ELECTRONIC LETTER

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

J S Müller*, A Abicht*, G Burke, J Cossins, P Richard, S K Baumeister, R Stucka, B Eymard, D Hantai, D Beeson, H Lochmüller

J Med Genet 2004;41:e104 (<http://www.jmedgenet.com/cgi/content/full/41/8/e104>). doi: 10.1136/jmg.2004.021139

Only recently, mutations of the RAPSIN gene have been recognised as causing acetylcholine receptor deficiency at the motor endplate resulting in early and late onset forms of congenital myasthenic syndromes (CMS).^{1–9} In most studies a single missense mutation of RAPSIN (N88K) was detected either homozygously or compound heterozygously in numerous, unrelated patients of European and North American origin. Based on the analysis of a small number of intragenic single nucleotide polymorphisms (SNPs) and/or extragenic, polymorphic repeat markers, we hypothesised that RAPSIN (N88K) may derive from a common founder.⁷ However, this hypothesis was disputed in a recent report in the *Journal of Medical Genetics*.¹⁰ In this study, 12 independent RAPSIN (N88K) alleles from white North American CMS patients were compared to 37 wild type chromosomes. Five intragenic SNPs and two extragenic microsatellite markers appeared to be in linkage disequilibrium with the mutation, whereas four extragenic microsatellite markers failed to prove a significant association.¹⁰ To resolve this question we analysed 41 independent RAPSIN (N88K) alleles using 21 SNPs flanking RAPSIN on chromosome 11p11.¹¹

METHODS

We collected 21 CMS patients harbouring RAPSIN (N88K) either homozygously (n = 20) or compound heterozygously (n = 1). Screening for the mutation N88K (264C→A) in exon 2 of the RAPSIN gene was performed as described previously.⁷ 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 RAPSIN (N88K) and show clinical features typical of CMS (data not shown). For the second Indian subcontinent patient (Ind2) with a second, heteroallelic mutation (46insC⁶), 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 RAPSIN (N88K). Primer sequences and restriction enzymes used to screen the selected SNPs are given in table 1. Optimal SNPs flanking RAPSIN 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 RAPSIN gene (SNPs 9–12). In addition to the 21 CMS patients, we analysed 20 European controls for all SNPs. We defined a core founder

Key points

- Mutations in various genes of the neuromuscular junction cause congenital myasthenic syndromes (CMS). The protein rapsyn is encoded by the RAPSIN gene and clusters acetylcholine receptors (AChR) at the motor endplate. Recessive mutations of RAPSIN result in AChR deficiency and impaired neuromuscular transmission.
- A single missense mutation of RAPSIN (N88K) detected frequently in patients of European ethnic origin results in early and late onset forms of CMS. Three studies suggested that RAPSIN (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 RAPSIN (N88K) alleles.
- Analysis of 21 single nucleotide polymorphisms (SNPs) flanking RAPSIN on chromosome 11p11 revealed a common conserved haplotype encompassing a distance of about 360 kb. Our results support the hypothesis that RAPSIN (N88K) derives from a single founder event in an ancient Indo-European population.

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 χ^2 test was used to compare mutant alleles with normal control alleles.

RESULTS AND DISCUSSION

All 41 RAPSIN (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 RAPSIN (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 RAPSIN (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

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

Table 1 Primer sequences and restriction enzymes used to screen the selected SNPs

