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Long-term follow-up in patients with congenital myasthenic syndrome due to *RAPSN* mutations

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Abstract

Rapsyn (*RAPSN*) mutations are a common cause of postsynaptic congenital myasthenic syndromes. We present a comprehensive description of the clinical and molecular findings of ten patients with CMS due to mutations in *RAPSN*, mostly with a long-term follow-up. Two patients were homozygous and eight were heterozygous for the common p.Asn88Lys mutation. In three of the heterozygous patients we have identified three novel mutations (c.869T > C; p.Leu290Pro, c.1185delG; p.Thr396Profs*12, and c.358delC; p.Gln120Serfs*8). In our cohort, the *RAPSN* mutations lead to a relatively homogeneous phenotype, characterized by fluctuating ptosis, occasional bulbar symptoms, neck muscle weakness, and mild proximal muscle weakness with exacerbations precipitated by minor infections. Interestingly, episodic exacerbations continue to occur during adulthood. These were characterized by proximal limb girdle weakness and ptosis, and not so much by respiratory insufficiency after age 6. All patients presented during neonatal period and responded to cholinergic agonists. In most of the affected patients, additional use of 3,4-diaminopyridine resulted in significant clinical benefit. The disease course is stable except for intermittent worsening.

Keywords: Rapsyn; Congenital myasthenic syndrome; Congenital myasthenia; Neuromuscular junction; Pyridostigmine; 3,4-diaminopyridine

1. Introduction

Congenital myasthenic syndromes (CMS) are a heterogeneous group of genetic disorders, all of which impair neuromuscular transmission. At present, 25 different genes have been identified in association with CMS. These genes code for proteins involved in neuromuscular junction structure or function [1–4]. All CMSs share the clinical feature of fatigable weakness, but age of onset, presenting symptoms, distribution of weakness, and response to treatment differ depending on the molecular mechanism that results from the genetic defect.

Rapsyn is a 43-kDa postsynaptic protein that binds to the long cytoplasmic loop of the AChR subunits and is essential for clustering and anchoring the AChR in the postsynaptic membrane. Mutations in rapsyn compromise the safety margin of neuromuscular transmission by causing endplate AChR deficiency [5]. Rapsyn is composed of several functionally distinct regions: a myristoylated N-terminal is required for membrane interaction; seven tetratricopeptide repeats are involved in rapsyn self-aggregation and binding to the cytoplasmic portion of the muscle-specific kinase MuSK; the coiled-coil domain

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interacts with the cytoplasmic loops of AChR subunits; and the C-terminal domain binds to cytoskeletal protein beta-dystroglycan and thereby links the rapsyn–AChR complex to the cytoskeleton.

Today it is estimated that *RAPSN* mutations account for approximately 14–27% of all CMS patients [3,6]. Mutations in *RAPSN* were first described in recessive forms of CMS by Ohno et al. in 2002 [7].

Two distinct phenotypes are described: an early-onset phenotype that presents at birth or in infancy with arthrogryposis, hypotonia, apnoeic crisis, and feeding difficulties; and a less common late-onset phenotype that presents during childhood, or even in adulthood, with weakness and sometimes wasting of distal upper limb muscles. The response to anticholinesterase medication is good in both phenotypes [8].

We describe the clinical and molecular genetic findings as well as long-term follow-up data of 10 patients with CMS due to *RAPSN* mutations. We also aimed to establish reliable clinical signs which might suggest this specific diagnosis.

2. Patients and methods

Patients with genetically confirmed CMS due to mutations in the *RAPSN* gene followed-up in the departments of Paediatric Neurology and Neurology of six Spanish Centers (Hospital Sant Joan de Déu, Barcelona; Hospital San Jorge, Huesca; Hospital de Cruces, Bilbao; Hospital La Fe, Valencia; Hospital 12 de Octubre, Madrid; Hospital Rey Juan Carlos, Madrid) were included.

