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Phenotypic Variability in a Large Czech Family with a Dynamin 2–Associated Charcot-Marie-Tooth Neuropathy

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Abstract: Mutations in the *Dynamin 2* gene (*DNM2*) cause autosomal dominant centronuclear myopathy or autosomal dominant (AD) Charcot-Marie-Tooth (CMT) disease. Here the authors report one large Czech family with 15 members affected with an AD CMT phenotype of extraordinary variability. Genetic linkage analysis using SNP arrays revealed a locus of about 9.6 Mb on chromosome 19p13.1–13.2. In this critical interval, 373 genes were located. The only gene herein known to be associated with an intermediate type of CMT was *Dynamin 2* (*DNM2*). Subsequent sequence analysis of the *DNM2* gene in the index patient revealed a novel missense mutation p.Met580Thr. This missense mutation segregated with the neuropathy, indicating the causal character of this mutation. The phenotype of CMT in this family shows mild to moderate impairment with relatively preserved upper limbs and a very broad range of the onset of clinical symptoms from an early onset around the age of 12 to the late onset during the fifth decade. Electrophysiology showed an intermediate type of peripheral neuropathy. The motor median nerve conduction velocity varied from 36 m/s to normal values with signs of asymmetrical affection of peripheral nerves. No additional symptoms such as cranial nerve involvement, cataract, and signs of neutropenia or myopathy syndrome were observed in any member of the family yet. The progression was slow with no loss of ambulation. The authors suggest that the characterization of clinical variability in a single family may help to direct the genetic analysis directly to the rarely observed *DNM2* mutations.

Keywords: CMT, Dynamin 2, DNM2, peripheral neuropathy

INTRODUCTION

Dynamin 2 (*DNM2*) gene mutations have been associated with two distinct clinical entities: autosomal dominant centronuclear myopathy (Bitoun et al., 2005), and a rare form of autosomal dominant Charcot-Marie-Tooth (CMT) peripheral neuropathy (Zuchner et al., 2005). Only few mutations in *DNM2* have been reported yet, therefore our knowledge on neurological phenotypes associated with *DNM2* mutations is very limited.

Dynamin 2 belongs to the family of large GTPases and is mainly involved in intracellular membrane trafficking, endo- and exocytosis, actin network, and centrosome cohesion (Durieux et al., 2010b; Thompson et al., 2004). It is a ubiquitously present protein. With regard to neuromuscular disorders expression in peripheral nerves and

in the skeletal muscle, it is of special interest (Durieux et al., 2010b). It is composed of five protein domains, an N-terminal GTPase domain, which is responsible for GTP binding and hydrolysis, a middle domain (MD) with self-assembly function, a pleckstrin homology (PH) domain that mediates membrane binding, GTPase effector domain (GED), and a C-terminal proline-rich domain that mediates multiple protein-protein interactions (Durieux et al., 2010b). Yet eight CMT-associated mutations are predominantly in PH and GED protein domains (Bitoun et al., 2005; Fabrizi et al., 2007; Zuchner et al., 2005). Recently mutation in the MD domain was also reported (Claeys et al., 2009). These mutations are heterozygous missense mutations or small deletions (Durieux et al., 2010a, 2010b). In autosomal dominant centronuclear myopathy, mutations are mainly situated in MD and PH protein domain of

DNM2 (Bitoun et al., 2005; Durieux et al., 2010a). The molecular pathways via which *DNM2* mutations result in two distinct neuromuscular disorders are not yet clear.

CMT caused by *DNM2* mutations was reported in families with intermediate or axonal forms of hereditary neuropathies (Bitoun et al., 2008; Claeys et al., 2009; Fabrizi et al., 2007; Zuchner et al., 2005). In some patients, cataract and neutropenia together with ptosis and ophthalmoparesis in addition to peripheral neuropathy were reported (Bitoun et al., 2008; Claeys et al., 2009).

Here we report the clinical and electrophysiological variability in patients of a large Czech family with a novel *DNM2* mutation, c.1739T→C, p.Met580Thr.

METHODS AND PATIENTS

Pedigree and Genetic Testing

Overall 21 family members were examined clinically and electrophysiologically. Fifteen family members were finally diagnosed as being affected by CMT of variable age of onset and severity (Figure 1). Eight of these patients have been examined in more detail at the Neurological Departments of the University Hospital Prague-Motol and the Thomayer University Hospital Prague-Krč. We performed a standard neurological examination, motor and sensory nerve conduction studies, and needle electromyography. Electrophysiology was performed using standard techniques.

