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ORIGINAL ARTICLE

Vincristine exacerbates asymptomatic Charcot–Marie–Tooth disease with a novel *EGR2* mutation

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Abstract Neurotoxicity is a common side effect of vincristine (VCR) treatment. Severe exacerbations of neuropathy have been reported in patients with Charcot–Marie–Tooth disease (CMT) 1A with duplication of the *peripheral myelin protein 22* (*PMP22*) gene. However, whether or not VCR exacerbates neuropathies through mutations in other CMT-associated genes besides *PMP22* duplication has not been well studied. The purpose of this study was to identify mutations in any CMT-associated genes in a patient with hypersensitivity to VCR. We performed clinical, electrophysiological, and genetic examinations of a 23-year-old woman, who was hypersensitive to low-dose VCR, and her healthy mother. DNA analysis was performed using our specially designed resequencing array that simultaneously screens for 28 CMT-associated genes. Electrophysiological studies revealed that the patient and her healthy mother had demyelinating polyneuropathy. Furthermore, they showed the same novel mutation in the *early growth response 2* (*EGR2*) gene. Recognizing pre-existing asymptomatic CMT by electrophysiological studies and genetic analysis before VCR treatment allowed us to prevent severe VCR-induced neuropathy.

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Introduction

Vincristine (VCR) is a vinca alkaloid drug that is an essential part of the chemotherapeutic regimens used to treat Hodgkin's and non-Hodgkin's lymphoma, acute lymphocytic leukemia, and several types of solid tumors. Neurotoxicity, the most frequently predominant distal axonal sensorimotor neuropathy, is a well-known dose-limiting side effect of VCR [1]. VCR disrupts microtubule functions in axons and inhibits axonal transport by binding and inactivating tubulin, thereby leading to axonal degeneration. VCR-induced neuropathy is usually observed after cumulative doses of 6–8 mg of VCR, and significant toxicity occurs at doses greater than 15–20 mg in neurologically normal individuals [2]. The symptoms of toxicity usually include paresthesia and muscle weakness in the distal extremities. Deep tendon reflexes often diminish or disappear. In most cases, neuropathy gradually improves as VCR is discontinued, but neuropathy can persist in some cases of severe sensorimotor dysfunction. Patients with pre-existing neuropathy are generally at increased risk of developing severe neuropathy after chemotherapy [2, 3]. Charcot–Marie–Tooth disease (CMT), a hereditary motor and sensory neuropathy, is one of the most common types of inherited neuropathies, with a prevalence rate of 1 in 2,500 [4], and it is clinically and genetically heterogeneous [5]. Until date, at least 30 genes are known to be associated with CMT and related inherited neuropathies (http://www.molgen.ua.ac.be/CMTMutations/Mutations). The most common type is CMT1A, which is an autosomal dominant demyelinating neuropathy associated with duplication of the *peripheral*

myelin protein 22 (*PMP22*) gene. Some anticancer drugs such as vinca alkaloids, platinum agents, taxanes, and thalidomide are potentially toxic to patients with CMT [3, 6]. There are many reports of cases of CMT1A that deteriorated or were revealed after VCR treatment [7–12]. However, whether or not VCR exacerbates neuropathies in other types of CMT besides CMT1A is unclear. There is insufficient data to comment on the neurotoxicity of VCR in less common subtypes of CMT that affect other genes [13–15]. In order to identify the genetic risk of severe VCR-induced neuropathy, we screened for mutations in 28 CMT disease-causing genes using a custom resequencing DNA chip. Our DNA chip can screen 28 genes in 2 days and is relatively cost-effective. Using this chip, we identified a mutation in the *early growth response 2* (*EGR2*) gene in a 23-year-old woman with hypersensitivity to low-dose VCR. *EGR2* encodes a transcription factor that regulates the expression of peripheral myelin protein genes [16]. Although the risk of VCR-induced neuropathy in patients with an *EGR2* mutation is unknown, our high-throughput mutation screening method revealed a novel risk of developing drug-induced neuropathy.

Materials and methods

Patient

A 23-year-old woman was referred to our hospital with primary mediastinal large B-cell lymphoma. She presented no subjective clinical symptoms except mediastinal lymphadenopathies and was diagnosed with clinical stage IA (Ann Arbor Classification). At that time, she had not developed any neurological abnormalities. Her family seemed healthy and had no history of inherited or acquired neuropathies. She was treated with chemotherapy following the administration of rituximab, cyclophosphamide, doxorubicin, VCR, and prednisolone (day 1, 750 mg/m^2 cyclophosphamide, 50 mg/m^2 adriamycin, 1.4 mg/m^2 VCR; days 1–5, 100 mg prednisolone; and day 5, 375 mg/m^2 rituximab). After two courses (total VCR administered, 3.9 mg), she developed muscular weakness and paresthesia with pain in the distal extremities and was hardly able to walk. On day 49, she demonstrated distal predominant muscular weakness and paresthesia on neurological examination. No obvious muscular atrophy or pes cavus was evident. In addition, she had developed areflexia. Her Babinski reflex was negative, and there were no signs of cerebellar or cranial nerve disturbances.

Electrophysiological studies

On day 54, nerve conduction studies were performed using the standard procedure. Skin temperature was maintained above 32°C.

DNA analysis

Genomic DNA was extracted from the peripheral blood leukocytes of the patient using the Gentra Puregene Blood Kit (Qiagen, Tokyo, Japan). The purpose-built GeneChip® CustomSeq® Resequencing Array (Affymetrix, Santa Clara, CA) was designed to screen for CMT and related diseases such as ataxia with oculomotor apraxia type 1, ataxia with oculomotor apraxia type 2, spinocerebellar ataxia with axonal neuropathy type 1, and hereditary motor neuropathies. The resequencing array was designed to screen for the following 28 genes: *EGR2*, *PMP22*, *myelin protein zero* (*MPZ*), *gap junction protein beta 1* (*GJB1*), *periaxin* (*PRX*), *lipopolysaccharide-induced TNF factor* (*LITAF*), *neurofilament light polypeptide* (*NEFL*), *ganglioside-induced differentiation associated protein 1* (*GDAP1*), *myotubularin-related protein 2* (*MTMR2*), *SH3 domain and tetratricopeptide repeats 2* (*SH3TC2*), *SET-binding factor 2* (*SBF2*), *N-myc downstream regulated 1* (*NDRG1*), *mitofusin 2* (*MFN2*), *rab-protein 7* (*RAB7*), *glycyl-tRNA synthetase* (*GARS*), *heat shock 27 kDa protein 1* (*HSPB1*), *heat shock 22 kDa protein 8* (*HSPB8*), *lamin A/C* (*LMNA*), *dynamin 2* (*DNM2*), *tyrosyl-tRNA synthetase* (*YARS*), *alanyl-tRNA synthetase* (*AARS*), *lysyl-tRNA synthetase* (*KARS*), *aprataxin* (*APTX*), *senataxin* (*SETX*), *tyrosyl-DNA phosphodiesterase 1* (*TDP1*), *desert hedgehog* (*DHH*), *gigaxonin 1* (*GAN1*), and *K-Cl cotransporter family 3* (*KCC3*). We designed 363 primer sets to cover all the coding exons and splice sites. The 363 polymerase chain reactions (PCRs) were amplified in 32 multiplex reactions using the Qiagen Multiplex PCR system (Qiagen). Each reaction used 120 ng of genomic DNA, 10 pmol of the primer set, dNTP, and the Qiagen Multiplex PCR reaction mix (Qiagen). We generated each multiplex PCR product using the following conditions: 15 min at 95°C; 42 cycles of amplification (94°C for 30 s, 60°C for 3 min, and 72°C for 1 min 30 s); and 15 min at 68°C. Pooling, DNA fragmentation, labeling, and chip hybridization were performed using the Affymetrix CustomSeq Resequencing protocol instructions. The chips were washed using the Affymetrix fluidics station using the Customseq Resequencing wash protocols. Analysis of microarray data was performed using the GeneChip sequence Analysis Software version 4.0 (Affymetrix).

The mutations detected by our DNA chip method were confirmed by conventional DNA Sanger sequencing. Briefly, we amplified 50 ng of the patient's genomic DNA using primers and the hot start PCR method. Using a presequencing kit (USB, Cleveland, OH), we purified the patient's PCR products detected using our resequencing array method and sequenced them by dye–primer chemistry using an ABI Prism 377 Sequencer (Applied Biosystems, Foster City, CA). We then aligned the resulting sequences and evaluated the mutations using the Sequencher sequence alignment program (Gene Codes, Ann Arbor, MI).

