Neurological Sciences (2019) 40:503–507
https://doi.org/10.1007/s10072-018-3645-2

ORIGINAL ARTICLE

Clinical and genetic characterization of an Italian family with slow-channel syndrome

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Received: 15 May 2018 / Accepted: 9 November 2018 / Published online: 12 December 2018

Abstract

Introduction The slow-channel congenital myasthenic syndrome (SCCMS) is a postsynaptic form of congenital myasthenic syndromes (CMSs), a clinically heterogeneous group of disorders caused by genetic defects leading to an abnormal signal transmission at the endplate.

Methods We report clinical and molecular data of a multigenerational family in which the presentation of a progressive proximal-distal weakness with ocular involvement led to a number of different clinical diagnoses.

Results A comprehensive genetic study which included whole-genome linkage analysis and whole-exome sequencing identified a heterozygous missense substitution (c.721C>T, p.L241F) in the ε subunit of the acetylcholine receptor (*CHRNE*) that was consistent with clinical weakness in all patients.

Discussion SCCMS is characterized by a broad and heterogeneous clinical phenotype in which disease onset, symptoms, severity, and progression can be highly variable even between family members. The identification of a *CHRNE* mutation allowed to make the definitive diagnosis of CMS in this family and contributed to define the clinical spectrum of this disease.

Keywords Congenital myasthenic syndrome · Slow-channel congenital myasthenic syndrome · Linkage analysis · Whole-exome sequencing · *CHRNE* mutation

Introduction

Congenital myasthenic syndromes (CMSs) are a group of genetically heterogeneous disorders of the neuromuscular junction that can be classified according to the site of the transmission defect into presynaptic, synaptic, and postsynaptic. So far, more than 20 CMS genes have been identified [1], but further genetic heterogeneity is expected as a molecular diagnosis can be established only in about half of CMS patients [2]. The slow-channel congenital myasthenic syndrome (SCCMS; OMIM# 605809) is a postsynaptic form of CMS that was first described by Engel et al. in 1982 [3] and can be caused by mutations in the genes encoding the alpha, beta, delta, or epsilon (*CHRNE*) subunits of the acetylcholine receptor (AChR). The disease is characterized by dominant inheritance, although occasional recessive cases have been reported [4]. Some SCCMS patients have severe disability by the end of the first decade; others present clinical sings later in life and progress gradually with forearm weakness and decreased bulk of shoulder muscles. Most patients show severe involvement of cervical, wrist, and extensor muscles. In this study, we report a family with progressive proximal and distal weakness in which an accurate electrophysiological and molecular characterization led to a final diagnosis of SCCMS.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10072-018-3645-2) contains supplementary material, which is available to authorized users.

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Case reports

Informed consent was obtained from all subjects enrolled in this study. The pedigree of the family is shown in Fig. 1.

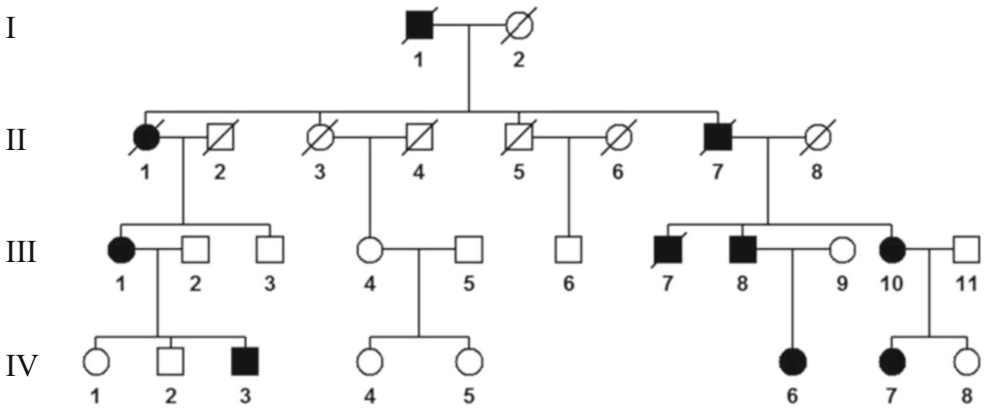

Fig. 1 Pedigree of the family

Patient 1 (III-7)

