Brain Advance Access published January 16, 2013

BRAIN
A JOURNAL OF NEUROLOGY

LETTER TO THE EDITOR

Czech family confirms the link between *FBLN5* and Charcot–Marie–Tooth type 1 neuropathy

Dana Šafka Brožková,[1] Petra Laššuthová,[1] Jana Neupauerová,[1] Marcela Krůtová,[1] Jana Haberlová,[1] David Stejskal[2] and Pavel Seeman[1]

1 DNA Laboratory, Department of Child Neurology, Charles University 2nd Medical School and University Hospital Motol, Prague, Czech Republic
2 Centre for Medical Genetics and Reproductive Medicine GENNET, Prague, Czech Republic

Correspondence to: Dana Šafka Brožková,
DNA Laboratory,
Department of Child Neurology,
Charles University 2nd Medical School and University Hospital Motol,
V Úvalu 84,
Prague 150 06,
Czech Republic
E-mail: dana.brozkova@lf2.cuni.cz

Sir,

The article by Auer-Grumbach *et al.* (2011) reporting that *fibulin 5* (*FBLN5*) mutations are also linked to inherited neuropathies helped us to clarify the cause of autosomal dominant, demyelinating hereditary motor and sensory neuropathy (HMSN I) in our long-time known, but unsolved, Czech family.

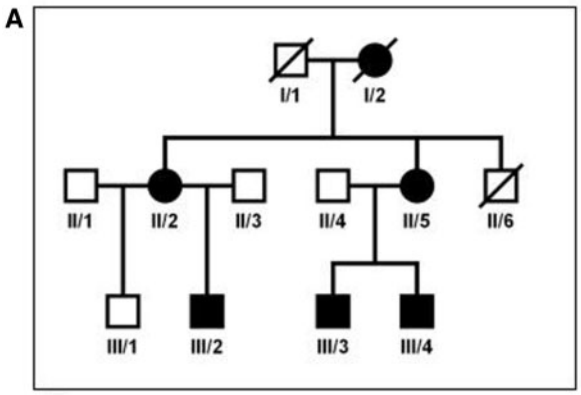
Before this study was published, mutations in *FBLN5* were associated only with the connective tissue disorder cutis laxa (Loeys *et al.*, 2002; Markova *et al.*, 2003; Lotery *et al.*, 2006; Nascimento *et al.*, 2010) and age-related macular degeneration leading to vision loss in those aged >50 years (Stone *et al.*, 2004; Lotery *et al.*, 2006). Auer-Grumbach *et al.* (2011) were the first to describe a large family with demyelinating Charcot–Marie–Tooth (CMT1) and linkage with the interval on chromosome 14. Subsequent resequencing of this region revealed only one novel variant c.1117 C > T (p.R373C) segregating with the disease in the family. Additional screening of 112 patients with Charcot–Marie–Tooth disease showed two other sequence variations in two patients with pure motor neuropathy and hyperelastic skin. The linkage with log of odds score >3.31 indicates the *FBLN5* mutation as highly probably causal for HMSN I, but no functional study to confirm this was performed, and no other study on *FBLN5* mutations in a family with HMSN has been published to date. This Austrian HMSN I family is the only one reported worldwide.

We report a second family with non-syndromic inherited demyelinating motor and sensory neuropathy (HMSN I) with *FBLN5* mutation and confirm *FBLN5* mutations as the new cause of HMSN I.

We used exome sequencing for clarification of the cause of HMSN I in a Czech family recruited in 2001 (Fig. 1A). The most frequent causes of CMT1 (CMT1 duplication/HNPP deletion, *GJB1* and *MPZ* genes) were excluded.

The phenotype of affected patients is mild and close to the manifestation of CMT1A, but with later clinical onset. The Charcot–Marie–Tooth disease neuropathy score ranged from 9 to 17 (Table 1), and the onset of the distal muscle weakness is in the third decade. Atrophies of small feet and hand muscles, reduced vibration sensation and sensory symptoms (paraesthesias) can be seen in the two oldest affected sisters (Patients II/2 and II/5). Three younger patients (Patients III/2, III/3 and III/4) have only minor symptoms of CMT1 and thus feel healthy (Fig. 1B), but according to the electrophysiological examination (Table 2), they undoubtedly have HMSN I. Demyelinating neuropathy can be

Received September 4, 2012. Revised October 15, 2012. Accepted October 16, 2012.