Results

Electrophysiological studies

The motor nerve conduction studies revealed moderately slow motor nerve conduction velocities (MCV) with reduced compound muscle action potential (CMAP) amplitude in all examined nerves. The sensory nerve conduction studies showed moderately slow sensory nerve conduction velocities (SCV) with slightly reduced sensory nerve action potential (SNAP) amplitude (Table 1). No temporal dispersions or conduction blocks were observed. These results suggest demyelinating polyneuropathy complicated by axonal sensorimotor polyneuropathy. Because the patient showed hypersensitivity to low-dose VCR (total VCR administered, 3.9 mg), we suspected a pre-existing, inherited neuropathy. Furthermore, electrophysiological studies were performed on her healthy, 51-year-old mother. MCV of the mother was slower in the lower extremities than the upper extremities. CMAP amplitudes were within normal limits. Median nerve distal latency was slightly prolonged. SCV was moderately slow, but this finding was uniform in all examined nerves. SNAP amplitudes were moderately reduced in the upper extremities; SNAP amplitude of the sural nerve was at the lower limit of our normal control data. Temporal dispersions, conduction blocks, and entrapment neuropathies were not observed. These results indicate an electrophysiologically mild demyelinating polyneuropathy (Table 1). These findings suggest that this family may have an inherited demyelinating polyneuropathy.

Resequencing analysis of this family and a control study

The DNA chip resequencing analysis detected a novel c.1057 C>G (p.R353G) missense mutation in the *EGR2* gene. In contrast, the analysis was negative for mutations involving the other 27 CMT or related disease-causing genes. The patient was heterozygous for the c.1057 C>G mutation that substitutes an arginine for glycine at amino acid 353 (p.R353G) in exon 2 of *EGR2* by conceptual translation (Fig. 1a). The mother had the same mutation as the patient (Fig. 1a). We did not observe R353G in 200 control chromosomes or in the 850 chromosomes from 425 patients with inherited neuropathy. In addition, we did not find the R353G mutation in the 1000 Genomes website (http://browser.1000genomes.org), which catalogs human genetic variations using 1,197 samples including 300 East Asian (100 Japanese) samples.

Clinical course of the patient

We changed the chemotherapy regimen after we suspected that the patient had CMT. We chose radiotherapy and rituximab for the treatment of B-cell lymphoma. After 2 months, her symptoms had almost recovered, and she walked normally with only mild numbness in her distal lower limbs.

However, it was difficult to trace the causal agent because she was treated with a combination of chemotherapy agents. According to a previous report [3], there is uncertainty about the neurotoxicity of cyclophosphamide, prednisolone, and rituximab in patients with CMT, while VCR is classified as high risk for such patients. Furthermore, she and her mother's electrophysiological findings were consistent with inherited demyelinating polyneuropathy without the presence of conduction block or temporal dispersion. There were no findings indicated other inherited demyelinating polyneuropathy such as disturbance of lipid metabolism, peroxisomal disorders, hepatic porphyria and amyloidosis besides CMT. The results of her laboratory studies, including liver function tests, renal function tests, serum electrolyte and fasting blood glucose were normal. Her mother was healthy in the past periodic medical checkup, but laboratory

Table 1 Results of the nerve conduction studies

	Nerve	DL (ms)	CMAP amplitude (mV)	MCV (m/s)	SNAP amplitude (μV)	SCV (m/s)
Patient	Median	4.3	1.5	26.9	6.7	45.1
	Ulnar	3.9	2.7	31.8	7.3	45.8
	Tibial	8.2	3.9	23.0	–	–
	Sural	–	–	–	4.2	33.3
Patient's mother	Median	5.0	11.2	44.6	3.9	39.7
	Ulnar	3.3	9.1	50.1	3.1	38.7
	Tibial	4.7	23.6	37.9	–	–
	Sural	–	–	–	5.2	37.6
Control	Median	<4.5	>3.1	>49.6	>7.0	>47.2
	Ulnar	<3.6	>6.0	>50.1	>6.9	>46.9
	Tibial	<5.7	>4.4	>41.7	–	–
	Sural	–	–	–	>5.0	>40.8

DL distal latency, *CMAP* compound muscle action potential, *MCV* motor conduction velocity *SNAP* sensory nerve action potential, *SCV* sensory conduction velocity

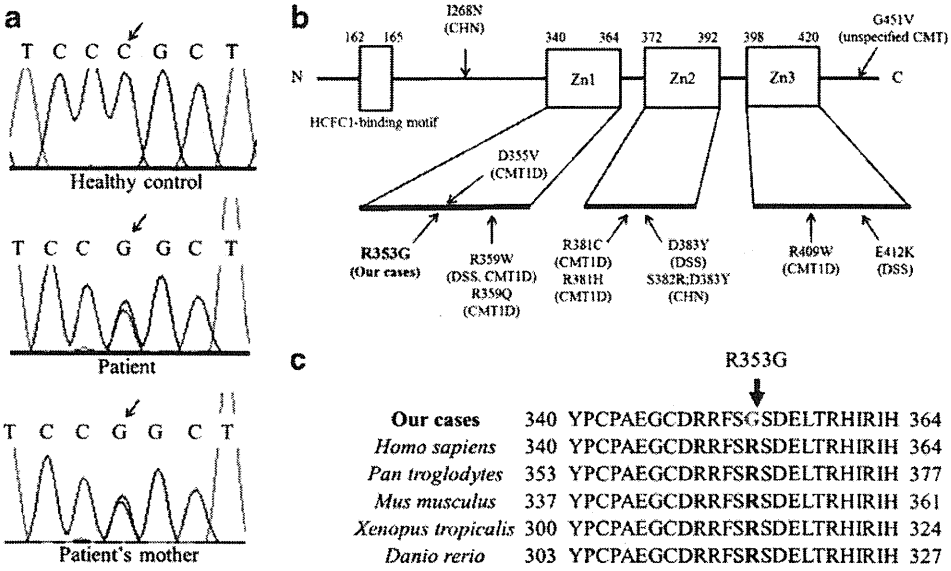

Fig. 1 **a** Chromatograms of the alterations in the *early growth response 2* (*EGR2*) gene that was identified in the patient and her mother, both of whom had the heterozygous transition c.1057 C>G that resulted in R353G. **b** Schematic diagram of the *EGR2* showing previously reported mutations and the R353G alteration. *CHN* congenital hypomyelination neuropathy, *DSS* Dejerine–Sottas disease, *Zn* zinc-finger domains. **c** Comparison of *EGR2* mutations in different species

screening tests were not examined in this report. We strongly suspected VCR-induced neuropathy in CMT with the *EGR2* mutation.

Discussion

This is the first report to describe an *EGR2* mutation that induced VCR hypersensitivity, similar to *PMP22* duplication. The *EGR2* gene located on human chromosome 10q21.1 has two exons that encode a 476 amino acid protein with three zinc finger domains, which is believed to be a transcription factor that regulates myelinogenesis [17, 18]. *EGR2* knockout mice exhibit severe hypomyelination of peripheral nerves due to the blocking of Schwann cell differentiation [19, 20]. Heterozygous mutations in *EGR2* cause myelinopathies, including congenital hypomyelinating neuropathy, Dejerine–Sottas disease, and mild to severe CMT1 [21–26]. Until date, 17 types of *EGR2* mutation have been found (http://www.molgen.ua.ac.be/CMTMutations/Mutations). *EGR2* induces high expression levels of myelin protein components such as *PMP22*, *MPZ*, *DHH*, and *PRX* in Schwann cells [27–30]. Vincristine inhibits axonal transport; thus, an insufficient supply of the myelin protein component necessary for the increased demand created by vincristine may induce a large degree of neurotoxicity. In the present study, we showed a novel R353G mutation in the first zinc finger domain of *EGR2* in a patient with late onset CMT1 who presented with

Table 2 Computational predictions of the pathogenicity on EGR2 mutation within the zinc finger domain

	Mutation	MUPro (SVM score[a])	PolyPhen[b]	PolyPhen2[c]	SIFT[d]
Our patients	R353G	−0.43[e]	2.57[e]	0.90[e]	0.00[e]
Reported mutations	D355V	1.00	2.75[e]	0.97[e]	0.00[e]
	R359W	−0.64[e]	2.79[e]	1.00[e]	0.00[e]
	R359Q	−1.00[e]	1.89[e]	0.92[e]	0.00[e]
	R381C	−0.11[e]	2.79[e]	0.99[e]	0.00[e]
	R381H	−0.24[e]	2.12[e]	0.99[e]	0.00[e]
	S382R	0.35	2.06[e]	0.81[e]	0.00[e]
	D383Y	0.09	2.75[e]	0.99[e]	0.00[e]
	R409W	−0.98[e]	2.69[e]	1.00[e]	0.00[e]
	E412K	−1.00[e]	1.69[e]	0.77[e]	0.00[e]