DNA extraction from blood was done according to standard protocols (Qiagen, Hildesheim, Germany). Mutation in the genes coding peripheral myelin protein 22 (PMP22) and myelin protein zero (MPZ/PO) were previously excluded by sequencing of all coding exons in two index patients. For genome-wide linkage analysis,

we genotyped DNA samples from nine affected and three none-affected family members using the Affymetrix GeneChip® Human Mapping 10K Array (Figure 2). Calculation was done assuming a reduced penetrance of 99%. We verified sample genders by counting heterozygous SNPs on the X chromosome. Relationship errors were evaluated with the help of the program Graphical Relationship Representation (Abecasis et al., 2001).

The program PedCheck was applied to detect Mendelian errors and data for single-nucleotide polymorphisms (SNPs) with such errors were removed from the data set. Non-Mendelian errors (O'Connell et al., 1998) were identified by using the program MERLIN (Abecasis et al., 2002) and unlikely genotypes for related samples were deleted. Linkage analysis was performed assuming autosomal recessive inheritance, full penetrance, and a disease gene frequency of 0.0001. Multipoint LOD scores were calculated using the program ALLEGRO (Gudbjartsson et al., 2000). Haplotypes were reconstructed with ALLEGRO and presented graphically with HaploPainter (Thiele & Nurnberg, 2005). All data handling was performed using the graphical user interface ALOHOMORA (Ruschendorf & Nurnberg, 2005).

Sequencing of the *DNM2* gene was done initially in all coding exons in two index patients and a novel missense mutation c.1739T→C, p.Met580Thr in the plekstrin domain was found. All available family members were subsequently tested by sequencing for the presence of this mutation in exon 16.

Molecular Modeling

Initial analyses were performed for the PH domain of Dynamin 1, since there is no crystal structure available
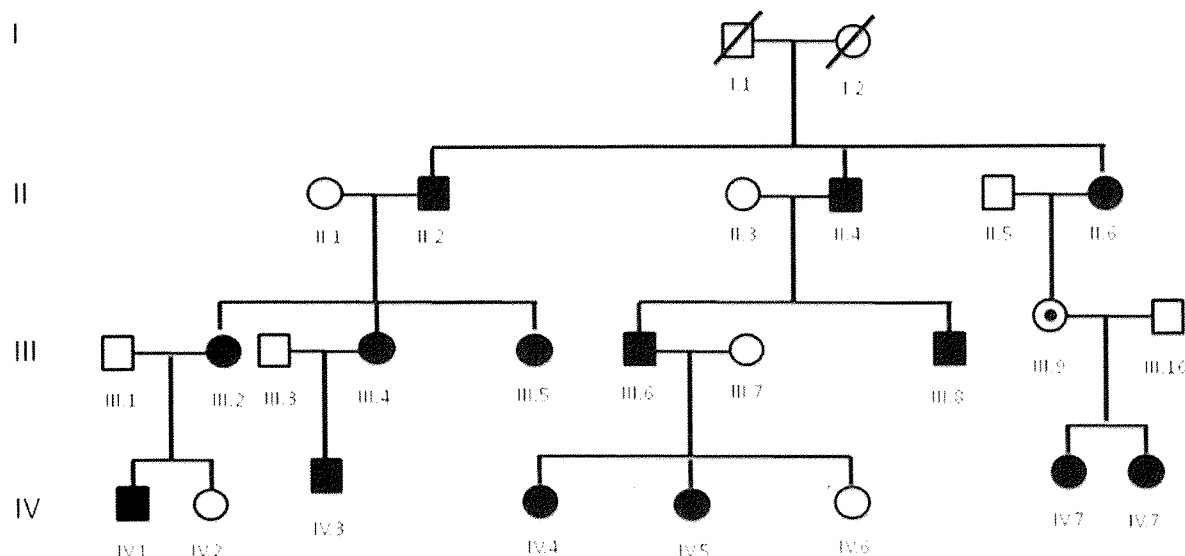

