The congenital myasthenic syndrome mutation RAPSN N88K derives from an ancient Indo-European founder


RESULTS AND DISCUSSION

All 41 RAPSN (N88K) chromosomes showed a shared core haplotype encompassing SNPs 7–16 (fig 1). Statistical analysis revealed that this haplotype was linked to N88K (p = 0.0006). This provides a strong indication that all RAPSN (N88K) alleles are derived from a common founder. Moreover, five patients of different European origin were homozygous for all analysed markers. For SNPs 3–6, only one RAPSN (N88K) allele of an Indian subcontinent patient revealed a different haplotype as compared to the other mutant chromosomes (shared haplotype encompassing SNPs 3–16). By contrast, several mutant chromosomes revealed distinct genotypes for SNPs 1–2 and/or SNPs 17–21 (fig 1). Statistical analysis indicated that these haplotypes may derive from a common founder event in an ancient Indo-European population.

Abbreviations: CMS, congenital myasthenic syndromes; SNPs, single nucleotide polymorphisms

Key points

- Mutations in various genes of the neuromuscular junction cause congenital myasthenic syndromes (CMS). The protein rapsyn is encoded by the RAPSN gene and clusters acetylcholine receptors (AChR) at the motor endplate. Recessive mutations of RAPSN result in AChR deficiency and impaired neuromuscular transmission.

- A single missense mutation of RAPSN (N88K) detected frequently in patients of European ethnic origin results in late onset forms of CMS. Three studies suggested that RAPSN (N88K) may derive from a common founder, while a fourth study could not corroborate this hypothesis. Therefore, we investigated 21 patients of European and Indian ethnic origin studying a total of 41 mutant RAPSN (N88K) alleles.

- Analysis of 21 single nucleotide polymorphisms (SNPs) flanking RAPSN on chromosome 11p11 revealed a common conserved haplotype encompassing a distance of about 360 kb. Our results support the hypothesis that RAPSN (N88K) derives from a single founder event in an ancient Indo-European population.

METHODS

We collected 21 CMS patients harbouring RAPSN (N88K) either homozygously (n = 20) or compound heterozygously (n = 1). Screening for the mutation N88K (264C→A) in exon 2 of the RAPSN gene was performed as described previously. Patients originated from Germany (n = 5), Austria (n = 1), Italy (n = 1), France (n = 2), the United Kingdom (n = 10), and the Indian subcontinent (n = 2). Consanguinity was not reported for any of the families. Most of the patients have been described, previously: patients G1–G4, Au1, UK10, and It1 (patients 1–7 in Müller et al); patients UK1–7 (patients 3, 9, 11, 12, 13, 15, and 16 in Burke et al); patient Ind2 (patient 1 in Burke et al), patient Fr1 (patient 1 in Richard et al), and patient Fr2 (in Yasaki et al). In addition, patients G5, UK8 and 9, and Ind1, who are included in this study, are homozygous for RAPSN (N88K) and show clinical features typical of CMS (data not shown). For the second Indian subcontinent patient (Ind2) with a second, heteroallelic mutation (46insC+), analysis of parental DNA was used to construct a haplotype for the N88K allele.

By PCR and restriction digest or sequence analysis, we analysed a total of 21 SNPs on chromosome 11p11 flanking RAPSN (N88K). Primer sequences and restriction enzymes used to screen the selected SNPs are given in table 1. Optimal SNPs flanking RAPSN were selected with a threshold of the minor allele frequency >20% according to the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP/). They are located within 4.012 Mb centromeric (SNPs 13–21; fig 1) and 2.688 Mb telomeric of the mutation (SNPs 1–8; fig 1). Four SNPs are located within the RAPSN gene (SNPs 9–12). In addition to the 21 CMS patients, we analysed 20 European controls for all SNPs. We defined a core founder haplotype encompassing SNPs 7–16, and an extended founder haplotype encompassing SNPs 3–16. For controls, DNA from the parents was not available which prevented exact phase determination for control haplotypes. Arbitrarily, haplotypes were constructed for the controls to result in the maximum possible number of core founder haplotypes (10/40) and extended founder haplotypes (5/40). Therefore, it appears likely that the number of founder haplotypes is overestimated for the controls. The χ² test was used to compare mutant alleles with normal control alleles.
0.36 Mb in European patients. Most of the data available for RAPSN (N88K) encompassing a genetic distance of about alleles. Our data demonstrate a core founder haplotype for region on chromosome 11p11.2 in a total of 41 mutant markers were not as appropriate to show linkage. Secondly, because the chromosomal region showing a statistical power to positively prove a founder effect was low. We looked at a relatively small number of mutant alleles, and the conclusion was hampered by two facts. Firstly, each study also revealed significant linkage to N88K (p = 0.0000174). Secondly, some 11 a low recombination rate of 0–0.1 Mb/cM was divided around 2000 BC giving rise to the related Indo-European languages.