[a] Support Vector Machine (SVM) scores <0 indicate a decrease in protein stability

[b] PolyPhen scores ≥1.5 indicates a prediction of pathogenic

[c] PolyPhen2 scores of ~1 indicate a prediction of pathogenic

[d] SIFT scores ≤0.05 indicate a prediction of pathogenic

[e] Denotes a pathogenic prediction

a very mild phenotypic expression. Most *EGR2* mutations within the first zinc finger domain cause Dejerine–Sottas disease or severe CMT1 phenotypes (Fig. 1b) [22, 24]. A sequence homology search was performed, which aligned protein sequences from multiple species, using a Constraint-based, Multiple-Alignment tool (COBALT) (http://www.ncbi.nlm.nih.gov/tools/cobalt/). Arginine 353 was conserved among all of the species analyzed (Fig. 1c). It was found that the R353G mutation identified in our patients was located in a remarkably well-conserved sequence of amino acids, suggesting that it may have a potential impact on *EGR2* function. Furthermore, we computationally predicted the effect of the R353G mutation on protein function using the MUpro (http://www.ics.uci.edu/~baldig/mutation.html), PolyPhen (http://genetics.bwh.harvard.edu/pph/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html) algorithms. The algorithms in these programs use evolutionarily conserved species as well as reference sequence alignments, physiochemical differences, and the proximity of various substitutions to predict functional domains and/or structural features. All these programs predicted that the R353G mutation is most likely pathogen-based on the degree of conservation of the affected residues (Table 2). Therefore, the R353G mutation could possibly disrupt various functions. Furthermore, different mutations in the same codon result in divergent CMT phenotypes [26]. The electrophysiological findings were the only abnormal results for the patient's asymptomatic mother with the same *EGR2* mutation. Her neurological findings were normal, including a normal handgrip, the absence of foot deformities, normal and prompt deep tendon reflexes, and normal sensations. It is difficult to diagnose late onset mild CMT based on clinical findings and family history because the disease is heterogeneous. Although we did not perform in vitro functional analysis of the R353G mutation in this study, such further functional studies would illuminate the details of the pathomechanism of the *EGR2* mutation and its relationship with vincristine toxicity in this patient. In order to clarify the pathogenic nature of the *EGR2* mutation and vincristine neurotoxicity, we need to continue the genetic analysis of vincristine-induced neuropathy patients who do not show the CMT phenotype.

VCR-induced neuropathy is a dose-limiting side effect observed in neurologically normal individuals, but it sometimes results in severe neuropathy in patients with CMT. Early recognition of CMT before VCR treatment can prevent severe neurotoxicity. It is very important to use electrophysiological studies to recognize pre-existing CMT before VCR treatment, even if there is no family history or neurological abnormalities. Moreover, the labor and reagent costs of molecular genetic testing have significantly increased along with the increase in the number of genes associated with CMT and related neuropathies that must be screened for mutations. Realistically, it is difficult to perform nerve conduction studies or genetic testing in all patients who receive chemotherapy because of the costs and effort. Because of recent progress in the development of a new generation of genomic sequencing technologies, it will be possible to screen the entire genome/exome sequence for potential risks in all patients before they undergo chemotherapy.

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特集■ニューロパチー

遺伝性ニューロパチー
——多様な原因遺伝子と遺伝子診断法の進歩

Hereditary Neuropathy——Variety of Disease-causing Genes and Progress of Molecular Genetic Diagnosis

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Abstract

Inherited neuropathies are clinically and genetically heterogeneous. At least 30 genes have been associated with Charcot-Marie-Tooth disease (CMT) and related inherited neuropathies. Genetic studies have revealed that abnormalities in the following factors are the cause of inherited neuropathies: myelin components, transcription factors controlling myelination, myelin maintenance system, differentiation factors related to the peripheral nerve, neurofilaments, protein transfer system, mitochondrial proteins, DNA repair, RNA/protein synthesis, ion channels, and aminoacyl-tRNA synthetase.

On the other hand, a precise molecular diagnosis is often needed to confirm a clinical diagnosis, offer genetic counseling to the patient and family, and provide prognostic information to the patient. Unfortunately, along with the increase in the number of genes that must be screened for mutations, the labor and reagent costs of molecular genetic testing have increased significantly. On the basis of the recent progress of DNA analysis methods, the use of resequencing microarray seems to be an economical and highly sensitive method to detect mutations. In this study, we attempted to screen for CMT patients mutations using these methods.

Key words：Charcot-Marie-Tooth disease (CMT), disease-causing genes, molecular genetic diagnosis, microarray, resequence, hereditary neuropathy

はじめに

遺伝性ニューロパチーの最も代表的な疾患はCharcot-Marie-Tooth病(CMT)で，遺伝性運動感覚性ニューロパチー (hereditary motor sensory neuropathy：HMSN)とも表現される。近縁疾患としては，運動神経障害のみの遺伝性運動性ニューロパチー (hereditary motor neuropathy：HMN) や感覚障害のみの遺伝性感覚性ニューロパチー (hereditary sensory neuropathy：HSN)，感覚神経と自律神経が障害される遺伝性感覚・自律神経性ニューロパチー (hereditary sensory and autonomic neuropathy：HSAN) などがある。これらは臨床的，遺伝学的に多くの型に分けられ，少なくとも30以上の原因遺伝子が報告されている。CMTは通常，少年期～中年期に，四肢遠位筋優位の進行性筋萎縮・筋力低下で発症するが，原因遺伝子の種類や変異部位によりさまざまで，発症時期の幅は広い。臨床症状は，大腿を高く上げて歩く鶏歩や，逆シャンペンボトル様下腿筋萎縮，凹足 (pes cavus) などにより特徴づけられる。さらに，進行により足趾が屈曲し槌状趾 (hammer toe) を形成することもある。上肢は手の骨間筋や母指球筋の萎縮が目立つ。正中神経の障害により母指球筋が萎縮し猿手(ape hand)，また尺骨神経障害のため骨間筋が萎縮

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Table　CMT の分類と原因遺伝子

	病型	連鎖部位	遺伝子	臨床的特徴
CMT 1 型　(脱髄型，AD)	CMT1A	17p.112	*PMP22*	脱髄型で最多（約 70%）
	CMT1B	1q22	*MPZ*	伝導速度著明低下
	CMT1C	16p13.3-p12	*LITAF/SIMPLE*	10 代発症
	CMT1D	10q21.1-q22.1	*EGR2*	重症型
	CHN	22q13	*SOX10*	Waardenburg 症候群
	CMT1E	1q22	*MPZ*	難聴
	CMT1F	8p21	*NEFL*	難聴，振戦
CMT 4 型（脱髄型，AR）	CMT4A	8q13-q21.1	*GDAP1*	嗄声
	CMT4B1	11q22	*MTMR2*	幼少時発症（平均発症 2 歳）
	CMT4B2	11p15	*SBF2*（*MTMR13*）	若年性緑内障
	CMT4C	5q23-q33	*SH3TC2*（*KIAA1985*）	緩徐進行性，側弯
	CMT4D	8q24	*NDRG1*	難聴
	CMT4E	10q21-q22	*EGR2*	重症型
	CMT4F	19q13	*PRX*	早期発症，感覚障害
	CMT4G	10q22	*HK1*	眼瞼下垂，顔面神経障害
	CMT4H	12p11.2	*FGD4*	2 歳以下発症，発達遅延
	CMT4J	6q21	*FIG4*	左右非対称
CMT X 連鎖型	CMTX1	Xq13	*GJB1*	薄いミエリン
	CMTX5	Xq22.3	*PRPS1*	難聴，視神経障害
CMT 2 型（軸索型，AD）	CMT2A1	1p35-p36	*KIF1B*	本邦に家系あり
	CMT2A2	1p35-p36	*MFN2*	軸索型で高頻度，視神経萎縮
	CMT2B	3q13-q22	*RAB7*	下肢皮膚潰瘍
	CMT2C	12q23-q22	*TRPV4*	横隔神経麻痺
	CMT2D	7p14	*GARS*	上肢優位筋力低下
	CMT2E	8p21	*NEFL*	難聴，振戦
	CMT2F	7q11-q21	*HSP27*（*HSPB1*）	distal HMN の病型
	CMT2G	12q12-113.3	不明	10 代以降発症
	CMT2H	8q21.3	不明	錐体路障害
	CMT2I/J	1q22	*MPZ*	難聴，Adie 瞳孔
	CMT2K	8q13-q21.1	*GDAP1*	嗄声
	CMT2L	12q24	*HSP22*（*HSBP8*）	distal HMN の病型
	CMT2M	19p12	*DNM2*	先天性白内障，眼筋麻痺
	CMT2N	16q22	*AARS*	
CMT 2 型（軸索型，AR）	AR-CMT2A	1q21.2-q21.3	*LMNA*	肩甲帯筋力低下
	AR-CMT2B	19q13.3	*MED25*	30 歳前後発症，感覚失調
	GAN	16q24.1	*GAN1*	顔面神経障害，呂律障害
	ACCPN	15q13-q15	*KCC3*	脳梁低形成，Andermann 症候群
CMT 2 型（中間型）	CMT-DIB	19p12-p13.2	*DNM2*	軽症，振戦
	CMT-DIC	1p34-p35	*YARS*	軽症