Figure 1. Pedigree of family Cz-CMT-J. Filled symbols indicate affected members who are also DNM2 mutation carriers. For III.9, detailed clinical data were not yet available, hence she is marked only as carrier.
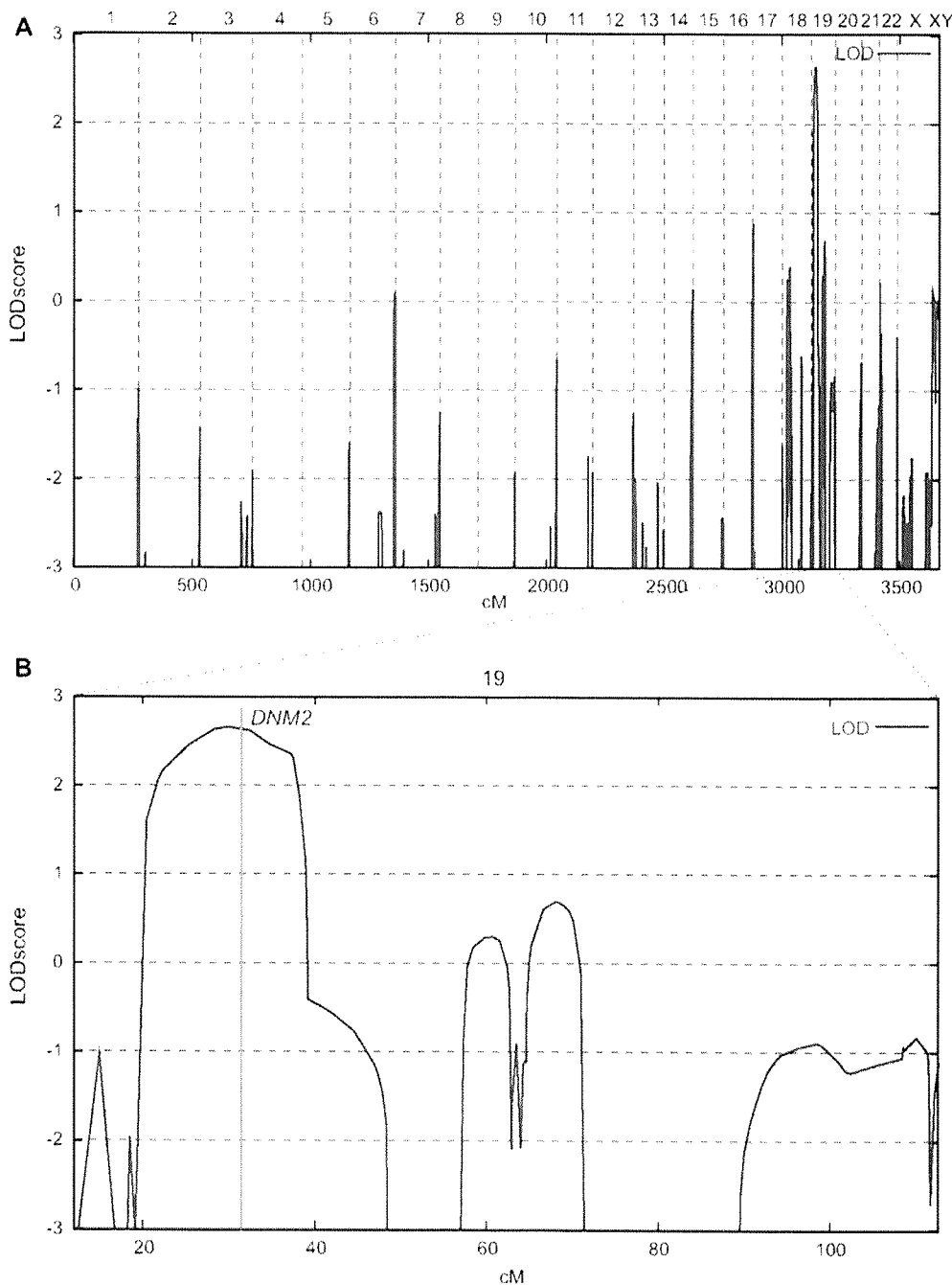
Figure 2. (A) Schematic representation of genome-wide LOD score calculations after 10K array SNP genotyping. LOD scores calculated with ALLEGRO are given along the *y*-axis relative to genomic position in cM (centiMorgan) on the *x*-axis. Note the highest peak (LOD = 2.66) in the region on chromosome 19. (B) Magnification of chromosome 19. The red line indicates the position of the DNM2 gene.

for the PH domain of Dynamin 2. The PH domains of Dynamin 1 and 2 share a sequence similarity of 97%. A model of the dimeric Dynamin 1 PH domain in complex with the membrane was obtained from the OPM server (Lomize et al., 2006). Molecular modeling of the Dynamin 2 PH domains and of the p.M580T mutation was performed using SwissModel (Arnold et al., 2006). RasMol (Sayle & Milner-White, 1995) was used for structure analysis and figure preparation (Figure 3).

