Late presented congenital myasthenic syndrome with novel compound heterozygous *CHRNE* mutations mimicking seronegative myasthenia gravis

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Running head: Late presented CMS with *CHRNE* mutations

Abstract

We found a late presented congenital myasthenic syndrome (CMS) patient with novel *CHRNE* gene mutations. Although our patient has shown blepharoptosis since youth, fatigable muscle weakness began at age 71. Genetic analysis revealed novel compound heterozygous *CHRNE* mutations (c.1032+2 T>G, c.1306_1307 delGA). His myasthenic symptoms were well managed by oral anti-cholinesterase drug until he died at 82-year old. The present case showed mild myasthenic symptoms with very late presentation and slow progression. Late presented CMS is often underdiagnosed, therefore, genetic testing is important to distinguish it from other myasthenic disease.

Key words: anti-cholinesterase drug, the *CHRNE* gene, congenital myasthenic syndrome, late presentation, mimicking seronegative myasthenia gravis.

Introduction

      Congenital myasthenic syndrome (CMS) is characterized by easy fatigability of skeletal muscle due to genetic disorders relating to neuromuscular transmission [1]. CMS is a heterogeneous disorder due to different gene mutations [1, 2]. Although 9.2 per million children under the age of 18 have been genetically diagnosed in the UK, the exact prevalence of CMS worldwide is uncertain [2, 3].

Case presentation

      The proband (71-year old male, II-9) became aware of muscle weakness in his bilateral upper extremities. At 76-year old, he came to feel physical fatigue with gradual progression of muscle weakness. A few months later, he developed bilateral blepharoptosis and double vision, and was admitted to our hospital.

      His neurological examination revealed bilateral blepharoptosis with easy fatigability, exotropia in his right eye, restriction of bilateral eye movement in all directions, mild facial muscle weakness, slight muscle atrophy and weakness in all extremities with proximal dominancy, normoreflexia, and normal gait. The pedigree (Fig. 1A) suggested an autosomal recessive inheritance and his portraits showed that the bilateral blepharoptosis was already present at age 19 with progression in the course of age (Fig. 1B, i=19, ii=45, iii=76, iv=80-year old).

      Both AChR antibodies and muscle specific kinase (MuSK) antibodies were negative. Edrophonium test showed improvement in the elevation of eyelids (Fig. 1C-i), eye movements (Fig. 1C-ii, iii, iv), and left arm strength (Fig. 1C-v, arrow). Repetitive nerve stimulation (RNS) at 3 Hz showed waning both on left ulnar (Fig. 1D-i) and left facial (Fig. 1D-ii) nerve analyses (13.5% and 27.2%, respectively). His left arm muscle

biopsy showed only pyknotic nuclear clumps (Fig. 1E-i, arrow). The cytochrome c oxidase (COX) activity did not decrease (Fig. 1E-iv), and there was no ragged-red fiber.

The whole exome DNA analysis was performed with written informed consent, and heterozygote mutations were identified for a single nucleotide substitution (c.1032+2 T>G) and a 2 bp deletion (c.1306_1307 delGA) of the *CHRNE* gene. These mutations were then confirmed by the Sanger direct sequencing method (Fig. 1F). The single nucleotide substitution (c.1032+2 T>G) was further confirmed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Fig. G-i). The 2 bp deletion (c.1306_1307 delGA) was also confirmed by allele specific PCR (AS-PCR) (Fig. 1G-ii).

Reverse transcription (RT)-PCR was performed on RNA samples extracted from the patient's muscle specimen, then two different size of PCR products were obtained by using primers recognizing the region including exon 8-11 in the *CHRNE* gene (Fig. 1H). As a result of sequence analysis of purified DNA from each PCR product, band 1 of 470bp showed a sequence of exon 8-11 with a mutation of c.1306_1307 delGA in exon 11 (Fig. 1I-i). Band 2 of 355bp showed the sequence skipping exon 9 without a mutation of c.1306_1307 delGA in exon 11 (Fig. 1I-ii). These results suggested compound heterozygous *CHRNE* mutations. Oral anti-cholinesterase (ChE) drug was administered from his age 80 and well managed myasthenic symptoms until he died at 82.

Discussion

CMS usually shows myasthenic symptoms in neonate or early childhood, but much less in late childhood. Therefore, adult onset CMS may often be mistaken for SNMG, myopathy, or motor neuron disease [1, 4, 5]. Our present case was initially

difficult to distinguish from SNMG because of the very late presentation of symptoms at age 71.

Because the first and last two bases of intron (GT and AG motives) are preserved across the species as a splice site, genetic mutations of them could result in a splicing defect such as exon skipping or intron retention [6]. In the present case, one novel mutation of the *CHRNE* gene is a point mutation at the 5' splice site of intron 9, which causes frameshift by skipping exon 9 (115 bp) with premature termination. Another novel mutation is 2 bp deletion, which results in a stop codon at the 454th amino acid. Because a stop codon is translocated in the last exon 12, nonsense-mediated mRNA decay (NMD) may not be induced in our case [7]. On the other hand, the downstream 470th cysteine residue (C470) is essential for surface expression of adult AChR [8]. Therefore, this mutation is also considered to be associated with AChR deficiency.

Our case showed mild myasthenic symptoms with very late presentation, probably because low level expression of the fetal AChR harboring γ subunit can partially compensate for the AChR deficiency [9]. Although adult onset type is often observed in autosomal dominant slow channel syndrome [1, 10, 11], clinical manifestations in our case suggested fast channel syndrome or post-synaptic AChR deficiency type.

Informed Consent

Informed consent was obtained from the patient family for publication of this case report including his medical photographs.

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Figure legends

Fig. 1. (A) The family tree of the patient, showing the proband (II-9, arrow) and his elder brother (II-5) with blepharoptosis.　(B) The chronological changes of blepharoptosis showing early onset and slow progression at ages of 19 (i), 45 (ii), 76 (iii), and 80 (iv). (C) Edrophonium test showed improvements of ptosis (i), eye movements (ii-iv), and left arm power (v, arrow).　(D) Repetitive nerve stimulation (RNS) at 3Hz, showing a waning in the left ulnar (i) and left facial (ii) nerves.　(E) Histopathological findings of his left arm muscle biopsy demonstrated (i) only pyknotic nuclear clumps in Hematoxylin and Eosin staining, (ii) no ragged red fiber in Modified Gomori trichrome staining, (iii) no fiber type grouping in NADH-tetrazolium reductase (NADH-TR) staining, and (iv) no decrease of COX activity in cytochrome c oxidase (COX) staining.　(F) DNA sequencing revealed a single nucleotide substitution (c.1032+2 T>G, i, arrow) and a 2 bp deletion (c.1306_1307 delGA) in the *CHRNE* gene (ii, arrowheads).　(G) The single nucleotide substitution (c.1032+2 T>G) was confirmed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) with uncut PCR product band (752 bp) in the patient (i).　The 2 bp deletion (c1306_1307 delGA) was confirmed by allele specific PCR (AS-PCR) showing a band of PCR products amplified with AS primer for variant only in the patient (ii).　(H) Reverse transcription (RT)-PCR on RNA from the patient's muscle using flanking primers for exon 8 and exon 11 in the *CHRNE* gene showed different two bands (band 1 of 470bp, and band 2 of 355bp).　(I) The DNA sequence of band 1 showed a chain of exon 8-11 with a mutation of c.1306_1307 delGA in exon 11 (i). The sequence of band 2 showed skipping exon 9 without a mutation of c.1306_1307 delGA (ii).　(J) The DNA sequence of each RT-PCR product showed compound heterozygous *CHRNE* mutations.