Clinical and Electrophysiological Studies of a Family with Probable X-linked Dominant Charcot-Marie-Tooth Neuropathy and Ptosis

Tony Wu, MD, DMS; Hung-Li Wang[1], PhD; Chun-Che Chu, MD; Jia-Ming Yu[2], MD; Jeng-Yeou Chen[3], MD; Chin-Chang Huang, MD

Background: The X-linked dominant Charcot-Marie-Tooth neuropathy (CMTX) is a hereditary motor and sensory neuropathy linked to a variety of mutations in the connexin32 (Cx32) gene. Clinical and genetic features of CMTX have not previously been reported in Taiwanese.

Methods: Clinical evaluations and electrophysiological studies were carried out on 25 family members of a Taiwanese family group. Molecular genetic analysis of the Cx32 gene was performed. A sural nerve biopsy was obtained from 1 patient.

Results: Nine patients had clinical features of X-linked dominant inheritance and a moderate Charcot-Marie-Tooth (CMT) neuropathy phenotype. Molecular genetic analysis showed no mutation of the Cx32 coding region, but revealed a G-to-A transition at position -215 of the nerve-specific promoter P2 of the Cx32 gene. Ptosis is 1 clinical manifestation of neuropathy in this probable CMTX family. Familial hyperthyroidism is an additional independent feature of the family. Electrophysiological and histological studies showed features of axonal neuropathy. Multimodality evoked potential studies revealed normal central motor and sensory conduction velocities.

Conclusions: The presence of ptosis in this family illustrates the existence of clinical heterogeneity among related family members with CMTX similar to that in CMT of autosomal inheritance. Electrophysiological and histological findings revealed normal central conduction and axonal neuropathy.
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Key words: X-linked dominant Charcot-Marie-Tooth neuropathy, electrophysiology, connexin32, ptosis, familial hyperthyroidism.

Charcot-Marie-Tooth (CMT) neuropathies are a heterogeneous group of disorders characterized by degenerative changes in peripheral nerves leading to progressive distal muscle weakness, atrophy, and sensory loss.[1,2] Based on electrophysiological and pathological findings, CMT neuropathies are classified into a demyelinating type (CMT 1) characterized by marked slowing of nerve conduction velocities (NCVs), and an axonal type (CMT 2) with preserved or only mildly reduced NCVs.[1,2] There are

From the First Section, Department of Neurology, Chang Gung Memorial Hospital, Taipei; [1]Department of Physiology, Chang Gung University; [2]Departmentof Neurology, Taipei Municipal Jen-Ai Hospital; [3]Department of Endocrine and Metabolism, Chang Gung Memorial Hospital, Taipei.
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Address for reprints: Dr. Tony Wu, Department of Neurology, Chang Gung Memorial Hospital. 5, Fushing Street, Gueishan Shiang, Taoyuan, Taiwan 333, R.O.C. Tel.: 886-3-3281200 ext. 8418; Fax: 886-3-3287226; E-mail: tonywu@adm.cgmh.org.tw

several modes of inheritance in both types: autosomal dominant, X-linked dominant, and autosomal recessive.[3,4] The most-frequent form, CMT1A, is due in most cases to a duplication of chromosome 17p11.2 containing the peripheral myelin protein 22 gene (PMP22).[5,6] CMT1B results from mutations in the myelin protein zero gene (MPZ) on chromosome 1.[7]

X-linked dominant CMT (CMTX) is linked to mutations within the connexin32 (Cx32) locus of chromosome Xq13.1.[8-11] Cx32 is thought to function as a gap junction protein, and to be involved in the exchange of information and metabolites in the nervous system. The Cx32 protein and mRNA are expressed in Schwann cells and oligodendrocytes that function as myelinated cells of the peripheral and central nervous system, respectively.[12,13] The severity of the CMTX phenotype is correlated with the location and type of mutation of the Cx32 gene.[14] Two CMTX families possessing mutations within the noncoding region of the Cx32 gene showed a moderate phenotype.[15] There is no consensus concerning the type of neuropathy.[16,17] Some related individuals suffering from CMTX showed electrophysiological and pathological findings of primary axonal degeneration,[3,18-21] while others were considered to have primary demyelinating neuropathy.[22,23] Most clinical and genetic studies of CMTX are based on families from Europe or North America, but the results of a genetic study have been described in a Taiwanese family.[24]

Two hereditary diseases may coexist in a single family. Independent presentations of CMT and myotonic dystrophy, facioscapulohumeral muscular dystrophy, or nephropathy in a single family group have been described in family studies.[25-27] The concurrence of CMT and familial hyperthyroidism in single families has not been reported. We encountered a family affected with the unusual combination of hereditary neuropathy, ptosis, and familial hyperthyroidism. This study investigates the clinical, electrophysiological, and molecular genetic characteristics in this Taiwanese family group.