### Table 1

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position on chr. 11 (Mb)</th>
<th>Primer sequences</th>
<th>Restriction enzyme</th>
<th>Frequency in controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs1446331 A/G</td>
<td>44.745543</td>
<td>5' - TCACCAGCCAGCTATATC -3'</td>
<td>Roa 40.0% A</td>
</tr>
<tr>
<td>2</td>
<td>rs728516 C/T</td>
<td>45.565115</td>
<td>5' - GAGAAATGAATGAGTTTGAG TAGTGA -3'</td>
<td>Mael 70.0% T</td>
</tr>
<tr>
<td>3</td>
<td>rs76474 A/C</td>
<td>46.307143</td>
<td>5' - CGCTATAGGGAAATCCCTA -3'</td>
<td>Hinfl 62.5% A</td>
</tr>
<tr>
<td>4</td>
<td>rs3136516 A/G</td>
<td>46.725065</td>
<td>5' - CTGTTGAAGGATCATCGGAGGG -3'</td>
<td>EcoRl 52.5% A</td>
</tr>
<tr>
<td>5</td>
<td>rs2279438 T/A</td>
<td>47.16848</td>
<td>5' - TCAAGCTTGCTGTTTGGCTAC -3'</td>
<td>Bsp1I 82.5% A</td>
</tr>
<tr>
<td>6</td>
<td>rs2278890 C/T</td>
<td>47.363911</td>
<td>5' - CTTCACCCCGCTGTTCTCTG -3'</td>
<td>Stul 37.5% T</td>
</tr>
<tr>
<td>7</td>
<td>rs229577 T/C</td>
<td>47.401511</td>
<td>5' - GTGCTGCCCAGTCCGCTAC -3'</td>
<td>HpolI 65.0% T</td>
</tr>
<tr>
<td>8</td>
<td>rs4282946 C/T</td>
<td>47.420511</td>
<td>5' - GAGCCAGTGAATCAGGACCAAG -3'</td>
<td>Mael 72.5% C</td>
</tr>
<tr>
<td>9</td>
<td>1143/T</td>
<td>RAPSN exon 7</td>
<td>5' - AAGTGGCTGAAGACCGAGGAGC -3'</td>
<td>Roa 72.5% C</td>
</tr>
<tr>
<td>10</td>
<td>IVS3-11/C/delC</td>
<td>RAPSN intron 3</td>
<td>5' - GGACAGCGGACTGCTAGGAGG -3'</td>
<td>Mael 92.5% G</td>
</tr>
<tr>
<td>11</td>
<td>456 C/T</td>
<td>RAPSN exon 2</td>
<td>5' - GAGGGTCCTGCAGGCTATG -3'</td>
<td>Roa 37.5% C</td>
</tr>
<tr>
<td>12</td>
<td>365 G/A</td>
<td>RAPSN promoter</td>
<td>5' - TACCAAGGAGCTGCTAGG -3'</td>
<td>Mael 25.0% T</td>
</tr>
<tr>
<td>13</td>
<td>rs2242081 T/C</td>
<td>47.64576</td>
<td>5' - TATCAGAGAACGCTGCTACG -3'</td>
<td>Roa 45.0% T</td>
</tr>
<tr>
<td>14</td>
<td>rs2280231 C/T</td>
<td>47.56474</td>
<td>5' - AAGGACGGACCCGCAAGAAGG -3'</td>
<td>Tail 45.0% T</td>
</tr>
<tr>
<td>15</td>
<td>rs3817334 T/C</td>
<td>47.61302</td>
<td>5' - GCAGAGGCTGAGACCGGAGC -3'</td>
<td>Stbl 107I 57.5% G</td>
</tr>
<tr>
<td>16</td>
<td>rs9099 C/G</td>
<td>47.764084</td>
<td>5' - GGTTGCTCCCTGCTATAGG -3'</td>
<td>Mael 52.5% G</td>
</tr>
<tr>
<td>17</td>
<td>rs2270994 A/G</td>
<td>48.122178</td>
<td>5' - GGACACGTGGTTGCTTGCCTATG -3'</td>
<td>Tail 70.0% T</td>
</tr>
<tr>
<td>18</td>
<td>rs229650 G/T</td>
<td>49.138316</td>
<td>5' - GGCACCTATATATGCTAC -3'</td>
<td>SstI 60.0% G</td>
</tr>
<tr>
<td>19</td>
<td>rs1800438 C/T</td>
<td>49.335007</td>
<td>5' - TATGTTCACTAGAATGGAAGG -3'</td>
<td>SstI 77.5% C</td>
</tr>
<tr>
<td>20</td>
<td>rs191207 C/T</td>
<td>50.500285</td>
<td>5' - TGACAGCAGCAGTTAGGTTGAT -3'</td>
<td>Hinfl 32.5% C</td>
</tr>
<tr>
<td>21</td>
<td>rs507015 G/A</td>
<td>51.45468</td>
<td>5' - TGGTTGAGAAGACCGGAGG -3'</td>
<td>Hinfl 62.5% A</td>
</tr>
</tbody>
</table>

