

ONLINE MUTATION REPORT

Possible founder effect of rapsyn N88K mutation and identification of novel rapsyn mutations in congenital myasthenic syndromes

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Congenital myasthenic syndromes (CMS) constitute a group of rare diseases heterogeneous both in terms of their mode of hereditary transmission (recessive and dominant forms) and their pathophysiology (with presynaptic, synaptic, and postsynaptic defects). They are responsible for dysfunction of neuromuscular transmission giving rise to a condition of muscle weakness which is accentuated by exertion. In most cases, CMS begin in early childhood but later onset in adulthood is possible. Severity also varies from severe with respiratory failure to mild expression.^{1,2}

The majority of CMS primarily affect postsynaptic function and are the result of mutations located in the muscle acetylcholine receptor (AChR) subunit genes that lead to kinetic abnormalities or to AChR deficiency. For example, an increased response occurs in the slow channel syndromes associated with dominant mutations in AChR subunits delaying channel closure or increasing the affinity of the receptor for acetylcholine. However, the most commonly encountered CMS is a deficiency in AChR which occurs with recessive mutations. Most of these mutations are located in the AChR ϵ subunit.^{3,4}

Recent advances have shown that mutations in rapsyn are also involved in recessive forms of postsynaptic CMS and cause AChR deficiency.⁵ Rapsyn is a 43 kDa postsynaptic protein involved in development and maintenance of the molecular architecture of the postsynaptic membrane by participating in the clustering of AChR after binding of neural agrin to its muscle specific receptor tyrosine kinase, MuSK.^{6,7} The rapsyn gene (*RAPSN*) has been mapped to chromosome 11p11.2 p11.1 and comprises eight exons.⁸ The primary structure of rapsyn is predicted to contain several functional domains such as an N-terminus myristoylation signal, seven tetratricopeptide repeats (residues 6-279), a coiled-coiled domain (298-331), a cysteine rich RING-H2 domain (363-402), and a serine phosphorylation site at codon 406.^{9,10}

The aim of this work was to search for mutations in *RAPSN* in 20 recessive forms of postsynaptic CMS in which the most frequently involved gene encoding the AChR ϵ subunit^{3,4} was excluded by direct sequencing of *CHRNE* promoter and exons.

MATERIALS AND METHODS

CMS was diagnosed on the basis of the following clinical criteria: first, the patients were suffering from a myasthenic syndrome with fluctuating muscle weakness and detection of a neuromuscular block on EMG examination; second, this myasthenic syndrome was of congenital origin with a family history of the disease and/or a neonatal onset. Tests for anti-AChR antibodies in the serum were always negative. Twenty patients affected by the recessive form of CMS and excluded for mutations in the AChR ϵ subunit were selected for analysis of *RAPSN*. Informed written consent was obtained

in accordance with a study protocol approved by the ethics committee of La Pitié-Salpêtrière Hospital (CCPPRB No 93-02).

Genetic analyses were performed on genomic DNA extracted from whole blood by standard methods. The screening for mutations was done with a direct sequencing approach on an automated laser fluorescent sequencer (ABI 3100, Applied Biosystems) after amplification of each exon separately with primers designed in intronic flanking sequences (GenBank accession number AC074195). A missense variant was considered as a mutation on the basis of three criteria: amino acid modification, conservation of the residue among species and

Key points

- Hereditary congenital myasthenic syndromes are heterogeneous disorders characterised by impaired neuromuscular transmission owing to specific defects classified as presynaptic, synaptic, or postsynaptic. Postsynaptic defects have been reported to be the result of anomalies of the acetylcholine receptor and particularly of mutations in the gene for the ϵ subunit. Recently, mutations in *RAPSN*, the gene for rapsyn, a protein involved in the maintenance of the postsynaptic membrane, were reported to lead to endplate acetylcholine receptor deficiency.
- Twenty patients with recessive forms of congenital myasthenic syndromes with no mutations in the ϵ subunit of acetylcholine receptor were selected and tested for mutations in *RAPSN*.
- Mutations in *RAPSN* were identified in five patients. All were carriers of the previously described N88K mutation, which was either homozygous or heteroallelic for novel mutations: a frameshift mutation (1083>1084dupCT), a splice site mutation (IVS4-2A>G), or a missense mutation (V165M). Haplotype analysis with microsatellite markers located on both sides of the gene indicated that four of the five patients and five healthy control heterozygous carriers of the N88K mutation shared a common haplotype owing to a possible ancestral founder effect.
- Mutations in *RAPSN* were found in 20% of a panel of 20 French patients with recessive forms of congenital myasthenic syndrome. In all patients the N88K mutation was identified either in the homozygous state or associated with another *RAPSN* variant. Moreover, the N88K variant was found in the heterozygous state in five out of 300 healthy controls of all origins.