し鷲手（claw hand）となる。

感覚障害を発症初期に自覚することは少ないが，診察すると手袋靴下型感覚消失や振動覚消失などが認められる。これらの症状は左右対称性で，腱反射なども左右対称性に低下または消失する。

CMT は，遺伝形式および電気生理学的に分類され，正中神経の神経伝導速度が 38 m/秒以下を脱髄型，神経伝導速度が 38 m/秒以上を軸索型と分類する。また，電気生理学的に脱髄型とも軸索型とも分類できないタイプを中間型としている。

脱髄型で常染色体優性遺伝（autosomal dominant：AD）のものを CMT1，常染色体劣性遺伝（autosomal recessive：AR）のものを CMT4，軸索型は AD も AR も CMT2 に分類される。CMT3 は，おおよそ 2 歳以下発症の Dejerine-Sottas 症候群（DSS）と同義であるが，CMT3 の名称はあまり使用されていない。X 染色体連鎖性の CMT は CMTX に分類される。

I．原因遺伝子

1991 年に Lupski らが，*peripheral myelin protein 22*（*PMP22*）の重複が CMT1A の原因であると同定してから，CMT の遺伝子研究は飛躍的に進歩してきた[1]。その後 CMT の原因遺伝子は数多く同定され，これまでに 30 以上同定されている（Table）。しかし，それでも原因未同定の CMT 患者が数多く存在し，今後さらに新規の原

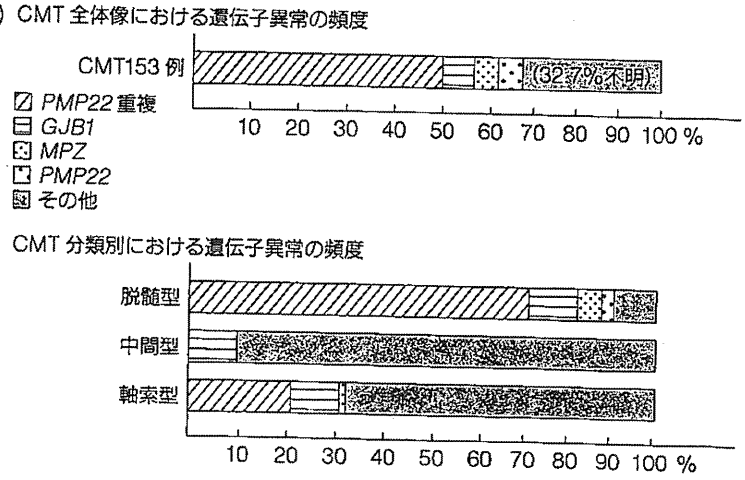

Fig. 1 CMT の原因遺伝子頻度
A：Boerkoel CF, Takashima H, Garcia CA, Olney RK, Johnson J, et al: Charcot-Marie-Tooth disease and related neuropathies: mutation distribution and genotype-phenotype correlation. Ann Neurol 51: 190-201, 2002（文献 2）より引用。B：Szigeti K, Nelis E, Lupski JR: Molecular diagnostics of Charcot-Marie-Tooth disease and related peripheral neuropathies. Neuromolecular Med 8: 243-254, 2006（文献 3）より改変。

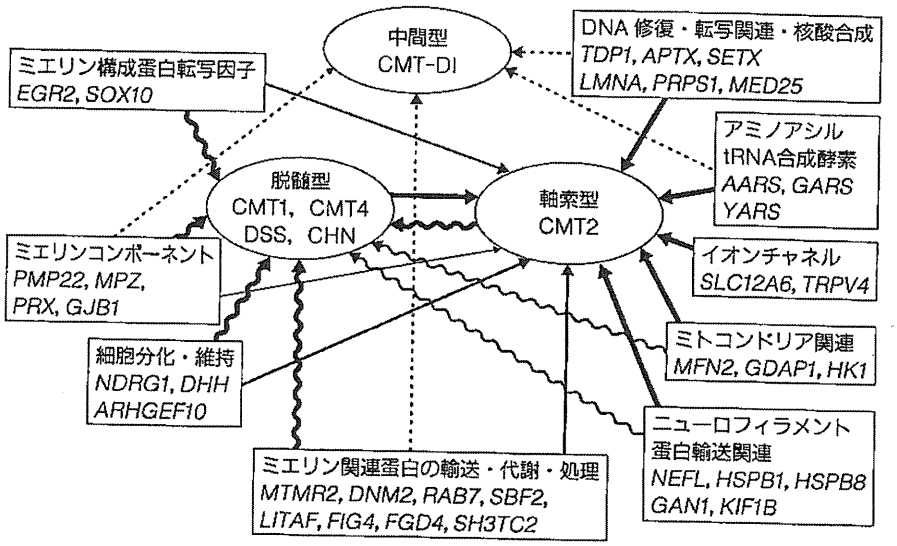

Fig. 2 病態別 CMT 原因遺伝子

因遺伝子を見つける努力が必要である。

CMT の原因遺伝子頻度で最も多いのは，CMT1A を引き起こす *PMP22* の重複であり，CMT 全体の約 50%，脱髄型 CMT の約 70%を占める。脱髄型の 1 番の原因として，*gap junction protein beta 1*（*GJB1*）が挙げられ，*myelin protein zero*（*MPZ*）がこれに続く。中間型 CMT の原因は *GJB1* の異常が最も多く，そのほかは少数である。軸索型 CMT の原因では *mitofusin 2*（*MFN2*），*GJB1* が多く認められるが，現在でも多くの例で原因が同定されていない[2,3]（Fig. 1）。

原因遺伝子は，機序的に分けると大きく以下の 9 つに分類される。①ミエリンコンポーネント，②ミエリン関連蛋白転写因子，③ミエリン関連蛋白の輸送・代謝・処理，④細胞分化・維持，⑤ニューロフィラメント・蛋白輸送関連，⑥ミトコンドリア関連，⑦DNA 修復・転写・核酸合成，⑧イオンチャネル，⑨アミノアシル tRNA（transfer ribonucleic acid）合成酵素，などである。これらの異常は，脱髄障害と軸索障害の両方を起こし得ることが知られている（Fig. 2）。これらについて，発症機序別に原因遺伝子とその特徴について概説する。

1．ミエリンコンポーネント

PMP22，*MPZ*，*periaxin*（*PRX*），*GJB1* などがこれにあたる。ミエリンコンポーネントの異常は髄鞘の形成不全のために脱髄型 CMT を起こす。前述の通り，CMT1A の原因となる *PMP22* の重複が最も多い。PMP22 はミエリン構成蛋白でミエリンの 20%を占める。

PMP22 が重複するメカニズムとしては，*PMP22* 領域を挟んで非常に類似した領域が存在するために，減数分裂時の遺伝子組み換えの際に誤った場所で組み換わり，*PMP22* を含む 1.5 Mb の領域が 2 重に組み込まれてし

