European Journal of Neurology 2007, 14: 1182–1185 doi:10.1111/j.1468-1331.2007.01917.x

SHORT COMMUNICATION

Charcot-Marie-Tooth neuropathy type 1A combined with Duchenne muscular dystrophy

P. Vondracek[a], M. Hermanova[b], J. Sedlackova[c], L. Fajkusova[c], D. Stary[d], A. Michenkova[e], R. Gaillyova[e], P. Seeman[f] and R. Mazanec[g]

Departments of [a]Paediatric Neurology, [b]Pathology, [c]Molecular Biology and Gene Therapy, [d]Paediatric Surgery, and [e]Clinical Genetics, University Hospital and Masaryk University, Brno, Czech Republic; Department of [f]Child Neurology and [g]Neurology, Second School of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic

Keywords:
Charcot-Marie-Tooth disease, CMT1A, Duchenne muscular dystrophy, hereditary neuropathy

Received 2 May 2007
Accepted 16 June 2007

We report a 24-year-old male with an unusual combination of two inherited neuromuscular disorders – Charcot-Marie-Tooth (CMT) disease type 1A and Duchenne muscular dystrophy (DMD). A phenotypic presentation of this patient included features of both these disorders. Nerve conduction studies revealed demyelinating peripheral neuropathy. Electromyography showed a profound myogenic pattern. The serum creatine kinase level was highly elevated. Muscle biopsy revealed a dystrophic picture with deficient dystrophin immunostaining. CMT1A duplication on chromosome 17p11.2 was found. The frame-shift mutation c.3609–3612delTAAAinsCTT (p.K1204LfsX11) was detected in the dystrophin gene by analysing mRNA isolated from the muscle tissue. The patient inherited both these mutations from his mother. The combination of CMT1A and DMD has not been reported as yet.

Introduction

Charcot-Marie-Tooth (CMT) disease, affecting one in 2500 people, is the most frequent hereditary peripheral neuropathy. CMT1A, the most common form, is autosomal dominantly inherited and caused by the duplication of a 1.5-Mb portion of chromosome 17 containing the *PMP22* gene (peripheral myelin protein 22) located within the region 17p11.2-p12. The affected individual has three instead of two functional copies of the *PMP22* gene [1–4]. *PMP22* overexpression leads to impaired myelination of peripheral nerves resulting in slowly progressive distal demyelinating sensorimotor polyneuropathy and the typical CMT phenotype. Patients display pes cavus, distal muscle weakness, peroneal muscle atrophy, inability to walk on the heels, deep tendon areflexia and distal sensory disturbance. Motor nerve conduction velocity is slowed in the typical range of 10–30 m/s.

Duchenne and Becker muscular dystrophies (DMD/BMD) are allelic variants of dystrophinopathy and rank among the most frequent and serious hereditary muscle diseases, leading to a serious physical handicap as early as in childhood. DMD is an allelic variant with a severe phenotype; afflicted boys are already wheelchair-bound around 12 years of age and usually die in the third decade because of respiratory insufficiency or pneumonia. BMD is a less frequent and milder variant; patients survive and are able to walk well into adulthood, but face a high risk of heart failure.

The disease has an X-recessive trait and is caused by mutations in the dystrophin gene located in the Xp21 region. Prevalent mutations are deletions of single or multiple exons responsible for about 60% of DMD/BMD cases. Duplications and point mutations are much less frequent (http://www.dmd.nl/dmdprobe.html) [5]. Because of the consequent dystrophin deficiency, patients suffer from progressive muscle weakness. Mutations disrupting the translational reading frame of the dystrophin transcript and thus leading to prematurely aborted dystrophin synthesis cause DMD, while in-frame mutations result in less severe BMD phenotype. Women are usually asymptomatic carriers. The incidence of DMD is estimated to be 1:3500 and that of less frequent BMD 1:17 000 newborn boys [5].

Case report

A male child was born at full term to unrelated parents. A failure to gain developmental motor milestones was observed at 6 months of age. He started to walk at 18 months and used the Gowers manoeuvre to rise. At age 4 years, his serum creatine kinase (CK) level was found to be grossly elevated (6000 IU/l) and the diagnosis of muscular dystrophy was made. Proximal muscle weakness and wasting deteriorated during the following years and bilateral pes equinovarus deformity developed. He has been wheelchair-bound since the age of 12 years. On recent examination at age 24 years, he displayed a profound proximal and distal muscle

Correspondence: P. Vondracek MD, PhD, Department of Paediatric Neurology, University Hospital, Cernopolni 9, 625 00 Brno, Czech Republic (tel.: +420-5-3223 4934; e-mail: pvondracek@fnbrno.cz).

weakness in the upper and lower extremities, and quadriceps atrophy in contrast with marked calf pseudohypertrophy (Fig. 1). Strength in proximal and distal muscles was ≤2 medical research council (MRC) grade. Bilateral pes equinovarus, contractures of the knees and Achilles tendons were prominent. Deep tendon reflexes were absent. A mild sensory loss in distal parts of the upper and lower extremities was noted. His cardiologic examination with electrocardiography and echocardiography revealed a mild dilated cardiomyopathy. He reported only mild occasional respiratory insufficiency. No scoliosis was observed. The patient was alert, intelligent and cooperative.

