by stimulation at the knee and ankle while recording CMAPs over the extensor digitorum brevis and adductor hallucis, respectively. Sensory conduction velocities (SCVs) were obtained over a finger-wrist segment from the median and ulnar nerves by orthodromic scoring and were also recorded for sural nerves.

Histopathological study

Pathological examinations of affected individuals included the light and electron microscopic analyses of a sural nerve. One sural nerve fragment was fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin, modified Masson's trichrome and Luxol fast blue. Another fragment was immediately fixed by immersion in 5% buffered glutaraldehyde and postfixed in 1% osmium tetroxide. Epon-embedded semi-thin and ultra-thin sections were prepared for light and ultrastructural examinations. The density of myelinated fibers, axonal diameter and myelin thickness, and the *g*-ratio of myelinated fibers were assessed directly from the toluidine blue-stained semi-thin transverse sections of the sural nerve using a computer-assisted image analyzer (analySIS, Soft Imaging System, GmbH, Germany).

Results

Rare CNVs on 17p12 by nonrecurrent rearrangement

We previously reported three CMT1A families with rare CNVs by nonrecurrent rearrangements on chromosome 17p12 by the haplotyping of microsatellites and aCGH application (Choi et al. 2011). The FC116 and FC388 families showed a single duplication, whereas the FC85 family showed a complex rearrangement with two discrete regions (Table 1). The breakpoint sequence analysis revealed an *Alu*-*Alu*-mediated rearrangement with 34-bp microhomology in FC116 and 3-bp microhomology-mediated rearrangement in FC388. However, we failed to determine the exact breakpoint sequence from FC85.

Clinical and electrophysiological findings

The clinical features of the 19 patients (eight males, 11 females) from the three families are shown in Table 2. Muscle weakness and atrophy started and were predominant in the distal portions of legs. The weakness was noted to a lesser extent distally in upper limbs. Paresis in the distal regions of lower limbs varied from asymptomatic or mild weakness to severe weakness of distal muscle groups (Figure 1).

The MCVs of median, ulnar, peroneal and tibial nerves and the SCVs of median, ulnar and sural nerves are shown in Table 3. Nerve conduction velocities (NCVs) were often markedly reduced, and we were unable to record amplitudes in two of the 17 patients (12%). All patients, except the two with absent NCVs, had at least one motor NCV of less than 38 m/s.

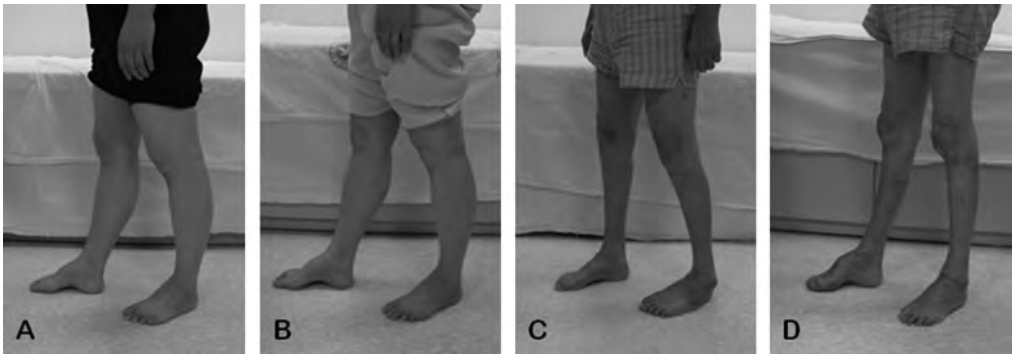

Figure 1. Distal leg muscle atrophy shown in the CMT1A patients with nonrecurrent duplication on 17p12 (FC116 family). Note the progressive leg muscle atrophy associated with disease duration (DD). A,DD = 26 years (F/34, IV-9); B, DD = 28 years (F/42, IV-39); C, DD = 57 years (M/67, III-7); D, DD = 67 years (M/75, III-22).

Table 3. Electrophysiological features in 17 CMT1A patients with rare rearrangements.

