ONLINE MUTATION REPORT

Lack of founder haplotype for the rapsyn N88K mutation: N88K is an ancient founder mutation or arises from multiple founders

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Mutations in *RAPSN*, a gene encoding rapsyn, a molecule that clusters acetylcholine receptors at the motor endplate,[1] cause endplate acetylcholine receptor deficiency.[2–7]

Müller and colleagues recently reported that N88K is a frequent mutation in *RAPSN*.[5] 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.[6] 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.[5 6]

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.

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).

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,[3] and patients 3, 4, 6, and 7 are cases 1–4 in another report.[2] Patients 2 and 9 have been reported in an abstract.[7]

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.[8] 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 R151P and A142D. We analysed six microsatellite markers at D11S4174, D11S1385, D11S1344, D11S1252, D11S4109, and D11S4117 (fig 1A) using the GeneScan Analysis software (Applied Biosystems, Foster City, CA, USA) as previously described,[3] and traced five *RAPSN* polymorphisms (−365G/A, IVS1-15C/T, 456T/C, IVS3-11delC, and 1298C/T) by direct sequencing using fluorescently labelled dideoxy terminators. Position +1 for –365G/A represents the transcriptional start site,[3] whereas position +1 for 456T/C and 1298C/T corresponds to the translational start site. We used an ABI377 DNA sequencer (Applied Biosystems) for both direct sequencing and microsatellite marker analysis.

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: −27CG (patient 1),[3] 1177delAA (patient 2),[7] L14P (patient 3),[2] C97X (patient 5), 553ins5 (patient 7),[2] R151P (Pt 8), E333X (Pt 9),[7] and A142D (Pt 10) (fig 2). All missense mutations affect residues conserved across species (fig 2B). C97X, R151P, and A142D have not been reported to date. Neither R151P nor A142D was detected in 200 normal alleles.

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 D11S4174, D11S1385, D11S1252, D11S4109, and five *RAPSN* polymorphisms were not linked to N88K, whereas D11S1344 and D11S4117 were (fig 1A). The presence or absence of the linkage was independent of the distance to *RAPSN*. All the mutant chromosomes shared a common genotype at five *RAPSN*

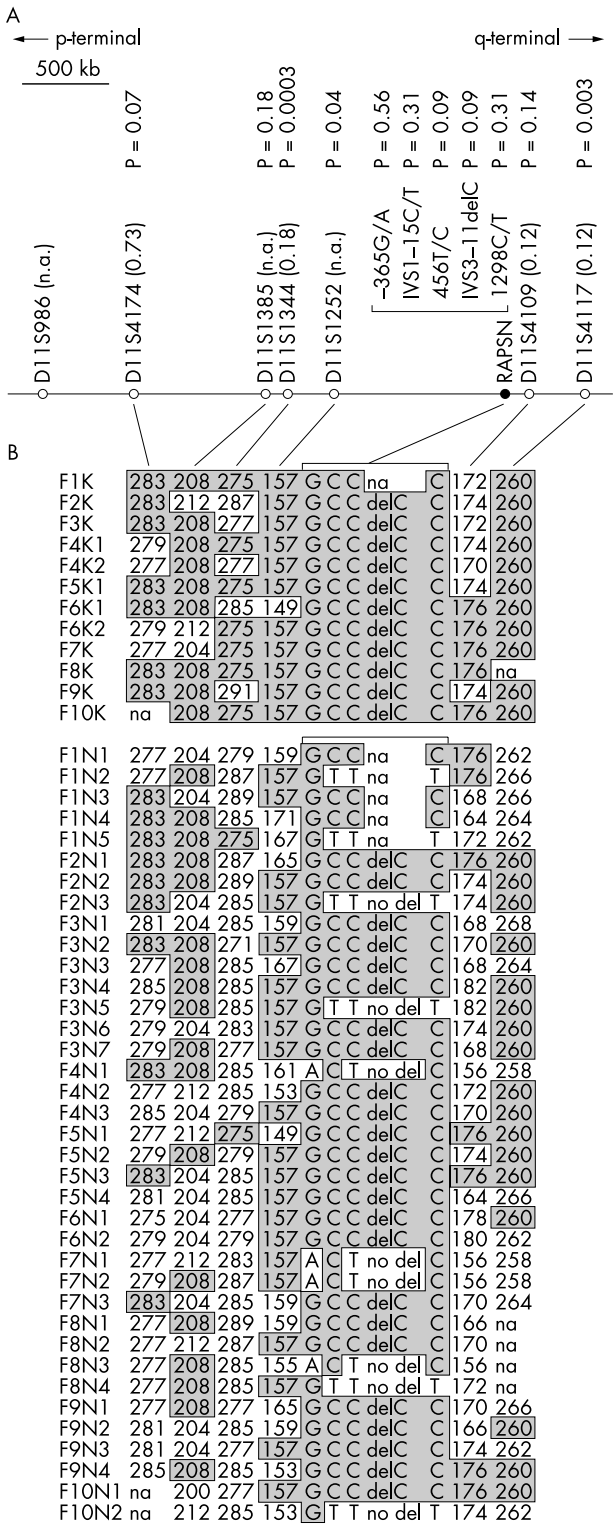

Figure 1 Haplotype analysis. (A) Physical map of microsatellite markers (open circles) flanking *RAPSN* (closed circle) according to the NCBI uniSTS database and Map Viewer Build 33. Positions of markers are slightly different from those reported by us[3] and by Müller *et al*,[5] because the Human Genome Project has recently been completed for this region (GenBank accession number NT_009237). Numbers in parentheses indicate genetic distances from *RAPSN* in centiMorgans according to the deCODE recombination map.[9] The genetic distances are approximate, because *RAPSN* has not been positioned in any recombination map. na, Not available. p values represent Fisher's two tailed exact test to see if a dominant genotype at each marker is linked to N88K. (B) Identified haplotypes. F1–F10 denote families 1 to 10. K and 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.