Since the age of 5–6 years, this man had difficulty extending his hands; the father and the grandfather had the same symptoms. He was diagnosed with distal myopathy since he had some difficulties walking. At the age of 55, a neurological exam showed atrophy of extensor forearm muscles, hypotrophy of thenar and hypothenar eminences in hands, reduced grip strength, and limitation in upward eye movements. Muscle biopsy at age 55 showed fiber size variability, central nuclei, fiber type grouping, and some lipid droplets indicative of mitochondrial metabolism dysfunction in oxidative fibers. Needle EMG examination showed myopathic changes in biceps brachii, anterior tibialis, and quadriceps. Motor conduction velocity of the sciatic nerve was 41–44 m/s with distal latency 5.8–22 msec and of the ulnar nerve 52 m/s with distal latency of 3.2–20 msec. Motor conduction velocity of the median nerve was 53 m/s with motor units action potential of 10 mV and distal latency of 3.6–23 msec. The increased distal latency with the *decreased* motor action potential and the slowed conduction velocity of sciatic nerve were suggestive of moderate distal neuropathy.

Patient 2 (III-8)

Since the age of 26, this man had distal atrophy of arms and weakness in neck extensor muscles. EMG showed myogenic changes, slow velocity in ulnar nerve, and pseudomyotonic discharges. At the age of 26, he was diagnosed with possible myotonic dystrophy. On neurological examination, he had ptosis, ophthalmoparesis in upward gaze, weakness in forearm muscles, and slight weakness of biceps. CK value was 117 IU/L. At the age of 65, he presented with atrophy of thenar and hypothenar muscles, impossible extension of fingers bilaterally, and severe weakness of biceps and deltoid muscles. Upward gaze ophthalmoparesis with partial paresis of lateral movements was found. Muscle biopsy of vastus lateralis showed atrophic myopathic fibers with few regenerating fibers, ring fibers, and type 2 fiber prevalence (85%). Muscle CT scan showed atrophy of the upper arm muscles and slight hypotrophy of gluteus muscles.

Patient 3 (III-10)

This woman had onset at 13 years old of hand and upper arm weakness. At the age of 45, she had some problems walking on heels, weakness in biceps and hand extensors, atrophy of interosseous muscles, and ophthalmoparesis (Fig. 2). At the age of 60, a neurological examination showed difficulty in rising from the floor with Gowers' maneuver performed using a hand on the knee; atrophy of hypothenar muscles bilaterally; weakness of biceps (4/5), shoulder rotators (4/5), and deltoid (3+/5); and total weakness of finger extensors and hands. Partial limitation of upward and lateral eye movements was observed. Muscle biopsy showed increased central nuclei, prevalence of type 1 fibers, and atrophy of single fibers. EMG showed second wave on ulnar CMAP (Fig. 3). At the age of 75, spirometry showed signs of respiratory insufficiency (FVC 61.5%) likely related to the neuromuscular disease. She was treated with salbutamol 6 mg for the following 3 years with modest benefit.

Patient 4 (IV-6)

At the age of 22, this woman had slight weakness of hand extensor muscles, more on the right side, and weak DTR. CK was normal. She was on fluoxetine 20 mg being depressed. Unfortunately, no follow-up data after treatment are available for this patient.

