Acta Myologica • 2017; XXXVI: p. 28-32

Congenital myasthenic syndrome: phenotypic variability in patients harbouring p.T159P mutation in *CHRNE* gene

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Congenital myasthenic syndromes (CMS) are rare and heterogeneous genetic diseases characterized by compromised neuromuscular transmission and clinical features of fatigable weakness; age at onset, presenting symptoms, distribution of weakness, and response to treatment differ depending on the underlying molecular defect. Mutations in one of the multiple genes, encoding proteins expressed at the neuromuscular junction, are currently known to be associated with subtypes of CMS. The most common CMS syndrome identified is associated with mutation in the CHRNE gene, causing principally muscle nicotinic acetylcholine receptor deficiency, that results in reduced receptor density on the postsynaptic membrane.
We describe the clinical, neurophysiological and molecular features of two unrelated CMS Italian families with marked phenotypic variability, carrying the already reported p.T159P mutation in the *CHRNE* gene. Our report highlights clinical heterogeneity, intrafamily variability in spite of the same genotype and a possible gender effect; it confirms the efficacy and safety of salbutamol in patients who harbor mutations in the epsilon subunit of acetylcholine receptor.

Key words: Congenital myasthenic syndromes, *CHRNE* gene, phenotype variability

Introduction

Congenital myasthenic syndromes (CMS) comprise heterogeneous genetic diseases characterized by compromised neuromuscular transmission. CMS can be classified as presynaptic, synaptic or postsynaptic, depending on the location of the primary defect within the neuromuscular junction (1, 2). Some patients present signs from birth, or shortly after, especially those with mild presentations, who remain undiagnosed until adolescence. To date, 31 causative genes in SMC have been identified including genes that code for the AChR subunits (CHRNE, CHRNA1, CHRNB1, CHRND and CHRNG), molecules expressed in the neuromuscular junction and, recently, proteins involved in abnormal glycosylation of AChR subunits (1-9). The most common CMS identified is associated with mutations in the *CHRNE* gene, encoding the epsilon subunit of the acetylcholine receptor (AChR).

We describe the clinical, neurophysiological and molecular features of two unrelated CMS Italian families with marked phenotypic variability, carrying the already reported p.T159P mutation in the *CHRNE* gene: in one family this mutation is present in homozygous state, whereas in the second family it is compound heterozygous associated with the known p.S235L mutation (10, 11).

Case reports

Family 1

Patient 1 is a female, 4 years old, second child of third cousins healthy parents. Since first months she presented bilateral ptosis, difficulties in sucking and dysphagia, leading to ab ingestis pulmonitis at 8 months of age. Psychomotor development was normal, but mild weakness, unsteady gait and fatigability since early infancy, and slight fluctuations of symptoms with worsening during the evening, were referred. Neurological examination at 4 years of age, showed bilateral ptosis (Fig. 1a), facial muscles weakness, nasal voice, generalized hypotonia, muscle weakness more marked at lower limbs, positive Gower's sign, anserine ambulation, and running inability. AChR antibodies were absent. Electromyography revealed mild

