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REFERENCES

[1] Llewelyn JG, Tomlinson DR, Thomas PK. Diabetin neuropathy. In: Thomas PK, Dyck PJ, editors. Peripheral neuropathy. Philadelphia: Elsevier Saunders; 2005 p. 1951–91.
[2] Dyck PJ, Sherman WR, Hallcher LM, Service FJ, O'Brien PC, Grina LA, et al. Human diabetic endoneurial sorbitol, fructose, and myo-inositol related to sural nerve morphometry. Ann Neurol 1980;8:590–6.
[3] Cornblath DR, Chaudhry V, Carter K, Lee D, Seysedadr M, Miernicki M, et al. Total neuropathy score: validation and reliability study. Neurology 1999;53:1660–4.
[4] Sumner CJ, Sheth S, Griffin JW, Cornblath DR, Polydefkis M. The spectrum of neuropathy in diabetes and impaired glucose tolerance. Neurology 2003;60:108–11.
[5] Pittenger GL, Ray M, Burcus NI, McNulty P, Basta B, Vinik AI. Intraepidermal nerve fibers are indicators of small-fiber neuropathy in both diabetic and nondiabetic patients. Diabetes Care 2004;27:1974–9.
[6] Dyck PJ, Karnes JL, Daube J, O'Brien P, Service FJ. Clinical and neuropathological criteria for the diagnosis and staging of diabetic polyneuropathy. Brain 1985;108(Pt 4):861–80.
[7] The DCCT Research Group. Factors in development of diabetic neuropathy: baseline analysis of neuropathy in feasibility phase of Diabetes Control and Complications Trial (DCCT). Diabetes 1988;37:476–81.
[8] Dyck PJ, Davies JL, Litchy WJ, O'Brien PC. Longitudinal assessment of diabetic polyneuropathy using a composite score in the Rochester Diabetic Neuropathy Study cohort. Neurology 1997;49:229–39.
[9] Smith AG, Ramachandran P, Tripp S, Singleton JR. Epidermal nerve innervation in impaired glucose tolerance and diabetes-associated neuropathy. Neurology 2001;57:1701–4.
[10] Dyck PJ, Kratz KM, Karnes JL, Litchy WJ, Klein R, Pach JM, et al. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. Neurology 1993;43:817–24.
[11] Dyck PJ. Detection characterization, and staging of polyneuropathy: assessed in diabetics. Muscle Nerve 1988;11:21–32.
[12] Yasuda H, Sanada M, Kitada K, Terashima T, Kim H, Sakaue Y, et al. Rationale and usefulness of newly devised abbreviated diagnostic criteria and staging for diabetic polyneuropathy. Diabetes Res Clin Pract 2007;77(Suppl. 1):S178–83.
[13] Group DNS. Staging of diabetic polyneuropathy. Peripher Nerve 2005;16:223–4.
[14] The Committee of the Japan Diabetes Society on the diagnostic criteria of diabetes mellitus. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. Diabetol Int 2010;1:2–20.
[15] Lauria G, Cornblath DR, Johansson O, McArthur JC, Mellgren SI, Nolano M, et al. EFNS guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy. Eur J Neurol 2005;12:747–58.
[16] Kennedy WR, Wendelschafer-Crabb G. The innervation of human epidermis. J Neurol Sci 1993;115:184–90.
[17] Dyck PJ, Karnes JL, O'Brien PC, Litchy WJ, Low PA, Melton 3rd LJ. The Rochester Diabetic Neuropathy Study: reassessment of tests and criteria for diagnosis and staged severity. Neurology 1992;42:1164–70.
[18] Levy DM, Karanth SS, Springall DR, Polak JM. Depletion of cutaneous nerves and neuropeptides in diabetes mellitus: an immunocytochemical study. Diabetologia 1989;32:427–33.
[19] Kennedy WR, Wendelschafer-Crabb G. Utility of the skin biopsy method in studies of diabetic neuropathy. Electroencephalogr Clin Neurophysiol Suppl 1999;50:553–9.
[20] Polydefkis M, Hauer P, Griffin JW, McArthur JC. Skin biopsy as a tool to assess distal small fiber innervation in diabetic neuropathy. Diabetes Technol Ther 2001;3:23–8.
[21] Quattrini C, Tavakoli M, Jeziorska M, Kallinikos P, Tesfaye S, Finnigan J, et al. Surrogate markers of small fiber damage in human diabetic neuropathy. Diabetes 2007;56:2148–54.
[22] Smith AG, Russell J, Feldman EL, Goldstein J, Peltier A, Smith S, et al. Lifestyle intervention for pre-diabetic neuropathy. Diabetes Care 2006;29:1294–9.
[23] Polydefkis M, Hauer P, Sheth S, Sirdofsky M, Griffin JW, McArthur JC. The time course of epidermal nerve fiber regeneration: studies in normal controls and in people with diabetes, with and without neuropathy. Brain 2004;127:1606–15.
[24] Gibbons CH, Freeman R. Treatment-induced diabetic neuropathy: a reversible painful autonomic neuropathy. Ann Neurol 2010;67:534–41.
[25] The effect of intensive diabetes therapy on the development and progression of neuropathy. The Diabetes Control and Complications Trial Research Group. Ann Intern Med 1995;122:561–8.
[26] Young MJ, Breddy JL, Veves A, Boulton AJ. The prediction of diabetic neuropathic foot ulceration using vibration perception thresholds. A prospective study. Diabetes Care 1994;17:557–60.
[27] Forrest KY, Maser RE, Pambianco G, Becker DJ, Orchard TJ. Hypertension as a risk factor for diabetic neuropathy: a prospective study. Diabetes 1997;46:665–70.
[28] Adler AI, Boyko EJ, Ahroni JH, Stensel V, Forsberg RC, Smith DG. Risk factors for diabetic peripheral sensory neuropathy. Results of the Seattle Prospective Diabetic Foot Study. Diabetes Care 1997;20:1162–7.
[29] Shun CT, Chang YC, Wu HP, Hsieh SC, Lin WM, Lin YH, et al. Skin denervation in type 2 diabetes: correlations with diabetic duration and functional impairments. Brain 2004;127:1593–605.
[30] Sorensen L, Molyneaux L, Yue DK. The relationship among pain, sensory loss, and small nerve fibers in diabetes. Diabetes Care 2006;29:883–7.
[31] Gandhi RA, Marques JL, Selvarajah D, Emery CJ, Tesfaye S. Painful diabetic neuropathy is associated with greater autonomic dysfunction than painless diabetic neuropathy. Diabetes Care 2010;33:1585–90.
[32] Chao CC, Tseng MT, Lin YJ, Yang WS, Hsieh SC, Lin YH, et al. Pathophysiology of neuropathic pain in type 2 diabetes: skin denervation and contact heat-evoked potentials. Diabetes Care 2010;33:2654–9.
[33] Atherton DD, Facer P, Roberts KM, Misra VP, Chizh BA, Bountra C, et al. Use of the novel Contact Heat Evoked Potential Stimulator (CHEPS) for the assessment of small fibre neuropathy: correlations with skin flare responses and intra-epidermal nerve fibre counts. BMC Neurol 2007;7:21.
[34] Luo KR, Chao CC, Hsieh PC, Lue JH, Hsieh ST. Effect of glycemic control on sudomotor denervation in type 2 diabetes. Diabetes Care 2012;35:612–6.

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ORIGINAL COMMUNICATION

A family with IVIg-responsive Charcot–Marie–Tooth disease

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Abstract We report a family of intravenous immunoglobulin (IVIg)-responsive X-linked Charcot–Marie–Tooth disease Type 1 (CMT1X) with a novel gap junction protein 1 mutation. Two of three siblings in the family complained of subacute motor and sensory impairment, and their symptoms improved after the administration of IVIg. Additional IVIg treatment also resulted in similar improvement. The other also showed a mild improvement on IVIg. It has been suggested that an immune-mediated process is involved in the progression of neuropathy in CMT1X. The finding in our report provides evidence of susceptibility to immune-mediated demyelinating neuropathy in some form of CMT1X. Superimposed demyelinating neuropathy as well as a gradual deterioration of neuropathy over decades can be a clinical manifestation of CMT1X.

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Keywords X-linked Charcot–Marie–Tooth disease type 1 · Chronic inflammatory demyelinating polyneuropathy · IVIg · GJB1 · Immune-mediated demyelination

Introduction

Charcot–Marie–Tooth disease (CMT) encompasses a genetically heterogeneous group of hereditary motor and sensory neuropathies characterized by slowly progressive weakness and atrophy, primarily in the distal leg muscles. X-linked CMT type 1 (CMT1X) is the second most common type of CMT, which accounts for 7–10 % of all CMT. The prevalence of CMT1X is estimated to be between 1 in 25,000 and 1 in 35,000 [1]. The gene for the gap junction protein, beta 1, 32 kDa (http://www.genenames.org/data/hgnc_data.php?hgnc_id=4283) (*GJB1*) is designated to be causative for CMT1X [2]. A nerve conduction study (NCS) in male patients with CMT1X usually shows a uniform intermediate slowing of the motor nerve conduction velocity (MCV; 25–45 m/s) without a conduction block [3]. There is also a wide variability of clinical presentations and nerve involvement in CMT1X patients, even in those with the same missense mutation of the *GJB1* gene [4–6].

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a clinically heterogeneous acquired polyneuropathy. CIDP evolves as a monophasic, relapsing, and progressive disorder that develops over a period of 8 weeks. The cause of CIDP is unknown and a single triggering antigen has yet to be determined.

Patients with CMT1X usually show a gradual deterioration in their symptoms. However, some patients with CMT1X experience a rapid progression of their motor or sensory symptoms and respond to immunotherapy,

including intravenous immunoglobulin (IVIg) [7–9]. These patients were diagnosed with coincidental CIDP and CMT.

We describe the clinical, electrophysiological, and genetic details of three affected offspring diagnosed with CMT1X and their mother. Two of three affected patients had a rapid aggravation of their symptoms, which were significantly alleviated with IVIg. The other also showed a mild improvement on IVIg. These findings indicate that CMT1X pathophysiology can trigger acquired demyelinating polyneuropathy, probably due to an immune-mediated process.