Table 1 Clinical features of the five patients with *RAPSN* mutations

Patients/ sex (M/F)	Family history	Onset	Initial symptoms	Symptoms during evolution	Presenting examination (age/signs)	Anticholinesterase (age at/effect)	Decrement
Patient 1/ M	Brother of patient 2	Childhood	Fluctuating ptosis	15 y/limb fatigability, girdle weakness	51 y/ptosis, neck fatigability	51 y/positive (ambenonium)	Yes
Patient 2/ M	Brother of patient 1	Childhood	Fluctuating ptosis, limb weakness	20 y/diplopia, stability of other symptoms	43 y/ptosis, ophthalmoplegia, limb weakness	43 y/negative (only parenteral neostigmine tested)	Yes
Patient 3/ F	No	Birth	Hypotonia, swallowing and sucking disturbances	First 4 mth/multiple hospitalisations for choking, hypotonia, vagal episodes, 5 mth: cardiac arrest, tracheostomy	5 mth/facial diparesis, hypotonia, limb weakness	3 m/positive (oral neostigmine)	Yes
Patient 4/ M	Sister: sudden death at 12 mth, arthrogryposis	Fetal	Hydranmios, arthrogryposis, hypotonia, respiratory distress, intubation day 1 to 8	Ptosis, ophthalmoplegia, facial diparesis, choking, scoliosis, 6 mth: respiratory distress	3 y/ptosis, fatigability, no limb weakness, open mouth	6 m/positive (pyridostigmine bromide)	Yes (20 Hz only)
Patient 5/ F	No	Fetal	Arthrogryposis, respiratory distress (intubation at birth), feeding problems	4 mth/tracheostomy, 7 mth/ejunosotomy, 2.5 y/cardiorespiratory arrest, severe neck + limb weakness	7.5 y/severe proximal weakness, no unaided walking	8 y/positive. After pyridostigmine bromide: walking alone, no more ventilation	Yes

Table 2 *RAPSN* mutations in the four white families*

	Patient	Nucleotide change	Amino acid change
Family 1	Patient 1	264C→A	N88K Homozygous
	Patient 2	264C→A	N88K Homozygous
Family 2	Patient 3	264C→A	N88K
		493G→A	V165M
Family 3	Patient 4	264C→A	N88K
		IVS4-2A→G	Splice mutation
Family 4	Patient 5	264C→A 1083_1084dupCT	N88K Frameshift mutation

*Nucleotide numbering following *rapsyn* cDNA sequence (GenBank accession number AF449218).

isoforms, and absence of the variant in the homozygous state in at least 200 normal chromosomes. Mutations inducing a disruption of the normal reading frame were considered as disease causing mutations.

A haplotype analysis was performed with four (CA)_n repeat microsatellite markers located on both sides of *RAPSN* (D11S986, D11S1252 upstream from the gene and D11S4117, D11S4109 downstream from the gene) by an automated fluorescent method on an ABI 310 analyser.

RESULTS AND DISCUSSION

Patients

Five patients from four non-consanguineous families were found to carry *RAPSN* mutations. Family 1 is composed of two affected brothers (patient 1 and patient 2) and in family 3 the sister of patient 4 was probably affected because she had arthrogryposis and died suddenly at the age of 12 months. Patients 3 and 5 were apparently sporadic patients. The disease was mild in patients 1 and 2, with onset in childhood, long standing fluctuating limb fatigability and ptosis, absence of bulbar and respiratory symptoms, and diagnosis was performed after 40 years of age. In contrast, the three other patients (patients 3, 4, and 5) were children presenting very severe and early onset diseases with arthrogryposis (patients 4 and 5) and major pharyngeal and respiratory involvement requiring assisted ventilation. Anticholinesterases were effective in patients 3, 4, and 5, allowing walking and cessation of ventilation when initiated at 8 years in patient 5. In the two mild patients 1 and 2, the parenteral prostigmin test was negative but ambenonium was effective. A decrement was seen in all patients, only at 20 Hz stimulation in patient 4. Clinical data are summarised in table 1.

Mutation detection

Sequencing of the entire coding sequence of *RAPSN* allowed the identification of mutations in five patients, as summarised in fig 1 and table 2. In all patients the N88K mutation (264C>A in exon 2) was either homozygous or associated with another mutation. In family 1, patients 1 and 2 were homozygous for the N88K mutation. Patient 3 was a carrier of this N88K mutation associated with the missense mutation V165M. Patient 4 was heteroallelic for the N88K mutation associated with the mutation IVS4-2A>G. This second variant is an acceptor splice site mutation predicted to introduce a splicing error during mRNA maturation. Patient 5 was heteroallelic for the N88K mutation associated with the dinucleotide CT duplication in exon 7 (1083>1084dupCT). This duplication leads to the disruption of the reading frame during translation and creation of a premature termination at codon 371. The N88K and V165M mutations are located in the TPR3 and TPR5 domain respectively, both domains involved in rapsyn self-association. The predicted consequences of the two