METHODS

Patients

Detailed histories and neurological examinations were obtained from the family members shown in the pedigree (Fig. 1). Most of the family members received electrophysiological and molecular genetic studies, and thyroid function tests (including T3, T4, TSH, antimicrosomal Ab, and TBII). All participants provided written informed consent according to the

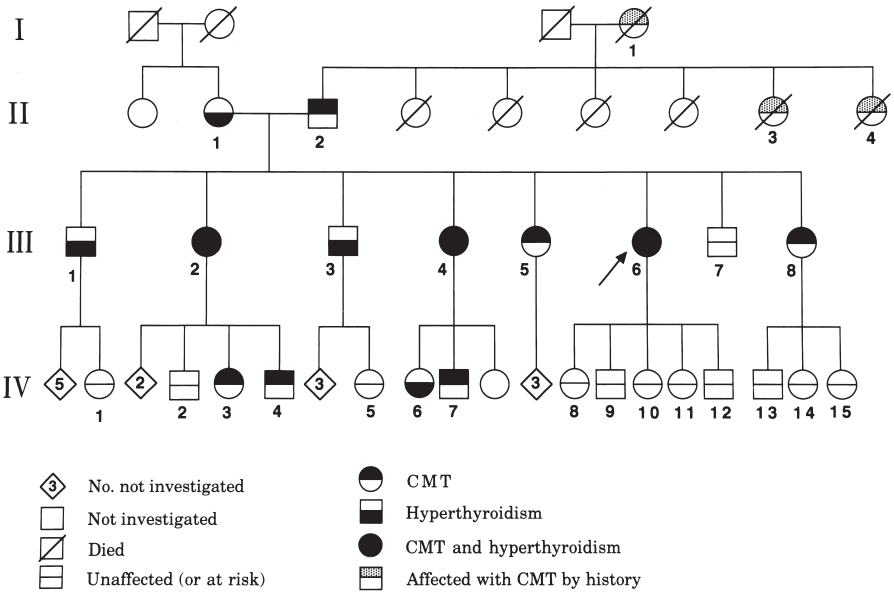

Fig. 1 Pedigree of this family with probable X-linked dominant Charcot-Marie-Tooth neuropathy and familial hyperthyroidism. The propositus is indicated by the arrow.

protocol approved by the Ethics Committee of Chang Gung Memorial Hospital, Taipei, Taiwan. Genomic DNA was extracted from peripheral blood lymphocytes using standard methods. A sural nerve biopsy was obtained from patient III-4.

Electrophysiological evaluation

Motor nerve conduction studies including median, ulnar, peroneal, and tibial nerves and sensory nerve conduction studies including median, ulnar, and sural nerves were performed as standard methods described previously.[28] Evoked potential studies including brainstem auditory evoked potentials (BAEPs), pattern-reversal visual evoked potentials (VEPs), somatosensory evoked potentials (SEPs), and motor evoked potentials (MEPs) were carried out according to techniques described previously.[29-31] In brief, VEPs were obtained by pattern reversal to monocular full-field stimulation using 30-min checks, reversing at a rate of 3.1 Hz. The BAEP studies were performed using monaural stimulation with rarefaction clicks with a 10-ms duration at a rate of 11.1 Hz. The SEPs were elicited by unilateral percutaneous stimulation of the median nerve at a threshold of just above the motor threshold, and were recorded from the brachial plexus (Erb potential), the cervical spine at C2 (N13), and the contralateral parietal area (N20) with a frontal (Fz) reference.

Molecular genetic analysis

The human Cx32 gene contains 2 tissue-specific promoters, P1 and P2, which function in the liver and nervous system, respectively.[32] CMTX mutations in the coding region of the Cx32 gene can lead to functional impairment of Cx32 gap junctions, and in the P2 promoter, to reduced Cx32 expression in myelinated Schwann cells.

Methods used for the genetic study in these family members were published elsewhere.[24] In brief, genomic DNA was isolated from venous blood with QIAGEN blood and cell culture DNA kits. A DNA fragment containing the Cx32 coding region or nerve-specific P2 promoter region of the Cx32 gene was obtained by performing PCR amplification using genomic DNA as the template. PCR was carried out with the following oligonucleotide primers: (a) the forward primer for human connexin32 was 5'TGTTTGCAGGTGTGAATGAGGCAG3' and corresponded to nucleotides 473 to 496 of the human Cx32 gene; (b) the reverse primer for the human Cx32 coding region was 5'TGGCAGGTTGCCTGGTATGTGGCA3' and corresponded to nucleotides 1350 to 1373 of the Cx32 gene; (c) the sense primer for the proximal P2 promoter was 5'GCCTCAGGGAAAATCCTGGTGACC3' and corresponded to nucleotides -353 to -330 of the human Cx32 gene; and (d) the antisense primer for the DNA fragment containing the P2 promoter region was 5'TGTCCAGTTCATCCTGCCTCATTC3' and corresponded to nucleotides 486 to 509 of the Cx32 gene.[30] The PCR DNA products were purified using a JETsorb kit (GENOMED) and were used as the template for the DNA sequencing by the dideoxy DNA sequencing method (Thermo Sequenase cycle sequencing kit, Amersham).