Case reports

The eldest brother

A 32-year-old male had trouble with sprinting due to drop foot in his early childhood. At the age of 28, he noticed rapidly progressing finger weakness and difficulty with fastening buttons. He also felt numbness of the bilateral soles and paresthesia of the distal extremities. At presentation, distal muscle weakness and atrophy were evident with a CMT disease neuropathy score (CMTNS) of 18 and a score of 20 for manual muscle testing (MMT) of ten selected muscles. All of the deep tendon reflexes were also absent. Cerebrospinal fluid (CSF) protein was slightly elevated at 44 mg/mL with a normal cell count. NCS revealed sensorimotor demyelinating polyneuropathy of all four limbs, with intermediate slowing of the MCV, temporal dispersion in the left median nerve, and A waves (Table 1). A sural nerve biopsy showed a significant reduction of large myelinated fibers with clusters of regenerating fibers. Onion bulbs and infiltration of inflammatory cells were not observed (Fig. 1). On the basis of his clinical presentation and intermediate low MCV, CMT1X was suspected, and a *GJB1* mutation was confirmed by a genetic test. However, the coincidental occurrence of acquired inflammatory neuropathy and CMT was also suspected based on the rapid deterioration of muscle weakness and sensory impairment, elevated CSF protein, and the NCS findings.

IVIg (0.4 g/kg for 5 days) was administered for the treatment of suspected acquired inflammatory neuropathy. His muscle weakness improved from 18 to 17 for the CMTNS, and the MMT score of ten selected muscles increased from 20 to 36. His sensory impairment was also alleviated. The effect of IVIg lasted only for 1 month. Three additional IVIg treatments showed a similar improvement each time. No significant improvement on NCS was seen during the course of the IVIg treatments. He has also improved on oral steroid therapy (30 mg/day) for 6 months.

The second-eldest brother

A 27-year-old male started to trip on stairs due to drop feet in his early childhood. It was not until 26 years old that he rapidly developed muscle weakness of the distal upper extremities and paresthesia of the fingers. At the age of 27, he visited a local orthopedic surgery because of his left hand paresthesia. Surgery for his left carpal-tunnel

Table 1 Nerve conduction studies of the patients

Location	Motor nerve				Sensory nerve	
	Distal latency (ms)	Amplitude (mV) distal/proximal	Conduction velocity (m/s)	*F* wave	Amplitude (μV) distal/proxymal	Conduction velocity (m/s)
The eldest brother						
Lt. median	4.9	1.2/0.4 (with TD)	33	A waves	3.6/0.9	35.7
Lt. ulnar	3.3	3.8/3.3	41.5	NE	4.9/4.2	31.3
Lt. tibial	6.7	0.2/0.2	33	NE		
Lt. sural					1.9	31
The second-eldest brother						
Lt. median	5	1.1/0.6	33.1	NE	1.7/NE	32.7
Lt. ulnar	3.8	2.0/1.6	37.1	A waves	7.2/NE	38.7
Lt. tibial	5.7	1.8/0.7	22.2	NE		
Lt. sural					2.9	32.9
The youngest sister						
Lt. median	5.5	1.0/0.9	30	NE	7.9/NE	33
Lt. ulnar	4.5	2.6/2.4	39	NE	4.9/NE	32
Lt. tibial	7.1	1.6/0.5	35	93.7		
Lt. sural					NE	NE

TD temporal dispersion, *NE* not evoked

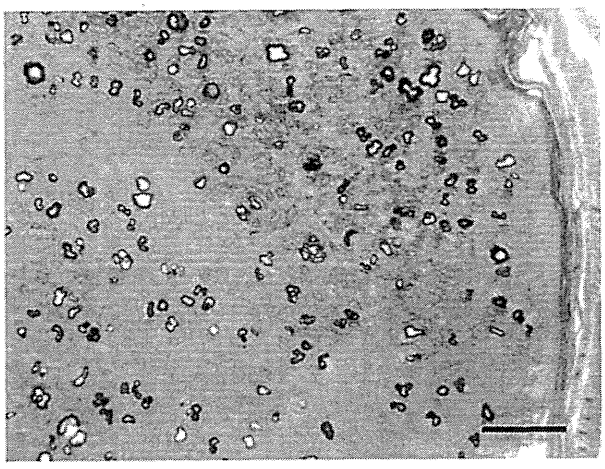

Fig. 1 The biopsied sural nerve of the eldest brother. A significant reduction of large myelinated fibers was observed with clusters of regenerating fibers. Formation of onion bulbs and infiltration of inflammatory cells were not seen. *Bar* = 50 μm

syndrome failed to alleviate the paresthesia. At presentation, a neurological examination revealed distal muscle weakness and atrophy of the limbs with an absence of all deep tendon reflexes and decreased vibration perception of the bilateral lower extremities. The patient had a CMTNS of 12 and an MMT score of 34 for ten selected muscles. An NCS showed sensorimotor demyelinating polyneuropathy with a non-uniform slowing of the MCV. Furthermore, variability of the MCV among nerves was also evident (Table 1). The outcome of a genetic test showed the same *GJB1* mutation as his elder brother.

Due to the acute emergence of his left hand paresthesia and NCS findings, he was also suspect of having acquired inflammatory neuropathy superimposed on CMT1X. The patient was treated with IVIg, resulting in an improvement of his muscle weakness, while his MMT score and CMTNS changed from 34 to 42 and from 12 to 11, respectively. An NCS showed no significant change after IVIg treatment.

The youngest sister

A 24-year-old female visited our outpatient clinic in order to have a genetic examination. At the age of 7, she had difficulty in uncorking a bottle. She never felt a stepwise deterioration. At the first visit, she could not perform dorsiflexion of her toes, but distal muscle weakness of her limbs was mild and graded as MMT 4 or 4+. Her CMTNS and MMT score of ten selected muscles were 10 and 44, respectively. An NCS demonstrated diffuse sensorimotor demyelinating polyneuropathy (Table 1). We confirmed that she had the same *GJB1* mutation as her brothers.

After the administration of IVIg, only extension of her large toes was observed.

Mother of the three patients

She complained of no clinical symptoms and her NCS findings were normal. We confirmed that she had the same GJB1 mutation as her children.

Mutation screening

After written informed consent was given from all family members, we screened for mutations in all *GJB1* coding exons in comparison to the published sequence (NM_000166) using the standard Sanger method. We also screened for duplication of the peripheral myelin protein 22 (*PMP22*) gene using fluorescent in situ hybridization (FISH).

Mutation analysis of *GJB1* in this family and a control study

From the family history and clinical information, we screened for mutations in the *GJB1* gene and detected a novel c.227T > G (p.L76R) missense mutation. In contrast, analysis using an original gene chip was negative for mutations involving the other 27 CMT genes or related disease-causing genes [10]. The patients do not have a *PMP22* duplication as determined by FISH.

The eldest brother was hemizygous for the c.227T > G mutation, which substitutes a leucine for arginine at amino acid 76 (p.L76R) in exon 2 of *GJB1* according to conceptual translation (Fig. 2). The younger brother had the same hemizygous mutation, and his mother and sister were heterozygous for this mutation (Fig. 2). We did not observe p.L76R in 112 controls or in 446 patients with inherited neuropathy. In addition, we did not find the p.L76R 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. Family segregation studies suggest that it is reasonable to assume that the L76R mutation was the cause of CMT in this family.

Discussion

Since Dyck's first report of seven prednisone-responsive CMT patients with stepwise deteriorating neuropathy, it has long been recognized that rapid progression and non-uniform demyelination with a conduction block or temporal dispersion could also occur in some patients with CMT. These patients responded to immunotherapy, including corticosteroids or IVIg [7, 11]. In fact, some patients with CMT1X are erroneously diagnosed as CIDP when an NCS shows non-uniform nerve conduction [12].

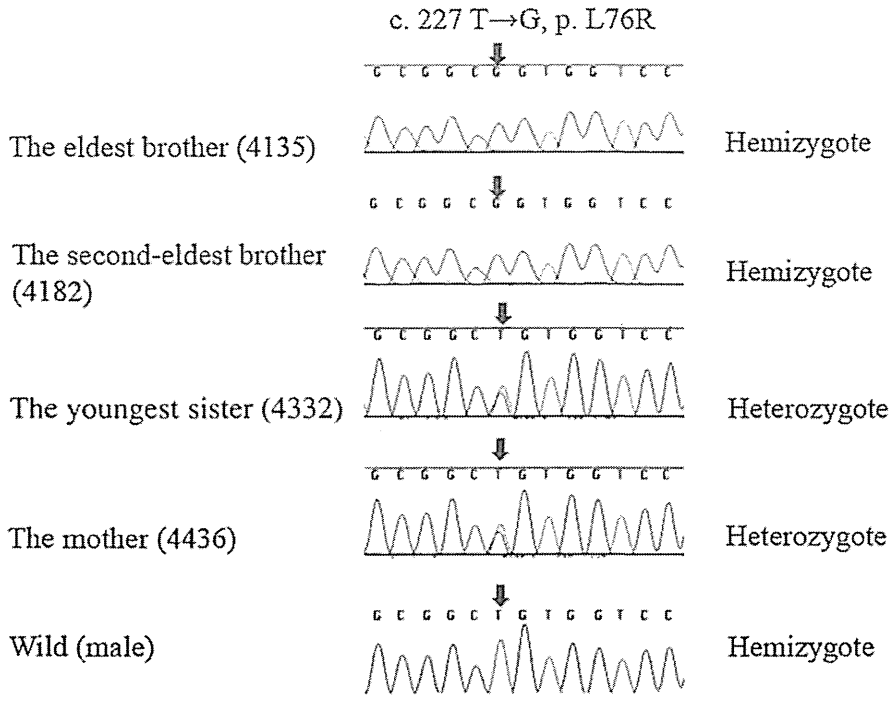

Fig. 2 *GJB1* alterations identified in the family. Chromatograms of the alterations in the *GJB1* gene. A hemizygous mutation was identified in the eldest brother (4135) and second-eldest brother (4182). A heterozygous mutation was identified in the youngest sister (4332) and mother (4436). The transition c.227T > G resulted in p.L76R

There have been three reports in which all of the CMT1X patients experienced a stepwise deterioration in neuropathy and responded to IVIg [4, 5, 9]. Thus, the superimposition of neuropathy and CMT was thought to be linked by some common mechanism. The condition is, however, reported as the coincidental occurrence of CIDP and CMT because the number of previous reports is limited, and there is only one report describing the occurrence of acquired inflammatory polyneuropathy along with the gradual deterioration of CMT1X in a single family [9].