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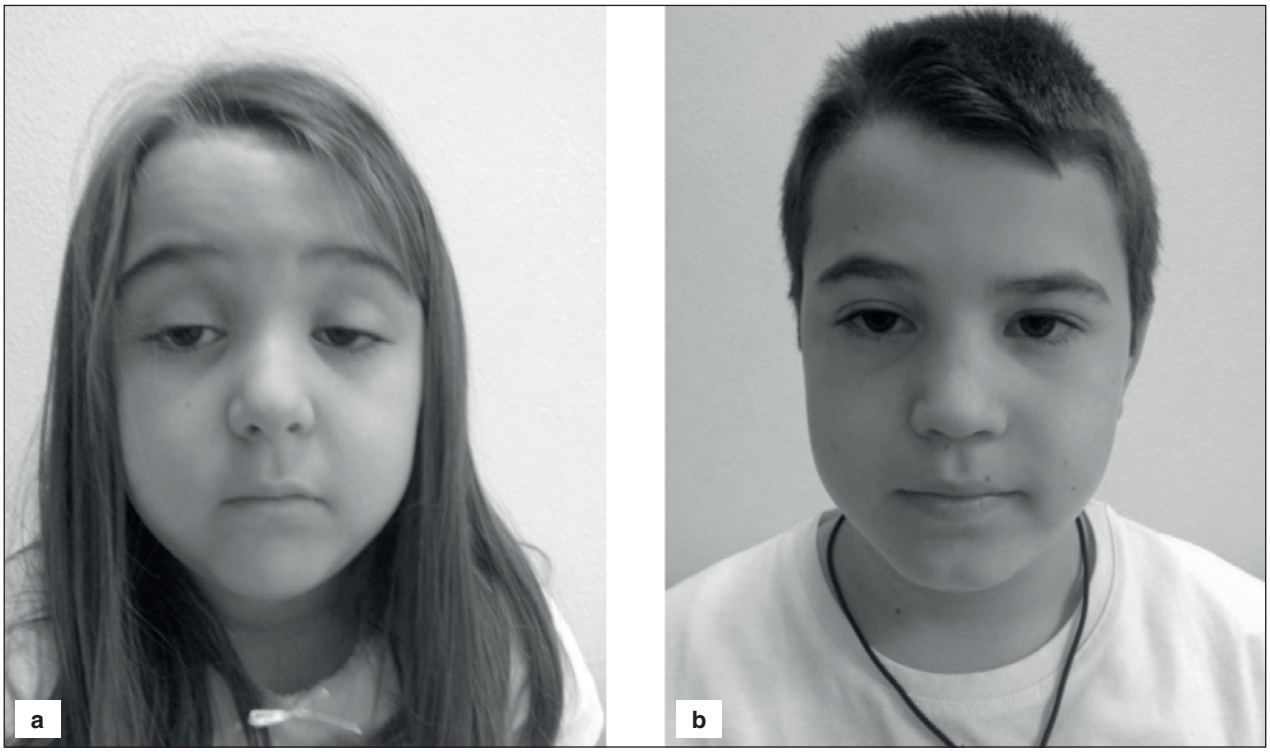

Figure 1. Patient 1 and patient 2 from family 1. The female presents marked bilateral ptosis (a) while the older brother shows slight bilateral ptosis (b).

myopathic alterations; single fibre test was not performed because of patient's poor compliance. Muscle biopsy revealed aspecific myopathic features. At follow up, 6 months later, clinical evolution was stable. Parents noticed substantial improvement during treatment with salbutamol for a trivial respiratory disease; a post synaptic CMS was suspected. Oral treatment with salbutamol was started: a marked improvement of ptosis, weakness and activities of daily living was reported, without side effects.

Patient 2, now 10 years old, is the older brother of Patient 1. After his sister's hospitalization, he underwent neurological examination showing only slight bilateral ptosis (Fig.1b), and very mild lower limb girdle weakness (MRC: 4+).

All 12 exons of the *CHRNE* gene were sequenced following the already reported protocol (12). The analysis in family 1 revealed a previously identified c.475A>C mutation in exon 6 (p.T159P), in homozygous form (10). Genetic analysis in her older brother (Patient 2) revealed the same homozygous p.T159P mutation. The healthy parents carry one mutant allele each (Fig. 2).

Family 2

Patient 3 is a girl, now 20 years old, second child of healthy non consanguineous parents. Since first months of life she presented with bilateral ptosis and axial weakness. Subsequently ophthalmoparesis, diurnal fluctuations of ptosis, facial weakness, fatigability, difficulties in running and climbing stairs were reported. At age 4 years a diagnosis of CMS was reached. Clinical conditions remained stable during adolescence; electromyographic study revealed mild myopathic changes in upper and lower limbs and repetitive nerve stimulation (RNS) of facial nerve showed a pathological decremental response. At last observation, 20 years of age, she presented with marked bilateral ptosis, almost complete ophthalmoparesis, axial weakness, positive Gower's sign, and running inability.