Single nucleotide polymorphisms selected for genotyping of the RAPSN N88K founder allele. Information about the position of the SNP on chromosome 11 and the flanking sequences for PCR primer design and selection of an appropriate restriction enzyme have been retrieved from the NCBI SNP database (accessed December 2003). The last column represents the frequency of the polymorphisms in a group of 40 European control alleles.

Analysis for the extended haplotype spanning SNPs 3–16 also revealed significant linkage to N88K (p = 0.0000174).

A possible founder effect for RAPSN (N88K) was discussed in detail in three previous studies. An unsurprising conclusion was hampered by two facts. Firstly, each study looked at a relatively small number of mutant alleles, and the statistical power to positively prove a founder effect was low. Secondly, because the chromosomal region showing a conserved haplotype is relatively small, remote repeat markers were not as appropriate to show linkage. Therefore, we analysed 21 SNPs of the suspected founder region on chromosome 11p1.2 in a total of 41 mutant alleles. Our data demonstrate a core founder haplotype for RAPSN (N88K) encompassing a genetic distance of about 0.36 Mb in European patients. Most of the data available for North American patients are also compatible with an old founder effect. All North American patients are of Caucasian descent and show identical intragenic SNPs. The microsatellite marker D11S1252, which showed the same value for 11 of the 12 North American chromosomes, lies between SNPs 3 and 4 and hence within the extended founder haplotype fragment spanning SNPs 3–16. D11S4117, linked to N88K in all three previous studies, is directly adjacent to SNP 16 and might also be part of the core founder haplotype. D11S986 was not linked to N88K in previous studies, and lies clearly outside of the founder haplotype defined by SNP analysis. However, microsatellite marker D11S4109 exhibits four different genotypes in North American patients, but is located within the core haplotype defined by SNP analysis (between SNP 14 and 15). This may indicate additional founder events in North American patients. Alternatively, the variation in D11S4109 may have occurred after the mutation event and may reflect the higher divergence rate of nucleotide repeat markers as compared to SNPs. This question could be resolved by SNP analysis of the North American patients.

The gene RAPSN is located pericentromeric (6 Mb from the centromere). Previous studies revealed that the rate of mitotic exchange is reduced across centromeres of human X chromosomes and of autosomes as compared to more telomeric regions. For the pericentromeric region of chromosome 11 a low recombination rate of 0–0.1 Mb/cM was observed (http://genome.ucsc.edu). Therefore, estimation of the age of the founder mutation is difficult. According to historical and linguistic evidence ancient tribes migrated and divided around 2000 BC giving rise to the related Indo-European populations and languages. Therefore, the RAPSN (N88K) mutation may have originated prior to these divisions. This is in good agreement with two findings. Firstly, RAPSN (N88K) was detected in patients of different
European and Indian subcontinent origin. Secondly, RAPSN (N88K) has not been reported in CMS patients of sub-Saharan African or East Asian ethnic origin. Similarly, an age of approximately 4000 years has been assigned to the founder mutation of myotonic dystrophy type II detected in various European and Afghan patients.14

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Figure 1 Haplotype analysis of 21 unrelated CMS patients (41 RAPSN N88K chromosomes). The 21 SNPs flanking the mutation have been used for analysis. Vertical columns represent individual haplotypes of the analysed patients, whereas each row represents the genotypes at one SNP. The SNPs are listed from telomeric to centromeric corresponding to table 1; SNPs 9–12 are intragenic. The distance of each SNP refers to the position of the mutation N88K. Genotypes dominant in mutant chromosomes have been coloured orange (representing a putative founder haplotype), while a differing genotype has been coloured white. Yellow has been assigned to a heterozygous constellation at one SNP. All patients share a common haplotype stretching from SNP 7–16. *Patient Ind2 carries only one K88 allele. Au, Austria; Fr, France; G, Germany; Ind, Indian subcontinent; It, Italy; UK, United Kingdom.
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