The two brothers in our report showed stepwise deterioration after periods of clinical stability. Their symptoms showed a significant improvement with IVIg therapy. Repetitive IVIg relieved the stepwise deterioration in the eldest brother; furthermore, a mild motor improvement was also seen in the sister without any sign of neuropathy mimicking CIDP. Of particular note is that all of the affected siblings diagnosed with CMT1X responded to IVIg. It seems stochastically unlikely that two out of three family members with CMT1X, with a prevalence of 1 in 35,000, experienced CIDP, with a prevalence of 1 in 100,000 [13]. These findings provide strong evidence that patients with CMT1X might be susceptible to IVIg-responsive demyelination during the course of CMT1X. NCS did not show any conduction blocks and improvement of the temporal dispersion in the eldest brother after IVIg treatment in examined nerves of the affected siblings. We speculate that conduction blocks might have existed and been improved in more distal nerves after IVIg administration.

How immunoregulation by steroids and IVIg alleviates the stepwise deterioration in patients with CMT1X remains to be elucidated. Evidence from animal models of CMT1X, however, suggests that an immune-mediated process is involved in the progression of neuropathy in CMT1X. In the gap junction component connexin32 deficient mice, numerous macrophages are found in the peripheral nerves before a migration of T cells. Once the macrophages become activated, they attract T cells. CD8-positive T cells presented with antigens, possibly of myelin origin, cause further activation of macrophages [14]. Kobsar et al. [15] showed that cross-bred connexin32-deficient mice with recombination activating gene-1-deficient mice, which lack mature T- and B-lymphocytes, showed a significant reduction of demyelinated peripheral nerves. Recently, a null mice mutant for colony-stimulating factor, a pivotal macrophage activator, demonstrated significant amelioration of demyelination [16]. Although no infiltration of inflammatory cells was seen in the left sural nerve biopsy of the eldest brother in the present study, the *GJB1* mutation might have resulted in a predisposition to develop immune-mediated demyelinating polyneuropathy in addition to the underlining CMT1X pathology, potentially as the result of the activation of macrophages. IVIg might have beneficial immunomodulating effects on stepwise deterioration of neuropathy in our patients.

In this study, we clearly demonstrated a family of IVIg-responsive patients with CMT1X. Immune-mediated demyelination can be partially responsible for the pathogenesis of CMT1X, not merely the coincidental occurrence

of CIDP with CMT. Some patients with CMT1X can experience rapid deterioration due to immune-mediated demyelination along with a steady decline in their symptoms. Both stepwise and gradual deterioration can be clinical manifestations of CMT1X.

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Conflicts of interest We declare no conflict of interest.

Ethical standard This study was conducted with the approval of the Aomori Prefectural Central Hospital ethical committee.

References

1. Pareyson D, Marchesi C (2009) Diagnosis, natural history, and management of Charcot–Marie–Tooth disease. Lancet Neurol 8:654–667
2. Bergoffen J, Scherer SS, Wang S et al (1993) Connexin mutations in X-linked Charcot–Marie–Tooth disease. Science 262:2039–2042
3. Hattori N, Yamamoto M, Yoshihara T et al (2003) Demyelinating and axonal features of Charcot–Marie–Tooth disease with mutations of myelin-related proteins (PMP22, MPZ and Cx32): a clinicopathological study of 205 Japanese patients. Brain 126: 134–151
4. Gutierrez A, England JD, Sumner AJ et al (2000) Unusual electrophysiological findings in X-linked dominant Charcot–Marie–Tooth disease. Muscle Nerve 23:182–188
5. Kuntzer T, Dunand M, Schorderet DF et al (2003) Phenotypic expression of a Pro 87 to Leu mutation in the connexin 32 gene in a large Swiss family with Charcot–Marie–Tooth neuropathy. J Neurol Sci 207:77–86
6. Tabaraud F, Lagrange E, Sindou P et al (1999) Demyelinating X-linked Charcot–Marie–Tooth disease: unusual electrophysiological findings. Muscle Nerve 22:1442–1447
7. Ginsberg L, Malik O, Kenton AR et al (2004) Coexistent hereditary and inflammatory neuropathy. Brain 127:193–202
8. Ryan MM, Jones HR Jr (2005) CMTX mimicking childhood chronic inflammatory demyelinating neuropathy with tremor. Muscle Nerve 31:528–530
9. Sakaguchi H, Yamashita S, Miura A et al (2001) A novel GJB1 frameshift mutation produces a transient CNS symptom of X-linked Charcot–Marie–Tooth disease. J Neurol 258:284–290
10. Nakamura T, Hashiguchi A, Suzuki S et al (2012) Vincristine exacerbates asymptomatic Charcot–Marie–Tooth disease with a novel EGR2 mutation. Neurogenetics 13:77–82
11. Dyck PJ, Swanson CJ, Low PA et al (1982) Prednisone-responsive hereditary motor and sensory neuropathy. Mayo Clin Proc 57:239–246
12. Michell AW, Laura M, Blake J et al (2009) GJB1 gene mutations in suspected inflammatory demyelinating neuropathies not responding to treatment. J Neurol Neurosurg Psychiatry 80:699–700
13. Lunn MP, Manji H, Choudhary PP et al (1999) Chronic inflammatory demyelinating polyradiculoneuropathy: a prevalence study in south east England. J Neurol Neurosurg Psychiatry 66:677–680
14. Martini R, Toyka KV (2004) Immune-mediated components of hereditary demyelinating neuropathies: lessons from animal models and patients. Lancet Neurol 3:457–465
15. Kobsar I, Berghoff M, Samsam M et al (2003) Preserved myelin integrity and reduced axonopathy in connexin32-deficient mice lacking the recombination activating gene-1. Brain 126:804–813
16. Groh J, Weis J, Zieger H et al (2012) Colony-stimulating factor-1 mediates macrophage-related neural damage in a model for Charcot–Marie–Tooth disease type 1X. Brain 135:88–104

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DYSF mutation analysis in a group of Chinese patients with dysferlinopathy

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ABSTRACT

Objective: Dysferlinopathies belong to heterogeneous group of autosomal recessive muscular disorders caused by mutations in the gene encoding dysferlin. The classifications of the dysferlinopathies mainly include limb-girdle muscular dystrophy 2B (LGMD2B) with predominantly proximal weakness, Miyoshi myopathy (MM) with calf muscle weakness and atrophy, and distal myopathy with anterior tibial onset (DMAT) with tibialis muscle atrophy. We describe the genetic character of dysferlinopathies in a group of Chinese patients.
Methods: DYSF mutations screening were done after muscle biopsy and immunohistochemical staining.
Results: Eight patients showed an absence or drastic decrease of dysferlin expression in biopsied muscle. We identified 6 different mutations, including one nonsense mutation, two insertion mutation, two deletion mutations and one splice site mutation. Five of them were novel mutations.
Conclusion: We described 8 Chinese patients with dysferlinopathy (four had a distal phenotype of MM; one had a phenotype of DMAT and three presented with LGMD2B). It is the first report of genetic confirmed DMAT in China. Mutations c.3112C>T and c.1045dup, may be recurrent mutations in China.

1. Introduction

Dysferlin is a 230 kDa transmembrane protein and has been shown to be involved in the process of membrane repair [1], myoblast differentiation [2], T tubulogenesis [3] and muscle regeneration [4] through mechanisms of membrane organisation. Dysferlinopathies refer to a group of autosomal recessive muscular dystrophies caused by mutations in *DYSF*. Mutations in *DYSF* lead to different clinical phenotypes of muscular dystrophy according the distribution of affected muscles. It encompasses limb-girdle muscular dystrophy (LGMD2B), Miyoshi myopathy (MM), distal myopathy with anterior tibial onset (DMAT), isolated hyperCK and rigid spine syndrome. LGMD2B is characterized by predominant weakness and atrophy of muscles of the pelvic and shoulder girdle, onset in the late teens or later. LGMD2B is the second more frequent form of LGMD in Western countries [5] and Japan [6]. In China, LGMD has not been adequately studied, and its epidemiology remains unclear. MM is an early-adult onset, distal muscular dystrophy, characterized by predominant involvement in the calf muscles [7]. DMAT is a distal muscular dystrophy, characterized by predominant involvement in the anterior compartment group [8]. There are very few reports regarding dysferlinopathy in the Chinese population. In this study, we performed mutation analysis of *DYSF* in 8 Chinese patients with a dysferlin protein deficiency; the clinical and pathological features were collected and analyzed.

2. Materials and methods

We analyzed 8 Chinese patients from 8 unrelated families. Inclusion criteria for patients were: (i) clinical phenotype consistent with LGMD or distal myopathy, and (ii) metabolic myopathies or congenital myopathies were excluded, and (iii) loss or strong reduction of dysferlin expression evidenced by immunohistochemistry on muscle biopsy.

2.1. Histological analysis

After informed consent, muscle biopsy specimens were obtained from the biceps brachii, gastrocnemius or anterior tibial muscle of the patients. Biopsied skeletal muscles were flash frozen in isopentane chilled by liquid nitrogen. Hematoxylin and eosin (H&E), modified Gomori trichrome, myosin ATPase, acid phosphatase, NADH-TR and oil red O (ORO), periodic acid-Schiff (PAS), were performed for histopathological analysis.

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2.2. Protein analysis

Immunohistochemistry on muscle biopsies was carried out, using monoclonal antibodies to dysferlin (Novocastra, Newcastle, United Kingdom; dilution 1:50). All immunohistochemical procedures were performed as reported previously [9].