Patient 5 (IV-7)

At 33 years old, this woman had been presenting fatigability and poor sleep for several years, with onset at the age of 19. She worked in a firm, and recently lost the ability to cope both physically and mentally with her job. She complained of cramps in lower limbs and had moderate extensor hand weakness. At the age of 31, she fainted and was thought to suffer from epilepsy. On last neurological examination, cranial

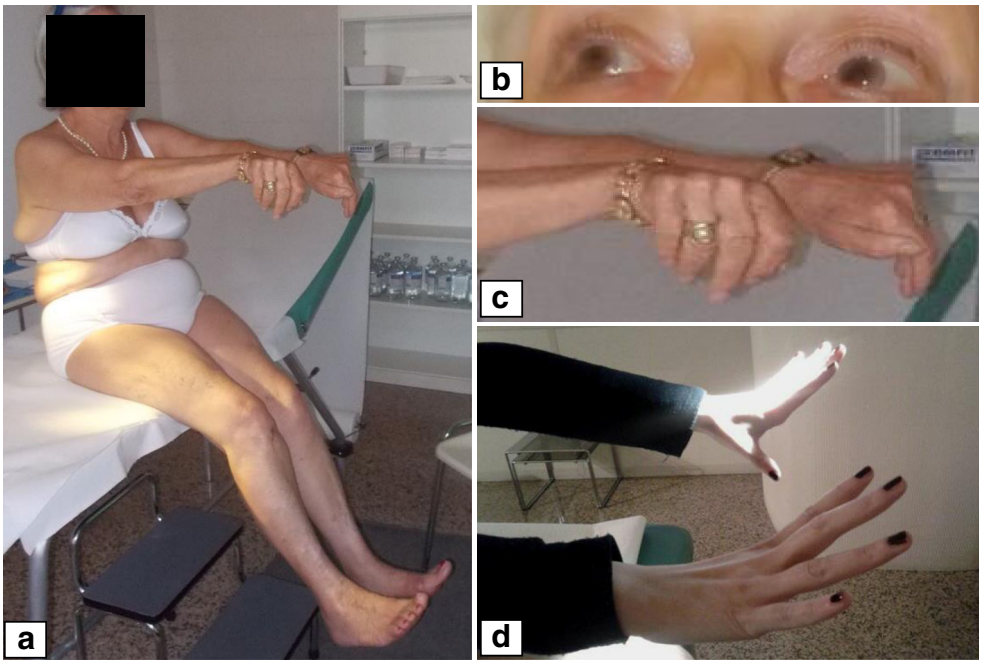

Fig. 2 Patient III-10. **a** Distal atrophy. **b** Ophthalmoparesis. **c** Severe weakness of finger extension. Patient IV-7. **d** Inability to fully extend fingers

nerves were normal except for limited lateral eye movements. She was able to walk on toes but had difficulty walking on heels and absent deep tendon reflexes. There was weakness of hand flexors (4/5) and extensors (3/5) (Fig. 2) and pes cavus. Neurophysiological diagnostic testing was performed: NCS and EMG were normal except that single stimuli elicited repetitive CMAPs (Supplementary Fig. 1). She was treated with fluoxetine 20 mg and salbutamol 6 mg but showed only a partial clinical response to this drug regimen and it was substituted by methylphenidate 10 mg per day.