RESULTS

Family pedigree

There was no consanguinity among the 4 generation of family members evaluated. Analysis of the pedigree showed a segregation pattern in generation III. All 5 daughters (III-2, III-4, III-5, III-6, and III-8) but none of the 23 sons (III-1, III-3, or III-7) of

Table 1. Summary of Clinical, Nerve Conduction Velocity (NCV), and Genetic Studies in Generations II, III, and IV

Generation	No. of total patients	Clinical and NCV studies			Genetic study	
		No. of patients	No. of patients with an abnormal neurologic examination	No. of patients with an abnormal NCV study	No. of patients	No. of patients with mutations
II	9	2 (II-1,2)	1 (II-2)	1 (II-2)	2 (II-1, 2)	1 (II-2)
III	8	8 (III-1~8)	5 (62.5%) (III-2, 4, 5, 6, 8)	5 (62.5%) (III-2, 4, 5, 6, 8)	8 (III-1~8)	5 (62.5%) (III-2, 4, 5, 6, 8)
IV	29	15 (IV-1~15)	3 (20%) (IV-3, 4, 7)	2 (13.3%) (IV-4, 7)	7 (IV-1~7)	3 (42.9%) (IV-3, 4, 7)

the affected father (II-2) had clinical pictures of CMT. There was no male-to-male transmission observed. These features suggested CMT in this family, probably with an X-linked dominant pattern of inheritance. Hyperthyroidism was noted in the members of generations II, III, and IV, probably with dominant transmission. The presence of hyperthyroidism was an independent feature in this CMT family. The unusual combination of CMT and familial hyperthyroidism in this family was caused by marriage between a man (II-2) with inherited neuropathy and a woman (II-1) with familial hyperthyroidism.

Clinical and electrophysiological findings

Twenty-five family members (II-1 and II-2, III-1 through III-8, and IV-1 through IV-15) received a full neurological examination and electrophysiological

Table 2. Clinical Features of Affected Family Members

Pedigree no. and gender	Age (yr) at examination	Neuropathy	Ptosis	Hyperthyroidism
I-1 (F) *	*	+	?	?
II-1 (F)	72	-	-	+
II-2 (M)	72	+	+	-
III-1 (M)	53	-	-	+
III-2 (F)	51	+	+	+
III-3 (M)	46	-	-	+
III-4 (F)	43	+	+	+
III-5 (F)	39	+	+	-
III-6 (F)	37	+	+	+
III-8 (F)	31	+	+	-
IV-3 (F)	20	+	-	-
IV-4 (M)	15	+	-	-
IV-6 (F)	16	-	-	+
IV-7 (M)	14	+	+	-

*: Clinical data from the history; +: presence; -: absence; ?: unknown.

Table 3. Clinical Features of Neuropathy in Probable CMTX Patients

Patient	Age (yr)	Gender	Onset (yr)	Initial symptom	Hand	Weakness Arm	Weakness Thigh	Leg	Pes cavus	Areflexia	Sensory impairment	Ptosis	Ophthalmoplegia
II-2	72	M	30s	G	+	+	+	+	+	+	+	mild	-
III-2	51	F	20s	G	+	+	+	+	+	+	+	mild	-
III-4	43	F	20s	P	+	+	+	+	+	+	+	severe	+
III-5	39	F	30s	G	+	-	-	+	-	+	+	mild	-
III-6	37	F	21	P	+	+	-	+	-	+	+	severe	+
III-8	31	F	20s	G	+	+	+	+	-	+	+	mild	-
IV-3	20	F	19	G	-	-	-	+	-	-	-	-	-
IV-4	15	M	13	G	-	-	-	+	-	-	-	-	-
IV-7	14	M	12	G	-	-	-	+	-	-	-	mild	-

Abbreviations: G: gait disturbance; P: ptosis.
+: presence of clinical signs; -: absence of clinical signs.

Table 4. Nerve Conduction Studies in Patients with Probable CMTX

Patient	Age (yr)	Gender	Onset (yr)	Peroneal A (mV)	(motor) MNCV	Tibial A (mV)	(motor) MNCV	Sural A (μV)	Median A (mV)	(motor) MNCV	Median A (μV)	(sensory) SNCV	Ulnar A (mV)	(motor) MNCV	Ulnar A (μV)	(sensory) SNCV
II-2	72	M	30s	NR	-	NR	-	4.0	4.6	36	24	43	4.3	36	25	43
III-2	51	F	20s	0.2	34	0.5	29	4.8	1.8	33	17	47	3.4	44	20	50
III-4	43	F	20s	0.7	35	0.2	34	4.0	6.5	43	18	53	7.6	50	13	56
III-5	39	F	30s	0.1	43	1.1	40	2.2	7.2	46	10	53	6.7	45	17	48
III-6	37	F	21	2.7	36	3.6	31	3.0	11.1	51	37	49	9.2	43	33	51
III-8	31	F	20s	0.2	33	1.3	30	NR	10.1	45	20	56	6.6	49	25	52
IV-3	20	F	19	2.9	53	3.5	50	18.0	9.0	57	25	61	9.4	60	22	62
IV-4	15	M	13	2.7	42	4.6	42	5.0	8.7	52	14	50	8.2	50	10	50
IV-7	14	M	12	2.7	42	4.0	41	15.0	7.4	51	19	50	8.2	53	17	51
Controls				> 3.9	> 41	> 4.5	> 42	> 11.0	> 6.6	> 49	> 13	> 57	> 6.5	> 53	> 11	> 52

Abbreviations: A: distal motor or sensory amplitude; MNCV: motor nerve conduction velocity (m/s); SNCV: sensory nerve conduction velocity (m/s); NR: no response.

