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Phenotypic heterogeneity in two large Roma families with a congenital myasthenic syndrome due to *CHRNE* 1267delG mutation. A long-term follow-up

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Abstract

Congenital myasthenic syndromes (CMS) are a heterogeneous group of genetic disorders. Mutations in CHRNE are one of the most common cause of them and the ε1267delG frameshifting mutation is described to be present on at least one allele of 60% of patients with *CHRNE* mutations. We present a comprehensive description of the heterogeneous clinical features of the CMS caused by the homozygous 1267delG mutation in the AChR Ɛ subunit in nine members of two large Gipsy kindreds. Our observations indicate that founder Roma mutation 1267delG leads to a phenotype further characterized by ophthalmoplegia, bilateral ptosis, and good response to pyridostigmine and 3,4-DAP; but also by facial weakness, bulbar symptoms, neck muscle weakness, and proximal limb weakness that sometimes entails the loss of ambulation. Interestingly, we found in our series a remarkable proportion of patients with a progressive or fluctuating course of the disease. This finding is in some contrast with previous idea that considered this form of CMS as benign, non progressive, and with a low impact on the capacity of ambulation.

Keywords: CHRNE; Acetylcholine receptor; Congenital myasthenic syndrome; Congenital myasthenia; Roma gypsies; Neuromuscular junction; Pyridostigmine; 3,4-Diaminopyridine; Founder mutation

1. Introduction

Congenital myasthenic syndromes (CMS) are a heterogeneous group of genetic disorders, all of which impair neuromuscular transmission. To date, more than 20 different genes are known to cause CMS, coding for proteins involved in neuromuscular junction structure and function. CMS are characterized by an impaired neuromuscular signal transduction [1–5].

Acetylcholine receptors (AChR) at neuromuscular junction are pentameric protein complexes composed of 4 subunits in the ratio of 2 alpha subunits to 1 beta, 1 epsilon, and 1 delta subunit. Embryonic acetylcholine receptors contain the gamma subunit in place of the epsilon subunit [6,7]. The transition from embryonic to adult AChR occurs in neonates during the first 2 weeks after birth [8].

Mutations with a reduced expression of the acetylcholine receptor are predominantly located in the epsilon subunit. Mutations in the gene encoding the AChR ε subunit (CHRNE; OMIM 608931) have been reported to be the cause of approximately 40–50% of all CMS, although frequencies of mutations can vary considerably between different populations [3] The main reason for this is that expression of the fetal type γ subunit, although at a low level, partially compensates for absence of the ε subunit whereas patients harboring null mutations in subunits other than ε might not survive for lack of a substituting subunit [6,9,10]. Mutations in *CHRNE* were first described in recessive forms of CMS by Ohno et al. in 1995 [11]. *CHRNE* is located on 17p13.1 [11]. In approximately 60% of patients with *CHRNE* mutations, the founder ε1267delG mutation is present on at least one allele, depending on series [3,12]. This frameshifting ε1267delG mutation occurring at

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homozygosity is endemic in Roma families where it derives from a common founder [13].

Clinical phenotype of CMS due to ε1267delG mutation has been reported to be similar to that of patients with other epsilon subunit mutations [14–16]. A mild clinical heterogeneity has been described. The classic presentation occurs in infancy [12] but there have been patients with other mutations in *CHRNE* and a later onset reported [14]. Most patients develop ophthalmoparesis and bilateral ptosis. Bulbar and proximal limb weaknesses are also common. CHRNEc.1267delG patients reported so far showed a mild and non progressive course of the disease. Response to anticholinesterase inhibitors (ACEIs) is favorable but incomplete. Additional use of 3,4-diaminopyridine (3,4-DAP) results in further improvement but the limited ocular ductions, pronounced in most patients with AChR deficiency, are typically refractory to any therapy [1,5,12,14,17].

We here present a series of nine patients of two large unrelated Roma kindreds with a CMS caused by a *CHRNE* 1267delG mutation. Moderately heterogeneous clinical features are observed. Moreover, we report five additional family members with similar symptoms who were not homozygous for this mutation. We discuss the clinical clues that may help to distinguish them.

