SHORT REPORT

Charcot-Marie-Tooth families in Japan with MPZ Thr124Met mutation

S Kurihara, Y Adachi, C Imai, H Araki, N Hattori, C Numakura, Y Lin, K Hayasaka, G Sobue, K Nakashima

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Background: The MPZ Thr124Met mutation is characterised by a late onset, pupillary abnormality, deafness, normal or moderate decreased motor nerve conduction velocity, and axonal damage in sural nerve biopsy.

Objective: To investigate the clinical manifestations of the axonal or demyelinating forms of the Japanese MPZ Thr124Met mutation originating in four different areas: Tottori, Nara, Aichi, and Ibaragi.

Results: Genotyping with DNA microsatellite markers linked to the MPZ gene on chromosome 1q22–q23 showed shared allelic characteristics between 12.65 cM and revealed a common haplotype in all Tottori families. Aichi and Ibaragi families shared parts of the haplotype around the MPZ gene. However, there was no consistency with a Nara family.

Conclusions: The high frequency of this peculiar genotype in the Tottori CMT population is presumably due to a founder effect, but in Thr124 it might constitute a mutation hotspot in the MPZ gene.

About one in 2500 persons have a form of Charcot-Marie-Tooth disease (CMT), making it the most common inherited neuromuscular disorder.¹ CMT falls into two large subtypes—the demyelinating form, CMT1, and the axonal form, CMT2.² CMT1B can be caused by mutations in peripheral myelin protein zero (MPZ or P0), especially mutations located in the extracellular domain of the protein.³ Interestingly, CMT2 patients carry distinct point mutations in the MPZ gene initially thought to be exclusively involved in the pathogenesis of demyelinating hereditary neuropathies.^{4–9} The MPZ Thr124Met mutation is included in this group.^{10–11} The mutation is associated with a clinically distinct phenotype characterised by late onset, marked sensory abnormalities, and in some families deafness and pupillary abnormalities. Sendrek *et al.*, investigating a general European population, considered that Thr124Met might constitute a mutation hotspot in the MPZ gene.¹² In the present study, we clarified the clinical features of Japanese CMT families with the MPZ Thr124Met mutation and the haplotype analysis of these Japanese ancestries.¹³

METHODS

Sixteen patients from Tottori, Nara, Aichi, and Ibaragi prefectures were examined by direct sequence analysis, which revealed a C→T mutation at position 371 in the MPZ gene, resulting in a substitution of methionine for threonine (Thr124Met). After identifying the mutation, the clinical symptoms were studied.

Electrophysiological and pathological studies were done using standard protocols. To diagnose CMT2, we used the criteria of European CMT consortium, 1997.¹⁴ Amplification

of repeat-containing regions was undertaken by polymerase chain reaction (PCR) for amplifying the microsatellite markers D1S2771, D1S2705, D1S2675, D1S1677, and D1S1595. These primers were as published by the Genome Database (<http://www.gdb.org>), and sense primers were labelled with Cy5 fluorophores (SIGMA genosist, The Woodlands, Texas, USA). In order to determine accurate repeats numbers, PCR amplification was done and an aliquot of the product electrophoresed on a denaturing 6% polyacrylamide gel with an automated DNA sequencer (ALF Express, Pharmacia LKB, Uppsala, Sweden). The data were processed using fragment analysis software (Fragment Manager, Pharmacia).

RESULTS

Clinical features

The clinical features of 16 patients are shown in table 1. The mean (SD) age of symptom onset was at 51.1 (13.4) years. A pupillary abnormality was found in 14 patients and deafness in 11, all with sensory deafness. Pes cavus is a frequent sign of CMT, but from this study we found that pes planus was also present in MPZ Thr124Met patients. Neuropathic symptoms occurred later than usual for CMT1, and the patients were able to manage most aspects of their lives. Electrophysiologically, median motor nerve conduction velocities (MNCV) of MPZ Thr124Met were normal (42.3 (15.6) m/s) and median nerve compound muscle action potentials (CMAP) were decreased (3.9 (3.9) mV). Sural nerve biopsies showed a marked decrease of large myelinated fibres and the presence of regenerating fibres with small onion bulb formation. Teased nerves mainly showed axonal degeneration.

Haplotype analysis

The haplotypes of the disease gene bearing chromosomes are shown in fig 1. In 13 cases from the six Tottori originating CMT families there was a shared haplotype (“11-30-17-21-13” repeats) for all five markers investigated (D1S1595, D1S2771, D1S2705, D1S2675, and D1S1677). Aichi and Ibaragi families shared parts of repeats between 2.57 cM and 8.66 cM. However, the Nara originating family did not share contiguity markers (D1S2771, D1S2705).