Table 5. Thyroid Function in Patients with Hyperthyroidism

Patient	Age (yr)	Gender	Age (yr) at diagnosis of HT	Thyroid-ectomy	Thyroid scan	T3 (ng/dl)	T4 (μg/dl)	TSH (μIU/ ml)	Antimi-crosomal Ab	TBII	Current treatment and thyroid status	Other endocrine anomaly
II-1	72	F	60s	60s	ND	< 10	< 1.0	45.4	1:6400(+)	ND	thyroxin, euthyroid	NIDDM
III-1	53	M	30s	30s	ND	77.0	6.0	7.2	ND	ND	hypothyroid	none
III-2	51	F	48	ND	DI	209	> 24.9	< 0.1	1:6400(+)	40.3 %	ATD, euthyroid	none
III-3	46	M	37	37	ND	59.4	4.2	7.6	ND	ND	hypothyroid	none
III-4	43	F	26	26 and 27	ND	57.4	5.9	2.8	ND	ND	euthyroid	NIDDM
III-6	37	F	34	ND	DI	320.0	17.6	< 0.1	1:100(+)	27.7 %	ATD, euthyroid	none
IV-7	16	F	16	ND	ND	221	14.1	< 0.1	ND	ND	hyperthyroid	none
Normal limits						52-175	4.8-12.8	0.4-4.5		< 15 %		

Abbreviations: HT: hyperthyroidism; ND: not done; DI: diffusely increased; TBII: TSH-binding inhibitory immunoglobulin; ATD: anti-thyroid drug; NIDDM: non-insulin-dependent diabetic mellitus.

Table 6. Evoked Potential Studies in Patients with Probable CMTX (ms)

Patient	Age (yr)	Gender	Onset (yr)	MEP ADM CMCT	MEP TA CMCT	SEP Median N13-N20	SEP Tibial N22-P40	BAEP latencies I	BAEP latencies III	BAEP latencies V	VEP latency Right	VEP latency Left
II-2	72	M	30s	8.4	15.6	5.4	-	NR	NR	6.2	NR	NR
III-2	51	F	20s	7.8	12.4	5.7	-	1.6	3.8	5.5	99	100
III-4	43	F	20s	7.2	14.0	5.1	14.4	1.6	3.6	5.6	108	109
III-5	39	F	30s	7.6	14.4	5.7	-	1.5	3.6	5.5	95	97
III-6	37	F	21	7.6	14.0	5.1	17.4	1.5	3.6	5.7	104	104
III-8	31	F	20s	6.8	14.0	5.1	-	1.5	3.8	5.8	104	104
IV-3	20	F	19	ND	ND	5.7	-	1.6	3.7	5.5	100	100
IV-4	15	M	13	7.6	15.4	ND	ND	ND	ND	ND	ND	ND
IV-7	14	M	12	9.6	16.0	6.0	16.8	2.2	4.4	5.6	104	105
Controls	< 10.2	< 18.6	< 8.1	< 20.5	< 2.0	< 4.5	< 6.4	< 112	< 112			

Abbreviations: MEP: motor evoked potential; SEP: somatosensory evoked potential; EAEP: brainstem auditory evoked potential; VEP: visual evoked potential; ADM: abductor digiti minimi; TA: tibialis anterior; CMCT: central motor conduction time; NR: no response: absence of N22 response; ND: not done.

studies. The general results of clinical, electrophysiological, and genetic studies are summarized in Table 1. Their clinical features are summarized in Table 2. The onset and distribution of neuropathic findings are shown in Table 3. The results of NCV studies are shown in Table 4. Mean MNCVs in the median, ulnar, tibial, and peroneal nerves and mean SNCVs in the median, ulnar, and sural nerves were uniformly reduced. Mean CMAPs and SNAPs were normal in the arms, but mildly reduced in the lower legs. The findings of the thyroid function tests are shown in Table 5 and of the evoked potential studies in Table 6.