Family/patient	Age at exam (yrs)	Side	Motor nerve conduction studies				Sensory nerve conduction studies		
			Median	Ulnar	Peroneal	Tibial	Median	Ulnar	Sural
FC116:III-7	67	right	16.7 (0.4)	16.8 (2.9)	NR	NR	NR	NR	NR
	67	left	18.6 (5.3)	15.9 (2.7)	NR	NR	NR	NR	NR
FC116:III-11	82	right	23.3 (7.2)	26.8 (5.4)	NR	14.8 (0.9)	NR	NR	NR
FC116:III-22	75	right	NR	NR	NR	NR	NR	NR	NR
FC116:III-29	58	right	22.6 (9.7)	20.1 (10.2)	17.6 (1.3)	17.1 (4.4)	14.9 (4.7)	16.7 (3.9)	14.3 (10.5)
	58	left	22.0 (8.5)	19.0 (8.7)	20.1 (1.0)	13.1 (4.5)	15.9 (3.9)	NR	15.8 (9.9)
FC116:IV-2	41	right	15.5 (5.3)	13.6 (3.2)	NR	NR	NR	NR	NR
	41	left	12.8 (3.9)	11.6 (1.5)	NR	NR	NR	NR	NR
FC116:IV-9	34	right	13.1 (0.5)	11.9 (2.7)	NR	NR	NR	NR	NR
	34	left	16.7 (1.0)	15.2 (2.8)	NR	NR	NR	NR	NR
FC116:IV-14	29	right	28.9 (11.9)	22.9 (12.0)	NR	18.4 (0.4)	22.5 (7.1)	23.1 (1.8)	11.7 (12.4)
	29	left	30.7 (11.3)	23.4 (9.6)	18.2 (0.1)	19.2 (0.4)	20.6 (6.5)	21.0 (3.9)	16.6 (12.7)
FC116:IV-17	66	right	22.2 (8.2)	23.8 (5.3)	NR	NR	NR	NR	NR
	66	left	25.0 (7.2)	25.9 (5.6)	NR	NR	NR	NR	NR
	67	right	25.0 (8.8)	28.2 (3.1)	NR	NR	NR	NR	NR
FC116:IV-25	52	right	22.7 (9.2)	18.8 (3.2)	NR	NR	NR	NR	NR
	52	left	21.4 (4.7)	20.4 (6.8)	NR	NR	NR	NR	NR
	53	right	21.8 (8.4)	20.4 (3.3)	NR	NR	NR	NR	NR
FC116:IV-38	42	right	27.7 (5.6)	26.1 (14.3)	21.5 (0.7)	23.1 (2.2)	NR	NR	NR
	45	right	27.2 (4.2)	27.1 (13.9)	17.7 (1.2)	19.0 (1.4)	NR	NR	NR
	45	left	27.9 (10.7)	27.8 (12.8)	19.5 (0.2)	NR	NR	17.3 (2.9)	NR
FC116:IV-39	42	right	28.7 (8.7)	26.7 (12.4)	NR	NR	19.1 (4.1)	17.3 (3.7)	NR
	42	left	26.0 (10.9)	23.0 (11.1)	NR	15.4 (0.2)	17.0 (3.1)	15.2 (2.9)	NR
FC116:V-1	11	right	17.3 (7.9)	15.3 (11.6)	15.2 (1.5)	15.1 (4.4)	16.2 (20.4)	15.6 (8.8)	16.3 (8.9)
	11	left	17.8 (5.8)	14.6 (11.8)	16.8 (0.5)	16.4 (3.4)	15.3 (10.8)	14.9 (8.1)	14.1 (7.3)
FC116:V-19	23	right	25.0 (12.5)	22.9 (9.0)	19.1 (4.9)	19.3 (6.1)	NR	NR	NR
FC116:V-23	19	right	23.7 (6.7)	19.9 (11.7)	18.7 (3.3)	21.4 (7.2)	17.5 (10.6)	15.8 (8.8)	NR
FC388:II-2	36	right	NR	NR	NR	NR	NR	NR	NR
	36	left	19.3 (1.4)	19.4 (1.8)	NR	NR	NR	NR	NR
FC388:III-1	6	right	11.0 (6.5)	10.4 (7.3)	11.4 (1.1)	11.8 (1.4)	NR	NR	NR
FC85:II-2	23	right	26.4 (6.6)	22.2 (11.7)	21.2 (0.5)	20.1 (1.7)	NR	NR	NR