Nerve conduction study results were consistent with a diffuse motor and sensory demyelinating neuropathy. Motor nerve conduction velocities were markedly slow (28 m/s in the median nerve and 31 m/s in the ulnar nerve). Compound muscle action potentials (CMAPs) of tibial and peroneal nerves were not elicited bilaterally. Distal motor latencies were prolonged (10.4 ms for the median nerve and 7.6 ms for the ulnar nerve). Sensory nerve potentials were not elicited in both upper and lower extremities. Electromyograph of deltoid and quadriceps muscles showed a typical myopathic pattern with motor unit potentials of short duration and low amplitude, and also some long-duration polyphasic motor unit potentials because of the chronicity of the myopathic process, as well as superimposed neuropathy.

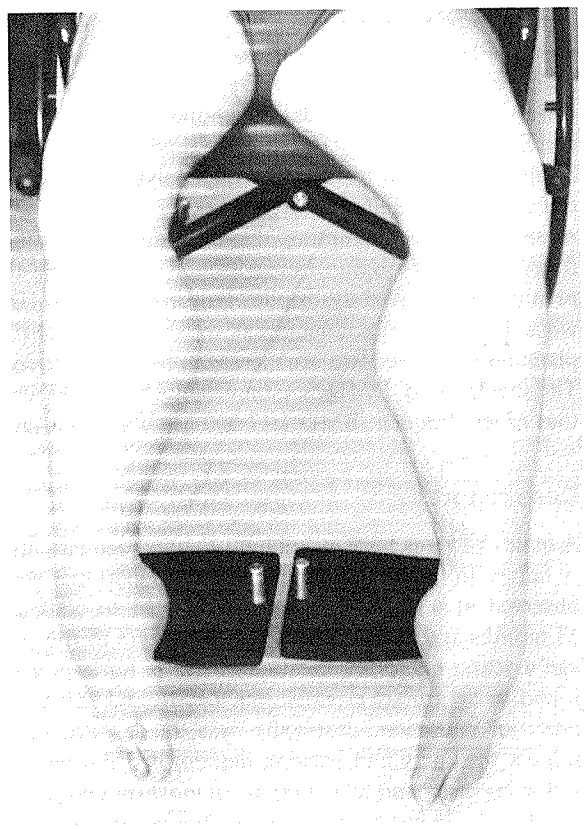

Figure 1 Quadriceps atrophy and calf pseudohypertrophy are seen in the lower extremities.

A needle muscle biopsy from the right deltoid was performed at age 24 years. Part of the tissue obtained from the needle biopsy was homogenized for Western blot analysis, but low concentrations of proteins did not enable a valid performance of this examination. The rest of the muscle biopsy specimen was snap-frozen in a propane–butane mixture, chilled in liquid nitrogen and then stored at −80°C until process. Five-micrometre-thick tissue cryosections were examined using conventional histological techniques. Haematoxylin and eosin-stained slides were examined and almost total replacement of muscle tissue by adipose and fibrous tissue with residual muscle fibres was revealed (Fig. 2a).

Commercial antibodies obtained from Novocastra Laboratories Ltd (Newcastle upon Tyne, UK) and Upstate Cell Signaling Solutions (Lake Placid, NY, USA) were used for immunohistochemical analysis of muscle biopsy sections using standard methodologies. Expressions of dystrophin (NCL-DYS1, dilution 1:10; NCL-DYS2, 1:10; NCL-DYS3, 1:20), N-utrophin (NCL-DRP2, 1:10), sarcoglycans (NCL-a-SARC, 1:100; NCL-b-SARC, 1:100; NCL-c-SARC, 1:100; NCL-g-SARC, 1:50), spectrin (SPEC1, 1:100), and alpha-dystroglycan (anti-a dystroglycan, clone VIA4-1, 1:500) were examined. The low amount of the tissue did not allow for a more extensive examination. A deficient expression of dystrophin was observed and mutational analysis of the dystrophin gene was suggested based on these results (Fig. 2b). Upregulation of N-utrophin was not observed in the examined fibres, and the expression of other proteins examined was normal.

Family history showed that CMT was a familial trait on the maternal side of the pedigree (Fig. 3). The proband, his mother, two of his aunts, and his grandfather were affected by hereditary neuropathy. The proband's mother displayed a typical CMT phenotype, motor nerve conduction velocities were markedly slowed in the range of 24–36 m/s, the EMG showed no signs of myopathy, and her serum CK level was normal. The patient had a healthy brother, who showed normal electrophysiological findings, and a normal CK level.