Patients were systematically assessed every six months for the duration of follow-up. Each patient was reassessed and underwent a detailed clinical examination during the six months prior to the conclusion of this work. None of these patients have been previously reported. The study complies with the ethical guidelines of the institutions involved.

Medical Research Council score (MRC) was used for strength testing. Quantitative myasthenia gravis score (QMG) was used to assess fatigability in different muscle groups, and objectify disease severity. 6-minute walk test (6MWT) was used to measure walking endurance.

Electrophysiological studies including motor and sensory nerve conduction studies were performed in all patients. Repetitive nerve stimulation (RNS) at 3 Hz of proximal (deltoid or trapezius) and distal (abductor pollicis brevis) muscles were performed in all patients.

Muscle biopsy specimens were obtained from 2 patients and processed according to standard histological and histochemical techniques.

Molecular genetic analyses were carried out in all 10 patients and, when available, in their parents and siblings. Genomic DNA was isolated from venous blood samples using a blood DNA extraction kit according to the manufacturer's recommendations (Promega, Mannheim, Germany).

The full coding regions of the *RAPSN* gene were amplified and sequenced by bi-directional Sanger Sequencing by the respective research laboratories. Patients 6, 7 and 8 were subjected to whole exome sequencing. DNA samples were sequenced in deCODE genetics (Iceland) as part of NeurOmics Project, using Illumina Nextera Rapid Capture Expanded Exome (62 Mb). Alignment and variant caller were done using Burrows Wheeler Aligner and Genome Analysis Tool Kit. Data were analyzed on the proprietary interface Clinical Sequence Analyser. Publicly available databases (LOVD: http://www.dmd.nl/; ExAc: http://exac.broadinstitute.org/ and EVS: http://evs.gs.washington.edu/EVS/) were interrogated to identify previously reported variants and also to determine the frequency of the novel variants observed. For the missense changes, a batch of in silico software (Polyphen: http://genetics.bwh.harvard.edu/cgi-bin/ggi/ggi2.cgi; UMD-Predictor: http://umd-predictor.eu/analysis.php and Mutation taster: http://www.mutationtaster.org/) were used to assess the deleteriousness of the variants.

3. Results

Ten patients (6 males, 4 females) from 8 unrelated families were followed up serially in our clinics over a mean period of 14.5 years (range from 1 to 28 years).

All patients were Spanish with a Caucasian origin. None of the cases were born from consanguineous marriages. Patients 7, 8 and 9 are siblings. The mean age at the first examination was 6 years (7 of 10 were examined for the first time during neonatal period). The patients were aged between 2–53 years old when they were last reviewed.

Individual clinical features of the ten patients harboring mutations in *RAPSN* are summarized in Table 1. Representative photos of patients are shown in Figs. 1–3.

3.1. Age of onset and clinical phenotype

Symptoms within the first hours of life were presented in all the patients. Decreased fetal movements were reported by 67% of mothers who were asked. Polyhydramnios was not found in any of the cases. Muscle hypotonia and sucking difficulties were consistent signs in all the ten cases. Respiratory insufficiency was reported as an associated presenting symptom in the neonatal period in six of ten cases, and respiratory support was required in four of them. Mechanical ventilation was needed in three. Arthrogryposis was noted in two of the patients.

Delayed motor developmental milestones were rarely observed. Eight of ten patients were able to walk autonomously before the age of 18 months. Mildly delayed acquisition of motor milestones in two patients was probably secondary to other conditions they presented (schizencephaly, middle cerebral artery stroke) and not secondary to CMS.

Repeated clinical examinations revealed a variable bilateral ptosis in all the patients, although in 50% of cases ptosis was undetectable for most of the day. Mild *orbicularis oculi* weakness was identified in only two of the patients. Ophthalmoparesis was observed only in one patient: he showed an inability to obtain full horizontal deviation of the eyes. None of the patients displayed pronounced and fixed ophthalmoplegia. The rest of the patients were apparently able to move their eyes without limitations, but all of them reported intermittent diplopia on attempted lateral gaze. Intermittent strabismus was reported in one. Diplopia and strabismus were partially resolved with medication.