Charcot–Marie–Tooth neuropathy due to a novel EGR2 gene mutation with mild phenotype – Usefulness of human mapping chip linkage analysis in a Czech family

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Abstract

Charcot–Marie–Tooth neuropathies (CMT) are a group of clinically and genetically heterogeneous disorders of the peripheral nervous system. Selection of candidate disease genes for mutation analysis is sometimes difficult since more than 40 genes and loci are known to be associated with CMT neuropathies. Hence a Czech family Cz-CMT with demyelinating type of autosomal dominant CMT disease was investigated by genome-wide linkage analysis by means of single-nucleotide polymorphism (SNP) arrays. Among 35 regions with linkage, five carried known CMT genes. In the final result a novel early growth response 2 – missense mutation c.1235 A>G, p.Glu412Gly was found. Surprisingly, the more severely affected proband carried an additional heterozygous myelin protein zero variant p.Asp246Asn detected previously, which may modify the phenotype. However, this MPZ variant is benign in heterozygous state alone, because it is also carried by the patient’s healthy father.

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1. Introduction

Hereditary neuropathy Charcot–Marie–Tooth is a heterogeneous disease which can be caused by mutations in more than 40 genes. All Mendelian types of inheritance are observed. Therefore, the selection of candidate genes for mutational analysis is sometimes difficult. A suitable family linkage analysis is a powerful tool to identify the critical regions and already known disease causing genes within these intervals.

Charcot–Marie–Tooth type 1D is a rare type of the demyelinating neuropathy caused by mutations in the EGR2 gene [1]. Only 12 pathogenic mutations have been reported [2], but among them a broad spectrum of clinical manifestations can be found; the severe and early onset Dejerine–Sottas neuropathy or congenital hypomyelinating neuropathy and moderate with late onset CMT1. The EGR2 encodes transcription factor with three Cys2His2 zinc-finger domains [3] and plays an important role in the myelination of the peripheral nerves [4].

2. Patients and methods

A Czech family Cz-CMT with demyelinating hereditary motor and sensory neuropathy (HMSN I) and dominant
inheritance was chosen for linkage analysis (Fig. 1) to narrow the candidate genes for further sequence analysis. The family initially tested negative for CMT1A duplication/HNPP deletion using microsatellite markers [5]. No pathogenic mutations were found in the GJB1, PMP22, MPZ genes using Sanger sequencing. However p.Asp246Asn mutation was found in the MPZ gene, which was considered a benign polymorphism [6].

Fig. 1. (a) Family pedigree Cz-CMT with filled out symbols for the affected members. Mutations in the EGR2 and MPZ genes are indicated. (b) Photographs of the affected members from family Cz-CMT. In patient II/2 (65 years old) only mild muscle atrophy of lower limbs with higher instep and no atrophies on the upper limbs are visible. Patient III/1 (37 years old) has pronounced atrophy of the lower limbs and also atrophy of upper limbs. Orthopaedic surgery was performed on both ankles. Patient III/2 (45 years old) has no atrophy of the lower limbs.
Electrophysiological testing was performed by standard procedures. A Medical Research Council (MRC) scale was used for muscle weakness estimation. All members of the family signed informed consent for DNA testing for CMT.

Genomic DNA from five family members (II.1, II.2, III.1, III.2, IV.1) was genotyped on Affymetrix GeneChip Human Mapping 10K arrays XbaI 142 2.0 (Affymetrix, Santa Clara, CA, USA). Linkage analysis was performed using Merlin 1.1.2 software assuming the autosomal dominant model and complete penetrance.

All coding exons and flanking intron sequences of NEFL, YARS, EGR2, HSPB1 genes were sequenced using the Big Dye Terminator v3.1 sequencing kit and analyzed on an ABI3130 Genetic Analyzer (Applied Biosystems, USA). The reference sequence used for EGR2 was the NM_000399.2, for MPZ D10537. Overall 176 ethnically matched DNA controls were examined. The 130 DNA controls were tested for the presence of EGR2 mutation with a restriction enzyme BccI which digest the mutated sequence of EGR2 and the additional 46 DNA samples were examined by sequencing of all coding parts of EGR2.

