LETTER TO THE EDITOR

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

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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 probable causal for HMSN I, but 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
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 10K 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 10K Array Xba 142 2.0).
detected (electrophysiologically) several years before clinical mani-
festation 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 10K 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.

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at onset/clinical examination</th>
<th>Sensory symptoms</th>
<th>Motor symptoms</th>
<th>Pin sensibility</th>
<th>Vibration</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Legs</td>
<td></td>
<td>Arms</td>
</tr>
<tr>
<td>III/2</td>
<td>AS/12</td>
<td>NO</td>
<td>Slaps feet (1)</td>
<td>NO</td>
<td>NE</td>
</tr>
<tr>
<td>III/3</td>
<td>AS/11</td>
<td>NO</td>
<td>Pedes planovalgi</td>
<td>NO</td>
<td>NE</td>
</tr>
<tr>
<td>III/4</td>
<td>AS/24</td>
<td>NO</td>
<td>Pedes plani, hammer fingers Canes (3)</td>
<td>NO</td>
<td>Reduced in fingers and toes (1)</td>
</tr>
<tr>
<td>II/5</td>
<td>38/61</td>
<td>Paraesthesias on hands and feet (2)</td>
<td>NO</td>
<td>Difficulty with buttons (1)</td>
<td>Normal</td>
</tr>
<tr>
<td>II/2</td>
<td>36/57</td>
<td>Paraesthesias of fingers and toes (1)</td>
<td>Slaps feet (1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

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.
Auer-Grumbach disease not only in Austrian patients with CMT1, as shown by we would suggest that Tooth disease did not reveal any other pathogenic mutation.

Additional unrelated patients with demyelinating Charcot–Marie–Tooth disease samples, but also in Czech patients with CMT1 where sequencing of all coding mutations. Because it is a C to T change at the nucleotide level, and 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.

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References


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


