Mutations in RAPSN, a gene encoding rapsyn, a molecule that clusters acetylcholine receptors at the motor endplate, cause endplate acetylcholine receptor deficiency. Müller and colleagues recently reported that N88K is a frequent mutation in RAPSN. By genotyping 17 mutant K88 chromosomes for two RAPSN polymorphisms (IVS3-11delC and 456T/C) and a microsatellite marker D11S4117 (fig 1A) in 12 patients from 10 independent central or western European families, they found that 14 of 17 mutant chromosomes shared a common haplotype and concluded that N88K arises from a common founder.

Richard and colleagues also recently reported that N88K arises from a common founder; they genotyped 10 mutant K88 chromosomes in five patients from four families and from five healthy controls heterozygous for N88K. They showed that 9/10 mutant chromosomes shared a common haplotype for D11S1252, D11S4109, and D11S4117 (fig 1A). Both groups also demonstrated that a distant microsatellite marker, D11S986, was not linked to N88K.

We also searched for a founder effect for N88K in our cohort of patients using six microsatellite markers and five RAPSN polymorphisms, and found that there is no founder haplotype for N88K.

MATERIALS AND METHODS

Our cohort comprised 10 unrelated white North American families consisting of 12 affected and 32 unaffected members, who carried a total of 12 mutant K88 chromosomes and 37 wild type N88 chromosomes. Patient 1 is case 1 in one previous report, and patients 3, 4, 6, and 7 are cases 1–4 in another report. Patients 2 and 9 have been reported in an abstract. Patients 5, 8, and 10 (all boys, 10, 4, and 8 years of age, respectively) have had mild to moderate myasthenic symptoms since birth, negative tests for anti-acetylcholine receptor antibodies, and respond partially to anti-cholinesterase medications.

DNA was isolated from muscle and blood as described. To identify RAPSN mutations, we directly sequenced eight RAPSN exons and their flanking noncoding regions. We employed allele specific PCR to screen 200 normal alleles for mutations in RAPSN, a gene encoding rapsyn, a molecule that clusters acetylcholine receptors at the motor endplate, cause endplate acetylcholine receptor deficiency. N88K is a frequent mutation in RAPSN.

Key points

- Mutations in RAPSN, a gene encoding rapsyn, a molecule that clusters acetylcholine receptors at the motor endplate, cause endplate acetylcholine receptor deficiency. N88K is a frequent mutation in RAPSN.
- Two recent reports from Europe demonstrate a founder effect for N88K.
- By analysing six microsatellite markers and five intragenic single nucleotide polymorphisms of 12 mutant K88 chromosomes and 37 wild type N88 chromosomes in 12 affected and 32 unaffected members from 10 white North American families, we found that there is no founder haplotype for N88K.
- Our studies indicate that N88K is an ancient founder mutation, but subsequent multiple recombination events and divergence of microsatellite markers have obscured a founder effect. Alternatively, N88K occurred independently and repeatedly in humans.
- We also report three novel RAPSN mutations (C97X, R151P, and A142D).

For statistical analysis, we employed Fisher’s two tailed exact test.

RESULTS

Patients 4 and 6 are homozygous for N88K, whereas other patients are heterozygous for N88K. Some patients also have a heteroallelic second mutation: N277C (patient 1), 1177delAA (patient 2), L14P (patient 3), C97X (patient 5), 553ins5 (patient 7), R151P (Pt 8), E333X (Pt 9), and A142D (Pt 10). All missense mutations affect residues conserved across species.

Mutations in RAPSN, a gene encoding rapsyn, a molecule that clusters acetylcholine receptors at the motor endplate, cause endplate acetylcholine receptor deficiency. N88K is a frequent mutation in RAPSN.

Haplotype analysis of the 12 mutant chromosomes revealed 11 distinct haplotypes (fig 1B). One mutant haplotype was shared between families 8 and 10, although one respective marker was not informative in each family. No haplotype was shared among the 37 wild type chromosomes, or between the wild type and mutant chromosomes. No recombination event was observed in any family.

We next performed Fisher’s exact test using the wild type chromosomes in family members as controls to see if individual microsatellite markers and polymorphisms were linked to N88K. We found that D11S1252, D11S1385, D11S1252, D11S4109, and five RAPSN polymorphisms were not linked to N88K, whereas D11S1344 and D11S4117 were linked to RAPSN. All the mutant chromosomes shared a common genotype at five RAPSN polymorphisms.
polymorphisms and D11S4117, whereas other microsatellite markers showed 2–5 divergent genotypes. Even when only RAPSN polymorphisms were taken into account, the shared haplotype was not linked to N88K (p = 0.09). The tightly linked marker D11S1344 demonstrated five divergent genotypes. D11S4109, which is only 142 kb from RAPSN towards the q terminal, showed four divergent genotypes.

DISCUSSION

Our data demonstrate that there is no founder haplotype for N88K. Shared genotypes at the five RAPSN polymorphisms and divergent genotypes at the flanking microsatellite markers indicate that N88K is an ancient mutation that occurred on a founder chromosome, but subsequent multiple recombination events and divergence of microsatellite markers have obscured a founder effect. Lack of statistical linkage of the five RAPSN polymorphisms to N88K, however, argues against this view. An alternative possibility is that N88K occurred independently and repeatedly in humans.

We analysed six microsatellite markers and five RAPSN polymorphisms, whereas Müller et al analysed one microsatellite and two RAPSN polymorphisms, and Richard et al analysed three microsatellite markers. Our analysis covered all the previously reported markers. Our data reveal a founder effect (p = 0.006), when only the D11S4117 marker and the two polymorphisms used by Müller et al are taken into account. Analysis including more markers, however, excludes a founder effect. Our data do not demonstrate a founder effect (p = 0.18) when the three microsatellite markers used by Richard et al are employed. This is partly because our cohort shows four divergent genotypes at D11S4109, whereas Richard et al observed a single genotype at D11S4109, which indicates that those patients probably originated from a restricted community.

Both Müller et al and Richard et al omitted genotypes at D11S986 on the basis that D11S986 is distant from RAPSN, and that D11S986 had no linkage to N88K. As D11S986 occurs 2.7 Mb towards the p terminal from RAPSN, which is not exceptionally distant compared with other markers (fig 1A), divergent genotypes at D11S986 may represent lack of a founder effect in their cohorts.

Lack of a founder haplotype for N88K in our cohort leads to two possibilities: N88K is an ancient founder mutation or N88K has multiple founders. The accumulating availability of single nucleotide polymorphisms will enable further dissection of a founder effect for N88K in the future.

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N in the haplotype names represent mutant K88 and wild type N88, respectively. Genotypes dominant in the mutant K88 chromosomes are shaded. Numbers in haplotypes represent the size of PCR product. na, Not available, because all family members were heterozygous for two morphs. In F8, all members carried morphs 260 and 264 at D11S4117. In F10, all members carried morphs 277 and 283 at D11S4174.
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Lack of founder haplotype for the rapsyn N88K mutation: N88K is an ancient founder mutation or arises from multiple founders
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