Amplitudes of evoked responses are given in parentheses (for motor NCSs as mV and for sensory NCSs as μV). Normal NCV values: motor median $\geq$ 50.5 m/s, ulnar $\geq$ 51.1, peroneal $\geq$ 41.2, tibial $\geq$ 41.1, sensory median $\geq$ 39.3, ulnar $\geq$ 37.5, sural $\geq$ 32.1; normal amplitude values: motor median $\geq$ 6.0, ulnar $\geq$ 8.0, peroneal $\geq$ 2.0, tibial $\geq$ 6.0, sensory median $\geq$ 8.8, ulnar $\geq$ 7.9, sural $\geq$ 6.0. NR: not recordable.

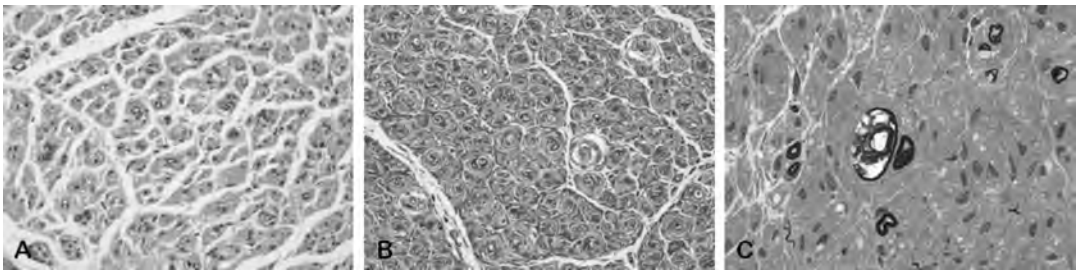

Figure 2. Histological examination for the sural nerve biopsy. The nerve biopsy was done in a 52-year-old female (IV-25 in FC116). Luxol fast blue stain (A) and modified Masson's trichrome stain (B) revealed a markedly decreased number of myelinated fibers with diffuse onion bulb formations surrounding myelinated and unmyelinated axons and endoneurial fibrosis. (C) Semithin transverse section with toluidine blue stain showed severe loss of myelinated fibers of all calibers and remaining myelinated fibers with abnormalities of myelin. Original magnification: $\times 40$ (A) $\times 40$ (B), and $\times 100$ (C).

Histopathological findings

A sural nerve biopsy was done oin a 52-year-old female (FC116: IV-25). Histopathological features showed severe loss of myelinated fibers (MFs) of all calibers, diffuse onion bulb formation, and endoneurial fibrosis (Figure 2). The remaining myelinated fibers were measured to be 381/mm^2 (normal distal sural nerve in 45-year-old female: 7,300/mm^2), and the range and average of the diameters of myelinated fibers were 3.8–16.1 μm and 7.4 μm, respectively (normal distal sural nerve of 45-year-old female: 1.8–14.8 μm and 5.4 μm, respectively). A histogram showed a unimodal distribution pattern with myelinated fiber diameters of less than 3 μm (Figure 3). The myelinated fibers with axonal diameters larger than 8 μm constitutisted of 29.5% of myelinated fibers. The percentage of MF area in this case was 1.8% (normal distal sural nerve of 45-year-old female: 26.9%). The range and average of the g-ratio were 0.58–0.86 and 0.73 ± 0.06, respectively. A g-ratio of more than 0.7 (indicating hypomyelination) and less than 0.4 (indicating hypermyelination) consisted of 62.8% and 0% of myelinated fibers had a g-ratio of more than 0.7 (indicating hypomyelination) and less than 0.4 (indicating hypermyelination), respectively (Figure 3).