2.3. Genomic mutation screening and mutational data analysis

After informed consent, genomic DNA was extracted from peripheral blood obtained from all patients. Control genomic DNA samples were obtained from 50 healthy Chinese unrelated individuals. Using the Primer v3 program, we designed primers to amplify exons and intronic splice junctions. By PCR we amplified the coding exons from 50 ng of patient genomic DNA using these primers and hot start PCR method as defined for amplification of polymorphic markers. Using the pre-sequencing kit (USB), we purified patient PCR products and sequenced them with dye terminator chemistry using an ABI377 automated sequencer (Applied Biosystems). Sequencher 3.1.1 software was used for analysis of the sequences. The reference sequence used was derived from GenBank ID NM_003494.2. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. The initiation codon is codon 1.

All novel nucleotide changes were sequenced in 50 control individuals (100 chromosomes) using the GS Junior pyrosequencing system (Roche 454 Life Sciences, Branford, CT, USA) by standard methods. Analysis of the pyrosequencing data was performed using 454 Sequencing System Software V 2.5 (Roche 454 Life Sciences).

3. Result

3.1. Mutational analysis of the DYSF gene

We performed a genomic analysis of the *DYSF* coding sequence in 8 unrelated Chinese patients based on a marked or total loss of dysferlin expression in muscle, demonstrated by immunohistochemistry on muscle sections. We identified 6 different mutations that included one nonsense mutations; two deletions, two insertions and one splice site mutations. Five of them were novel mutations. Three patients carried a single homozygous mutation; one patient had two compound heterozygous mutations; three patients, only one heterozygous mutation could be identified. One homozygous mutation and one heterozygous mutation were identified in more than one patient. The patients who had the same mutations came from different provinces (Fig. 1).

3.2. Clinical analysis

Among the 8 patients analyzed in this study, 6 are male and 2 female. Age of disease onset ranged from 15 to 43 years, average age of onset was 24.1 years. One patient has an affected brother with similar phenotype; other patients have no family history. Consanguinity was not reported. Four patients had a distal phenotype of MM; one patient had a phenotype of DMAT (Fig. 2) and three patients presented with LGMD2B (Table 1).

3.3. Histological and immunohistochemical analysis

Muscle biopsies from all patients showed active necrotic and regenerating processes. Perimysial lymphocytic infiltration was noted in one patient (Fig. 3A). Immunohistochemical analysis of all patients showed complete absence of dysferlin expression in 6 patients and strong reduction of dysferlin expression in 2 patients (Fig. 3B and C).

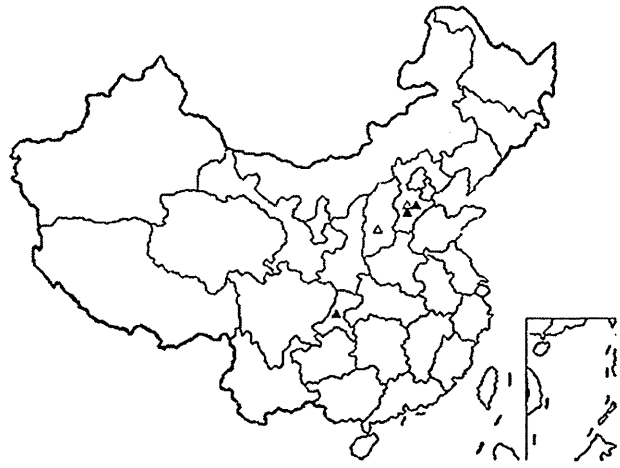

Fig. 1. Patients with same *DYSF* mutations came from different province of China.

4. Discussion

Mutations in *DYSF* cause different clinical phenotypes of muscular dystrophy and the distribution of affected muscles is different between patients. Some patients show onset in proximal muscles, while others show initial selective atrophy and weakness of calf muscles or anterior tibial muscles. *DYSF* gene mutation analysis was carried out in 8 Chinese patients, who had drastic or complete protein deficiency on muscle sections. We have identified 6 variations in the *DYSF* gene, 5 of them being novel (Table 2), according Leiden Muscular Dystrophy pages database (www.dmd.nl updated June 16, 2012) and UMD-DYSF database (Universal Mutation Database for Dysferlin www.umd.be/DYSF/) [10]. Mutations being distributed over the entire coding-sequence, no hot spot was observed. Previous reports on mutations in *DYSF* in China revealed two novel compound heterozygous mutations (c.1310+1G>A, c.1651_1652delinsTC; c.447delC, c.6576_6578delinsCTG) and one novel homozygous mutation(c.6429delG) [11,12].

Three non-consanguineous patients carry homozygous mutations, and we think these mutations are pathogenic variations since they are truncating mutations (one nonsense mutation, one splice site mutations). Two unrelated patients carry the same c.3112C>T (p.Arg1038X) homozygous nonsense mutation, which was reported compounded with another nonsense mutation in a France patient with MM phenotype [13]. In theory, this change caused a stop codon. The codon 1038(arginine) was replaced by a stop codon. Since a nonsense mutation causes premature translation termination codons, they degrade immediately by the process of nonsense-mediated decay (NMD). A novel homozygous mutation c.1638+1G>T was identified in a patient with LGMD2B phenotype. This mutation affects acceptor splicing site, likely to produce aberrantly spliced transcripts. Further research

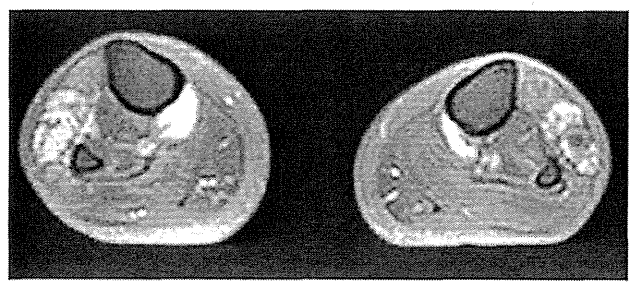

Fig. 2. Muscle MRI T1 transverse images at calf level in patient 8. The anterior tibial muscle is mainly involved.

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Table 1
Description of clinical, pathological information of 8 Chinese patients with dysforlinopathy.

Patient no.	Sex	Family history	Age of onset (years)	CK level (IU/L)	Initial affected muscle	Phenotype	Muscle biopsy A: degeneration B: inflammation C: dysferlin
1	F	–	26	2163	Calf muscle	MM	A: active B: no C: deficiency
2	M	–	19	13,864	Calf muscle	MM	A: active B: no C: deficiency
3	M	–	27	7121	Proximal muscle	LGMD	A: active B: no C: deficiency
4	M	–	21	4490	Calf muscle	MM	A: active B: no C: deficiency
5	M	–	19	2559	Proximal muscle	LGMD	A: active B: yes C: strong reduction
6	M	–	15	3029	Proximal muscle	LGMD	A: active B: no C: strong reduction
7	M	+	23	5425	Calf muscle	MM	A: active B: no C: deficiency
8	F	–	43	9640	Anterior tibial muscle	DMAT	A: active B: no C: deficiency

M, male; F, female; –, negative; +, positive; MM, Miyoshi myopathy; LGMD, limb-girdle muscular dystrophy; DMAT, distal myopathy with anterior tibial onset; CK, creatine kinase.

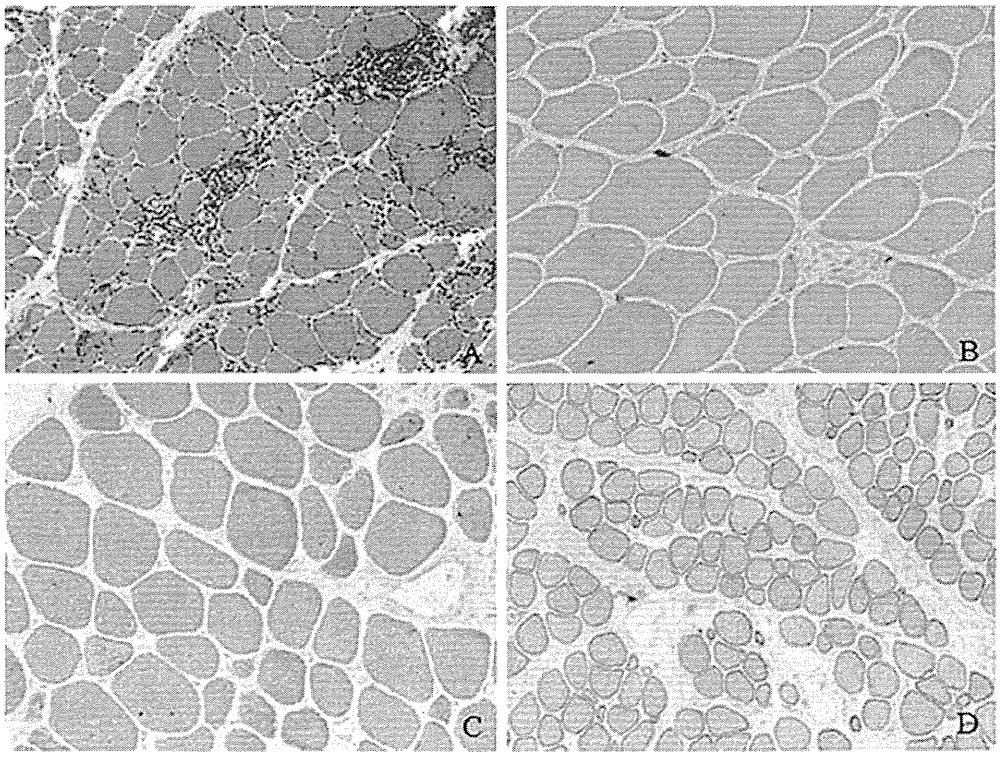

Fig. 3. (A) Hematoxylin and eosin staining. Lymphocytic infiltration was noted around small vessel in one patient 5 (200×). (B–D) Anti-dysferlin immunohistochemical staining. (B) Complete absence of dysferlin expression (400×); (C) strong reduction of dysferlin expression (400×); (D) normal control (200×).