C

Physical position on chromosome 14	Number from db SNP	Affected haplotype Czech family	Affected haplotype Austrian family
87 138 729	rs1959290	B	B
87 138 772	rs1959291	B	B
87 727 149	rs1958843	B	B
87 949 260	rs1951507	B	B
88 165 569	rs951706	A	A
88 165 690	rs951705	A	A
88 365 902	rs1349377	A	A
88 485 777	rs2008686	B	A
89 376 271	rs727725	A	A
89 502 023	rs2401787	A	B
89 635 129	rs756470	A	B
89 673 252	rs997897	B	A
90 123 909	rs2401841	B	B
92 293 518	rs2402074	A	A
92 641 692	rs1985201	B	A
94 718 520	rs2007879	A	B
94 905 511	rs2011604	B	B

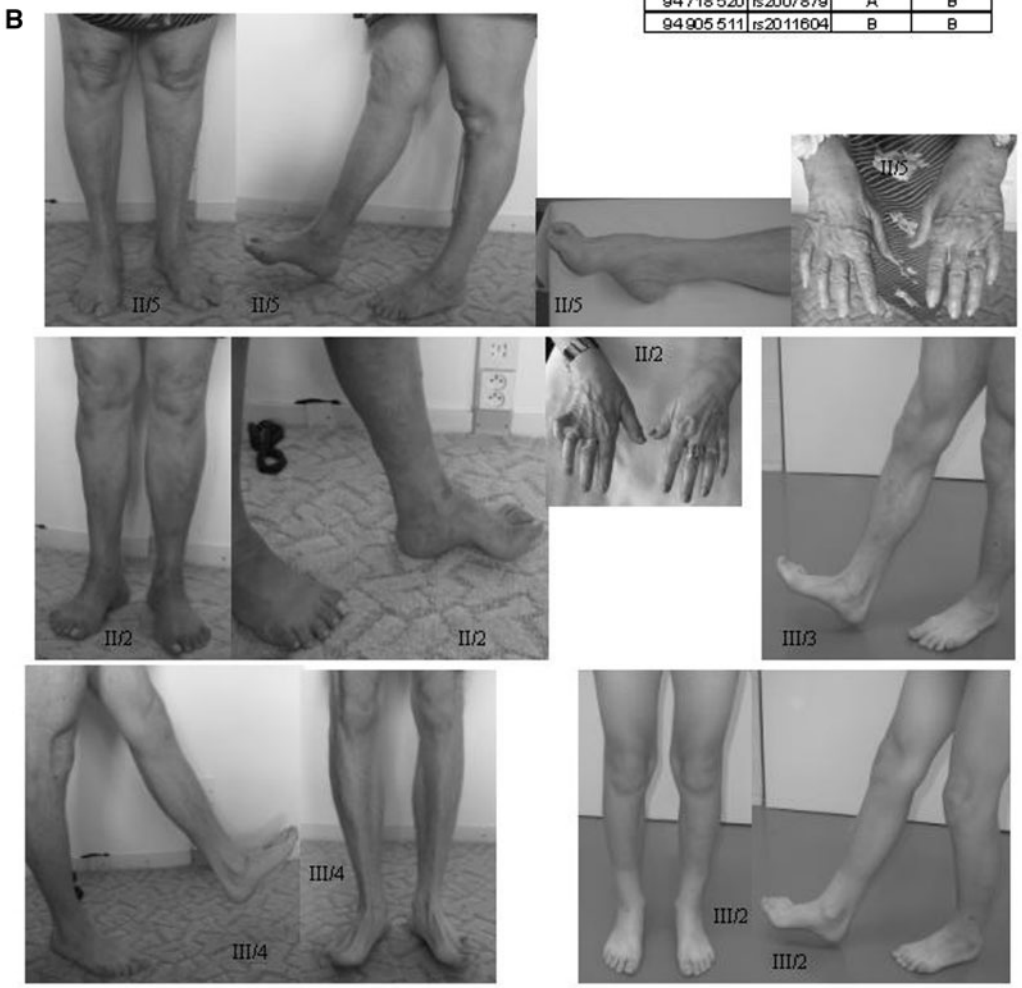


Figure 1 (**A**) Pedigree of the family with p.R373C mutation. Square = male; circle = female; filled symbol = affected patient; clear symbol = healthy person; slash symbol = deceased person. Patients II/2, II/3, II/4, II/5, III/1, III/2, III/3 and III/4 were genotyped using the GeneChip® Human Mapping 10 K Array Xba 142 2.0 for the linkage analysis. (**B**) Photos of the affected persons from the Czech family. Photos of Patients II/2 and II/5 present the pes cavus deformity, mild muscle atrophies on the lower limbs and mild atrophies of small hand muscles. Contracture of IV and V finger (Patient II/2) on the right side is due to the ulnar nerve injury with osteofyt in the elbow. Photo of Patient III/4 shows only atrophies of small feet muscles. Photos of Patients II/2, II/5 and III/4 are from the last examination in 2012; photos of Patients III/2 and III/3 are from 2001, and only higher foot arch and pedes planovalgi could be seen in Patients III/2 and III/3, respectively. (**C**) Comparison of haplotypes of Czech and