3. Results

Genome-wide linkage analysis initially revealed 35 regions with an identical maximal LOD score of 0.6. Seven of these regions have been previously associated with autosomal dominant CMT disease, but only five of these seven regions comprised known genes associated with CMT neuropathy. The DNA from affected patient III.2 was sequenced for the presence of mutations in these candidate genes NEFL, YARS, EGR2, and HSPB1. The fifth gene within the linked intervals, MPZ, was already sequenced in the family before this study and mutation p.Asp246Asn was found in the proband, but also in proband's healthy sister. This variant is not listed in the Exome Variant Server (EVS) [9]. Prediction programs Polyphen2 [10] and MutationTaster [11] scored the p.Asp246Asn mutation as possibly damaging (score 0.766) and disease causing (score 2.67), respectively. The position p.412 is highly conserved among species (Fig. 2 – Supplementary material).

3.1. Clinical and electrophysiological features of patients carrying the EGR2 p.Glu412Gly mutation

The 37-year-old proband (III.1) is the most severely affected member of the family. The first symptoms, stumbling and ankle distortions, started at the age of 7 years. The diagnosis of demyelinating CMT was established at age 10 after electrophysiological and neurological examination of the proband and also of his mother and sister. Subsequent sural nerve biopsy at age 10 completed the diagnosis of the demyelinating motor and sensory neuropathy. Clinical examination at age 37 showed pronounced muscle atrophies below the knees, bilaterally reduced distal muscle strength and plegia of foot extension. Foot flexion was MRC 2/5 (Fig. 1). Two orthopaedic surgeries were performed on both his ankles. Pronounced atrophies of the small hand muscles were found with reduced distal muscle strength and contracture of II–IV fingers. Proximal muscle strength was normal above the knee and wrist. He could not walk on his heels. Pin sensitivity was reduced in the fingers and toes; vibration sensation was reduced below the knees. Neurological examination showed no cranial nerve involvement. Electrophysiological examination showed the primary demyelinating motor and sensory neuropathy with pronounced secondary axonopathy (Table 1 – electrophysiological examination). According to the CMT neuropathy score (CMTNS), the value was 21, indicating a severe type of CMT neuropathy.

The first symptoms of the proband’s mother II.2, stumbling, appeared around age 45. Prior to this she was able to do sports without any problems. Clinical examination at age 65 revealed mild to moderate muscle atrophies up to the knees with bilateral shortening of the Achilles tendons (90°) and pes cavus. Muscle strength was reduced to MRC 4/5 on the foot flexion and extension. She could not walk on her heels because of Achilles tendon shortening. Muscle strength in the upper limbs was normal with no atrophies. She reported only minimal awkwardness when picking up coins or needles. Vibration sensation was reduced at the wrist and ankles; mild hypaesthesia was distal from the knees and fingers. Neurological examination showed no cranial nerve involvement. The patient’s CMTNS value was 11 and she was clearly less affected compared to her son despite the age difference.

The proband’s sister, patient III.2, has not yet reported any problems. She was examined at age 45. Shortening of the Achilles tendons (90°) was found, but only mild atrophies and impaired foot extension (MRC 4/5) were present. She could not walk on her heels. No atrophies in the upper limbs were present and muscle strength was also normal. Vibration sensation was reduced at the wrists and ankles; mild hypaesthesia was distal from the knees and fingers. Cranial nerve involvement was not present and BAEP examination did not show any pathology. The value of her CMTNS was 7, signifying mild disability with CMT neuropathy.

Patient IV.1 was not available for our clinical examination but according to clinical and electrophysiological data from the past he was only very mildly affected with demyelinating neuropathy.
Table 1
Electrophysiological findings of patients from family Cz-CMT. Abnormal values are in bold. The nerve conduction velocities are decreased on lower and upper limbs. The sensory neurogram for the ulnar nerve is unrecordable for patients III/1 and II/2. All three patients have unrecordable neurograms on tibial nerve and sural nerve. Patient III/1 has decreased amplitudes giving evidence of secondary axonopathy.