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Table 2
The *DYSF* mutations information of the 8 patients.

Patient no.	Phenotype	Nucleotide change	Protein change	State
1	MM	c.3112C>T	Arg1038X	Homozygous
2	MM	c.3112C>T	Arg1038X	Homozygous
3	LGMD	**c.1638+1G>C**	Splice change	Homozygous
4	MM	**c.1045dup**	Glu349fsX11	Heterozygous
		c.5563_5564delGA	Asp1855fsX9	Heterozygous
5	LGMD	**c.1045dup**	Glu349fsX11	Heterozygous
6	LGMD	**c.1045dup**	Glu349fsX11	Heterozygous
7	MM	**c.3954_3955insAT**	Tyr1319IlefsX28	Heterozygous
8	DMAT	**c.5670_5671delTG**	Ala350SerfsX10	Heterozygous

Bold text, novel mutation.

by RT-PCR from muscle tissues should do in order to confirm its pathogenic consequence on the transcript. A novel heterozygous frameshift mutation, c.1045dup (Glu349fsX11) was identified in three unrelated patients including LGMD2B and MM phenotypes. This suggests the absence of relationship between the type of mutation and the phenotype, as previously reported [14,15]. Among these mutations, two (c.3112C>T, c.1045dup) were identified each in two or three unrelated patients from different provinces. This suggests these mutations may be recurrent mutations in China.

Patient 8 presented the distal anterior dysferlinopathy phenotype (DMAT). The clinical examination revealed severe distal weakness, predominantly in the anterior compartment, with a steppage gait. DMAT is a relatively uncommon and recently recognized form of dysferlinopathy [8]. Previous report suggested patients had symptom in their early adulthood [8,16–19]. But patient 8 experienced weakness of anterior tibial muscle in 43 years. This suggests a wide range of disease onset. Only one novel heterozygous frameshift mutation, c.5670_5671delTG (Ala350SerfsX10) was identified, although all 55 exons were sequenced directly. This is the first report of genetic confirmed DMAT in China. Other three patients (patients 5, 6, 7) also identified only one allelic mutation. Further research by RT-PCR from muscle tissues should do in order to detect truncated transcript, and there is possibility that these patients may have mutations in the promoter region or a regulatory region of an intron of *DYSF*.

In this study no pathogenic missense mutation was detected, while previous reported 22–26% [13,20]. This difference may partially be caused by the exclusion of patients with mild or moderate reduction of dysferlin expression evidenced by immunohistochemistry on muscle biopsy. Interestingly, we found higher proportion of nonsense mutation, out-of-frame insertion, out-of-frame deletion, may generate premature translation termination.

In conclusion, we found 6 *DYSF* mutations in 8 Chinese patients. Among them, 5 were novel mutations. We first reported genetic confirmed DMAT in China. Mutations c.3112C>T and c.1045dup were identified twice or three times, may be recurrent mutations in China.

References

[1] Bansal D, Miyake K, Vogel SS, Groh S, Chen CC, Williamson R, et al. Defective membrane repair in dysferlin deficient muscular dystrophy. Nature 2003;423:168–72.
[2] De Luna N, Gallardo E, Soriano M, Dominguez-Perles R, de la Torre C, Rojas-García R, et al. Absence of dysferlin alters myogenin expression and delays human muscle differentiation "In vitro". Journal of Biological Chemistry 2006;281:17092–8.
[3] Klinge L, Harris J, Sewry C, Charlton R, Anderson L, Laval S, et al. Dysferlin associates with the developing T-tubule system in rodent and human skeletal muscle. Muscle and Nerve 2010;41:166–73.
[4] Chiu YH, Hornsey MA, Klinge L, Jørgensen LH, Laval SH, Charlton R, et al. Attenuated muscle regeneration is a key factor in dysferlin-deficient muscular dystrophy. Human Molecular Genetics 2009;18:1976–89.
[5] Guglieri M, Magri F, D'Angelo MG, Prelle A, Morandi L, Rodolico C, et al. Clinical, molecular, and protein correlations in a large sample of genetically diagnosed Italian limb girdle muscular dystrophy patients. Human Mutation 2008;29(February (2)):258–66.
[6] Tagawa K, Ogawa M, Kawabe K, Yamanaka G, Matsumura T, Goto K, et al. Protein and gene analyses of dysferlinopathy in a large group of Japanese muscular dystrophy patients. Journal of the Neurological Sciences 2003;211(July (1–2)):23–8.
[7] Miyoshi K, Kawai H, Iwasa M, Kusaka K, Nishino H. Autosomal recessive distal muscular dystrophy as a new type of progressive muscular dystrophy. Seventeen cases in eight families including an autopsied case. Brain 1986;109(February (Pt 1)):31–54.
[8] Illa I, Serrano-Munuera C, Gallardo E, Lasa A, Rojas-García R, Palmer J, et al. Distal anterior compartment myopathy: a dysferlin mutation causing a new muscular dystrophy phenotype. Annals of Neurology 2001;49:130–4.
[9] Saito A, Higuchi I, Nakagawa M, Saito M, Hirata K, Suehara M, et al. Miyoshi myopathy patients with novel 5′ splicing donor site mutations showed different dysferlin immunostaining at the sarcolemma. Acta Neuropathologica 2002;104(6):615–20.
[10] Blandin G, Beroud C, Labelle V, Nguyen K, Wein N, Hamroun D, et al. UMD-DYSF, a novel locus specific database for the compilation and interactive analysis of mutations in the dysferlin gene. Human Mutation 2012;33(March (3)):E2317–31.
[11] Shunchang S, Fan Q, Huacheng W, Leturcq F, Yongjian S, Bingfeng Z, et al. Dysferlin mutation in a Chinese pedigree with Miyoshi myopathy. Clinical Neurology and Neurosurgery 2006;108(June (4)):369–73.
[12] Ro LS, Lee-Chen GJ, Lin TC, Wu YR, Chen CM, Lin CY, et al. Phenotypic features and genetic findings in 2 Chinese families with Miyoshi distal myopathy. Archives of Neurology 2004;61(October (10)):1594–9.
[13] Krahn M, Béroud C, Labelle V, Nguyen K, Bernard R, Bassez G, et al. Analysis of the DYSF mutational spectrum in a large cohort of patients. Human Mutation 2009;30(February (2)):E345–75.
[14] Argov Z, Sadeh M, Mazor K, Soffer D, Kahana E, Eisenberg I, et al. Muscular dystrophy due to dysferlin deficiency in Libyan Jews. Clinical and genetic features. Brain 2000;123(June (Pt 6)):1229–37.
[15] Kawabe K, Goto K, Nishino I, Angelini C, Hayashi YK. Dysferlin mutation analysis in a group of Italian patients with limb-girdle muscular dystrophy and Miyoshi myopathy. European Journal of Neurology 2004;11:657–61.
[16] Vilchez JJ, Gallano P, Gallardo E, Lasa A, Rojas-García R, Freixas A, et al. Identification of a novel founder mutation in the DYSF gene causing clinical variability in the Spanish population. Archives of Neurology 2005;62:1256–9.
[17] Saito H, Suzuki N, Ishiguro H, Hirota K, Itoyama Y, Takahashi T, et al. Distal anterior compartment myopathy with early ankle contractures. Muscle and Nerve 2007;36:525–7.
[18] Khadilkar SV, Singh RK, Agrawal P, Krahn M, Levy N. Twenty-two year follow-up of an Indian family with dysferlinopathy-clinical, immunohistochemical, western blotting and genetic features. Neurology India 2008;56:388–90.
[19] Liewluck T, Pongpakdee S, Witoonpanich R, Sangruchi T, Pho-Iam T, Limwongse C, et al. Novel DYSF mutations in Thai patients with distal myopathy. Clinical Neurology and Neurosurgery 2009;111(September (7)):613–8.
[20] Klinge L, Aboumousa A, Eagle M, Hudson J, Sarkozy A, Vita G, et al. New aspects on patients affected by dysferlin deficient muscular dystrophy. Journal of Neurology, Neurosurgery and Psychiatry 2010;81(September (9)):946–53.

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ORIGINAL ARTICLE

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

Tomonori Nakamura · Akihiro Hashiguchi · Shinsuke Suzuki · Kimiharu Uozumi · Shoko Tokunaga · Hiroshi Takashima

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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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Keywords Charcot–Marie–Tooth disease · Early growth response 2 · Vincristine-induced neuropathy · DNA chip

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

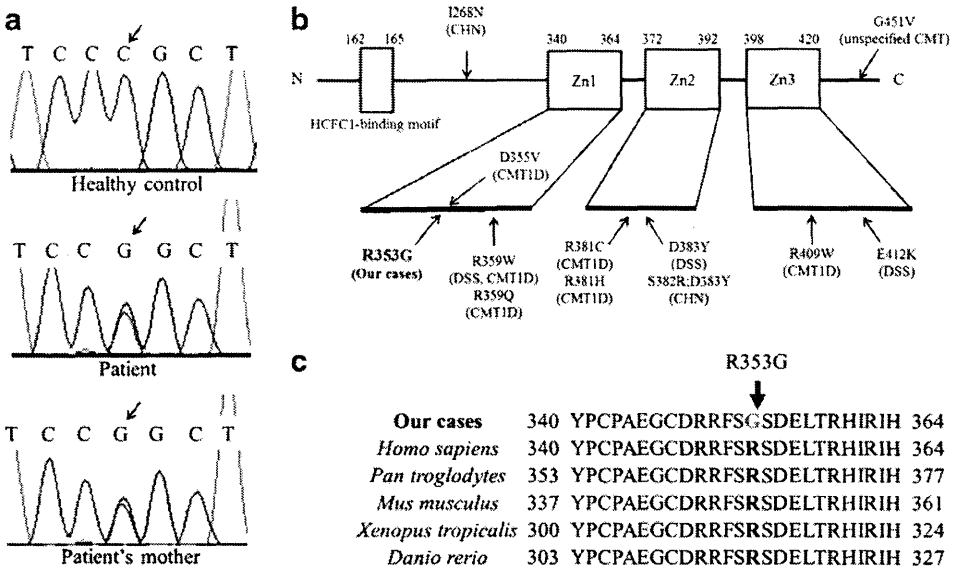

Fig. 1 **a** Chromatograms of the alterations in the *early growth response 2* (*EGR2*) gene that was identified in the patient and her mother, both of whom had the heterozygous transition c.1057 C>G that resulted in R353G. **b** Schematic diagram of the *EGR2* showing previously reported mutations and 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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References