RESULTS

Clinical Data

Pre- and perinatal history in all affected family members has been normal. There was no delay of motor development during the first 2 years of life. First symptoms appeared during the second decade of life in most of the patients (5/8); however, age of onset was variable. One
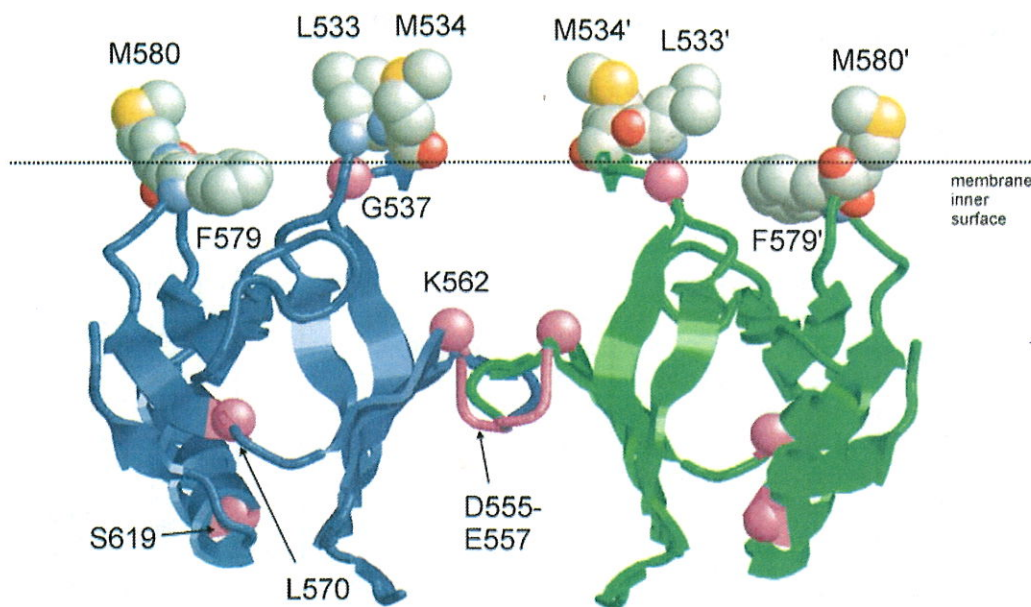
Figure 3. Model of the Dynamin 2 PH domain in complex with the membrane. In each subunit of the dimeric PH domain (blue, green), two loops interact with the membrane (the membrane inner surface is shown as a dashed line). Each loop exhibits a hydrophobic dipeptide motif (L533–M534, F579–M580) that penetrates into the membrane. The respective residues are shown in space-filled presentation (heavy atoms only) and colored according to the atom type (carbon, grey; oxygen, red; nitrogen, blue; sulfur, yellow). In addition, those residues for which CMT2-related mutations have been reported in the past are shown in magenta and are labeled in one of the subunits. The types of mutations reported are: G537C, D555–E557del, K562del, K562E, L570H, S619L, and S619W (see http://omim.org/entry/602378 for details). For the point mutations, the Cα carbon is shown as magenta sphere, and for residues D555–E557 found to be deleted in one mutant, the backbone is colored magenta. Some of these mutations have also been observed in the context of the shorter isoform 2 that lacks residues 516–519. Therefore, the respective positions differ by four in some reports (e.g., G537C of isoform 1 corresponds to G553C of isoform 2).

patient developed symptoms after the age of 46 years. One patient at the age of 24 years did not show any muscular weakness but suffered from muscle cramps after long stay. One patient at the age of 41 years was subjectively asymptomatic. The most typical first symptoms were difficulties in heel walking and in running.