Case III-6

The propositus, a 35-year-old woman, came to our clinic for ptosis, which had not improved after medical treatment and a thymectomy at the age of 20 years. A neck goiter was noted at the age of 34 years, and thyroid function tests revealed hyperthyroidism with T3 of 320 (normal, 52 - 175) ng/dl, T4 of 17.6 (normal, 4.8 - 12.8) μg/dl, and TSH of < 0.1 (normal, 0.4 - 4.5) μIU/ml. A Tc-99m thyroid scan showed diffusely increased uptake. She was treated with propylthiouracil and propranolol, and remained in an euthyroid status. An examination revealed severe ptosis, external ophthalmoplegia, diffuse hyporeflex-

ia, a flexor plantar response, decreased distal sensation, and mild distal weakness in the extremities. There was no pes cavus or peroneal muscle atrophy. Her ptosis did not show diurnal fluctuations. Intramuscular injection of 10 mg edrophonium chloride produced no improvement in the ptosis. Serum acetylcholine receptor antibodies were absent. Repetitive stimulation tests in the abductor pollis brevis, deltoid, and orbicularis oculi muscles at 3 Hz produced no amplitude decrement of the motor responses. Electroneurography revealed normal or slightly slowed motor nerve conduction velocities (MNCVs) of the median (51 m/s), ulnar (43 m/s), peroneal (36 m/s), and tibial (31 m/s) nerves. Sensory nerve conduction velocity (SNCV) studies showed mildly slowed SNCV of the median (49 m/s) and ulnar (51 m/s) nerves. The amplitudes of compound muscle action potentials (CMAPs) of the peroneal (2.7 mV) and tibial (3.6 mV) nerves, and sensory nerve action potentials (SNAPs) of the sural (3 μV) nerve were reduced. The amplitudes of CMAPs and the SNAPs of the median and ulnar nerves were normal. Electromyography (EMG) revealed chronic neurogenic changes that were more prominent in the distal muscles. Brainstem auditory evoked potential (BAEP) and pattern-reversal visual evoked potential (VEP) studies were normal. Somatosensory evoked potential (SEP) and motor evoked potential (MEP) studies demonstrated normal central sensory and motor conduction velocities. Peripheral conduction of the SEPs had a delayed N9 latency of 11.4 (normal, < 10.9) ms to median nerve stimulation and a delayed N22 latency of 27.6 (normal, < 24.1) ms to tibial nerve stimulation. MEPs to magnetic stimulation over the cervical and lumbar spinal column revealed normal peripheral motor conduction to the abductor digiti minimi of 14.4 (normal, < 15.9) ms, but mildly slowed peripheral motor conduction to the tibialis anterior of 14.8 (normal, < 14.6) ms.

Case III-4

This 52-year-old woman had noted ptosis since the age of 25 years and gait disturbance since the age of 30 years. These symptoms slowly progressed to the point that she had drop foot and severe ptosis over the past 6 years. She was diagnosed as having hyperthyroidism at the age of 26 years, and had twice received a thyroidectomy at the ages of 26 and 27 years, respectively. An examination showed severe ptosis (Fig. 2A), external ophthalmoplegia, pes cavus, distal muscle atrophy, decreased distal sensation, diffuse hyporeflexia, and the absence of Babinski's sign. A thyroid function test revealed a euthyroid status. MNCVs were mildly slowed for the median (43 m/s), ulnar (50 m/s), peroneal (35 m/s), and tibial (34 m/s) nerves. SNCVs were also mildly slowed for the median nerve (53 m/s) but were normal for the ulnar nerve (56 m/s). Repetitive stimulation test in the abductor pollis brevis, deltoid, and orbicularis oculi muscles at 3 Hz produced no decrement in the motor responses. EMG revealed diffuse chronic neurogenic changes particularly in the distal muscles. The BAEP and VEP studies were normal. The SEP and MEP studies revealed normal central sensory and motor conduction velocities. Peripheral conduction in the SEP study revealed a delayed latency of the N9 of 11.7 (normal, < 10.9) ms to median nerve stimulation and delayed latency of the N22 of 28.2 (normal < 24.1) ms to tibial nerve stimulation. MEPs to magnetic stimulation over the cervical and lumbar spinal column had normal peripheral motor conduction to the abductor digiti minimi of 14.4 (normal, < 15.9) ms but slowed peripheral motor conduction to the tibialis anterior of 17.8 (normal, < 14.6) ms. A sural nerve biopsy showed a decreased number of large myelinated fibers and clusters of thinly myelinated regenerating fibers. Morphometric analysis revealed a normal density of myelinated fibers (6810 fibers/mm^2). The axon diameter histogram was unimodal with a loss of large myelinated fibers and a concomitant increase in smaller-sized fibers, reflecting the prominent number of thinly myelinated sprouts. Ultrastructural examination revealed some axons with inappropriately thin myelin sheaths in relation to axon diameter in a cluster of regenerating fibers. No onion bulb formation or giant axonal swelling was noted. Single teased fiber examination confirmed the absence of paranodal or segmental demyelination and remyelination. The above histopathologic findings were consistent with axonal neuropathy. Her 14-year-old son (IV-7) had had gait abnormality and right ptosis (Fig. 2B) since the age of 12 years. An examination revealed mild pes cavus, weakness of foot dorsiflexion, diffuse hyporeflexia, and decreased distal sensation. Extraocular movement and thyroid function were normal. Her 16-year-old daughter (IV-6) had abnormal thyroid function tests with T3 of 221 (normal,