Austrian families with the p.R373C mutation. Position of the *FBLN5* gene is chr14:92 335 755-92 414 046 according to reference assembly GRCh37(hg19) and is highlighted with shading in the haplotype. The haplotype analysis was performed manually with genotypes obtained from the previous single nucleotide polymorphism genotyping used for the linkage analysis (GeneChip® Human Mapping 10 K Array Xba 142 2.0).

Table 1 Clinical examination of a CMT1 family with *FBLN5* mutation

Patient	Age at onset/clinical examination	Sensory symptoms	Motor symptoms		Pin sensibility	Vibration
			Legs	Arms		
III/2	AS/12	NO	Slaps feet (1)	NO	NE	NE
III/3	AS/11	NO	Pedes planovalgi	NO	NE	NE
III/4	AS/24	NO	Pedes plani, hammer fingers	NO	Normal	Reduced up to ankles (2)
II/5	38/61	Paraesthesias on hands and feet (2)	Canes (3)	NO	Reduced in fingers and toes (1)	Reduced up to knee (3)
II/2	36/57	Paraesthesias of fingers and toes (1)	Slaps feet (1)	Difficulty with buttons (1)	Normal	Reduced above elbow/knee (4)

Patient	Strength		Patellar tendon reflexes	Hyperelastic skin	AMD	Charcot–Marie–Tooth disease neuropathy score
	Legs	Arms				
III/2	4 + on foot dorsiflexion (1)	Normal	Diminished	NE	NE	NE
III/3	4 + on foot dorsiflexion (1)	Normal	Absent	NE	NE	NE
III/4	Normal	Normal	Absent	NO	NE	9
II/5	4 on foot dorsiflexion (1)	4 + on finger extensors (1)	Absent	NO	NO	16
II/2	4 on foot dorsiflexion (1)	4 on finger extensors (1)	Absent	NO	NO	17

The overview is based on the CMT neuropathy score (values in brackets correspond to CMTN scores).
AMD = age-related macular degeneration; AS = asymptomatic; NE = not examined; NO = not present.