	SNP	Position on chr. 11 (Mb)	Primer sequences	Restriction enzyme	Frequency in controls
1	rs1446331 A/G	44.745543	5'- TCCATCCACCCAGTCATTTTC -3' 5'- GAGAAATGGATGGATGGGTAGTGTA -3'	RsaI	40.0% A
2	rs728516 C/T	45.565115	5'- GGCTCCAGAAATCCACCCTG -3' 5'- CTTCTGAGTCGTTTGGCTC -3'	MseI	70.0% T
3	rs7476 A/C	46.307143	5'- CACTGTACAGAGACCAAGAAC -3' 5'- ATGTGCTGAGCTGATGAAGAG -3'	HinfI	62.5% A
4	rs3136516 A/G	46.725065	5'- CCTGTGAAGGTGACAGTGGGG -3' 5'- TCAATGACCTTCTGTATCCAC -3'	EcoNI	52.5% A
5	rs2279438 T/A	47.168484	5'- CTACTGTCTAGGGGAGGAGCCGA -3' 5'- TCCTCCACCCGCTGTACCGTG -3'	Bsp143I	82.5% A
6	rs2278890 C/T	47.363911	5'- GGTTTCTCCCTGGAACCTCTG -3' 5'- CTCTTACCACCCGCTTAC -3'	AvaI	37.5% T
7	rs2293577 T/C	47.401511	5'- GCGCTGTCCCCAGCCATGC -3' 5'- GTCCAGCAGGGCAAGGCCTC -3'	HpaII	65.0% T
8	rs4282946 C/T	47.420511	5'- GGCGCAGTGATGCAACAG -3' 5'- GAGCCCATCCAAGCTGGCTCC -3'	MseI	72.5% T
9	1143C/T	RAPSN exon 7	5'- AAGTGGCTGAGACCGGAGCCTA -3' 5'- TTAAGCCAGCTGGGCGCTAGAGT -3'	Sequencing	72.5% C
10	IVS3-11C/delC	RAPSN intron 3	5'- GCAGAGGAGTTGGCTGAGCCACAGG -3' 5'- TGCATCCCGGTGACCTCACCTC -3'	StuI	72.5% delC
11	456 C/T	RAPSN exon 2	5'- GAGAGCTTCGAGAAGGCCCTGCGGTA -3' 5'- GCCACAGGGTGTGTGCCTCA -3'	RsaI	72.5% C
12	-365 G/A	RAPSN promoter	5'- TGAGTGACCCACGGTGCTCAG -3' 5'- AGGCCTTGGGCTAGAGGTGCTC -3'	MnII	92.5% G
13	rs2242081 T/C	47.464576	5'- TATCAGAGAACAGCCTCAGTC -3' 5'- CCAATGGGAGGTAAGTTTTC -3'	RsaI	37.5% C
14	rs2280231 C/T	47.564747	5'- ACCGCTTGGTCTTCTCAGCGTC -3' 5'- AGGAGGGAACGAGGTGAGAAAG -3'	Eco47I	25.0% T
15	rs3817334 C/T	47.615302	5'- ACTATTCATACTGTAAGCAGG -3' 5'- CCATTCTTGCTTTGTAATTTTTCAC -3'	TaqI	45.0% T
16	rs9909 C/G	47.764084	5'- CTTTATTGGTCAGATTAGAAGC -3' 5'- AAGTATATAATGTAATATGTTATG -3'	BstI 107I	57.5% C
17	rs2270994 A/G	48.122178	5'- GAGACAGTTAGAGTGGCTGTTC -3' 5'- GGCACTTATATAACATCAC -3'	MnII	52.5% G
18	rs2299650 G/T	49.138316	5'- ACAAATGAAAGTAATGTGATG -3' 5'- TGCTATCAGAAAAGCATGGAG -3'	SclI	60.0% G
19	rs1880438 C/T	49.335007	5'- TCCTTCACTAACTGAAGGTGAG -3' 5'- TACGTATCTCTGATTCAGTACG -3'	SfaNI	77.5% C
20	rs1916207 C/T	50.500285	5'- GTGTAATCTGAGAGACTAATG -3' 5'- GGGGTTTGGAAACACTGTTGTGG -3'	HinfI	32.5% C
21	rs507015 G/A	51.454648	5'- ACAGCCACTGGATGAAATGTTT -3' 5'- TTTTGTATCCAAGGTTCTCC -3'	HpaII	62.5% A

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.^{7,8,10} An unambiguous 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 11p11.2 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.¹⁰ 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.^{7,8,10} 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,¹⁰ 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 meiotic exchange is reduced across centromeres of human X chromosomes¹² and of autosomes¹³ 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

SNP	Distance to N88K (Mb)	G4	Fr1	UK8	UK9	UK10	Fr2	UK4	UK5	UK7	UK1	UK3	UK6	G3	G2	Au1	UK2	G5	G1	It1	Ind1	Ind2*
1	2.688	A	A	A	A	A	A/G	A/G	A/G	A/G	G	G	G	A	G	A/G	A/G	A/G	A/G	G	G	G
2	1.869	T	T	T	T	T	T	T	T	T	T	T	T	T	C/T	C	C/T	T	C/T	C/T	T	C
3	1.127	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
4	0.709	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
5	0.266	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
6	0.070	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	C
7	0.032	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
8	0.013	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
9	0.009	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
10	0.006	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC	delC
11	0.000	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
12	0.001	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
13	0.030	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
14	0.131	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
15	0.181	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
16	0.330	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
17	0.688	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G/A	G/A	A	C
18	1.704	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G/T	G/T	G/T	G/T	G
19	1.901	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C/T	C/T	T	C	C
20	3.066	C	C	C	C	C	C	C	C	C	C	C	C	C/T	C	C	C/T	C/T	C/T	C/T	T	T
21	4.021	A	A	A	A	A	A	A	A	A	A	A	A	A/G	A	A	A/G	A	G	G	A	A

Figure 1 Haplotype analysis of 21 unrelated CMS patients (41 RAPSIN 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.

European and Indian subcontinent origin. Secondly, RAPSIN (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

ACKNOWLEDGEMENTS

We thank the patients for participating in this study and Ursula Klutzny for expert assistance.