In the clinical examination (Table 1), the main features were mild to moderate distal muscle weakness and atrophies in lower limbs predominantly on extensor maximum with relatively preserved L2/4 reflexes (6/8). Reflexes L5/S2 were usually absent (6/8). The muscle weakness and atrophy of upper limbs usually appeared after the third decade of life and were mild. The reflexes in upper limbs were mostly preserved (7/8). The pin sensibility was abnormal only in 2 out of 8 patients and was mild. Foot deformity type pes cavus was present only in 2/8 patients. The progression was slow with no loss of ambulation; patients in their 60s usually use a cane.

There was no cranial nerve involvement, no symptoms of myopathy, no cataract, and no history of leuko- or neutropenia in any of the affected members.

Seven members who do not carry the p.Met580Thr mutation were also examined and had no subjective symptoms of CMT and no abnormality in neurological examination.

Electrophysiological Results

Nerve conduction studies revealed in most of the patients a primary axonal motor and sensory peripheral neuropathy (7/8) (Table 2). One patient was classified to suffer from an intermediate type of CMT. Median nerves were more often (3/8) more severely affected than ulnar nerves and sensory fibers were in general more affected (6/8) than motor nerves. However, in 2 patients (mother and her daughter) the motor fibers of the peripheral nerves were predominantly affected and the diagnosis of distal spinal muscular atrophy (dSMA) was originally considered for them.

Needle electromyography (EMG) in all examined patients resulted in a chronic neurogenic pattern. In case of the clinically asymptomatic patient, the electrophysiology, with abnormal sensitive nerve conduction studies and chronic neurogenic pattern in needle EMG, was the only abnormality related to the *DNM2* mutation.

Seven family members that are not carriers of this mutation were examined and found to have no abnormality in electrophysiology.

The DNM2 mutation p.Met580Thr was found in all affected family members and in none of the unaffected family members. Only III.9 did not yet undergo a detailed

Table 1. Subjective and objective symptoms of carriers.

	Gender/age of examination/DNA number							
	F/24 y/IV.4	F/24 y/IV.3	F/34 y/IV.5	M/41 y/III.8	M/54 y/III.7	F/54 y/III.3	M/59 y/III.5	F/67 y/III.2
Age of onset	14 y	Crampi after long stay	14 y	No symptoms	11 y	46 y	19 y	17 y
Sensory symptoms	No	No	Limited to toes and fingers	No	No	No	Limited to toes and fingers	Limited to toes and fingers
Motor symptoms arms	No	No	Reduced in fingers	No	Reduced in fingers	No	Reduced in fingers	Reduced in fingers
Motor symptoms legs	Slaps feet	No	Slaps feet	No	Ankle surgery	Use cane	Use cane	Use cane
Pin sensibility	Normal	Normal	Reduced in fingers/ toes	Normal	Reduced up to wrist/ankle	Normal	Normal	Normal
Vibration	Reduced at wrist/ankle	Normal	Normal	Normal	Normal	Reduced at elbow/knee	Normal	Normal
Strength of arms (MRC)	Normal	Normal	Normal	Weak about elbow	Weak about elbow	4 on intrinsics muscles	4 on intrinsics muscles	Weak about elbow
Strength of legs (MRC)	4 on foot dorsiflexion	Normal	4 on foot dorsiflexion	Proximal weakness	Proximal weakness	Proximal weakness	Proximal weakness	Proximal weakness

MRC = Medical Research Council scale.

Table 2. Electrophysiologic examination of carriers.

	Gender/age of examination/DNA number							
	F/24 y/IV.4	F/24 y/IV.3	F/34 y/IV.5	M/41 y/III.8	M/54 y/III.7	F/54 y/III.3	M/59 y/III.5	F/67 y/III.2
MNCV/CMAP n.medianus	40/0.8	55/7	42/1	51/7	Not done	Not done	36/3	Not done
MNCV/CMAP n.ulnaris	39/6	59/7	46/6	56/10	34/4	53/6	39/6	46/4
MNCV/CMAP n.tibialis	Absent	50/0.8	Absent	40/5	Absent	Absent	Absent	Absent
MCV/CMAP n.peroneus	Absent	46/3	Absent	44/9	Absent	Absent	Absent	Absent
SNCV/SNAP n.medianus	Not done	53/17	58/4	37/9	Not done	Not done	Absent	Not done
SNCV/SNAP n.ulnaris	Not done	Not done	Not done	44/12	39/4	42/7	Absent	38/3
SNCV/SNAP n.suralis	Absent	51/4	Not done	37/6	Absent	42/8	Absent	Absent
Needle EMG	Chronic neurogenic pattern	Chronic neurogenic pattern	Chronic neurogenic pattern	Chronic neurogenic pattern	Chronic neurogenic pattern	Chronic neurogenic pattern	Chronic neurogenic pattern	Chronic neurogenic pattern