Table 2 Nerve conduction study in patients with *FBLN5* mutation

Patient—age at testing	Motor (DML/NCV/ampl.)			
	Median nerve	Ulnar nerve	Peroneal nerve	Tibial nerve
Normal	3.6 ms/50 m/s/5 mV	2.5 ms/50 m/s/5 mV	4.8 ms/41.7 m/s/4 mV	5.1 ms/40.6 m/s/5 mV
III/3—18	7.1/32.4/–	4.9/23.8/–	8.3/25/–	7.5/23.1/–
III/4—13	6.9/32.9/–	4.2/31.3/–	9.2/28.1/0.83	7.8/26.3/0.1
III/4—24	6.8/31.6/2.7	4.6/25/0.5	7.9/21.3/0.9	7.5/19.8/0.7
II/5—50	7.5/21/–	4.4/19.6/–	NR	NR
II/5—61	6.0/22.9/0.8	3.2/20.3/1.5	NR	NR
II/2—46	9.0/19.9/–	6.5/26.7/–	7.7/17.3/–	8.0/15.3/–
II/2—57	ne	4.8/16.5/0.1	ne	4.3/17.7/0.1
III/2—15	6.1/28.8/1.3	4.5/33.3/3.2	9.6/23/1.6	12.2/26.8/0.9

The development of the electrophysiological findings and progress of the nerve conduction velocities in Patients II/2, II/5 and III/4 can be seen. The difference between two examinations is 11 years.
Sensory nerve examinations were not recordable in all patients on median, ulnar and sural nerves.
DML/NCV/ampl. = distal motor latency/nerve conduction velocity/amplitude. The amplitudes were not included in the old reports and are not accessible in previous examination of Patients III/3, III/4, II/5 and II/2.
ne = not examined; NR = no response.

detected (electrophysiologically) several years before clinical manifestation of distal weakness.

Because the cause of HMSN I could not be clarified by direct DNA tests, in 2004, we decided to continue with linkage analysis using microsatellite markers for 12 genes or loci associated with the dominant neuropathy. This helped us to virtually exclude almost all the known Charcot–Marie–Tooth disease genes and pointed to a possibly novel, not yet known form of Charcot–Marie–Tooth disease.

In 2009, the eight family members (Fig. 1A) were genotyped on single nucleotide polymorphism chips (GeneChip® Human Mapping 10 K Array Xba 142 2.0, Affymetrix). Multipoint linkage revealed 21 areas with a positive log of odds score; only 10 reached the maximal log of odds score 1.2. Three genes and one region already associated with hereditary neuropathy were located in the linkage regions. Subsequent sequencing of *MFN2* and *RAB7* genes, located in regions with linkage, has not shown any mutation. Again we could exclude all the known causes of autosomal dominant Charcot–Marie–Tooth disease.

Finally, in 2011, exome sequencing as a method for finding a mutation in a novel Charcot–Marie–Tooth disease gene was performed. Two affected members, genetically the most distant (Patients III/2 and III/3), were examined. Samples were processed from the preparation of the libraries to the final variant report by Axeq Technologies (www.axeq.com, Axeq Asia). The TrueSeq Exome Enrichment Kit was used as a capture method

for isolating the desired target exonic regions. The captured libraries were sequenced using an Illumina HiSeq 2000 Sequencer (Illumina).

Almost 18 000 coding variants (single nucleotide polymorphisms) were detected in each exome. The filtering of the variants to only heterozygous, coding, not presented in the dbSNP 132 database and shared by both affected members, reduced the number of variants to 336. Application of the linkage regions from genotyping on single nucleotide polymorphism chips reduced the possible variants to 64. The last filtering removed the synonymous variants, presented in the Exome Variant Server and with bad quality of aligned data presented in the integrative genomics viewer (Robinson *et al*., 2011). Finally, only five probable variants in different genes remained. Predicting programs and conservation scores ranked the variant in the *FBLN5* gene as the most probable disease causing. This *FBLN5* variant c.1117 C > T (p.R373C) was confirmed by Sanger sequencing and subsequently was found in all affected family members and absent in all healthy members.

Auer-Grumbach *et al*. (2011) published the same missense change c.1117 C > T in an Austrian family, and associated *FBLN5* with HMSN.