1. Weiss HD, Walker MD, Wiernik PH (1974) Neurotoxicity of commonly used antineoplastic agents (second of two parts). N Engl J Med 291:127–133
2. Trobaugh-Lotrario AD, Smith AA, Odom LF (2003) Vincristine neurotoxicity in the presence of hereditary neuropathy. Med Pediatr Oncol 40:39–43
3. Weimer LH, Podwall D (2006) Medication-induced exacerbation of neuropathy in Charcot–Marie–Tooth disease. J Neurol Sci 242:47–54
4. Birouk N, Gouider R, Le Guern E, Gugenheim M, Tardieu S, Maisonobe T, Le Forestier N, Agid Y, Brice A, Bouche P (1997) Charcot–Marie–Tooth disease type 1A with 17p11.2 duplication. Clinical and electrophysiological phenotype study and factors influencing disease severity in 119 cases. Brain 120:813–823
5. Boerkoel CF, Takashima H, Garcia CA, Olney RK, Johnson J, Berry K, Russo P, Kennedy S, Teebi AS, Scavina M, Williams LL, Mancias P, Butler IJ, Krajewski K, Shy M, Lupski JR (2002) Charcot–Marie–Tooth disease and related neuropathies: mutation distribution and genotype–phenotype correlation. Ann Neurol 51:190–201
6. Yerushalmi R, Levi I, Wygoda M, Ifergane G, Wirguin I (2007) Are platinum-based chemotherapeutic drugs safe for patients with Charcot–Marie–Tooth disease? J Peripher Nerv Syst 12:139–141
7. Neumann Y, Toren A, Rechavi G, Seifried B, Shoham NG, Mandel M, Kenet G, Sharon N, Sadeh M, Navon R (1996) Vincristine treatment triggering the expression of asymptomatic Charcot–Marie–Tooth disease. Med Pediatr Oncol 26:280–283
8. Mercuri E, Poulton J, Buck J, Broadbent V, Bamford M, Jungbluth H, Manzur AY, Muntoni F (1999) Vincristine treatment revealing asymptomatic hereditary motor sensory neuropathy type 1A. Arch Dis Child 81:442–443
9. Uno S, Katayama K, Dobashi N, Hirano A, Ogihara A, Yamazaki H, Usui N, Kobayashi T, Inoue K, Kuraishi Y (1999) Acute vincristine neurotoxicity in a non-Hodgkin's lymphoma patient with Charcot–Marie–Tooth disease. Rinsho Ketsueki 40:414–419
10. Hildebrandt G, Holler E, Woenkhaus M, Quarch G, Reichle A, Schalke B, Andreesen R (2000) Acute deterioration of Charcot–Marie–Tooth disease IA (CMT IA) following 2 mg of vincristine chemotherapy. Ann Oncol 11:743–747

11. Naumann R, Mohm J, Reuner U, Kroschinsky F, Rautenstrauss B, Ehninger G (2001) Early recognition of hereditary motor and sensory neuropathy type 1 can avoid life-threatening vincristine neurotoxicity. Br J Haematol 115:323–325
12. Cil T, Altintas A, Tamam Y, Battaloglu E, Isikdogan A (2009) Low dose vincristine-induced severe polyneuropathy in a Hodgkin lymphoma patient: a case report (vincristine-induced severe polyneuropathy). J Pediatr Hematol Oncol 31:787–789
13. Ajitsaria R, Reilly M, Anderson J (2008) Uneventful administration of vincristine in Charcot–Marie–Tooth disease type 1X. Pediatr Blood Cancer 50:874–876
14. Nishikawa T, Kawakami K, Kumamoto T, Tonooka S, Abe A, Hayasaka K, Okamoto Y, Kawano Y (2008) Severe neurotoxicities in a case of Charcot–Marie–Tooth disease type 2 caused by vincristine for acute lymphoblastic leukemia. J Pediatr Hematol Oncol 30:519–521
15. Porter CC, Carver AE, Albano EA (2009) Vincristine induced peripheral neuropathy potentiated by voriconazole in a patient with previously undiagnosed CMT1X. Pediatr Blood Cancer 52:298–300
16. Nagarajan R, Svaren J, Le N, Araki T, Watson M, Milbrandt J (2001) EGR2 mutations in inherited neuropathies dominant-negatively inhibit myelin gene expression. Neuron 30:355–368
17. Scherer SS (1997) The biology and pathobiology of Schwann cells. Curr Opin Neurol 10:386–397
18. Niemann A, Berger P, Suter U (2006) Pathomechanisms of mutant proteins in Charcot–Marie–Tooth disease. Neuromolecular Med 8:217–242
19. Swiatek PJ, Gridley T (1993) Perinatal lethality and defects in hindbrain development in mice homozygous for a targeted mutation of the zinc finger gene Krox20. Gene Dev 7:2071–2084
20. Topilko P, Schneider-Maunoury S, Levi G, Baron-Van Evercooren A, Chennoufi AB, Seitanidou T, Babinet C, Charnay P (1994) Krox-20 controls myelination in the peripheral nervous system. Nature 371:796–799
21. Warner LE, Mancias P, Butler IJ, McDonald CM, Keppen L, Koob KG, Lupski JR (1998) Mutations in the early growth response 2 (EGR2) gene are associated with hereditary myelinopathies. Nat Genet 18:382–384
22. Timmerman V, De Jonghe P, Ceuterick C, De Vriendt E, Lofgren A, Nelis E, Warner LE, Lupski JR, Martin JJ, Van Broeckhoven C (1999) Novel missense mutation in the early growth response 2 gene associated with Dejerine–Sottas syndrome phenotype. Neurology 52:1827–1832
23. Warner LE, Svaren J, Milbrandt J, Lupski JR (1999) Functional consequences of mutations in the early growth response 2 gene (EGR2) correlate with severity of human myelinopathies. Hum Mol Genet 8:1245–1251
24. Boerkoel C, Takashima H, Bacino C, Daentl D, Lupski J (2001) EGR2 mutation R359W causes a spectrum of Dejerine–Sottas neuropathy. Neurogenetics 3:153–157
25. Yoshihara T, Kanda F, Yamamoto M, Ishihara H, Misu K, Hattori N, Chihara K, Sobue G (2001) A novel missense mutation in the early growth response 2 gene associated with late-onset Charcot–Marie–Tooth disease type 1. J Neurol Sci 184:149–153
26. Mikesova E, Huhne K, Rautenstrauss B, Mazanec R, Barankova L, Vyhnalek M, Horacek O, Seeman P (2005) Novel EGR2 mutation R359Q is associated with CMT type 1 and progressive scoliosis. Neuromuscul Disord 15:764–767
27. Jang SW, LeBlanc SE, Roopra A, Wrabetz L, Svaren J (2006) In vivo detection of Egr2 binding to target genes during peripheral nerve myelination. J Neurochem 98:1678–1687
28. LeBlanc SE, Ward RM, Svaren J (2007) Neuropathy-associated Egr2 mutants disrupt cooperative activation of myelin protein zero by Egr2 and Sox10. Mol Cell Biol 27:3521–3529
29. Jang SW, Svaren J (2009) Induction of myelin protein zero by early growth response 2 through upstream and intragenic elements. J Biol Chem 284:20111–20120
30. Jones EA, Lopez-Anido C, Srinivasan R, Krueger C, Chang LW, Nagarajan R, Svaren J (2011) Regulation of the PMP22 gene through an intronic enhancer. J Neurosci 31:4242–4250

CHARCOT-MARIE-TOOTH DISEASE TYPE 4C IN JAPAN: REPORT OF A CASE

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ABSTRACT: *Introduction:* The distribution of documented cases of Charcot-Marie-Tooth disease type 4C (CMT4C) is mainly limited to the Mediterranean region. We report the first documented case of CMT4C in East Asia. Furthermore, we estimate the proportion of CMT4C in Japan and compare the same with that in European countries. *Case Report:* A 72-year-old Japanese woman presented with early-onset motor and sensory neuropathy associated with scoliosis, deformities of the hands and feet, and carpal tunnel syndrome. A genetic screen detected a homozygous p.R529Q mutation in *SH3TC2*, the causative gene of CMT4C. The *SH3TC2* mutation identified here is unique among 426 unrelated Japanese CMT patients, excluding those with CMT1A. *Conclusions:* Although CMT4C also occurs in Japan, it is less common than in European countries.

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Charcot-Marie-Tooth (CMT) disease is the most common inherited motor and sensory neuropathy.[1] CMT is usually inherited as an autosomal dominant trait and is subdivided into a demyelinating form (CMT type 1) and an axonal form (CMT type 2).[2] At present, gene analysis can identify mutations in approximately 50% of CMT cases.[3,4] The most common type of CMT, CMT type 1A (CMT1A), is an autosomal dominant demyelinating neuropathy with duplication of peripheral myelin protein 22 (PMP22).[4,5] The proportion of CMT1A cases relative to the total CMT or CMT1 population reported in East Asia is estimated to be lower than that reported in Europe and the United States.[3,6]

CMT type 4 (CMT4) is an autosomal recessive demyelinating form of CMT. One genetic subtype of CMT4, CMT type 4C (CMT4C), is linked to a locus on chromosome 5q23–33[7–9] and is characterized clinically by demyelinating peripheral neuropathy frequently associated with spinal deformities.[10]

Abbreviations: ABR, auditory brainstem response; CMT, Charcot-Marie-Tooth disease; CMT1A, Charcot-Marie-Tooth disease type 1A; CMT4, Charcot-Marie-Tooth disease type 4; CMT4C, Charcot-Marie-Tooth disease type 4C; MUP, motor unit potential; NCS, nerve conduction study; NEE, needle electromyographic examination; PMP22, peripheral myelin protein 22; SNP, single nucleotide polymorphism

Key words: autosomal recessive inheritance; elderly; hereditary neuropathy; Charcot-Marie-Tooth disease type 4C; SH3TC2 gene

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The gene implicated as the cause of CMT4C, *SH3TC2* (*KIAA1985*), was identified in 2003.[11] The gene product, SH3TC2, is a small protein with 2 Src homology 3 domains and 10 tetratricopeptide repeat motifs and is expressed specifically in Schwann cells. *SH3TC2* knockout mice develop progressive peripheral neuropathy with reduced nerve conduction velocity and hypomyelination.[12]

Of interest, the distribution of documented cases of CMT4C is limited mainly to Europe,[8–11,13–20] French Canada,[21] North Africa,[7,9,10] and West Asia.[8,11,14,18,22] Although variable prevalence of CMT4C in different ethnic groups may explain this distribution, the evidence for this proposal is inconclusive.