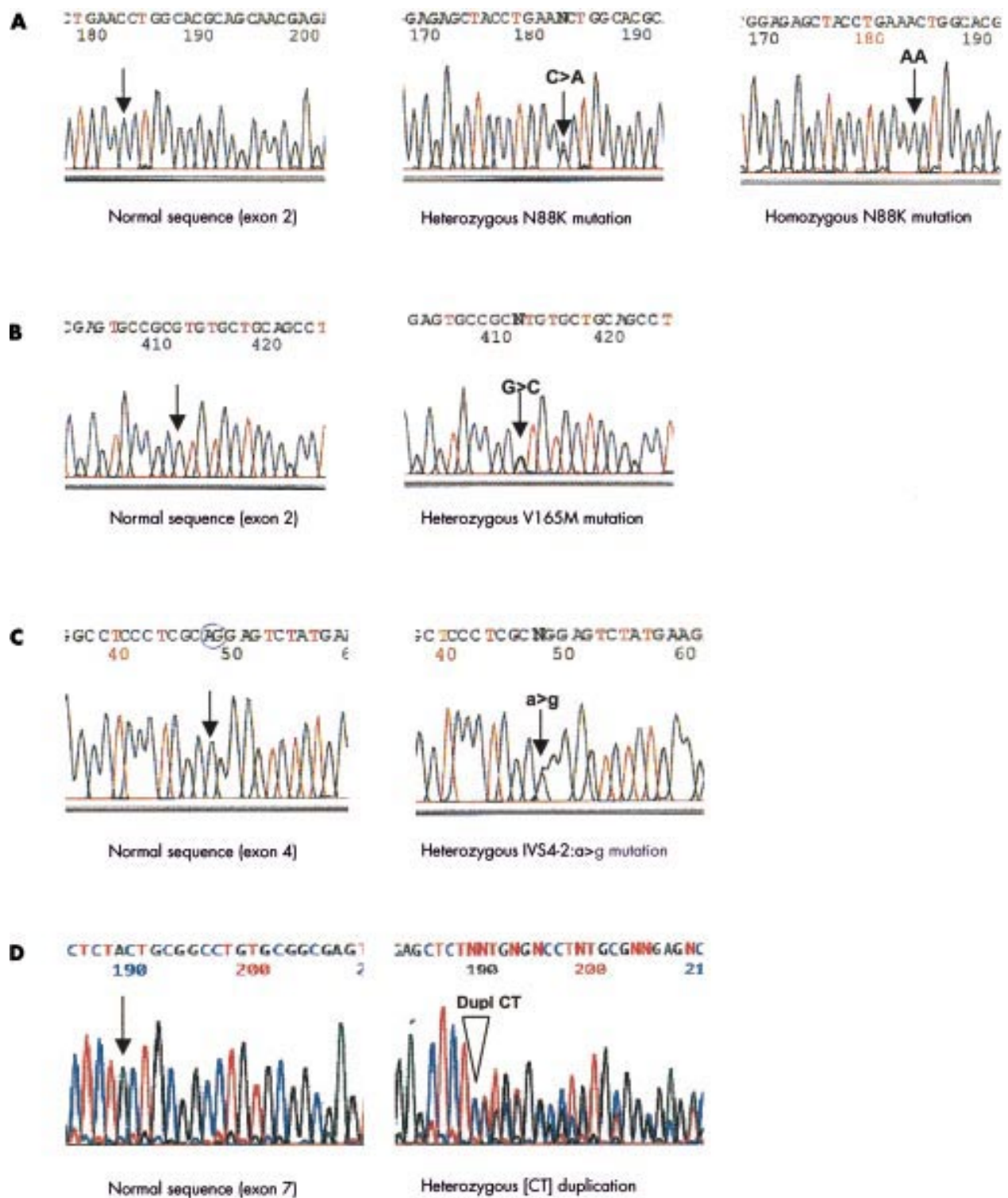


Figure 1 Electropherograms of normal and mutated exons. The arrow indicates the position and nature of the modified nucleotides. (A) Segments of exon 2 indicating the C to A variation responsible for N88K mutation with heterozygous status or homozygous status. (B) Normal and heterozygous variant for V165M mutation. (C) Normal and heterozygous mutated fragment of exon 4 indicating the a to g mutation at position IVS4-2. (D) Normal and mutated sequence for exon 7 indicating a CT duplication disrupting the reading frame.

nonsense mutations (patient 4 and patient 5) are the synthesis of truncated proteins lacking their carboxy-terminus part corresponding to the cysteine rich RING-H2 domain¹¹ and the actin-binding synaptic nebulin-related anchoring protein (S-NRAP) domain. The second hypothesis is an absence of the mutant protein through a nonsense mediated mRNA decay mechanism.¹²

Amino acids N88 and V165 are highly conserved among species. Testing of 200 control chromosomes from healthy subjects did not show the V165M variant but the N88K mutation was found in the heterozygous state in five of them. Thus, 400 additional chromosomes were tested for the N88K mutation. This mutation was not detected indicating that the allelic frequency of this particular mutation is probably low (<1%).