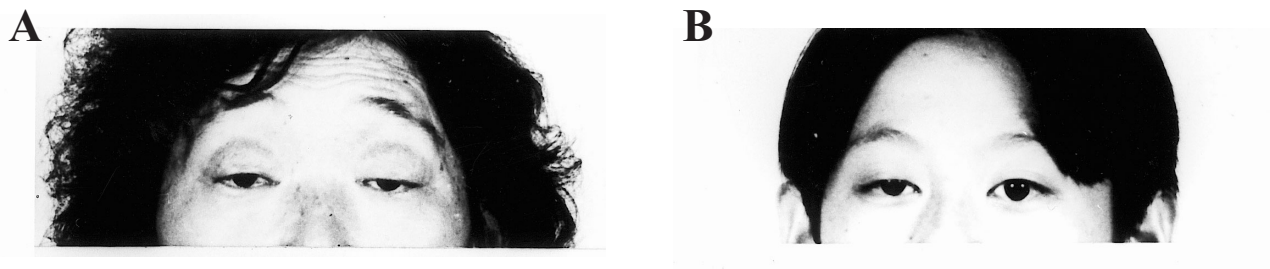

Fig. 2 (A) Severe ptosis of case III-4; (B) mild right eye ptosis of case IV-7 (son of case III-4).

52 - 175) ng/dl, T4 of 14.1 (normal, 4.8 - 12.8) μg/dl, and TSH of < 0.2 (normal, 0.4 - 4.5) μIU/ml. She had a normal neurological examination and NCV study.

Other family members

Cases I-1, II-3, and II-4 were reported to have abnormal gait according to their histories. II-2 had noted pes cavus, drop foot, and mild ptosis since he was in his 30s. A neurological examination revealed ptosis, steppage gait, pes cavus, diffuse hyporeflexia, distal muscle atrophy, weakness in the extremities, decreased distal sensation, and hearing impairment. He had normal mental function, and could still walk without assistance. Thyroid function tests revealed a euthyroid status. II-1 had hyperthyroidism and had received a thyroidectomy at the age of 60 years. Unfortunately, she developed symptoms of myxedema, cold intolerance, thin hair, hoarseness, and unsteady gait thereafter. Thyroid function tests revealed hypothyroidism with T3 of < 10 (normal, 52 - 175) ng/dl, T4 of < 1.0 (normal, 4.8 - 12.8) μg/dl, and TSH of 45.4 (normal, 0.4 - 4.5) μIU/ml. The symptoms and signs of hypothyroidism had been corrected with thyroxin treatment since she was 70 years old. She had a normal neurological examination and NCV study. III-1 had hyperthyroidism and had received a thyroidectomy when he was in his 30s. III-3 also had hyperthyroidism and had received a thyroidectomy at the age of 37 years. Both III-1 and III-3 had normal neurological examinations and thyroid function tests. III-2 had had peripheral neuropathy and ptosis since she was in her 20s, and had been diagnosed as having hyperthyroidism at the age of 48 years. III-5 was noted to have ptosis and peripheral neuropathy in a family screening. The neurological examination, as well as thyroid function and nerve conduction studies were all normal in III-7. III-8 had noted symptoms of peripheral neuropathy and ptosis when she was in her 20's. IV-3 had noted mild leg weakness at 19 years of age. A neurological examination revealed diffuse hyporeflexia, mild weakness on feet dorsiflexion, and mildly decreased distal sensation. IV-4, a 15-year-old male, had experienced mild gait abnormality for 2 years. An examination showed pes cavus, weakness on foot dorsiflexion, diffuse hyporeflexia, and decreased distal sensation. Neither IV-3 nor IV-4 had ptosis or external ophthalmoplegia, and their thyroid function tests were normal.

Molecular genetic findings

Direct sequencing of the PCR DNA product revealed that the Cx32 coding region of 9 affected members exhibited no mutation. Instead, analysis of the PCR products revealed that CMT patients had a G-to-A point mutation at position -215 relative to the transcription initiation site in the nerve-specific Cx32 P2 promoter.[24] This mutation occurred in male (II-2, IV-4, and IV-7) and heterozygous female (III-2, III-4, III-5, III-6, III-8, and IV-3) CMT patients, but was not present in unaffected family members. The G-to-A transfusion in the P2 promoter is located 200 base pairs (bp) upstream of the TATA box.

DISCUSSION

Three genes commonly causing CMT encode myelin-related proteins: peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ), and Cx32. PMP22 duplication mainly causes demyelinating phenotypes with variable axonal features. Patients with MPZ mutations fall into 2 distinctive phenotypic subgroups: one showing preserved MNCV and exclusively axonal pathological features, with the other exhibiting exclusively demyelinating features. Patients with Cx32 mutations show intermediate

slowing of MNCVs, predominantly axonal features, and relatively mild demyelinating pathologies.

