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


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. 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.1

Recent advances have shown that mutations in rapsyn are also involved in recessive forms of postsynaptic CMS and cause AChR deficiency.2 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.3,4 The rapsyn gene (RAPSN) has been mapped to chromosome 11p11.2-p11.1 and comprises eight exons.5 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 406.5

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 was excluded by direct sequencing of CHRNA10 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 (CCPRRB 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
### Table 1  Clinical features of the five patients with RAPSN mutations

<table>
<thead>
<tr>
<th>Patients/ sex</th>
<th>Family history</th>
<th>Onset</th>
<th>Initial symptoms</th>
<th>Symptoms during evolution</th>
<th>Presenting examination (age/signs)</th>
<th>Anticholinesterase (age at/effect)</th>
<th>Decrement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1/ M</td>
<td>Brother of patient 2</td>
<td>Childhood</td>
<td>Fluctuating ptosis</td>
<td>1.5 y/limb fatigability, girdle weakness</td>
<td>51 y/ptosis, neck fatigability</td>
<td>51 y/positive (ambenonium)</td>
<td>Yes</td>
</tr>
<tr>
<td>Patient 2/ M</td>
<td>Brother of patient 1</td>
<td>Childhood</td>
<td>Fluctuating ptosis, limb weakness</td>
<td>20 y/diplopia, stability of other symptoms</td>
<td>43 y/ptosis, ophthalmoplegia, limb weakness</td>
<td>43 y/negative</td>
<td>Yes</td>
</tr>
<tr>
<td>Patient 3/ F</td>
<td>No</td>
<td>Birth</td>
<td>Hypotonia, swallowing and sucking disturbances</td>
<td>First 4 mth/multiple hospitalisations for choking, hypotonia, vaginal episodes, 5 mth: cardiac arrest, tracheostomy</td>
<td>5 mth/facial diparesis, hypotonia, limb weakness</td>
<td>3 m/positive (oral neostigmine)</td>
<td>Yes</td>
</tr>
<tr>
<td>Patient 4/ M</td>
<td>Sister: sudden death at 12 mth, arthrogryposis</td>
<td>Fetal</td>
<td>Hydramnios, arthrogryposis, hypotonia, respiratory distress, intubation day 1 to 8</td>
<td>7.5 y/severe proximal weakness, no unaided walking</td>
<td>7.5 y/severe proximal weakness, no unaided walking</td>
<td>8 y/positive (pyridostigmine bromide)</td>
<td>Yes (20 Hz only)</td>
</tr>
<tr>
<td>Patient 5/ F</td>
<td>No</td>
<td>Fetal</td>
<td>Arthrogryposis, respiratory distress (intubation at birth), feeding problems</td>
<td>4 mth/tracheostomy, 7 mth/jejunostomy, 2.5 y/cardiorespiratory arrest, severe neck + limb weakness</td>
<td>4 mth/tracheostomy, 7 mth/jejunostomy, 2.5 y/cardiorespiratory arrest, severe neck + limb weakness</td>
<td>8 y/positive. After pyridostigmine bromide: walking alone, no more ventilation</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### RESULTS AND DISCUSSION

**Clinical data are summarised in table 1.**

- Patients 1 and 2 were affected by RAPSN mutations. Patients 3, 4, and 5 were children presenting very severe and early onset diseases with arthrogryposis (patients 3, 4, and 5) and major pharyngeal and respiratory involvement (patients 1 and 2).

**Mutation detection**

- Sequencing of the entire coding sequence of RAPSN allowed the identification of mutations in five patients, as summarised in table 1 and fig 1.

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

**Clinical data are summarised in table 1.**

**Patients**

- Patient 1 was affected by the same mutation, N88K, in both his brother (patient 2) and his sister (patient 3).

**Mutation**

- Patient 4 was heteroallelic for the 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 frameshift targeting the 3' UTR of the RAPSN transcript.

**Clinical features**

- Patient 1 had a mild disease with onset in childhood and long-lasting fluctuating limb fatigability and absence of bulbar and respiratory symptoms, and diagnosis was performed after 40 years of age.

**For the four non-consanguineous families, five patients from four non-consanguineous families were affected by RAPSN mutations. Patient 1 is a carrier of two affected brothers (patient 1 and patient 2) and in family 3 the affected brother (patient 1) and sister (patient 4) were probably affected because she had a sister with arthrogryposis and died suddenly at the age of 12 months.**

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>264C→A</td>
<td>N88K</td>
</tr>
<tr>
<td>Patient 2</td>
<td>493G</td>
<td>V165M</td>
</tr>
<tr>
<td>Patient 3</td>
<td>IVS4-2A→G</td>
<td>Splice mutation</td>
</tr>
<tr>
<td>Patient 4</td>
<td>1083_1084dupCT</td>
<td>Frameshift mutation</td>
</tr>
</tbody>
</table>

- **Clinical data are summarised in table 1.**

- **Clinical features**

- Patient 1 and patient 2 were affected by the same mutation, N88K, in both their parents (patient 1) and in family 3 the affected brother (patient 1) and sister (patient 4) were probably affected because she had a sister with arthrogryposis and died suddenly at the age of 12 months.

**Mutation**

- Patient 4 was heteroallelic for the 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 frameshift targeting the 3' UTR of the RAPSN transcript.

**Clinical features**

- Patient 1 had a mild disease with onset in childhood and long-lasting fluctuating limb fatigability and absence of bulbar and respiratory symptoms, and diagnosis was performed after 40 years of age.
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 domain 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.

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%).
tions. Their clinical pattern was similar to that of three severe
mutations associating N88K + missense or frameshift muta-
tions, which caused distress requiring assisted ventilation, harboured heteroallelic
involvement. With fetal onset in two of them and respiratory
involvement. These two patients are quite
characterised by ocular and mild limb weakness, without bul-
gatory complications. Patients 1 and 2 from the same sibship had a mild disease
form of the CMS with no mutations in the AChR ε subunit were
found in patients with the recessive form of CMS with no mutations in the AChR ε subunit were
found for this gene and five patients were found to carry
mutations. Three novel mutations were found compound
heterozygous with the N88K variant in three patients. The
N88K mutation was found homzygous in the two other
patients.

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