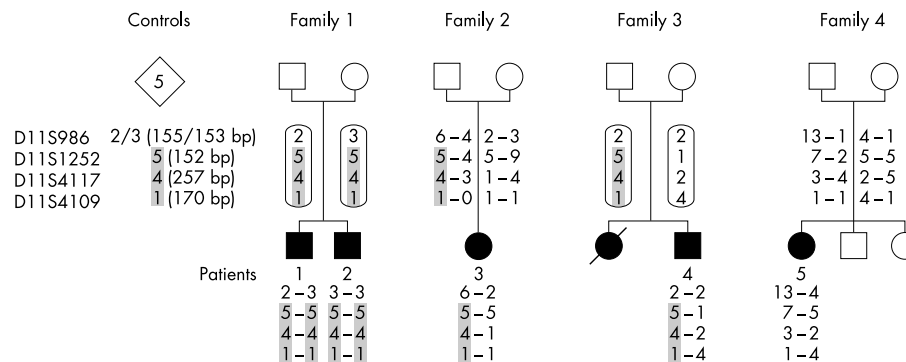


Figure 2 Pedigrees of the families carrying the N88K mutation and haplotype results with four microsatellite markers (D11S986, D11S1252 upstream and D11S4117, D11S4109 downstream of the *RAPSN* gene). Affected patients are indicated by black symbols and "affected" haplotypes are indicated by shadows. Molecular size of the common allele for each marker is indicated in parentheses. Haplotype analysis showed that all patient carriers of the N88K mutation shared the same (5-4-1) haplotype indicating a possible ancestral founder effect for this mutation.

This N88K mutation was previously reported³ and also found in the homozygous state or associated with a frameshift or a missense mutation. To date, all patients with *RAPSN* mutations have carried this particular mutation on at least one allele.

Genotype-phenotype correlation

Analysis of phenotype and disease severity showed that patients 1 and 2 from the same sibship had a mild disease characterised by ocular and mild limb weakness, without bulbar or respiratory involvement. These two patients are quite similar to the 27 year old patient reported by Ohno *et al*⁵ and furthermore all three were carrying the same homozygous N88K variant. Our three other patients, who were severely affected, with fetal onset in two of them and respiratory distress requiring assisted ventilation, harboured heteroallelic mutations associating N88K + missense or frameshift mutations. Their clinical pattern was similar to that of three severe neonatal cases in the report by Ohno *et al*.⁵ In this latter series, two infants also had heteroallelic mutations associating N88K + missense or frameshift mutations, but the third one, also severely affected, had the homozygous N88K variant, found in mild cases (see above). On the whole, the results obtained in two series including nine patients (five in this report) do not militate in favour of a clear genotype-phenotype correlation.

Possible founder effect of the N88K mutation

A haplotype analysis with four extragenic markers located on both sides close to *RAPSN* was performed to search for an ancestral founder effect. D11S986, D11S1252, D11S4117, and D11S4109 were analysed in all patients, parents when available, and controls. A number corresponding to its size was used to designate each allele and a haplotype was constructed (fig 2). Despite a low informativeness of D11S1252 and D11S4117 markers and a genetic recombination between the D11S986 and D11S1252 markers, three families shared the same (5-4-1) haplotype for the three closest markers (D11S1252, D11S4117, and D11S4109) indicating a possible ancestral founder effect for the N88K mutation. Family 4 did not share this particular haplotype, suggesting that in this family the mutation arose independently. Surprisingly, all five controls with this mutation were carriers of this haplotype on one allele.

Until now, in postsynaptic defects with AChR deficiency, the most frequent genetic defect was a mutation in one of the AChR subunits. In our experience, the involvement of the *RAPSN* gene in CMS is greater than the involvement of the AChR ϵ subunit. In all patients, previous sequencing of the entire coding sequence of the AChR ϵ subunit failed to detect any mutation. The prevalence of the N88K mutation in CMS

patients and in the whole population may have consequences in terms of genetic counselling.

In conclusion, we report here the results of an analysis of the *RAPSN* gene in CMS. Twenty patients with the recessive form of CMS with no mutations in the AChR ϵ subunit were tested for this gene and five patients were found to carry mutations. Three novel mutations were found compound heterozygous with the N88K mutation in three patients. The N88K mutation was found homozygous in the two other patients.

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