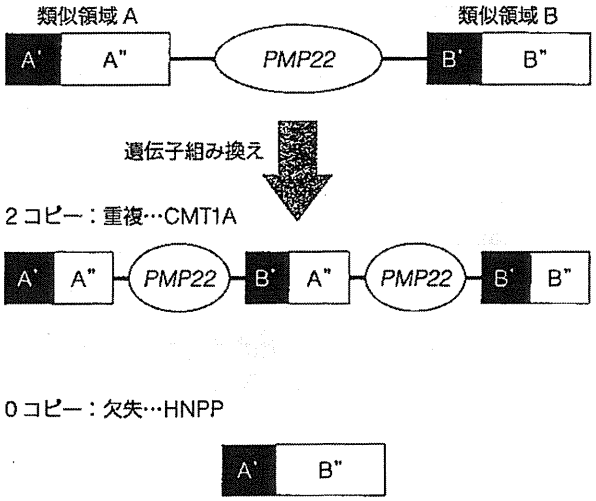

Fig. 3 *PMP22* 遺伝子組み換え（重複・欠失メカニズム）

まうためである（Fig. 3）。そのため1つの染色体上に *PMP22* が2コピー存在することとなる。

PMP22 の重複は，fluorescence *in situ* hybridization (FISH) 法で確認できる。*PMP22* を含む領域を蛍光色素で染めて核内に何コピーの *PMP22* 遺伝子があるかを調べるこの方法では，同一核内に正常な1コピーの染色体と異常な2コピーの染色体という計3コピーが認められた場合にCMT1Aと診断できる。逆に，1つの核に1コピーしか認めない場合は *PMP22* の欠失による遺伝性圧脆弱性ニューロパチー（hereditary pressure sensitive neuropathy）となる[4]。また，*PMP22* の1塩基のアミノ酸置換によってもCMTやDSSが発症する[5]。

MPZ は，ミエリンを構成する主要蛋白であるP0蛋白をコードしている。ミエリン構成成分の約半分を占めるコンパクトミエリンの接着に関与し，CMT1Bの原因遺伝子として同定された[6]。脱髄型CMTの約10%を占める。また，軸索型CMTのCMT2I/Jの原因となることも確認された。臨床的にDSSを呈する症例もみられ，発症年齢や重症度など，臨床型に多様性が認められる。

PRX は，Schwann細胞がミエリンを形成する際のミエリンと軸索の接着に関与し，CMT4Fの原因遺伝子として同定された[7]。ミエリンループと軸索の結合が弱まり，異常なミエリン形成が引き起こされる[8]。早期の発症で感覚障害が強い点が特徴的である。

GJB1 はconnexin32（Cx32）をコードしており，CMTX1の原因遺伝子として同定された[9]。Cx32はSchwann細胞でミエリン鞘を形成する際のギャップ結合を構成する蛋白として重要である。病理学的には，菲薄化したミエリンが観察されることが多い。たまねぎ形成の顕著な例とそうでない例がある。通常は，ミスセンス変異により疾患が引き起こされ，X染色体性遺伝形式であるため男性がより重症であるが，女性も発症する。一方，*GJB1* の欠失の例も報告されるが，ミスセンス変異の例に比べ重症ではない。欠失変異をもつ家系の母親にも軽症のニューロパチーがあると報告されている[10]。

2．ミエリン構成蛋白転写因子

Early growth response 2（*EGR2*），*sex determining region Y-box 10*（*SOX10*）などがこれにあたる。主に脱髄型CMTの原因となるが軸索型の原因にもなる。*EGR2* と *SOX10* は胎生期の蛋白転写制御因子で，ミエリン構成蛋白の遺伝子発現に関与する[11,12]。胎生期の異常のため重症型となることが多い。*EGR2* は，CMT1D，CMT4E，DSS，先天性低ミエリン形成性ニューロパチー（congenital hypomyelinating neuropathy：CHN）などの原因遺伝子として同定され，常染色体優性および劣性遺伝形式を呈する。*SOX10* はCHNやWaardenburg-Shah症候群やWaardenburg-Hirschsprung病の原因遺伝子として同定され，さまざまな神経系の発達の異常を呈する。

3．ミエリン関連蛋白の輸送・代謝・処理

ミエリンの維持に関連するCMTの原因遺伝子は多く，*myotubularin related protein 2*（*MTMR2*），*SET binding factor 2*（*SBF2*），*dynamin 2*（*DNM2*），*rab-protein 7*（*RAB7*），*lipopolysaccharide-induced TNF factor*（*LITAF*），*FIG4 homolog*（*FIG4*），*five*（*FGD4*），*SH3 domain and tetratricopeptide repeats 2*（*SH3TC2*）などがこれにあたる。

MTMR2 は，tyrosine phosphataseをコードし，ミエリン形成に関するシグナル伝達に関連すると考えられ，*CMT4B1* の原因遺伝子として同定された[13]。*MTMR2* は *SBF2* と複合体を形成することでホスファターゼ活性が上昇し，共同で働いていると考えられている。*SBF2* は，*MTMR2* 同様シグナル伝達に関連し，CMT4B2の原因遺伝子として同定された[14]。CMT4B2は若年性緑内障を合併することが知られている。

DNM2 は，細胞分裂・融合に関連し，中間型CMTであるCMT-DIBの原因遺伝子として同定された[15]。

RAB7 は小胞輸送と膜貫通の調節機構により細胞内物質輸送と関連し，CMT2Bの原因遺伝子として同定された[16]。下肢に皮膚潰瘍を合併しやすい。

LITAF/SIMPLE は蛋白分解，特にライソゾームの分解に関与すると考えられ，CMT1Cの原因遺伝子で，10

代で発症する[17]。

SH3TC2/KIAA1985 はミエリン形成やRanvier絞輪の維持に関連し，CMT4Cの原因遺伝子として同定された[18]。

FIG4 は，CMTの原因として確認された遺伝子である。ホスファチジルイノシトール代謝に関連し，CMT4Jの原因遺伝子として同定された[19]。*FGD4* は *FIG4* 同様2007年に確認された原因遺伝子で，Schwann細胞の分化調節に関連すると考えられており，CMT4Hの原因遺伝子として同定された[20]。

4．細胞分化・維持

N-myc downstream regulated 1（*NDRG1*），*desert hedgehog*（*DHH*），*Rho guanine nucleotide exchange factor 10*（*ARHGEF10*）がこれにあたる。

NDRG1 は，細胞原形質蛋白の1つでストレス反応・ホルモン反応・細胞分化および成長に関連すると考えられており，CMT4Dの原因遺伝子として同定された[21]。本症は，難聴を合併しやすい。

DHH は，神経周膜や性腺の分化に関連し神経束形成不全の原因となる。46XY性腺形成不全を伴うminifascicular neuropathyの原因遺伝子として同定された[22]。

ARHGEF10 は細胞の分裂・結合に関与していると考えられ，ミエリン低形成と関連している。臨床的には症状がないかもしくは非常に軽症で，神経伝導検査の異常が報告されている。

5．ニューロフィラメント蛋白輸送関連

Neurofilament, light polypeptide（*NEFL*），*heat shock 27kDa protein 1*（*HSPB1*），*heat shock 22kDa protein 8*（*HSPB8*），*gigaxonin 1*（*GAN1*），*kinesin family member 1B*（*KIF1B*）などがこれにあたる。

NEFL はニューロフィラメント関連蛋白であり，CMT2EとCMT1Fの原因遺伝子として同定された[23,24]。難聴や振戦を合併しやすい。

HSPB1，*HSPB8* は小熱ショック蛋白質（small heat-shock protein）に関連すると考えられている。小熱ショック蛋白質はストレスから細胞を守る分子シャペロンの働きがあり，細胞骨格の維持のための中間フィラメントに関連すると考えられている。*HSPB1* はCMT2Fの，*HSPB8* はCMT2Lの原因遺伝子として同定された[25,26]。

GAN1 は，細胞形態の調節，特に微小管ネットワークに関連し，巨大軸索ニューロパチー（giant axonal neuropathy：GAN）の原因遺伝子として同定された[27]。巨大な軸索を呈し，臨床的には失調や毛髪異常を合併する。

KIF1B は軸索輸送に関連し，CMT2A1の原因遺伝子として報告されたが，染色体上の同部位にマップされるCMT2Aのほとんどは，次に述べる *MFN2* の異常によることが知られている。

6．ミトコンドリア関連

MFN2, ganglioside-induced differentiation associated protein 1（*GDAP1*），*hexokinase 1*（*HK1*）などがこれにあたる。

MFN2 は軸索型CMTの最も多い原因遺伝子である。*MFN2* はミトコンドリア外膜に存在し，ミトコンドリアの融合に関連している。CMT2A2の原因遺伝子として同定された[28]。亜急性の視神経障害を合併することが知られている。

GDAP1 もまたミトコンドリア外膜に存在し，*MFN2* とは逆にミトコンドリアの分裂を促進させる。嗄声を伴うCMT4Aの原因遺伝子として同定された[29]。*GDAP1* と *MFN2* はともにミトコンドリア外膜にあり，ミトコンドリアの分裂・融合を調節し，ミトコンドリア数や分布を調節している。これらの遺伝子の異常により，ミトコンドリアの数や細胞内局在に異常が起こり，細胞内のミトコンドリアネットワークが障害を受ける。