Here, we report an elderly Japanese patient with CMT4C. We also estimate the proportion of CMT4C in Japanese CMT, in comparison with other regions mentioned above.

CASE REPORT

A 72-year-old Japanese woman of consanguineous parentage complained of nocturnal numbness in her hands for several years and was examined in our hospital. Her parents were first cousins. Her history included hepatitis C, hypertension, and ovarian cystoma. She consumed neither alcohol nor any drug that could have potentially caused the neurological symptoms. Furthermore, she had no family history associated with peripheral neuropathy. She had been living in a small village in the Chiba prefecture (near Tokyo) where she could trace her ancestry for over 13 generations.

Careful historical analysis revealed several unique events. She was a slower runner than her peers in childhood, and she developed scoliosis in high school. She observed a deformity in her hands at the age of 20 years. Because of gait instability, she developed bone fractures in her ankles at the ages of 56 (on the right) and 67 years (on the left). It became challenging to walk up a flight of stairs without a banister by her middle sixties, and she has required a cane for walking since her

Table 1. Nerve conduction studies.

Nerve stimulated (left side)	Stimulation site	Recording site	Amplitude* (NL)	Latency (ms) (NL)	Conduction velocity (m/s) (NL)	F-Wave latency (ms) (NL)
Median (m)	Wrist	APB	2.1 (≥ 6.0)	17.0 (≤ 4.0)		n.e.
	Elbow	APB	2.0 (≥ 6.0)	24.4	27 (≥ 50)	
Ulnar (m)	Wrist	ADM	8.3 (≥ 8.0)	7.4 (≤ 3.4)		n.e.
	Below elbow	ADM	7.6 (≥ 8.0)	13.0	32 (≥ 50)	
	Above elbow	ADM	7.3 (≥ 8.0)	15.0	25 (≥ 36)	
Tibial (m)	Ankle	AH	0.68 (≥ 10.0)	9.6 (≤ 5.0)		84.2 (≤ 51.0)
	Popliteal space	AH	0.71 (≥ 8.0)	22.0	28 (≥ 45)	
Median (s)	Index finger	Wrist	0.70 (≥ 10.0)	8.74 (≤ 2.40)	14 (≥ 50)	
Ulnar (s)	Small finger	Wrist	1.30 (≥ 8.0)	3.52 (≤ 2.30)	30 (≥ 48)	
Sural (s)	Calf	Posterior ankle	2.10 (≥ 8.0)	4.32 (≤ 3.30)	35 (≥ 45)	
Facial (m)	Stylomastoid foramen	Nasalis	1.0 (≥ 1.0)	4.6 (≤ 3.0)		

The F-wave latency represents the minimum F-wave latency.
**Amplitude: motor (in millivolts); sensory (in microvolts).*
m, motor study; s, sensory study; NL, normal; APB, abductor pollicis brevis; ADM, abductor digiti minimi; AH, abductor hallucis; n.e., not elicited.

late sixties. She observed foot deformities at the age of 69 years. She had observed nocturnal numbness of the hands.

Neurological examination revealed the following: hypoacusia; mild, symmetrical distal muscle weakness and atrophy of limbs; positive bilateral Tinel and Phalen signs at the wrists; areflexia; distal dominant hyperalgesia; diminished vibratory sensation (especially prominent in the legs); Romberg sign; and gait instability. Scoliosis and deformities of the hands (claw hand) and feet (pes planus) were also observed.

Electrophysiological studies yielded several significant findings. In a nerve conduction study (NCS), relatively diffuse and homogeneous slowing of conduction velocity without conduction block, so called "uniform conduction slowing," was observed (Table 1). In median NCS, distal latencies of motor and sensory nerves were more prolonged than the distal latencies of other nerves. This focal conduction delay was presumably caused by concomitant carpal tunnel syndrome. Needle electromyographic examination (NEE) was performed in the biceps brachii, extensor carpi ulnaris, vastus medialis, and tibialis anterior muscles. All muscles showed marked high-amplitude and long-duration motor unit potentials (MUPs) with reduced MUP recruitment. Fibrillation potentials were observed in the tibialis anterior muscle. These NEE findings reflected chronic denervation and reinnervation. The blink reflex showed prolonged R1 and R2 responses, and auditory brainstem response (ABR) analysis revealed diminished wave I with delayed wave III (latencies were 5.2 ms on the left and 5.3 ms on the right; normal < 4.3 ms) and wave V (latencies were 7.0 ms on the left and 7.6 ms on the right; normal < 6.4 ms) bilaterally, suggesting cranial nerve involvement.

Because uniform slowing of conduction velocities in NCS suggested the existence of an inherited peripheral neuropathy,[23–25] we analyzed mutations associated with CMT after obtaining informed consent. First, we confirmed that the subject was negative for *PMP22* duplication or deletion by fluorescence *in situ* hybridization, eliminating CMT1A or hereditary neuropathy with liability to pressure palsies as the relevant condition. Subsequently, a re-sequencing method was performed, using GeneChip® CustomSeq® Resequencing Array (Affymetrix, Santa Clara, CA) equipped with 28 genes, including *SH3TC2*, as we previously reported.[26]

Variants c.1586G>A and c.1587T>G were observed in the *SH3TC2* gene (Fig. 1). The former variant is a rare mutation reported previously in Turkish children[11] and a middle-aged woman in France,[20] and the latter variant is a single nucleotide polymorphism (SNP). These variants caused a homozygous p.R529Q mutation, therefore, the patient was diagnosed with CMT4C. Pathological investigation was not performed, because the subject refused nerve biopsy.

Intriguingly, the *SH3TC2* mutation identified here is unique among 427 unrelated Japanese CMT cases previously investigated. The details of the 427 cases are as follows: (1) duplication of *PMP22* causing CMT1A was previously excluded; (2) the number of subjects with autosomal inherited demyelinating CMT (motor nerve conduction velocity < 38 m/s), autosomal inherited axonal CMT, X-linked CMT, and unclassified CMT was 125, 182, 26, and 94, respectively; (3) 100 subjects with CMT1 and 25 subjects with CMT4 were included in the autosomal inherited demyelinating CMT category; (4) 1 subject was CMT1A due to missense mutation of *PMP22*.

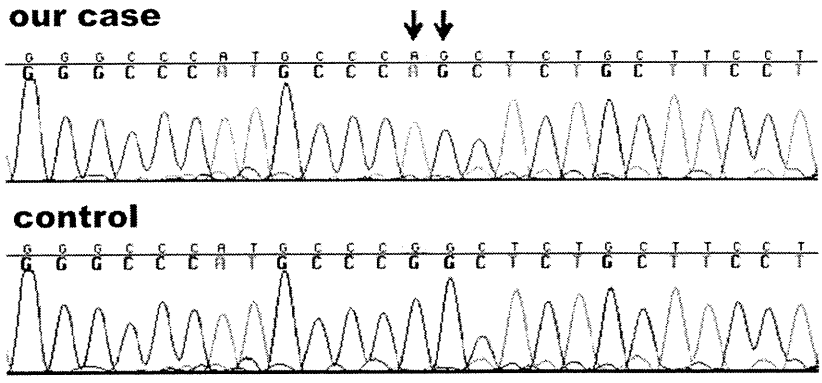

FIGURE 1. Sequence analysis showing c.1586G>A and c.1587T>G (p.R529Q, exon 11, arrow). Note that control data also show a single nucleotide polymorphism (SNP) at c.1587T>G. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

This report describes the first documented case of CMT4C in East Asia in a patient with an inherited peripheral neuropathy concurrent with scoliosis, hand and foot deformities, and carpal tunnel syndrome. DNA chip screening for the CMT causative gene detected a rare p.R529Q homozygous variant, resulting from mutations in the *SH3TC2* gene.

Mutations in *SH3TC2* tend to differ among ethnic groups, suggesting a founder effect. The mutation p.R954X appears most frequently in the Czech population[19] and in a French-Canadian cluster.[21] In contrast, the p.R1109X variant predominates in Gypsies.[13–15] Before the case of our Japanese patient, the p.R529Q mutation had been identified only in Turkish children[11] and a patient in France (the national origin was not described in the report).[20] Because the p.R529Q mutations arose from these entirely distinct ethnic and racial populations, the mutations may have distinct ancestral origins. On the other hand, we cannot deny the possibility that CMT4C in Turkish children and our Japanese patient share the same ancestry by means of the Silk Road.

The clinical aspects of CMT4C are early-onset demyelinating neuropathy with spinal deformity.[10,17] While only 8–29% of CMT1A patients develop spinal deformity,[22,27] most patients with CMT4C show spinal deformity.[10,20] As with other types of CMT,[17,28,29] foot deformity is often observed in patients with CMT4C. Usually, the deformities progress faster than the motor deficits.[10] Our patient showed early-onset spinal and hand deformities with slowly progressive motor deficits. Because her motor deficit was mild, the existence of CMT4C was less noticeable in our patient. Cranial nerves are broadly involved in CMT4C cases, with hearing loss being the most common manifestation.[10,11,13,14,16,17,19–22] Additionally, asymmetrical pupil size,[16] abnormality of pupillary reflexes,[14,16] nystagmus,[8,11] facial paresis,[10,16,17,20,21] tongue fasciculations,[14] atrophy[16,20] and weakness,[16] dysphagia,[20] vocal cord involvement,[20,22] and accessory nerve palsy[16] have been reported. In our patient, hypoacousia with diminished wave I in ABR indicated acoustic nerve involvement. Finally, combined with prolonged latency of the facial nerve in NCS, delayed R1 and R2 responses in the blink reflex suggest subclinical facial nerve involvement.