Authors' affiliations

J S Müller, A Abicht, S K Baumeister, R Stucka, H Lochmüller, Friedrich-Baur-Institute, Department of Neurology, and Gene Center, Ludwig-Maximilians-University, Munich, Germany
G Burke, J Cossins, D Beeson, Neurosciences Group, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK
P Richard, B Eymard, D Hantai, INSERM U.582, Institut de Myologie and Unité Fonctionnelle de Cardiogénétique et Myogénétique, Hôpital de la Salpêtrière, Paris, France

This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) and the Deutsche Gesellschaft für Muskelkranke (DGM) to HL and AA, by grants from the Medical Research Council (UK) and the Muscular Dystrophy Campaign/Myasthenia Gravis Association of Great Britain to DB, and by grants from the Institut National de la Santé et de la Recherche Médicale (INSERM), the Assistance Publique-Hôpitaux de Paris (APHP), and the Association Française contre les Myopathies (AFM) to PR, BE, and DH. JSM receives a scholarship from the Boehringer Ingelheim Fonds. SKB receives a scholarship through the program for molecular medicine of the Ludwig-Maximilians-University, Munich.

Conflict of interest: none declared.

*These authors contributed equally to this paper.

Correspondence to: Dr Hanns Lochmüller, Genzentrum München, Feodor-Lynen-Str. 25, 81377 München, Germany; hanns@lmb.uni-muenchen.de

Revised version received 4 May 2004
Accepted for publication 4 May 2004

REFERENCES

1 Ohno K, Engel AG, Shen XM, Selcen D, Brengman J, Harper CM, Tsujino A, Milone M. Rapsyn mutations in humans cause endplate acetylcholine-receptor deficiency and myasthenic syndrome. *Am J Hum Genet* 2002;**70**(4):875–85.
2 Ohno K, Sadeh M, Blatt I, Brengman JM, Engel AG. E-box mutations in the RAPSIN promoter region in eight cases with congenital myasthenic syndrome. *Hum Mol Genet* 2003;**12**(7):739–48.
3 Dunne V, Maselli RA. Identification of pathogenic mutations in the human rapsyn gene. *J Hum Genet* 2003;**48**(4):204–7.
4 Maselli RA, Dunne V, Pascual-Pascual SI, Bowe C, Agius M, Frank R, Wollmann RL. Rapsyn mutations in myasthenic syndrome due to impaired receptor clustering. *Muscle Nerve* 2003;**28**(3):293–301.
5 Banwell BL, Ohno K, Sieb JP, Engel AG. Novel truncating RAPSIN mutations causing congenital myasthenic syndrome responsive to 3,4-diaminopyridine. *Neuromusc Disord* 2004;**14**:202–7.
6 Burke G, Cossins J, Maxwell S, Owens G, Vincent A, Robb S, Nicolle M, Hilton-Jones D, Newsom-Davis J, Palace J, Beeson D. Rapsyn mutations in hereditary myasthenia: Distinct early- and late-onset phenotypes. *Neurology* 2003;**61**(6):826–8.
7 Müller JS, Mildner G, Müller-Felber W, Schara U, Krampfl K, Petersen B, Petrova S, Stucka R, Mortier W, Butler J, Kurlmann G, Huebner A, Merlini L, Lochmüller H, Abicht A. Rapsyn N88K is a frequent cause of congenital myasthenic syndromes in European patients. *Neurology* 2003;**60**(11):1805–10.
8 Richard P, Gaudon K, Andreux F, Yasaki E, Prioleau C, Bauché S, Barois A, loos C, Mayer M, Routon MC, Mokhtari M, Leroy JP, Fournier E, Hainque B, Koenig J, Fardeau M, Eymard B, Hantai D. Possible founder effect of rapsyn N88K mutation and identification of novel rapsyn mutations in congenital myasthenic syndromes. *J Med Genet* 2003;**40**(6):e81.
9 Yasaki E, Prioleau C, Barbier J, Richard P, Andreux F, Leroy JP, Darteville P, Koenig J, Molgó J, Fardeau M, Eymard B, Hantai D. Electrophysiological and morphological characterization of a case of autosomal recessive congenital myasthenic syndrome with acetylcholine receptor deficiency due to a N88K rapsyn homozygous mutation. *Neuromusc Disord* 2004;**14**(1):24–32.
10 Ohno K, Engel AG. Lack of founder haplotype for the rapsyn N88K mutation: N88K is an ancient founder mutation or arises from multiple founders. *J Med Genet* 2004;**41**(1):e8.
11 Buckel A, Beeson D, James M, Vincent A. Cloning of cDNA encoding human rapsyn and mapping of the RAPSIN gene locus to chromosome 11p11.2–p11.1. *Genomics* 1996;**35**(3):613–6.
12 Mahtani MM, Willard HF. Physical and genetic mapping of the human X chromosome centromere: repression of recombination. *Genome Res* 1998;**8**(2):100–10.
13 Puechberty J, Laurent AM, Gimenez S, Billault A, Brun-Laurent ME, Calenda A, Marçais B, Prades C, Ioannou P, Yurov Y, Roizes G. Genetic and physical analyses of the centromeric and pericentromeric regions of human chromosome 5: recombination across 5cen. *Genomics* 1999;**56**(3):274–87.
14 Liquori CL, Ikeda Y, Weatherspoon M, Ricker K, Schoser BG, Dalton JC, Day JW, Ranum LP. Myotonic dystrophy type 2: human founder haplotype and evolutionary conservation of the repeat tract. *Am J Hum Genet* 2003;**73**(4):849–62.