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,[5] and Richard *et al* analysed three microsatellite markers.[6] 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*[5] 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 makers used by Richard *et al*[6] 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*[5] and Richard *et al*[6] 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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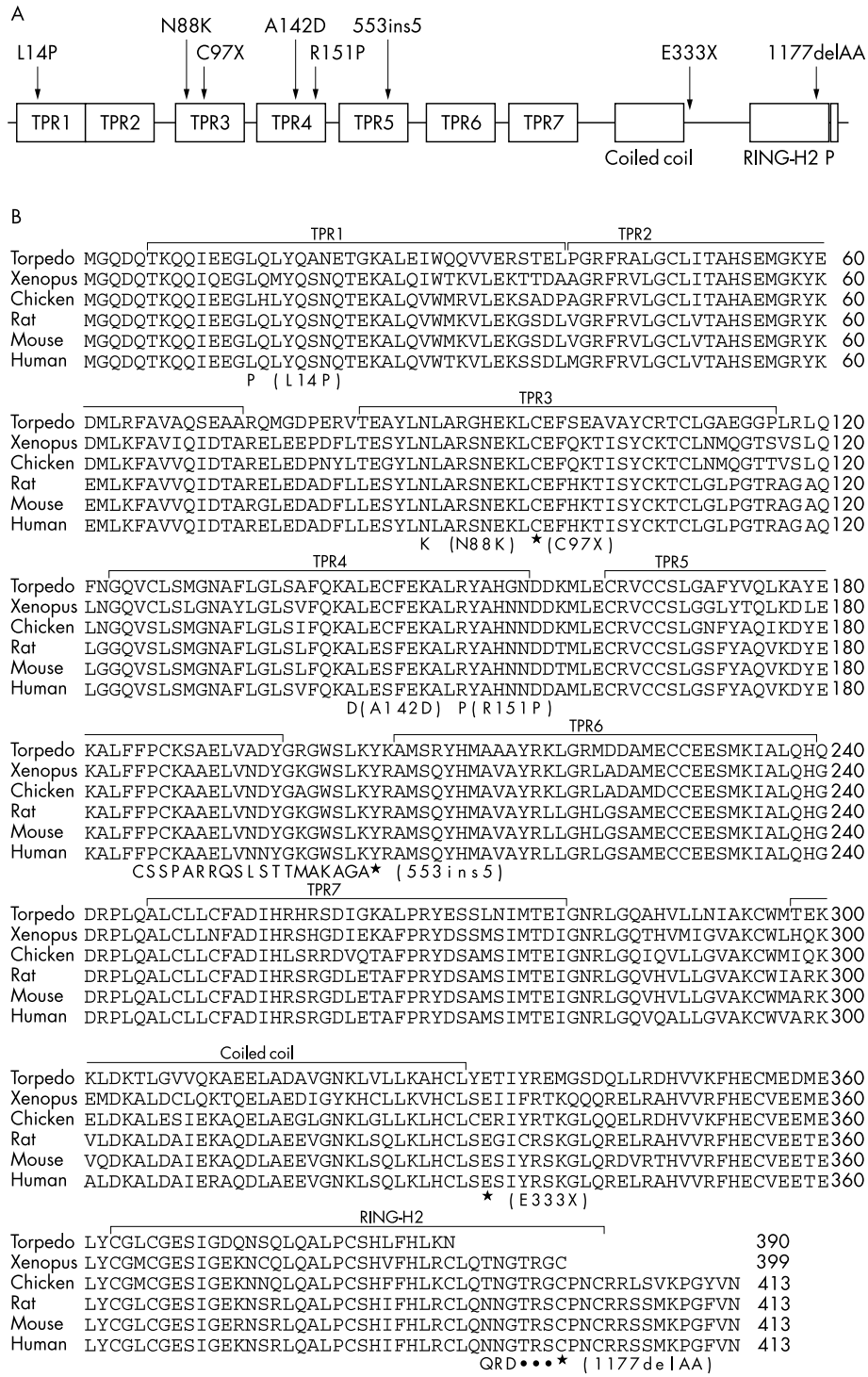

Figure 2 (A) Rapsyn domains and identified mutations. Seven tetratricopeptide repeats (TPRs) subserve rapsyn self association. A coiled coil domain binds to the acetylcholine receptor. A RING-H2 domain binds to β dystroglycan to anchor rapsyn clusters to the cytoskeleton. There is a serine phosphorylation site (designated by P) at codon 406. (B) Alignment of amino acid sequences of *Torpedo californica*, *Xenopus*, chicken, rat, mouse, and human rapsyn. Identified mutations (**bold** letters) and functional domains are indicated. Each missense mutation alters a conserved amino acid. The 553ins5 and 1177delAA mutations predict 19 and 82 missense codons followed by a stop codon (*), respectively. The right column indicates codon numbers of the rightmost amino acids.

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