HK1 は，ミトコンドリアの移動やグルコース代謝に関連し，2009年にHMSN-Russe/CMT4Gの原因遺伝子として同定された[30]。

7．DNA修復・転写関連・核酸合成

Tyrosyl-DNA phosphodiesterase 1（*TDP1*），*aprataxin*（*APTX*），*senataxin*（*SETX*），*lamin A/C*（*LMNA*），*phosphoribosyl pyrophosphate synthetase 1*（*PRPS1*），*mediator complex subunit 25*（*MED25*）などがこれにあたる。

TDP1，*APTX*，*SETX* はDNAの修復・転写に関連する。*TDP1* は軸索型ニューロパチーと脊髄小脳失調を合併するspinocerebellar ataxia with axonal neuropathy（SCAN1）の原因遺伝子として同定された[31]。DNAは転写複製過程で問題が生じると，トポイソメラーゼにより一時的に切断され一本鎖となる。切断されたDNAを修復する際にDNAに結合したトポイソメラーゼを取り除く酵素が *TDP1* である。*TDP1* を介する一本鎖DNA修復が障害され，SCAN1患者のリンパ球では，一本鎖DNA修復遅延が観察されている。

APTX は，*TDP1* 同様一本鎖DNA修復に関与しており，軸索型ニューロパチーに小脳失調と眼球運動失行を

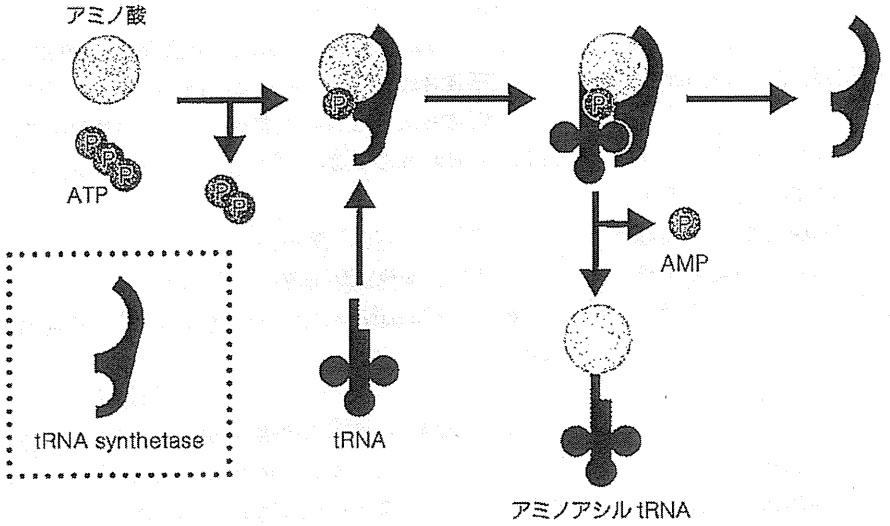

Fig. 4 tRNA synthetaseによるtRNAのアミノアシル化

合併するataxia with oculomotor apraxia type 1 (AOA1)の原因遺伝子として同定された[32]。アプラタキシンは複数の蛋白と複合体を形成し，一本鎖DNA修復で重要な役割を担う[33]。

*SETX*は，DNA/RNAヘリケースの構造を持ちDNAの転写・修復・複製に関与し，ataxia with oculomotor apraxia type 2 (AOA2)の原因遺伝子として同定された[34]。これらは小脳失調を合併することが特徴的である。*SETX*は若年性ALS(amyotrophic lateral sclerosis：ALS4)の原因遺伝子としても同定されている[35]。

*LMNA*は，核膜蛋白のlamin A/Cをコードしており，AR-CMT2Aの原因遺伝子として同定された[36]。*LMNA*はEmery-Dreifuss型筋ジストロフィーやHutchinson-Gilford progeria症候群の原因遺伝子であることも知られている。

*PRPS1*はX染色体にあり，プリン・核酸代謝に関連する。2007年に難聴や視神経障害を合併するCMTX5の原因遺伝子として同定された[37]。

*MED25*はARC (activator-recruited cofactor)のサブユニットをコードしておりRNA polymerase IIを介する転写に関与する。AR-CMT2Bの原因遺伝子として同定された[38]。

8．イオンチャネル

Solute carrier family 12, member 6 (*SLC12A6*), *transient receptor potential cation channel, subfamily V, member 4* (*TRPV4*)などがこれにあたる。

*SLC12A6*はK-Cl cotransporter (KCC) familyの1つで*KCC3*とも呼ばれる。K-Clを共輸送する蛋白をこのまま合成する。脳梁低形成と精神発達遅滞を特徴とするAndermann症候群(agenesis of the corpus callosum with peripheral neuropathy：ACCPN)の原因遺伝子として同定された[39]。

*TRPV4*はCa^{2+}浸透圧性カチオンチャネルをコードしており，全身の浸透圧調節に関与している。2010年，横隔神経麻痺により呼吸障害をきたしやすいCMT2Cの原因として同定された[40]。

9．アミノアシルtRNA合成酵素

Alanyl-tRNA synthetase (*AARS*), *glycyl-tRNA synthetase* (*GARS*), *tyrosyl-tRNA synthetase* (*YARS*)などがこれにあたる。特定のtRNAに対応するアミノ酸を結合させるアミノアシル化と関連する(Fig. 4)。それぞれ，*AARS*はアラニンtRNA合成酵素をコードしCMT2Nの原因遺伝子として，*GARS*はグリシルtRNA合成酵素をコードしCMT2Dの原因遺伝子として，*YARS*はチロシルtRNA合成酵素をコードし中間型CMTのCMT-DICの原因遺伝子として同定された[41-43]。このアミノアシルtRNA合成酵素は，それぞれのアミノ酸に対応しているため，この3種類以外にも数多く存在する。そのため，今後，この3種類以外にもCMTの原因遺伝子として確認されることが予想される。

II．遺伝子検査

*PMP22*の重複・欠失は前述のFISH法が三菱化学メ

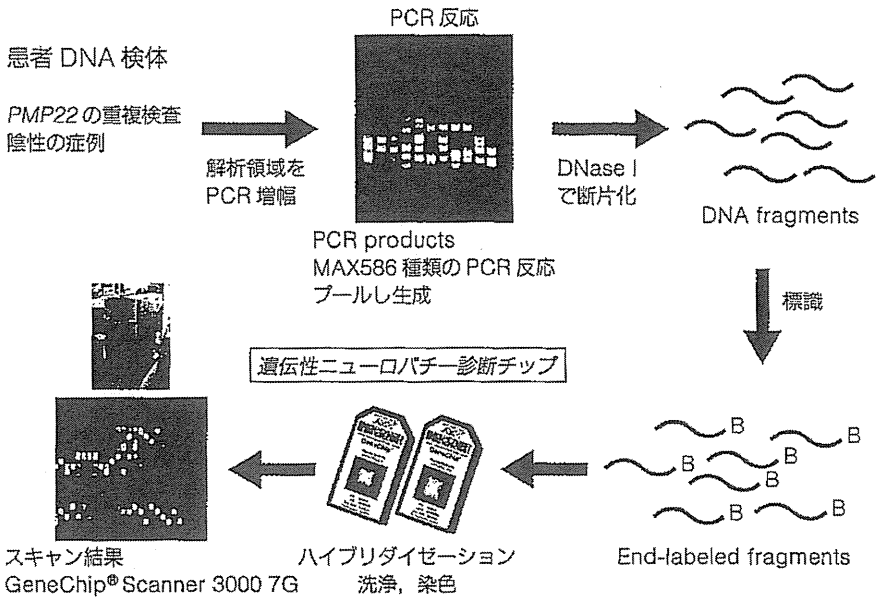

Fig. 5　遺伝性ニューロパチーマイクロアレイ遺伝子診断システム

ディエンス株式会社にて検査可能である。前述のように，その他の原因遺伝子は非常に多く，個々の症例すべての原因遺伝子について，従来の Sanger 法によるシークエンスで解析するには莫大な費用と時間を要するため，多数例に検査を思考することは不可能である。臨床症状から原因遺伝子を推測することは，一部の例では可能な場合もあるが，同じ原因でも脱髄型や軸索型を呈することもあり，通常は臨床症状から原因遺伝子を推測できない。