NCV = motor nerve conduction velocity (m/s); CMAP = compound muscle action potential (µV); SNCV = sensory nerve conduction velocity (m/s); SNAP = sensory nerve action potential (µV).

clinical examination (Figure 1). So the mutation segregates with the CMT phenotype in the family. It also was not found in 120 ethnically matched control chromosomes. Therefore, we concluded that the p.Met580Thr mutation in *DNM2* is causative for CMT in this family.

Molecular Modeling

Molecular modeling suggests that each PH domain of Dynamin 2 is anchored in the membrane via two loops (Figure 3). In each of the loops a hydrophobic dipeptide motif (L533–M534, F579–M580) penetrates into the membrane. These four residues are highly conserved between Dynamin 1 and Dynamin 2, suggesting that both PH domains interact in a highly similar fashion with the membrane (M534, F579, and M580 are strictly conserved and a conservative replacement of isoleucine by leucine is present at position 533).

This computationally predicted membrane orientation is also supported by the experimental finding that a mutation of I533 to cysteine in Dynamin 1 hampers membrane bending and subsequent membrane fission (Ramachandran et al., 2009). The observation that mutation of a single hydrophobic residue has a drastic effect on membrane interaction is also consistent with the fact that pairs of adjacent hydrophobic residues are frequently required for the membrane association of PH domains (Manna et al., 2007).

A replacement of M580 by a polar threonine will affect the conserved hydrophobic membrane interaction motif (F579–M580) and is therefore expected to affect membrane scission in a similar fashion than the experimental I533C mutation in Dynamin 1.

DISCUSSION

Here we report the phenotype for a novel DNM2 mutation p.Met580Thr within a large Czech family affected by autosomal dominant CMT of intermediate type. The family is probably one of the largest families ever reported for a DNM2 mutation and therefore enabled us to a more reliable genotype-phenotype correlation. We observed a broad variation of phenotype in the large number of carriers of the same mutation within this family.

The CMT phenotype within this family had mild-to-moderate impairment with relatively preserved upper limbs and only mild sensory disturbances, which were not even present in all affected members. The range of onset of clinical symptoms is very wide—from typical second decade of life to more rare late onset after the fifth decade of life. The wide range of clinical onset is similar to the findings of Claeys et al. (2009) however,

in that study some patients had an earlier age of onset during the first decade. The DNM2 mutation in the family presented here is located in the PH domain where the majority of mutations were reported, but mutations causing similar CMT phenotype were recently reported also for the middle domain and even the proline-rich domain of Dynamin 2 (Claeys et al., 2009). A strong genotype-phenotype correlation has not been reported in any of the yet published reports.

The electrophysiological pattern on peripheral nerve abnormality in individual patients of the family reported here is not diffuse as is typical for the most common CMT1 and CMT 2 types (Berciano et al., 2010). For example, motor fibers of median nerve are in some cases clearly more affected than motor fibers of ulnar nerve. Furthermore, in two cases there is a clear predominance of motor fiber affection over sensory fiber affection. This is in accordance with the clinical observation where the sensory impairment was even very mild. The overlap of *DNM2* CMT and distal spinal muscular atrophy has not yet been reported and will need confirmation in the future. Although the electrophysiological changes were predominantly axonal, the range for motor nerve conduction velocity in upper limbs varies from the normal values to 34 m/s and matches this novel *DNM2* mutation to the intermediate forms of CMT.

In our patients there were no clinical and electrophysiological signs of myopathy, cataract, or blood cell abnormalities present, as was observed in two families with mutations affecting the Lys558 aminoacid of the PH domain reported by Claeys et al. (2009).

Finally, the family reported here gives proof for the usefulness of linkage analysis in families with rare type of CMT.

In conclusion, DNM2 associated CMT is phenotypically heterogeneous and even one mutation in a single family may manifest at a very different age of onset. We propose to analyze the *DNM2* gene in patients with unclear intermediate or primary axonal CMT event with late onset of disease and to consider this diagnosis in patients with predominantly distal motor peripheral neuropathy.

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