Molecular genetic studies were performed on all family members. DNA was extracted from leucocytes after informed consent had been obtained. The diagnosis of CMT1A was verified in 1998, when the proband was 16 years old, and the CMT1A duplication on chromosome 17p11.2 was confirmed with a set of eight microsatellite markers in two multiplex polymerase

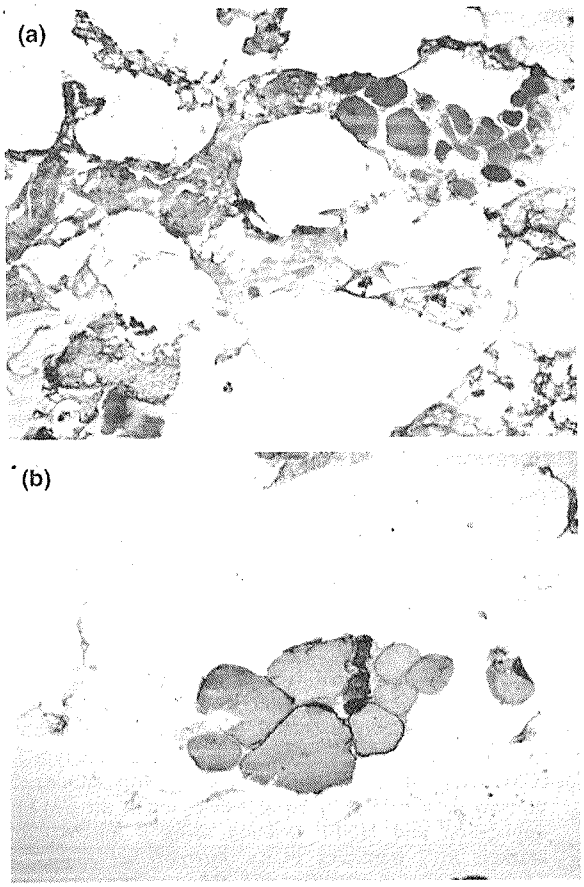

Figure 2 (a) Muscle biopsy with large amount of adipose and fibrous tissue and several residual muscle fibres with significant variation in fibre size (haematoxylin–eosin staining, original magnification ×100). (b) Deficient expression of dystrophin on residual muscle fibres, totally negative muscle fibres and fibres with partial expression of dystrophin in sarcolemmal localization (dystrophin immunohistochemistry (DYS2), original magnification ×200).

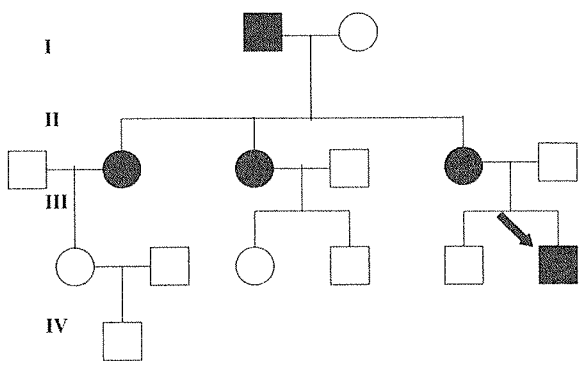

Figure 3 Family pedigree. Empty circles indicate unaffected females; empty squares, unaffected males; filled circles, affected females; filled squares, affected males; the proband is marked with an arrow.

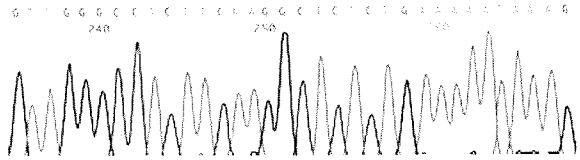

Figure 4 Sequence analysis of the part of DMD exon 27; sequence with mutation c.3609–3612delTAAAinsCTT: *...GTTGGGCCTCTTCAAGGCTCT...* (standard sequence: *...GTTGGGCCTCTTCTTTAGCTCT...*).

chain reactions (PCRs) [4]. The CMT1A duplication was detected in the proband and three other family members with a CMT phenotype. In the case of the patient's healthy brother, the CMT1A DNA analysis was negative.

Molecular genetic testing was completed in 2006, when the patient was 24 years old, by the mRNA analysis of the dystrophin gene using RNA isolation from the muscle tissue, reverse transcription, PCR, protein truncation test and sequencing. The frame-shift mutation c.3609–3612delTAAAinsCTT (p.K1204L fsX11) was detected and molecular diagnosis of DMD established. Analysis of mRNA was confirmed by analysis on the DNA level (sequencing of exon 27) (Fig. 4). The same mutation in a heterozygous state was found in the patient's mother. This mutation has not been reported in the Leiden DMD mutation database yet (http://www.dmd.nl).