近年の分子遺伝学的検査法の発展により，マイクロアレイ法を用いたリシークエンス技術を応用して網羅的で高速にかつ低コストで遺伝子配列を決定することが可能となった。マイクロアレイ法は，検体から抽出し増幅した DNA を，あらかじめ配列をデザインした DNA オリゴマーが配置されたチップに，ハイブリダイゼーションし，専用のスキャナ（GeneChip® Scanner 3000 7G・Affymetrix 社製）で信号を読むことで配列を決定する方法である（Fig. 5）。鹿児島大学では，2006 年時点で CMT の原因遺伝子として確認されていた 27 遺伝子と新規遺伝子候補 10 遺伝子を搭載した CMT-DNA チップを作成し，2007 年より解析を開始している。脱髄型 CMT 例では FISH 法で *PMP22* コピー数が正常だった症例を，軸索型・中間型 CMT は全例を対象とし，2010 年までに 350 例を超える症例と 50 例のコントロールを解析した。その結果，脱髄型 CMT の原因遺伝子は，既報告で *PMP22* の次に多いのは *GJB1* であったのに対して，本邦では *MPZ* が明らかに多かった。軸索型 CMT は既報告同様 *MFN2* が最も多かった。中間型も既報告同様 *GJB1* が最多であった。しかし，原因が特定できない症例も多く，近年新しく発見された遺伝子の追加や新規遺伝子の同定が重要と思われる。さらに今後は，マイクロアレイ法とともに，現在発展著しい次世代ゲノムシークエンス法が遺伝子診断に用いられると考えられる。次世代シークエンス法は，数千万～1,000 億塩基配列を 1 度に決定する方法で，1 ランでヒトゲノム全体を決定できる能力を持つ機械も登場している。しかし，本法のランニングコストは高いこと，データ量が膨大で，解析に計算能力の高いコンピュータが必要などの問題点があった。近年，症例あたりのコストが比較的安価な機械が登場しており，将来はゲノムシークエンス法が，遺伝子診断の主力になる可能性が高い。

III. 治　　療

CMT は原因遺伝子が多様なため病態も多様である。将来的には原因遺伝子別に遺伝子治療や酵素補充療法など病態に応じた治療も考え得るが，現状はそのような治療は困難である。これまでにはガングリオシド製剤である Cronassial® の筋肉注射や γ-リノール酸，ビタミン E，コエンザイム Q10 などが試験的に投与されたことはあるが，いずれも有効性の証明を得るまでには至らなかった。

現在もアスコルビン酸，クルクミン，neurotrophin-3 (NT-3)，プロゲステロン拮抗薬などの治療研究が進められている。

1．アスコルビン酸

アスコルビン酸は前述のCMT1Aの原因の*PMP22*の遺伝子発現量を下げる機序により，CMT1Aモデルマウスに対するアスコルビン酸投与は有効であったとの報告がある[44]。海外で小規模臨床試験も行われたがいずれもエビデンスを得るには至らなかった[45-47]。本邦においても厚生労働省精神・神経疾患研究委託費「難治性ニューロパチーの病態に基づく新規治療法の開発に関する研究」研究班で臨床試験が行われたが，残念ながら有効性を確認するには至っていない。しかし，握力や一部の指標に改善傾向がみられたため，今後，治療評価項目の検討を行い，さらに研究が発展する可能性はある。

2．クルクミン（curcumin）

ウコンに含まれる自然色素成分であるクルクミンは*PMP22*変異によるdominant negative effectを減少させると考えられている。クルクミンは変異PMP22蛋白を小胞体（endoplasmic reticulum：ER）に停滞させ，ERストレス性アポトーシスを誘発させる。同様の事象は*MPZ*変異によるCMTでも同様であり，さまざまなCMTに有効である可能性を秘めている。CMT1Aマウスを用いた動物実験では運動機能が改善している[48]。

3．NT-3

神経栄養因子であるNT-3によりSchwann細胞増加や軸索再生が得られることから，CMTに対する治療の有効性が期待される。今後，大規模で長期間のrandomized control studyが必要である。

4．プロゲステロン拮抗薬

プロゲステロンはSchwann細胞や神経細胞で産生され，*PMP22*や*MPZ*などミエリン構成蛋白の発現に必要である。プロゲステロン拮抗薬がCMT1Aモデルラットにおいて脱髄による軸索減少を改善させると報告されている[49]。

しかし，このラット実験に使用したプロゲステロン拮抗薬は肝毒性のためヒトには使用できない。安全性の高いプロゲステロン拮抗薬の開発が待たれるところである。

おわりに

遺伝性ニューロパチーの原因は，ミエリン構成蛋白の異常からDNA修復機能の障害，ミトコンドリア関連，tRNA合成酵素など多様である。それゆえ，根治的で，包括的な治療は容易ではなく，それぞれ原因別に対応する必要がある。CMTの約50%を占めるCMT1Aにおいても有効な治療法がなく，今後，研究が進展し，遺伝子異常に応じた治療法の早期開発が期待される。

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CASE REPORTS

A new phenotype of mitochondrial disease characterized by familial late-onset predominant axial myopathy and encephalopathy

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Abstract Axial myopathy is a rare neuromuscular disease that is characterized by paraspinal muscle atrophy and abnormal posture, most notably camptocormia (also known as bent spine). The genetic cause of familial axial myopathy is unknown. Described here are the clinical features and cause of late-onset predominant axial myopathy and encephalopathy. A 73-year-old woman presented with a 10-year history of severe paraspinal muscle atrophy and cerebellar ataxia. Her 84-year-old sister also developed late-onset paraspinal muscle atrophy and generalized seizures with encephalopathy. Computed tomography showed severe atrophy and fatty degeneration of their paraspinal muscles. Their mother and maternal aunt also developed bent spines. The existence of many ragged-red fibers and cytochrome *c* oxidase-negative fibers in the biceps brachii muscle of the proband indicated a mitochondrial abnormality. No significant abnormalities were observed in the respiratory chain enzyme activities; however, the activities of complexes I and IV were relatively low compared with the activities of other complexes. Sequence analysis of the mitochondrial DNA from the muscle revealed a novel heteroplasmic mutation (m.602C>T) in the mitochondrial $tRNA^{Phe}$ gene. This familial case of late-onset predominant axial myopathy and encephalopathy may represent a new clinical phenotype of a mitochondrial disease.

Keywords Mitochondrial disease · Predominant axial myopathy · Encephalopathy · Late-onset · Familial case

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Introduction

Camptocormia, a term coined by Souques and Rosanoff-Saloff from two Greek words (*kamptos* meaning bent and *kormos* meaning trunk), is characterized by involuntary trunk flexion in the erect position that disappears in the supine position. Camptocormia was initially described as a hysterical phenomenon that occurred in male soldiers during World Wars I and II [1, 16]. However, in the last 20 years camptocormia has been reported to be present with various organic diseases, including muscular dystrophies, inflammatory myopathies, dystonia, amyotrophic lateral sclerosis, myasthenia gravis, paraneoplastic syndrome, Parkinson's disease, multiple system atrophy, and spinal deformities, as well as in an idiopathic form. Camptocormia is also referred to as "bent spine syndrome" [1, 32].

Axial myopathy has been described as the selective involvement of the paraspinal muscles in camptocormia or dropped head. Axial myopathy has heterogeneous etiologies, including primary and various other neuromuscular disorders. Primary axial myopathy is characterized by the

insidious and progressive weakness of the extensor muscles of the spine, normal or slightly elevated serum creatine kinase (CK) levels, and a myogenic pattern on electromyography in the elderly. Muscle biopsies show nonspecific myopathic changes with fibrosis, fatty replacement, and variations in fiber size. In addition, some ragged-red fibers and complex I and III deficiencies have been observed; these findings are considered to be the age-related accumulation of various mitochondrial abnormalities [21, 31].

Some cases of autosomal dominant inheritance patterns of familial primary axial myopathy were reported several years ago; however, the genetic analyses that were used have not been described [31]. Recently, a novel heterozygous dominant mutation in the skeletal muscle ryanodine receptor gene was identified in the central cores of muscle biopsy specimens that were excised from sporadic cases of axial myopathy [15]. Furthermore, facioscapulohumeral muscular dystrophy with isolated axial myopathy has also been reported [19]. Five cases of axial myopathy that were associated with mitochondrial dysfunction have been previously reported; however, no familial cases of mitochondrial gene mutation have been reported [8, 11, 28, 30, 32].

In this paper, we have reported about a mitochondrial disease that is characterized by familial late-onset predominant axial myopathy and encephalopathy. In addition, the pathogenicity of a novel, familial, mitochondrial tRNA gene mutation is discussed.