The present study revealed distinct features of typical peripheral sensorimotor neuropathy (CMT), ptosis, and hyperthyroidism in 4 generations of a Taiwanese family. Their clinical, electrophysiological, and pathological features in 9 affected members with hereditary neuropathy are consistent with those seen in axonal CMT. In addition to the classic clinical features of CMT, ptosis in this family was an unusual manifestation of a hereditary neuropathy. The family pedigree showed a segregation pattern in which all 5 daughters and none of the 3 sons of the affected father (II-2) were affected. There was no male-to-male transmission, and there were more affected females than males (6:3). The onset ages in male patients (IV-4 and IV-7) were earlier than those in female patients. This evidence suggests that CMT in this family has an X-linked dominant inheritance pattern. The young age (≤10 years old) of the at-risk patients IV-8~15 may explain the low percentage (20%) of abnormal clinical and NCV findings in generation IV. A molecular genetic study confirmed that this CMTX1 family had a G-to-A transition at position -215 (in relation to the transcription initiation site) of nerve-specific promoter P2 of the Cx32 gene. Analysis of the family pedigree indicated that the familial hyperthyroidism of probable dominant transmission was an independent trait in this CMTX-afflicted family. The unusual combination of CMTX1 and familial hyperthyroidism in this family was caused by a marriage between a man with inherited neuropathy and a woman with familial hyperthyroidism.

Most CMTX families have distinct mutations in the Cx32 gene, and more than 160 mutations of the Cx32 gene coding region have been identified.[17,33] The severity of the CMTX clinical phenotype is correlated with both the location and type of mutation in the Cx32 gene.[14] Most missense mutations show mild clinical phenotypes, whereas nonsense mutations normally cause severe CMT phenotypes.[14] Families with CMTX without mutations in the open reading frame may have mutations in the Cx32 promoter, splice sites, or untranslated regions.[15] The present family might be the third CMTX family reported to possess mutations in the noncoding region of the Cx32 gene although another study had a different opinion.[34] According to the classification of the CMT phenotype by Ionassescu, [15] the clinical features of this family should be classified as a moderate CMT phenotype characterized by weakness of the peroneals and tibialis anterior (which required ankle foot orthoses), weakness of the palmar and dorsal interossei, and ptosis. This is consistent with a previous report of CMTX families with point mutations in promoter P2 of the Cx32 gene or 5' untranslated region of mRNA who had moderate CMT phenotypes.[15] Mutations of the P2 promoter may directly affect the rate of initiation of transcription and may decrease production of the Cx32 protein.

Previous reports of CMTX families with detailed clinical, electrophysiological, and histological descriptions produced no consensus concerning the type of neuropathy manifested. Some consider CMTX to primarily be an axonal neuropathy,[3,18-20,35] whereas others concluded it to primarily be a demyelinating neuropathy.[21,23] In this family, median MNCVs were normal or slightly reduced (≥45 m/s) in 6 of the 9 CMTX patients, and 4 of them had normal peroneal MNCVs. All of the CMTX patients had reduced CMAP amplitudes and MNCVs exceeding 30 m/s in the peroneal nerve. These NCV features suggest axonal damage more than a myelinopathy, and differ from results of a previous study of families with mutations in the noncoding regions of the Cx32 gene who had MNCVs which ranged from 20 to 30 m/s, although no details were given of the nerves tested.[15] From the aspect of the evoked potential study, patients with CMT of the demyelinating type usually have increased wave I latency of the BAEP or prolongation of the P100 peak latency in VEPs.[33,36-40] In the present family, all except for case II-2 among CMT patients had normal BAEP and VEP studies. Patient II-2 lacked a wave I but had a normal wave V peak latency in BAEP, while lacking the P100 waveform in the VEP study. These evoked potential characters suggest that the involvement of cochlear and optic nerves is mainly due to axonopathy. Furthermore, the histological features including morphological findings, morphometry, and electron microscopic examination also support primary axonal damage being responsible for the neuropathy in this probable CMTX family.

In peripheral nerves, connexin32 has been demonstrated in the Schmidt-Lanterman incisures and the paranodal portions of the node of Ranvier.[41-43] In the central nervous system, connex-

in32 is expressed throughout the internodal region as well as in oligodendrocytes.[41] The exact mechanism through which CMTX patients experience predominant disturbance of the peripheral myelin and to a lesser extent the central myelin is unknown. A possible explanation is that several other connexins expressed in the central nervous system may substitute for Cx32's function. Impairment of the central nervous system has been demonstrated in evoked potential and brain MRI studies from some CMTX families.[23,33,44,45] Patients with mutations in the coding region of the Cx32 gene show prolonged wave I-V interpeak latencies in BAEP, delayed P100 peak latencies in VEPs, and increased central motor conduction times in MEPs.[23,45,46] This may represent a functional defect of central gap junctions by dominant negative effects, in that chimeric connexins with mutant and wild-type connexins cannot properly be inserted into the cytoplasmic membrane.[47] On the other hand, mutation of the nerve-specific promoter P2 of the Cx32 gene may decrease the production of Cx32 protein without the formation of chimeric connexins. This might explain why our patients with probable CMTX had normal central sensory, motor, and auditory conductions in the SEP, MEP, and BAEP studies.