Electron microscopic examination revealed myelinated fibers with abnormalities of myelin (irregular myelin sheath, wrinkled myelinated fibers with abnormal myelin compaction, and focally folded myelin or tomacula) and single myelinated or unmyelinated fibers with surrounding loops of Schwann cell cytoplasmic processes (onion bulbs or pseudo-onion bulbs) (Figure 4A). Endoneurial fibroblast proliferation and collagen deposition were well documented (Figure 4B).

Wide clinical phenotypic variations

As shown in Tables 2 and 3, affected individuals revealed wide phenotypic variations. The mean age at onset was 18.3 ± 15.7 years (range 4–60 years), and the disease duration at the time of examination was 23.4 ± 17.8 years (range 2–67 years). Length-dependent sensory loss was found in 15 patients (79%), and vibration sense was reduced to a greater extent than pain in 10 of the 15 patients (67%). The mean FDS was 1.8 ± 1.5, and CMTNS was 11.2 ± 6.9 (Table 2).

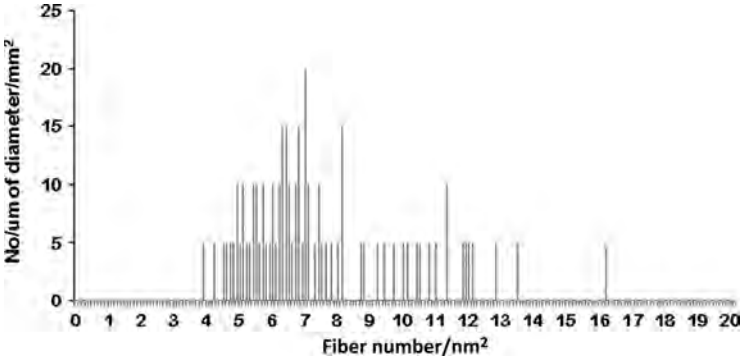

Figure 3. Diameter histogram of myelinated fibers. The histogram was obtained from a sural nerve biopsy done in a 52-year-old female (FC116: IV-25). The histogram revealed a unimodal distribution pattern with no myelinated fiber diameter of less than 3 μm.

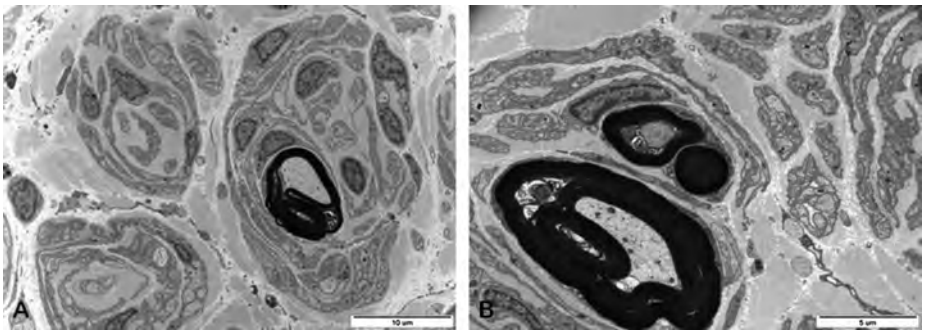

Figure 4. Electron microscopic analysis. (A) Electron microscopic examination revealed myelinated fibers with abnormalities of myelin and single myelinated or unmyelinated fibers with surrounding loops of Schwann cell cytoplasmic processes forming onion bulbs or pseudo-onion bulbs. (B) Endoneurial collagen deposition is well documented. Original magnification: $\times 4000$ (A), and $\times 8000$ (B).

All affected individuals except two showed low FDSs (score ≤ 3). Two patients were in the severe category (CMTNS ≥ 21), five in the moderate category, and ten in the mild category (CNTNS ≤ 10). Foot deformities were found in 16 patients (84%), and heel gait defects were more frequent than toe gait defects ($P < 0.05$) (Table 2). In particular, when the clinical phenotypes were compared among 15 affected members in the large FC116 family, they revealed broad intrafamilial variations, such as variable age at onset, asymptomatic to wheelchair-dependent, and normal to severe sensory impairment (Table 4).