<table>
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<tr>
<th>Patient</th>
<th>Median nerve MNCV (m/s)</th>
<th>Median nerve Amplitude (mV)</th>
<th>Ulnar nerve MNCV (m/s)</th>
<th>Ulnar nerve Amplitude (mV)</th>
<th>SNCV (m/s)</th>
<th>Amplitude (μV)</th>
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<td>&gt;50</td>
<td>&gt;5.5</td>
<td>&gt;48</td>
<td>&gt;15</td>
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<td>43.0</td>
<td>6.7</td>
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</tr>
<tr>
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<td>37.0</td>
<td>1.2</td>
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<tr>
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<td>41.0</td>
<td>6.9</td>
<td>35.0</td>
<td>6.0</td>
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</table>

4. Discussion

Generally, mutations in the EGR2 gene are a very rare cause of CMT in Czech patients. To date, only one EGR2 mutation was detected and reported from a Czech patient [12]. Therefore EGR2 is not routinely tested in Czech CMT patients.

In a Cz-CMT family with dominant demyelinating HMSN we previously excluded the most common causes, namely CMT1/HNPP by microsatellites and GJB1, MPZ, PMP22 by sequencing. One would have sequenced an additional seven candidate genes for demyelinating CMT, but without assurance of finding a disease associated mutation. We decided to proceed by linkage analysis using SNP chips to decrease the number of candidate genes. A novel mutation p.Glu412Gly in the EGR2 gene was found segregating with demyelinating CMT in this family (Fig. 1) which supported the pathogenicity of the mutation. The more severely affected proband (III.1), was the first clinically examined from the family. Due to the suspicion of hereditary neuropathy, other family members were examined. Demyelinating CMT was also diagnosed by nerve conduction study (NCS) in his mother and sister but clinically they presented only mild affection. Their NCS indicated demyelinating neuropathy with the range of values rather in the intermediate range on the upper limbs compared to the lower limbs, where the hereditary neuropathy is more pronounced (Table 1).

The more severe phenotype of the proband may be the combined influence of mutations in the EGR2 and MPZ genes. The MPZ mutation p.Asp246Asn was also found in the healthy father and is considered a polymorphism or a recessive mutation as we already reported [6]. The coincidence of these two mutations can be the reason for the more severe phenotype (CMTNS value of 21) in the proband compared to his mother (CMTNS = 11), sister (CMTNS = 7) and her son. The EGR2 is an important transcription factor and plays an important role in myelination and activation of the MPZ gene via EGR2 and SOX10 cooperation [13]. Recently, Jang et al. published new potential EGR2 binding sites which regulate the expression of the MPZ gene [14].

Although we did not prove the pathogenicity of our mutation by any functional test, we assume the effect of mutation p.Glu412Gly will be similar to the effect of another mutation p.Glu412Lys reported at the same codon [15]. This mutation is located in the third zinc-finger domain and reduces the transcriptional activity to 28% of the wild-type and decrease the ability to bind DNA. The reported phenotype of p.Glu412Lys is severe with onset in infancy, delayed age of walking (24 months) and cranial neuropathy. This phenotype undoubtedly differs from the mild phenotype of p.Glu412Gly reported here. Similar differences in the mutations of the same codon are on the position 381, where p.Arg381Cys is reported in patients with late onset mild neuropathy [16,17] and p.Arg381His is associated with early onset severe phenotype with cranial nerve deficits [18,19]. Another variance of phenotypes was described for p.Arg359Trp, where four patients suffer from early onset Dejerine–Sottas neuropathy [20,21] and two patients with the same mutations present the CMT1 neuropathy [22,23].

The p.Glu412 residue is highly conserved among species and together with the testing of controls and prediction programs these findings support the pathogenicity of the p.Glu412Gly mutation reported here. This variant is also not listed in the 1000 Genomes or EVS project.

We showed that SNP genotyping together with linkage analysis may successfully help to find the causal mutation even in a small family where the most frequently mutated genes were excluded. This approach seems to be an efficient tool for discovering causal mutations in heterogeneous disorders if familiar occurrence exist. Moreover, with the rapid development of next generation sequencing methods, the
targeted resequencing of CMT related genes seems to be a first choice for this type of study, since the cost and effectiveness are more favorable than at the beginning of this study. Another type of approach would be to perform exome sequencing. Exome sequencing was successful in discovering many novel causal genes for recessive and dominant disorders even if the number of homozygous variants is smaller compared to heterozygous variants throughout the exome [24]. In the presented study, linkage analysis pointed out the regions already associated with CMT and together with Sanger sequencing, the causal mutation was found. In the case of a gene not yet associated with CMT, the linkage regions could be used for filtering data from exome sequencing and for reducing the large number of found variants.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nmd.2012.04.002.

References