Methods

Subjects

Patient 1

A 73-year-old woman (Fig. 1, III-8) presenting with abnormal posture and gait disturbance. Since the age of 63, the patient had a slight stooping posture and a pushed-out waist. At 68 years of age, she started using a walking stick because of her unstable gait. She was diagnosed with hypothyroidism by her family physician and administrated with 25 μg/day levothyroxine; however, her symptoms did not improve. At 70 years of age, it gradually became more difficult for her to climb the stairs. At 71 years of age, she was admitted to another hospital. Doctors suspected myopathy because of elevated serum CK levels. She visited our hospital presenting with prominent paraspinal muscle atrophy and mild proximal weakness of limbs. Hypothyroidism-related myopathy was suspected in her, and hence, the levothyroxine dose was increased to 50 μg/day; however, her symptoms did not improve. She had a family history of bent spine, i.e., in her elder sister (patient 2, Fig. 1, III-5), mother (Fig. 1, II-3), and maternal aunt (Fig. 1, II-4). Physical examination on arrival revealed a marked atrophy of the paraspinal muscles and abnormal posture (Fig. 2a, b). She also presented with right ptosis, dysarthria, bilateral cataracts, and hearing loss. Her eye movements were normal. But there was moderate weakness of the neck flexion and mild weakness of the proximal limb muscles. Tendon reflexes were symmetrical, and Babinski's sign was absent. She had poor balance with tandem gait without limb ataxia. Sensory systems were intact and Romberg's sign was negative. She scored poorly on the attention and calculation tests that are a part of the Mini-Mental State Examination (score: 25 points).

Laboratory data were as follows: serum CK level was 290 IU/l (normal range 45–163 IU/l), resting blood and cerebrospinal fluid (CSF) lactate levels were normal, thyroid-stimulating hormone levels were slightly low at 0.47 μIU/ml (normal range 0.5–5.0 μIU/ml). Under the administration of 50 μg/day levothyroxine; antithyroglobulin antibody levels were high at 7.0 U/ml (normal range <0.3 U/ml), antithyroid peroxidase antibody levels were high at 46.5 U/ml (normal range <0.3 U/ml), rheumatoid factor levels were high at 152.3 IU/ml (normal value <15.0 IU/ml), antinuclear antibody levels were mildly elevated (titer of 1:80). Autoimmune analyses, including anti-Jo-1, anti-RNP, anti-SS-A, and anti-SS-B, were negative. The oral glucose tolerance test (75 g) was within normal limits, but Holter monitoring revealed high-frequency premature contractions. Pure-tone audiometry indicated sensorineural and high-frequency hearing loss.

Needle electromyographic findings of the biceps brachii and rectus femoris muscles indicated mild myopathic features. Computed tomography (CT) of the thoracic spinal nerve 10 (T10) revealed severe atrophy and fatty degeneration of the paraspinal muscles (Fig. 2c). Brain magnetic

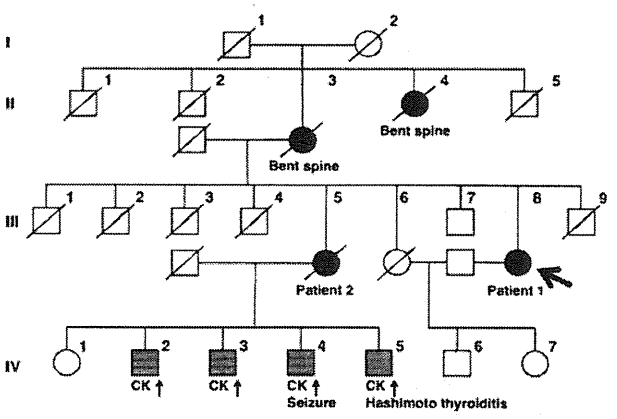

Fig. 1 Pedigree of the family. The *arrow* indicates the proband. The affected individuals are represented by the *solid black symbols*; *open symbols* represent healthy individuals. *Gray symbols* indicate individuals with elevated CK levels

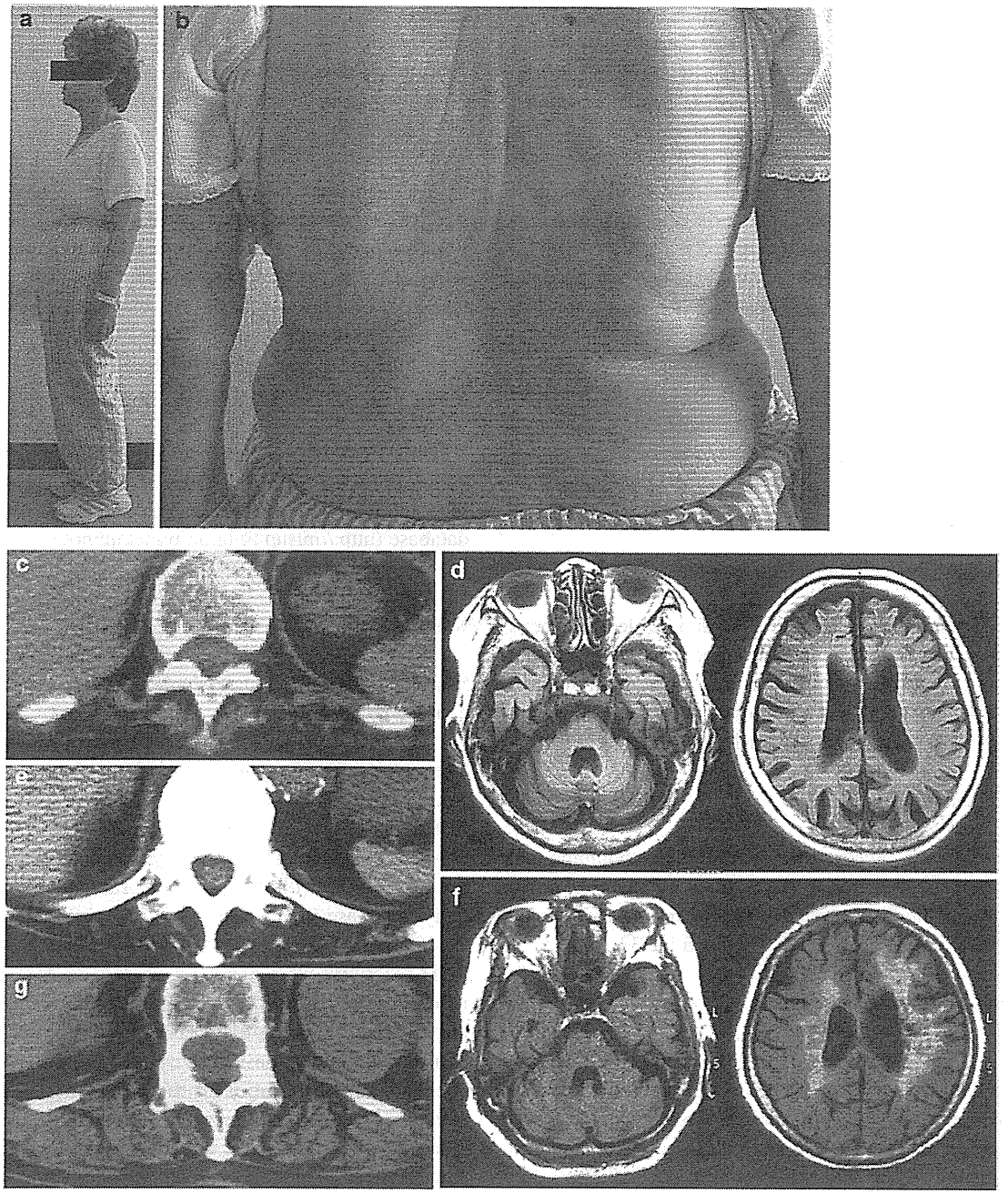

Fig. 2 **a** The full-length figure indicates the posture of patient 1 showing her pushed-out waist. **b** The dorsal view shows the marked atrophy of the paraspinal muscles in patient 1. CT of T10 of **c** patient 1 (age 71), **e** patient 2 (age 82), and **g** a healthy female (age 74) reveals the profound atrophy of the paraspinal muscles in **c** patient 1 and **e** patient 2, but not in **g** the healthy female. Brain MRI studies revealed several differences between the patients 1 and 2. **d** Axial FLAIR images of patient 1 show moderate cerebellar atrophy and some cerebral cortical atrophy. **f** The same images of patient 2 revealing hyperintense lesions around the white matter

resonance imaging (MRI) with fluid-attenuated inversion recovery imaging showed moderate cerebellar and temporo-parieto-occipital lobe atrophy (Fig. 2d). MR spectroscopy revealed the absence of increased lactate peaks. 123I-IMP single photon emission CT revealed hypoperfusion that was indicative of atrophic brain lesions.

Patient 2

The elder sister of patient 1 was an 84-year-old woman with a stooping posture presenting with tremors since the age of 60. In her 70s she started walking with the aid of a walking stick. At 82 years of age, she was hospitalized for