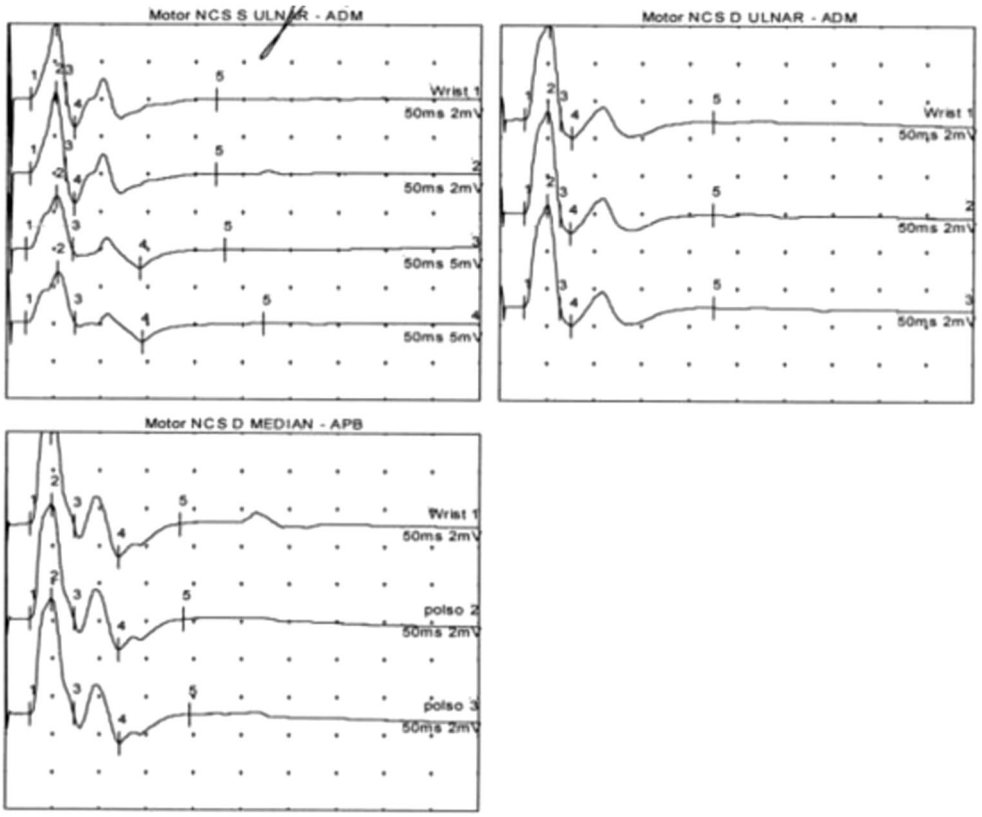

Fig. 3 An EMG of patient III-10 showing normal CMAP but presence of repetitive CMAP in ulnar and median nerves

Molecular findings

In order to identify the disease gene, a whole-genome linkage analysis was performed on six affected (III-1, III-7, III-8, III-10, IV-6, IV-7) and six unaffected (III-3, III-4, III-6, III-9, III-11, IV-8) family members whose DNA was available for the study. Over 200,000 single nucleotide polymorphisms (SNPs) were genotyped in each subject using the Illumina Human CNV370-Quad platform. Linkage analysis and haplotype reconstruction identified two genomic regions on chromosome 13q (49.6–59.9 Mb) and 17p (1.3–6.2 Mb) shared only by the affected subjects, thus perfectly co-segregating with the disease in the family. Whole-exome sequencing (WES) was then performed in patients III-1 and III-6 using the Agilent SureSelect Human All Exon v4 kit for the targeted enrichment and the Illumina Hiseq2000 platform for sequencing (mean coverage depth > 100X). No putative disease-causing variants were detected in any of the known genes associated with distal myopathies or with other related phenotypes.

The analysis of WES variants in the candidate region of chromosome 17p identified two heterozygous missense substitutions: c.583T>C, p.S195P in the *PLD2* gene (NM_001243108) and c.721C>T, p.L241F in the *CHRNE* gene (NM_000080.3). Sanger sequencing confirmed the presence of both variants in all patients and not in the healthy relatives. The *PLD2* gene encodes the phospholipase D2 protein involved in the hydrolysis of phosphatidylcholine to phosphatidic acid and choline. *PLD2* seems to play a role in several pathological conditions, including neurological diseases, but so far it has not been clearly associated to any particular inherited human disease [5].

Conversely, *CHRNE* encodes for the epsilon subunit of AChR of neuromuscular junctions, and its mutations are associated with CMS (autosomal recessive) and with SCCMS (autosomal dominant). The here identified L241F mutation (also annotated as L221F considering the position in the mature protein) affects a conserved residue in the first transmembrane domain (M1) and was previously reported in two non-Italian families affected by SCCMS [4]. These data support the causative effect of the L241F mutation in *CHRNE*, while further studies are needed to clarify the role, if any, of the *variant* of uncertain significance in the *PLD2* gene.

Discussion

In this work, we report the first Italian family with SCCMS. The marked difficulty in reaching a diagnosis in this family was due to the presentation of a permanent distal weakness; this led initially to several different possible diagnoses including peripheral neuropathy, myotonic dystrophy, and distal myopathy (Welander distal myopathy [6], Laing distal myopathy due to a myosinopathy [7], or Nonaka type [8]).