Patient 4 is the younger brother of Patient 3, now 13 years old. He similarly showed since birth presence of ptosis, ophthalmoparesis and mild axial weakness, that remained stable during subsequent years. The electrophysiological findings were similar to those observed in his sister. Treatment with Pyridostigmine was uneffective.

Direct sequencing of the *CHRNE* gene in both siblings revealed the known p.T159P mutation associated with a second already reported mutation c.704C>T (p.S235L) in exon 7, both mutations were present in heterozygous form (10, 11). The mother was the carrier of p.T159P mutation, and the father of the p.S235L mutation (Fig. 2).

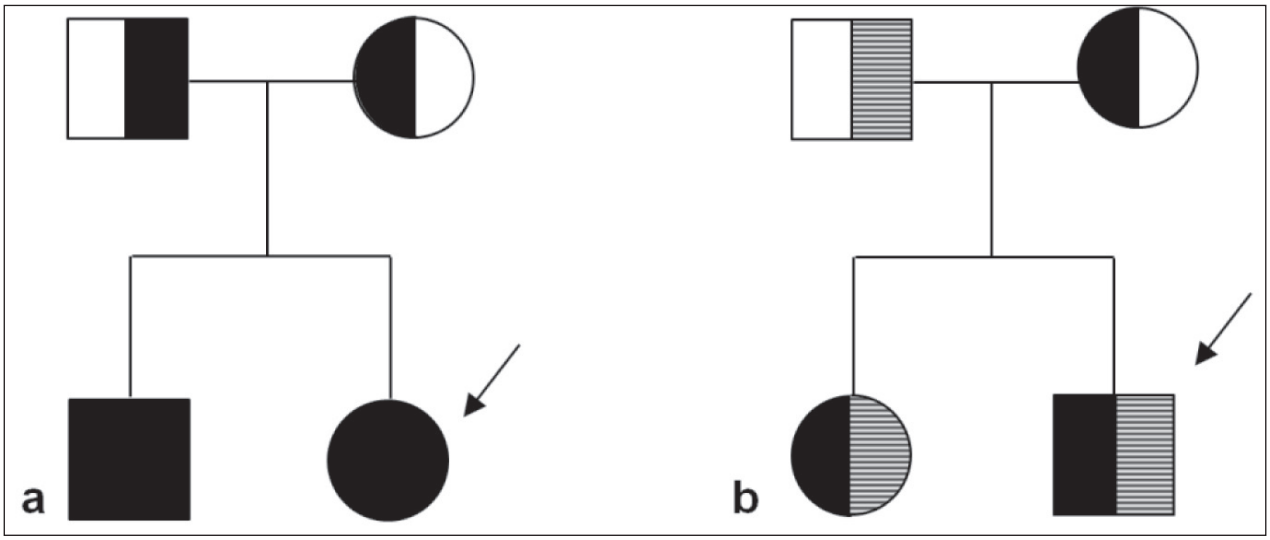

Figure 2. Families 1 and 2: genomic DNA of propositi (arrows) and family members. Closed symbols indicate affected individuals carrying two mutant alleles. Half shaded symbols represent asymptomatic carriers harbouring a single mutant allele. (a) Pedigree of the family 1: the p.T159P mutation is present in homozygous form in the sons, while in heterozygous form in carrier parents. (b) Pedigree of the family 2: the children present compound heterozygous mutations p.T159P and p.S235L, whereas the mother and the father are carrier of p.T159P and p.S235L, respectively.

Table 1 summarizes the clinical aspects of the 4 patients of 2 families described in this study.