Clinical findings of the Czech and the Austrian families are similar, although the age-related macular degeneration is not present in the Czech family. The difference may be because only one Austrian patient showed age-related macular degeneration at age 81 (Auer-Grumbach *et al*., 2011), while the oldest patient in the Czech family is 61 years old and age-related macular degeneration may develop later. Hand paraesthesias were present in two older Czech patients (corresponding to the carpal tunnel syndrome-like feature mentioned in the Austrian family). This is not common in other forms of Charcot–Marie–Tooth disease and can be a feature of *FBLN5* mutations.

The origin of the Czech family from South Bohemia close to the Austrian border led us to the idea of a common founder for the p.R373C mutation, but haplotype analysis showed completely different haplotypes around the mutation region (Fig. 1C), suggesting that the c.1117C > T (p.R373C) mutation arose independently in both families. The p.R373 position can be a hotspot for mutations because it is a C to T change at the nucleotide level, and in case of methylation of the CpG dinucleotide, cytosine can easily deaminate to thymine (Cooper *et al*., 2010).

Mutations in *FBLN5* are a rare cause of Charcot–Marie–Tooth disease not only in Austrian patients with CMT1, as shown by Auer-Grumbach *et al*. (2011) among 112 unclassified Charcot–Marie–Tooth disease samples, but also in Czech patients with CMT1 where sequencing of all coding *FBLN5* exons in 37 additional unrelated patients with demyelinating Charcot–Marie–Tooth disease did not reveal any other pathogenic mutation. Therefore, it is not reasonable to test this gene separately, but we would suggest that *FBLN5* be included in a panel for examining the Charcot–Marie–Tooth disease-associated genes or exome sequencing.

Our finding of a second CMT1 family with a mutation in the *FBLN5* gene confirms the causality of *FBLN5* mutations for HMSN I. To date, the p.R373C mutation is the most frequent mutation in *FBLN5* in patients with CMT1.

Acknowledgements

The authors thank the participating family for their assistance. They thank Michaela Auer-Grumbach for the clinical consultation and haplotypes of the Austrian family and Irena Bednářová and Vlastimil Vaněk for previous electrophysiological examination.

Funding

The study was supported by grant IGA MH CR NT 11521-4 and by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic).

References

Auer-Grumbach M, Weger M, Fink-Puches R, Papic L, Frohlich E, Auer-Grumbach P, et al. Fibulin-5 mutations link inherited neuropathies, age-related macular degeneration and hyperelastic skin. Brain 2011; 134(Pt 6): 1839–52.

Cooper DN, Mort M, Stenson PD, Ball EV, Chuzhanova NA. Methylation-mediated deamination of 5-methylcytosine appears to give rise to mutations causing human inherited disease in CpNpG trinucleotides, as well as in CpG dinucleotides. Hum Genomics 2010; 4: 406–10.

EVS. Exome Variant Server. NHLBI Exome Sequencing Project (ESP), Seattle, WA http://evs.gs.washington.edu/EVS/ (February 2012, date last accessed).

Loeys B, Van Maldergem L, Mortier G, Coucke P, Gerniers S, Naeyaert JM, et al. Homozygosity for a missense mutation in fibulin-5 (FBLN5) results in a severe form of cutis laxa. Hum Mol Genet 2002; 11: 2113–18.

Lotery AJ, Baas D, Ridley C, Jones RP, Klaver CC, Stone E, et al. Reduced secretion of fibulin 5 in age-related macular degeneration and cutis laxa. Hum Mutat 2006; 27: 568–74.

Markova D, Zou Y, Ringpfeil F, Sasaki T, Kostka G, Timpl R, et al. Genetic heterogeneity of cutis laxa: a heterozygous tandem duplication within the fibulin-5 (FBLN5) gene. Am J Hum Genet 2003; 72: 998–1004.

Nascimento GM, Nunes CS, Menegotto PF, Raskin S, Almeida N. Cutis laxa: case report. An Bras Dermatol 2010; 85: 684–6.

Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative genomics viewer. Nat Biotechnol 2011; 29: 24–6.

Stone EM, Braun TA, Russell SR, Kuehn MH, Lotery AJ, Moore PA, et al. Missense variations in the fibulin 5 gene and age-related macular degeneration. N Engl J Med 2004; 351: 346–53.