In a further examination, EMG highlighted repetitive CMAPs after a single nerve stimulus, thus indicating a diagnosis of either SCCMS or acetylcholinesterase deficiency. Genome-wide genotyping and whole-exome sequencing revealed the presence of the L241F substitution in the *CHRNE* gene, thus confirming a diagnosis of SCCMS.

CHRNE is the most mutated gene in CMSs; however, the majority of reported mutations are null alleles causing recessive AChR deficiency. By contrast, SCCMS is mainly inherited with an autosomal dominant pattern, due to gain-of-function alleles, that are distributed about equally in all the four AChR subunits. To date, only five SCCMS mutations have been published in *CHRNE*, including the L241F found in this study. This substitution was first identified in 2002 in two unrelated pedigrees [4], initially described by Oosterhuis in 1987 [9]. However, no other patients with this mutation have been subsequently published and, more in general, *CHRNE* mutations have been usually reported only in single patients. In this view, this report of a rare *CHRNE* mutation in an Italian family provides relevant information for molecular diagnosis and genetic counseling, as well as for the future assessment of mutation recurrence, phenotypic variability, and possible genotype-phenotype correlations.

According to Croxen et al., the L241F mutation we found seems to be associated to an atypical inheritance pattern and a mild phenotype, characterized by ocular findings and distal weakness [4]. In the here described family, the dominant inheritance with variable expressivity in proximal, distal, and ocular muscles is evident. Conversely, a sporadic patient carrying the L269F substitution (currently updated in L289F) [10] displayed a remarkably different clinical presentation. He was a 15-year-old case with an early CMS that did not respond to acetylcholinesterase medication, presented ptosis, ophthalmoplegia mostly in the upward gaze, difficulty in maintaining the head upright, mild scoliosis, and fatigability.

This variability may be due to different pathogenetic mechanisms underlying the slow-channel phenotype [11]. The L289P mutation has been shown to cause openings of unliganded channels, as well as a slower shutting rate that determine an excessive cation entry into the postsynaptic region [12]. This leads to an endplate myopathy and is thought to be the most common mechanism of gain-of-function mutations in SCCMSs [13]. By contrast, the L241F mutation (that seems associated to a milder phenotype) has limited effects on gating kinetics, but it has been shown to increase receptor affinity for ACh, thus increasing the chances of reopenings [13]. Indeed this substitution is located in the first transmembrane domain (M1), close to the extracellular domain responsible for ACh binding. Interestingly, mutations in the ligand binding domain of the α subunit (*CHRNA1*) have been reported to be associated with a less severe phenotype [14]. Further investigations are required to clarify whether this difference accounts for the different phenotypic expressivity of *CHRNE* mutations and thus for the specific disease symptoms in this form of SCCMS [15].

According to experimental and clinical studies, fluoxetine and quinidine are the first-line treatments for CMS since they act as AChR open-channel blockers [16]. In addition, the use of salbutamol or ephedrine is reported as beneficial for many CMS subtypes [1]. In this family, a modest improvement in patient 5 was observed with a treatment regimen that combined salbutamol and fluoxetine which seemed first to improve fatigability [9, 12] but was rather unsuccessful since it did not result in sustained benefit. A plausible explanation for differences in treatment outcomes may reside in the fact that open-channel blockers work better when the mutation affect the AChR channel gating rather than the ligand binding as the L241F [14]. In this view, the identification of the disease subtype as well as the molecular characterization of the involved pathogenic mechanisms has evident implication in refining the treatment strategy of CMS.

In conclusion, the misleading clinical features observed in this kindred, characterized by a progressive distal myopathy and followed by lack of response to most drugs, delayed the diagnosis of slow-channel syndrome.

Acknowledgements We are grateful to the members of the family who participated in the study.

Funding information This work was supported by the Fondazione Telethon-Italy (grant no. GEP12083 to G.V.)

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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