Genetic analysis

In patients all 12 exons and the adjacent splice donor and acceptor sequences of the *CHRNE* gene were sequenced, using genomic DNA isolated from blood, following the already reported protocol (5), while in their healthy parents the only mutated exons were analysed. The PCR products were purified by EuroSAP (Euroclone) and sequenced by bidirectional sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific), on an 3130xl Genetic Analyzer (Thermo Fisher Scientific). The obtained sequences were analysed with SeqScape v.3.0 software (Thermo Fisher Scientific) and compared with reference wild-type sequence (GenBank CHRNE accession numbers: NM_000080.3).

Informed consent

Written informed consent for genetic analysis and for photos from children of Family 1 was obtained from probands' relatives and their familial members.

Discussion

All CMS patients share same clinical features, but age at onset, presenting symptoms, distribution of weakness, and response to treatment differ depending on the molecular mechanism that results from the genetic defect (1, 2).

We report four Italian CMS patients harboring *CHRNE* mutations and showing marked clinical variability, ranging from isolated mild ptosis to marked ptosis associated with ophthalmoparesis, facial and lower limb-girdle weakness (Patients 3 and 4) and intrafamily phenotypic variability in both families.

Genotype is different in the two families. In Family 1 the known p.T159P mutation is present in homozygous state whereas in Family 2 the p.T159P mutation is associated with the known p.S235L mutation (10, 11).

The p.T159P mutation is localized on the long cytoplasmatic N-terminal portion of the epsilon protein, which contains several loop regions which are critical for receptor function (6). Expression study showed that this mutation causes principally AChR deficiency (10, 14). The p.T159P mutation was previously identified in one CMS proband in compound heterozygous whit a second one (p.A411P) (10).

The p.S235L mutation is localized at the end of the membrane-spanning M1 domain of the epsilon protein, which joins covalently the four a-helical segments M1-M4 to the extracellular domain, hence this mutation may change this structural link (13). The p.S235L mutation was previously found in one Portuguese CMS patient associated with a second p.70insG mutation, presenting the clinical signs of ptosis, ophthalmoparesis, dysphagia,

Table 1. Clinical characteristics of the patients.

Patient		Gender	Onset age/ symptoms	Evolution	Cinical findings at diagnosis	Treatment/ response
Family 1	1	Female	First months/ bilateral ptosis, difficulties in sucking and dysphagia	Worsened	Bilateral ptosis, facial muscles weakness, nasal voice, generalized hypotonia, limb girdle weakness more marked at lower limbs, positive Gower's sign, anserine ambulation	Salbutamol/effective
	2	Male	Early infancy/ mild ptosis	Stable	Mild bilateral ptosis and mild lower limb girdle weakness	No treatment
Family 2	3	Female	First months/ ptosis	Worsened in infancy/ stable in adolescence	Bilateral ptosis, ophthalmoparesis, axial weakness, positive Gower's sign	Pyridostigmine/ uneffective
	4	Male	First months/ ptosis	Worsened in infancy/ stable in adolescence	Bilateral ptosis, ophthalmoparesis and mild axial weakness	Pyridostigmine/ uneffective

proximal weakness, and electrophysiological studies revealed a RNS decrement (11). Also in our patients the p.S235L mutation in compound heterozygous state seems to aggravate the phenotype.

In siblings of Family 1, harbouring p.T159P mutation in homozygous state, a marked clinical variability is evident. Marked phenotypic variability has been already described in two siblings with CMS due to mutations in *MUSK* gene: the sister was reported to be much more severely affected than the brother and a gender-effect was hypothesized since menstrual periods and fever worsened her symptoms (15). Although Patient 1 was in a prepuberal age, our report confirmed the hypothesis of a gender effect in the phenotypic expression. Our report underlines intrafamily clinical variability in spite of the same genotype and a possible gender effect; confirms the efficacy and safety of salbutamol in patients who harbour mutations in the epsilon of AchR (16).

Acknowledgments

We are very grateful to the parents for providing consent for this study.

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