2. Materials and methods

Fourteen members (10 females, 4 males) from two unrelated kindreds were followed up over a mean period of 9.9 years (range from 1 to 26 years). Patients were systematically assessed every six months for the duration of follow-up. Each of these fourteen clinically affected family members was reassessed and underwent a detailed clinical examination during the six months prior to the conclusion of this work. Written informed consent was obtained from all patients or their legal representatives. The study complies with the ethical guidelines of the institutions involved. None of these patients have been previously reported.

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.

Electrophysiological studies including electromyography, motor and sensitive nerve conduction and repetitive nerve stimulation (RNS) at 3 Hz of proximal (deltoid or anconeus) and distal (abductor pollicis brevis and/or abductor digiti minimi) muscles were performed.

Molecular genetic analyses were carried out in all 14 patients. Genomic DNA was isolated from venous blood samples using a blood DNA extraction kit according to the manufacturer's recommendations (Promega, Mannheim, Germany). Exon 12 and intronic flanking sequences of the *CHRNE* were PCR amplified with primers 5′-AGAGGCCCACCACTGTCTC-3′ and 5′-GG CACACACCATTCTTGTGA-3′. PCR-amplified fragments were sequenced using the manufacturer's recommendations (Applied Biosystems) and the sequences obtained were compared with the consensus sequence NM_000080.3.

Muscle biopsy specimens were obtained from 3 patients (Patients A-VI.28, A-V.7 and A-V.11) and processed according to standard histological and histochemical techniques.

3. Results and representative case histories

We were able to trace 65 and 108 members in six and seven generations of each family, respectively (Fig. 1) and were able to interview and examine fourteen apparently affected family members. All of them were Spanish with a Roma origin and most of them were born from consanguineous marriages. Seven other family members with myasthenic symptoms were no available for the study: two had passed away and five could not be located.

Molecular genetic analyses were performed in all these fourteen individuals with clinical symptoms. Homozygous 1267delG mutation in *CHRNE* was found in nine of them. Six were women and three were men. The mean age at the first examination was 9.6 years old (range from newborn period to 30 years old). They were followed up over a mean period of 11.6 years (range from 1 to 26 years). The patients were 8–34 years old when they were last reviewed. Individual clinical features of the nine index patients homozygous for ε1267delG are summarized in Table 1. A representative photograph is shown in Fig. 2.

3.1. Age of onset and clinical phenotype

Disease onset ranged from first weeks after birth to 9 years (Mean 2.2 years; median 12 months). Arthrogryposis or other antenatal manifestations were not reported in any patient.

Onset in the first month of life was observed in four patients. Hypotonia, poor cry and suck, and ophthalmoparesis were constant signs in all the four cases. Respiratory insufficiency was reported as an associated presenting symptom in the neonatal period in three of them (one of them required respiratory support). Onset of the disease occurred between the ages of 1 and 2 years in three patients with ptosis. In two patients the initial symptoms were noted after 6 years with ptosis or ophthalmoparesis.

Delayed motor development was rarely observed. Seven of nine patients were able to walk autonomously before the age of 18 months. However, the mean and median age of acquisition of gait without support were significantly higher than those of the normal population (mean: 16.4 months, median: 18 months, range: 12–24 months). Ophthalmoparesis and bilateral symmetric ptosis were observed in all nine patients. Facial weakness and mild bulbar symptoms were encountered in seven patients. None of them required a minced or pureed diet.

Proximal muscle weakness was found in six patients. It was mild in three (patients A-VI.28, A-V.7, and A-V.11), moderate in one (patient B-V.5) and severe in two (patients B-V.1 and B-V.4), rendering one patient (patient B-V.4) wheelchair bound at age 12 years, and two others with restricted ambulation, defined as the inability to walk more than 350 meters autonomously. No significant distal weakness was found in any of the patients. Selective involvement of neck flexor muscles was noted in five patients. Scoliosis was found in three patients.