Thyrotoxic sensorimotor polyneuropathy is an infrequent neuromuscular complication of hyperthyroidism.[48-51] Clinical and electrophysiological features of thyrotoxic polyneuropathy may mimic those of CMT. Patients with thyrotoxic polyneuropathy present symptoms mostly affecting the legs (Basedow's paraplegia).[48,50] Nerve conduction studies are normal or mildly slowed in the lower limbs,[48,50,51] and nerve biopsies show features of axonal neuropathy.[50,52] Thyrotoxic polyneuropathy usually presents in the later stages of hyperthyroidism and improves with medical treatment.[50,53] In the present family, none of the 3 patients with both neuropathy and hyperthyroidism (III-2, III-4, and III-6) showed any improvement in the neuropathic symptoms following medical treatment or a thyroidectomy. Two (III-2 and III-6) of them had neuropathy which had occurred much earlier than the time at which they had been diagnosed as having hyperthyroidism. The polyneuropathy in this family was not secondary to hyperthyroidism. The unusual combination of CMTX and familial hyperthyroidism in this family was caused by the marriage between a man with neuropathy and a woman with hyperthyroidism. This is similar to previous reports of concurrent CMT and myotonic dystrophy, facioscapulohumeral muscular dystrophy, or nephropathy in single family groups.[25-27]

Several studies have described unusual clinical associations of ptosis and CMT similar to those in this family.[54,55] Patients with CMT presenting with ptosis or ophthalmoplegia may mimic ocular myasthenia gravis.[56] Although myasthenia gravis as a cause of ptosis was considered in our patients, all 7 patients with neuropathy and ptosis had negative repetitive stimulation and edrophonium tests. A therapeutic trial of pyridostigmine in the 2 patients with severe ptosis and external ophthalmoplegia (III-4 and III-6) was unsuccessful. The propositus had ptosis as an initial presentation and underwent a thymectomy. She had subsequent intermittent physostigmine treatment for more than 10 years; however, she had negative repetitive stimulation and edrophonium tests, and acetylcholine receptor antibodies were undetectable. Therefore, myasthenia gravis cannot be considered as the cause of ptosis in this family. Ptosis is an accompanying feature of CMTX, and may manifest before the classical signs of hereditary neuropathy as in patients III-4 and III-6. Two younger CMTX patients (IV-3 and IV-4) presenting with the symptom of mild gait abnormality for only 1 or 2 years may develop ptosis at some future point.

In conclusion, the presence of ptosis in this family illustrates the existence of clinical heterogeneity among related family members with CMTX similar to that in CMT of autosomal inheritance. Electrophysiological and histological features support primary axonal damage being responsible for the neuropathies in this family. Multimodality evoked potentials studies revealed normal central motor and sensory conduction velocities, with the possible involvement of the auditory and optic nerves. Familial hyperthyroidism is an independent feature in this probable CMTX family. Further cellular and molecular biology studies are essential to examine the influence of the genetic background on variations of CMTX phenotypes.

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疑是性染色體顯性遺傳型 Charcot-Marie-Tooth 神經疾病 (CMTX) 併眼瞼下垂家族之臨床、電生理、分子基因研究

吳禹利 王鴻利[1] 朱俊哲 游家銘[2] 陳正友[3] 黃錦章

背 景： 性染色體顯性遺傳型Charcot-Marie-Tooth神經疾病 (CMTX) 與connexin32 (Cx32, 隙接合蛋白32) 基因突變有關，目前尚未有台灣人CMTX的臨床與分子基因研究報告。

方 法： 評估一台灣人家族成員的臨床症狀與電生理檢查特徵，研究其Cx32基因是否突變，一位病人接受神經切片檢查。

結 果： 臨床上有9位病人表現中度Charcot-Marie-Tooth神經疾病症狀，分子基因學分析顯示在Cx32基因coding region沒有突變，而在神經特異promoter P2，亦即開始轉譯點-215的位置有G→A的突變。眼瞼下垂是遺傳性神經疾病之一臨床表徵。遺傳性神經疾病與家族性甲狀腺機能亢進的病徵是獨立出現的。神經電生理學與病理檢查顯示爲軸性 (axonal) 神經病變，誘發電位檢查顯示中樞運動與感覺神經傳導是正常的。

結 論： 此家族的眼皮下垂症狀顯示CMTX臨床表徵的多樣性，此特徵類似於體染色體遺傳性神經疾病。電生理與組織學檢查顯示正常中樞神經傳導與軸性周邊神經病變。
(長庚醫誌 2004;27:489-500)

關鍵字： 性染色體顯性遺傳型Charcot-Marie-Tooth神經疾病，電生理學，隙接合蛋白32，眼瞼下垂，家族性甲狀腺機能亢進。

長庚紀念醫院　台北院區　神經內科一科；[1]長庚大學　生理科；[2]台北市立仁愛醫院　神經內科；[3]長庚紀念醫院　台北院區內分泌及新陳代謝科
受文日期：民國92年10月14日；接受刊載：民國93年5月13日。
索取抽印本處：吳禹利醫師，長庚紀念醫院　神經內科一科。桃園縣333龜山鄉復興街5號。Tel: (03)3281200轉8418; Fax: (03)3287226; E-mail: tonywu@adm.cgmh.org.tw