DISCUSSION

Japanese CMT families with four isolated ancestries were studied. There are at least two founders and Thr124 was the hotspot in the MPZ gene. The disease usually manifested in about the fifth decade and showed slowly progressive

Abbreviations: CMAP, compound muscle action potential; CMT, Charcot-Marie-Tooth disease; CMT1, Charcot-Marie-Tooth disease type 1; CMT2, Charcot-Marie-Tooth disease type 2; DSS, Dejerine-Sottas disease; MNCV, motor nerve conduction velocity; MPZ, myelin protein zero; NCV, nerve conduction velocity

Table 1 Clinical, electrophysiological, and pathological data from Japanese MPZ Thr124Met patients

Patients, family	Age at onset (years), sex	Initial symptom	Gait disturbance	Pes cavus/pes planus	Dys-aesthesia	Pupillary abnormality	Deafness	Median n MNCV (m/s)	Median n CMAP (mV)	Sural nerve pathology
Tottori, A-1	57, M	Gait disturbance	+	C	+	+	+	34.9	10.0	OB, axonal loss
A-2	70, F	Gait disturbance	+	P	+	+	+	50.5	4.0	
A-3	57, M	Dysaesthesia	—	C	+	—	—			
Tottori, B-1	40, F	Dysaesthesia	+	C	+	+	+	ND	ND	Decreased MF, OB(—)
B-2	40, F	Dysaesthesia	—	C	+	+	—	51.2	10.1	
B-3	36, M	Dysaesthesia	+	P	+	+	—	47.5	2.5	Decreased MF, Myelin ovoid
B-4	20, M	Dysaesthesia, cramp	—	C	+	+	—	47.1	11.1	
Tottori, C-1	60, M	Gait disturbance	+	P	+	+	+	35.8	2.0	Decreased MF
C-2	50, M	Gait disturbance	+	C	+	+	+	45.4	0.66	Small OB
Tottori, D-1	50, M	Dysaesthesia	+	P	+	+	+	61.2	6.8	
D-2	60, F	Dysaesthesia	—	P	+	—	+	69.9		
Tottori, E-1	44, F	Muscle weakness	+	C	+	+	+	38.5	2.0	Decreased MF, OB(—)
Tottori, F-1	65, M	Gait disturbance	+	C	+	+	+	38.8	0.25	
Nara	50, F	Gait disturbance	+	C	+	+	+	31.0	1.6	Decreased MF, OB(—)
Aichi		Dysaesthesia	+	C	+	+	—	40.0	2.3	Sprouting, OB(—)
Ibaragi	67	Photophobia	+	C	+	+	+	40.0	1.5	Sprouting, OB(—)

Normal nerve conduction velocity values: motor median nerve, >45–65 m/s. Normal amplitude values: motor median nerve, >4–25 mV.
C, pes cavus; F, female; M, male; MF, myelinated fibres; MPZ, myelin protein zero; NCV, nerve conducting velocity; ND, not done; OB, onion bulb; P, pes planus.

neuropathic symptoms. Pupillary abnormalities and deafness are sometimes present in other inherited neuropathies, but they were a constant feature in this mutation. The NCVs varied widely from less than 38 m/s to normal in early stage patients, and the values tended to fall within the range of CMT1 in severely affected patients. Clusters of remyelinating axons in the sural nerve biopsy showed axonal involvement with axonal regeneration.¹¹ Phenotype–genotype correlations in 16 patients indicated that these were difficult to classify as CMT1B or CMT2. This tendency might derive from the character of the MPZ Thr124Met mutation.^{10–12 15} Haplotype analysis showed that the Nara CMT family was completely unrelated. However, other Japanese families shared a common founder. Thus more than two distinct ancestral Thr124 MPZ alleles exist in Japan. Our study strongly supports the hypothesis that the high frequency of the ACG to ATG transition in codon124 is not only due to a founder effect, but that Thr124 is a mutation hotspot.

In conclusion, CMT patients with slightly reduced or nearly normal NCVs should be screened for MPZ mutations,

particularly when additional clinical features such as pupillary abnormalities or deafness are also present.

Authors' affiliations

S Kurihara, Y Adachi, C Imai, H Araki, Department of Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University, Yonago, Japan
N Hattori, G Sobue, Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan
C Numakura, K Hayasaka, Department of Paediatrics, Yamagata University, School of Medicine, Yamagata, Japan
Y Lin, Department of Immunology, National Institute of Neuroscience, National Centre of Neurology and Psychiatry, Japan

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Correspondence to: Dr Saiko Kurihara, Department of Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University, 36-1 Nishimachi, Yonago, Tottori 683-8504 Japan; psycho@k3.dion.ne.jp

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Figure 1 Disease associated haplotypes in Japanese Charcot-Marie-Tooth disease (CMT) families. In 13 cases from the six Tottori originating CMT families, there was a shared haplotype ("11-30-17-21-13" repeats) for all five markers investigated. Aichi and Ibaragi families shared parts of repeats between 2.57 cM and 8.66 cM. The Nara originated family did not share contiguity markers (D1S2771, D1S2705).

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