Discussion

We present a 24-year-old male with an unusual combination of two inherited neuromuscular disorders – CMT disease type 1A and DMD. Two independent mutations responsible for a unique clinical phenotype were detected. The CMT1A duplication on chromosome 17p11.2 was found to be a familial autosomal dominant trait on the maternal side of the pedigree. DMD because of a frameshift mutation in the dystrophin gene was also inherited from the patient's mother, who was found to be a carrier.

The reported prevalence of DMD/BMD is 30 per 100 000 male births [6], and that of CMT1A can be 40 per 100 000 [1]. It can be calculated from these data that the probability of inheriting both these disorders is approximately 1 in 10 000 000. To the best of our knowledge, the combination of CMT1A and DMD has not been reported as of yet, and only two further cases of a combination of CMT disease with a muscular dystrophy have been described. Bütefisch *et al.* reported a female who inherited facioscapulohumeral muscular dystrophy and CMT1A from her parents [7]. Bergmann *et al.* described a young male with a combination of two

X-linked disorders – BMD and X-linked CMT neuropathy [6]. This patient inherited CMT disease from his mother, while a second mutation causing BMD was sporadic.

A phenotypic presentation of our patient included features of both peripheral neuropathy and a muscular dystrophy. Hereditary neuropathy manifested by distal muscle weakness, bilateral pes equinovarus, the absence of deep tendon reflexes, sensory loss and markedly reduced nerve conduction velocities. Muscular dystrophy presented with proximal muscle weakness and wasting, calf pseudohypertrophy, dilated cardiomyopathy and elevated serum CK level. A myopathic pattern on the EMG, a severe dystrophic picture revealed by a muscle biopsy, and deficient dystrophin immunostaining were indicative of DMD. The early onset of the disease and the young age at which our proband became wheelchair-bound were also consistent with a diagnosis of DMD.

This is probably the first time when the combination of CMT1A and DMD was observed, because CMT disease is highly variable and can present with a milder phenotype than the one described in this patient. It is therefore possible that other cases exist in which the CMT phenotype is completely masked by the concomitant severe DMD phenotype. In this patient both phenotypes were manifest. Another important feature is the failure to gain developmental motor milestones observed at 6 months of age as early motor milestones are usully normal in DMD and obvious in CMT1A patients. In this respect the association of DMD and CMT1A may have played a role in anticipating the clinical onset.

From the differential diagnostic point of view the association of a demyelinating sensory motor neuropathy and a muscular dystrophy could also occur in the case of merosin (laminin-α2) deficiency. Obviously this patient has a clear AD inheritance pattern for neuropathy, while laminin-α2 deficiency is an autosomal recessive (AR) disease.

Almost 20 years after the clinical diagnosis had been established, the molecular basis of both CMT1A and DMD was clarified in this patient. Our case is an instructive example of an aggravating effect of a muscular dystrophy on the course of hereditary neuropathy resulting in an unusual clinical syndrome.

Acknowledgements

The study was supported by Parent Project Czech Republic, the CMT Society Czech Republic, and with grants A8608-4, MZO 00064203/6506 (Internal Grant Agency of the Ministry of Health of the Czech Republic), and MSM0021622415 (The Ministry of Education of the Czech Republic).

References

1. De Jonghe P, Timmerman V, Nelis E, Martin JJ, Van Broeckhoven C. Charcot-Marie-Tooth disease and related peripheral neuropathies. *Journal of the Peripheral Nervous System* 1997; **4**: 370–387.
2. Grandis M, Shy ME. Current therapy for Charcot-Marie-Tooth disease. *Current Treatment Options in Neurology* 2005; **1**: 23–31.
3. Pleasure D. New treatments for denervating diseases. *Journal of Child Neurology* 2005; **3**: 258–262.
4. Seeman P, Mazanec R, Zidar J, Hrusakova S, Ctvrteckova M, Rautenstrauss B. Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP): reliable detection of the CMT1A duplication and HNPP deletion using 8 microsatellite markers in 2 multiplex PCRs. *International Journal of Molecular Medicine* 2000; **6**: 421–426.
5. van Deutekom JC, van Ommen GJ. Advances in Duchenne muscular dystrophy gene therapy. *Nature Review Genetics* 2003; **10**: 774–783.
6. Bergmann C, Senderek J, Hermanns B, *et al.* Becker muscular dystrophy combined with X-linked Charcot-Marie-Tooth neuropathy. *Muscle and Nerve* 2000; **5**: 818–823.
7. Bütefisch CM, Lang DF, Gutmann L. The devastating combination of Charcot-Marie-Tooth disease and facioscapulohumeral muscular dystrophy. *Muscle and Nerve* 1998; **6**: 788–791.