Discussion

We identified three unique CNVs associated with nonrecurrent rearrangements on 17p12 from three CMT1A families: two simple duplications and a complex duplication (Choi et al. 2011). Although nonrecurrent genomic rearrangements have been reported recently in the CMT1A or HNPP patients, this study might be the first full clinical examination study.

Table 4. Wide phenotypic variations among affected members within the FC116 family with rare CNVs.

Examination	Mean value	Range of variation
No. of patients	$n = 15$	–
Age at onset	20.8 yrs	4 to 60 yrs
Asymptomatic patients	n = 1 (6.7%)	–
FDS	2.0	0 to 5
CMTNS	11.4	2 to 27
Muscle weakness (lower limb)	$n = 12$ (80.0%)	No symptom to wheelchair dependent
Muscle atrophy	11 (73.3%)	No symptom to severe
Sensory impairment	12 (80.0%)	Normal to severe
Abnormal DTR	13 (86.7%)	Normal to absent
Foot deformity	13 (86.7%)	Normal to severe

FDS, functional disability scale; CMTNS, CMT neuropathy scale; DTR, deep tendon reflex.

For the present 19 patients with nonrecurrent duplication, muscle weakness and atrophy was predominant in distal lower limbs and varied from asymptomatic to severe forms. Sensory loss, mostly with a length-dependent pattern, was dominated by proprioception defects. The frequency of foot deformity was high, and heel gait defects were more frequent than the toe gait defects. The electrophysiological evaluations of 17 patients showed distinct conduction slowing on the tested motor and sensory nerves. All median motor nerve conduction velocities, except two harboring unrecordable amplitudes, were reduced and ranged from 11.0 to 30.7 m/s. Moreover, sensory nerve action potentials were not recordable in 71–82% of the cases. Actually, these data did not differ significantly from those of 149 CMT1A patients with common duplication on 17p12 (Choi et al. 2011). The histopathological studies on the sural nerve showed diffuse onion bulb formations surrounding myelinated and unmyelinated axons. A diameter histogram of myelinated fibers showed a unimodal distribution pattern and confirmed the hypertrophic or demyelinating changes. Those clinical, electrophysiological, and histopathological features were similar to the CMT1A patients with common duplication (Birouk et al. 1997; Lee and Choi 2006).

The exact duplication regions were different among the three families; however, the *PMP22* gene was included in all the duplications. When the clinical phenotypes were fully examined for all the involved affected members, no observable different feature was revealed in comparison with common CMT1A patients. This result is consistent with the idea that the copy number of *PMP22* is exclusively contributed to the CMT1A phenotype. However, we also found a wide

range of intrafamilial variation in the large FC116 family (Table 4). For example, the age at onset showed marked variation and ranged from 4 years to 60 years. Moreover, an individual with rare CNV was asymptomatic in the FC116. The wide variations of clinical phenotypes hinted at the presence of a genetic modifier or environmental factor(s).

Genotype-phenotype correlations are often less strict in CMT patients, and CMT displays clinically wide phenotypic heterogeneity depending on causative mutations within the same gene. Kleopa et al. suggested that mutations in the P*MP22* gene are relevant in the broad phenotypic spectrum (Kleopa et al. 2004). Some *MPZ* gene mutations have revealed considerable clinical heterogeneity in the affected individuals, not only among families, but also within the same family (Szabo et al. 2005; Mazzeo et al. 2008). Choi et al. (2011) suggested that the nonrecurrent rearrangements are stably inherited without alteration of the junction sequence; however, the nonrecurrent rearrangements may allow some alteration of the genomic contents in duplication across generations by recombination events. This may partially explain some of the broad phenotypic spectrum in the same CMT1A family.

CMT is one of the genetic diseases in which molecular-based therapies are progressing to the clinical trial phase; therefore, the exact manifestation of clinical phenotypes according to genotypes needs to be described in order to elucidate the phenotype-genotype correlation. This in-depth study for the clinical characteristics of CMT1A with partial duplication suggests that it has a similar phenotype to common CMT1A, but variable phenotypes within the same family. This study may broaden the understanding of the role of CNVs in the pathogenesis of CMT.

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