Carpal tunnel syndrome in CMT4C, which was also observed in our patient, is reported infrequently; 2 reports have been published regarding the p.R954X mutation of *SH3TC2* and carpal tunnel syndrome.[21,30] Gosselin et al.[21] reported that 2 of 17 CMT4C cases possessing the p.R954X mutation of *SH3TC2* had carpal tunnel syndrome. Recently, Lupski et al.[30] revealed by whole-genome sequencing in CMT patients that the p.R954X nonsense mutation—itself or accompanied by the missense variant (p.Y169H)—is associated with carpal tunnel syndrome and suggested that presumed loss-of-function nonsense variants of *SH3TC2* are associated with carpal tunnel syndrome. However, it is uncertain whether mutation of *SH3TC2* is related to carpal tunnel syndrome.

Our mutation analysis also revealed that *SH3TC2* mutation was detected in 1 of 426 Japanese CMT patients excluding those with CMT1A. As the proportion of CMT1A in Japanese CMT is approximately 18%,[3] the estimated proportion of CMT4C in total CMT is approximately 0.2% in Japan. Furthermore, we found that, in Japan, CMT4C accounts for approximately 4% of CMT4, an autosomal recessive demyelinating CMT.

Finally, we compared the proportion of CMT4C among different regions. Laššuthová et al.[19] detected *SH3TC2* mutation in 13 of 60 autosomal recessive demyelinating CMT patients and at least 8 of 412 CMT patients who were negative for

PMP22 duplication/deletion in Czechoslovakia. In addition, Baets et al.[17] examined 77 unrelated patients who developed motor and sensory neuropathy within the first year of life in various European countries, the Middle East, and United States. The *SH3TC2* mutation accounted for 12% of the identified mutations. Recently, Yger et al.[20] detected *SH3TC2* mutation in 16 of 102 unrelated autosomal recessive demyelinating or intermediate CMT cases in France. Although direct comparison is impossible because of varying experimental designs, these studies indicate that CMT4C in Japan is actually less common than in European countries.

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REFERENCES

1. Martyn CN, Hughes RA. Epidemiology of peripheral neuropathy. J Neurol Neurosurg Psychiatry 1997;62:310–318.
2. Pareyson D. Charcot-Marie-Tooth disease and related neuropathies: molecular basis for distinction and diagnosis. Muscle Nerve 1999;22: 1498–1509.
3. Abe A, Numakura C, Kijima K, Hayashi M, Hashimoto T, Hayasaka K. Molecular diagnosis and clinical onset of Charcot-Marie-Tooth disease in Japan. J Hum Genet 2011;56:364–368.
4. Braathen GJ, Sand JC, Lobato A, Høyer H, Russell MB. Genetic epidemiology of Charcot-Marie-Tooth in the general population. Eur J Neurol 2011;18:39–48.
5. Szigeti K, Luski JR. Charcot-Marie-Tooth disease. Eur J Hum Genet 2009;17:703–710.
6. Choi BO, Lee MS, Shin SH, Hwang JH, Choi KG, Kim WK, et al. Mutational analysis of PMP22, MPZ, GJB1, EGR2 and NEEL in Korean Charcot-Marie-Tooth neuropathy patients. Hum Mutat 2004;24: 185–186.
7. LeGuern E, Guilbot A, Kessali M, Ravisé N, Tassin J, Maisonobe T, et al. Homozygosity mapping of an autosomal recessive form of demyelinating Charcot-Marie-Tooth disease to chromosome 5q23-q33. Hum Mol Genet 1996;5:1685–1688.
8. Gabreëls-Festen A, van Beersum S, Eshuis L, LeGuern E, Gabreëls F, van Engelen B, et al. Study on the gene and phenotypic characterisation of autosomal recessive demyelinating motor and sensory neuropathy (Charcot-Marie-Tooth disease) with a gene locus on chromosome 5q23-q33. J Neurol Neurosurg Psychiatry 1999;66: 569–574.
9. Guilbot A, Ravisé N, Bouhouche A, Coullin P, Birouk N, Maisonobe T, et al. Genetic, cytogenetic and physical refinement of the autosomal recessive CMT linked to 5q31-q33: exclusion of candidate genes including EGR1. Eur J Hum Genet 1999;7:849–859.
10. Azzedine H, Ravisé N, Verny C, Gabreëls-Festen A, Lammens M, Grid D, et al. Spine deformities in Charcot-Marie-Tooth 4C caused by SH3TC2 gene mutations. Neurology 2006;67:602–606.
11. Senderek J, Bergmann C, Stendel C, Kirfel J, Verpoorten N, De Jonghe P, et al. Mutations in a gene encoding a novel SH3/TPR domain protein cause autosomal recessive Charcot-Marie-Tooth type 4C neuropathy. Am J Hum Genet 2003;73:1106–1119.
12. Arnaud E, Zenker J, de Preux Charles AS, Stendel C, Roos A, Médard JJ, et al. SH3TC2/KIAA1985 protein is required for proper myelination and the integrity of the node of Ranvier in the peripheral nervous system. Proc Natl Acad Sci U S A 2009;106: 17528–17533.
13. Gooding R, Colomer J, King R, Angelicheva D, Marns L, Parman Y, et al. A novel Gypsy founder mutation, p.Arg1109X in the CMT4C gene, causes variable peripheral neuropathy phenotypes. J Med Genet 2005;42:e69.
14. Colomer J, Gooding R, Angelicheva D, King RH, Guillén-Navarro E, Parman Y, et al. Clinical spectrum of CMT4C disease in patients homozygous for the p.Arg1109X mutation in SH3TC2. Neuromuscul Disord 2006;16:449–453.
15. Claramunt R, Sevilla T, Lupo V, Cuesta A, Millán JM, Vílchez JJ, et al. The p.R1109X mutation in SH3TC2 gene is predominant in Spanish Gypsies with Charcot-Marie-Tooth disease type 4. Clin Genet 2007;71:343–349.
16. Houlden H, Laura M, Ginsberg L, Jungbluth H, Robb SA, Blake J, et al. The phenotype of Charcot-Marie-Tooth disease type 4C due to SH3TC2 mutations and possible predisposition to an inflammatory neuropathy. Neuromuscul Disord 2009;19:264–269.
17. Baets J, Deconinck T, De Vriendt E, Zimoń M, Yperzeele L, Van Hoorenbeeck K, et al. Genetic spectrum of hereditary neuropathies with onset in the first year of life. Brain 2011;134: 2664–2676.
18. Fischer C, Trajanoski S, Papić L, Windpassinger C, Bernert G, Freilinger M, et al. SNP array-based whole genome homozygosity mapping as the first step to a molecular diagnosis in patients with Charcot-Marie-Tooth disease. J Neurol 2012;259:515–523.
19. Laššuthová P, Mazanec R, Vondráček P, Sišková D, Haberlová J, Sabová J, et al. High frequency of SH3TC2 mutations in Czech HMSN I patients. Clin Genet 2011;80:334–335.
20. Yger M, Stojkovic T, Tardieu S, Maisonobe T, Brice A, Echaniz-Laguna A, et al. Characteristics of clinical and electrophysiological pattern of Charcot-Marie-Tooth 4C. J Peripher Nerv Syst 2012;17: 112–122
21. Gosselin I, Thiffault I, Tétreault M, Chau V, Dicaire MJ, Loisel L, et al. Founder SH3TC2 mutations are responsible for a CMT4C French-Canadians cluster. Neuromuscul Disord 2008;18: 483–492.
22. Deymeer F, Matur Z, Poyraz M, Battaloglu E, Oflazer-Serdaroglu P, Parman Y. Nerve conduction studies in Charcot-Marie-Tooth disease in a cohort from Turkey. Muscle Nerve 2011;43:657–664.
23. Lewis RA, Sumner AJ. The electrodiagnostic distinctions between chronic familial and acquired demyelinative neuropathies. Neurology 1982;32:592–596.
24. Kaku DA, Parry GJ, Malamut R, Lupski JR, Garcia CA. Uniform slowing of conduction velocities in Charcot-Marie-Tooth polyneuropathy type 1. Neurology 1993;43:2664–2667.
25. Lewis RA, Sumner AJ, Shy ME. Electrophysiological features of inherited demyelinating neuropathies: A reappraisal in the era of molecular diagnosis. Muscle Nerve 2000;23:1472–1487.
26. Nakamura T, Hashiguchi A, Suzuki S, Uozumi K, Tokunaga S, Takashima H. Vincristine exacerbates asymptomatic Charcot-Marie-Tooth disease with a novel EGR2 mutation. Neurogenetics 2012;13: 77–82.
27. Horacek O, Mazanec R, Morris CE, Kobesova A. Spinal deformities in hereditary motor and sensory neuropathy: a retrospective qualitative, quantitative, genotypical, and familial analysis of 175 patients. Spine 2007;32:2502–2508.
28. Nagai MK, Chan G, Guille JT, Kumar SJ, Scavina M, Mackenzie WG. Prevalence of Charcot-Marie-Tooth disease in patients who have bilateral cavovarus feet. J Pediatr Orthop 2006;26:438–443.
29. Guthmundsson B, Olafsson E, Jakobsson F, Lúthvígsson P. Prevalence of symptomatic Charcot-Marie-Tooth disease in Iceland: a study of a well-defined population. Neuroepidemiology 2010;34:13–17.
30. Lupski JR, Reid JG, Gonzaga-Jauregui C, Rio Deiros D, Chen DC, Nazareth L, et al. Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy. N Engl J Med 2010;362: 1181–1191.