BJMG 22 (1), 2019 • 95-98
DOI: 10.2478/bjmg-2019-0010
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CASE REPORT

A NOVEL c.973G>T MUTATION IN THE ε-SUBUNIT OF THE ACETYLCHOLINE RECEPTOR CAUSING CONGENITAL MYASTHENIC SYNDROME IN AN IRANIAN FAMILY

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ABSTRACT

Congenital myasthenic syndrome (CMS) constitutes a group of inherited disorders of neuromuscular junctions. The majority of postsynaptic syndromes result from mutations in the *CHRNE* gene that causes muscle nicotine acetylcholine deficiency. In this study, we report on a 2 and a half-year-old boy with normal developmental milestones and bilateral ptosis. Clinical courses, electrophysiological studies and molecular genetic analysis were assessed. Polymerase chain reaction (PCR) and direct DNA sequencing of the *CHRNE* gene were performed for the proband and all the family members. A novel homozygous missense mutation of c.973G>T was found in the *CHRNE* gene. Segregation studies were suggested to be the genetic cause of the disease. Using three *in silico* tools and the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) variant classification guidelines indicated that the novel variant c.973G>T was likely pathogenic. Our results recommended first screening of the *CHRNE* gene for pathogenic mutations in Iranian origin.

Keywords: Congenial myasthenic syndrome (CMS); Neuromuscular junction; *CHRNE* gene.

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INTRODUCTION

Congenital myasthenic syndromes (CMSs) [MIM 100725] are a heterogeneous group of rare disorders arising from aberrations in the structure and function of proteins in neuromuscular junction. Symptoms of CMS generally emerge at the time of birth, or shortly after birth. Cases with mild symptoms can remain undiagnosed until adolescence or adulthood [1]. The disease is often characterized by the feature of fatigable weakness, but some typical presentations including ptosis and extraocular muscle, facial, bulbar and general weakness also occur. The subtypes of CMSs are classified as presynaptic, synaptic or postsynaptic, based on the localization of the defect [2]. Postsynaptic CMS is more common than the presynaptic type or the synaptic basal lamina. The postsynaptic subgroup is often caused by a kinetic abnormality of the acetylcholine receptor (AChR), a deficiency of AChR or both [3]. Mutations are dispersed over different adult subunits of AChR mainly (*CHRNE*; OMIM 608931), the gene encoding the ε-subunit. The *CHRNE* gene mutations are predominantly one of the most common causes in post-synaptic CMS patients [4]. The frequent identification of CMS patients worldwide with *CHRNE* mutations prompted us to clinically test our patient to see if there were any mutations in the candidate gene. We detected a novel *CHRNE* mutation in an Iranian family with the clinical phenotype of CMS.

A homozygous missense mutation in the *CHRNE* gene was detected in the patient under study, belonging to this family. It is likely that the *CHRNE* gene in the patient was inherited from his parents who were genetically unaffected by this mutation.

CLINICAL REPORT

The finding of a clinical report was established in a 5 and half-month-old boy referred to the Department of Pediatric Neurology at the Mofid Children's Hospital, Tehran, Iran due to bilateral ptosis. He was the product of consanguineous marriage with an uneventful birth history. Ptosis started when he was 2 months old and was exacerbated by crying and breast feeding, which led to fatigue and lack of sleep during this period. The family history for neuromuscular disorders tested negative.

In a neurological examination, the patient showed hypotonia bilateral ptosis but normal mental development in terms of normal facial phenotypic expression followed by a social smile (Figure 1). In order to rule out the etiology of intracranial involvement, at first, we did a brain magnetic resonance imaging (MRI) and the result was within normal limits. According to the above history and physical examination, the top list of our diagnosis was CMS. Therefore, electromyography (EMG) and nerve conduction velocity (NCV), in the right and left deltoid, biceps, triceps, extensor digitrum communis and first dorsal interosseous in upper extremities and in right and left gluteus medius, vastus medialis, tibialis anterior and gastrocnemius (medial head in lower extremities evaluated a 2+ fibrillation, normal NCV and low-amplitude pattern were detected, although it showed some myopathic pattern but the repetitive nerve stimulation (RNS) test in abductor digiti minimi muscle did not show any decremental pattern. This response could be due to the young age of the patient. Creatine phosphokinase was normal and antibody against the AChR was negative. Neurometabolic disorders such as mitochondrial disease would probably be given little attention because of the early symptoms of ptosis and motor delay. We evaluated serum urine and cerebral spinal fluid and the results were within normal limits. Metabolic screenings, tandem mass spectro chromatography, high performance liquid chromatography of amino acids, urine organic acids, ammonia and venous blood gas (VBG), were all within normal limits. For confirmation of our diagnosis, we did a genetic study of CMS.

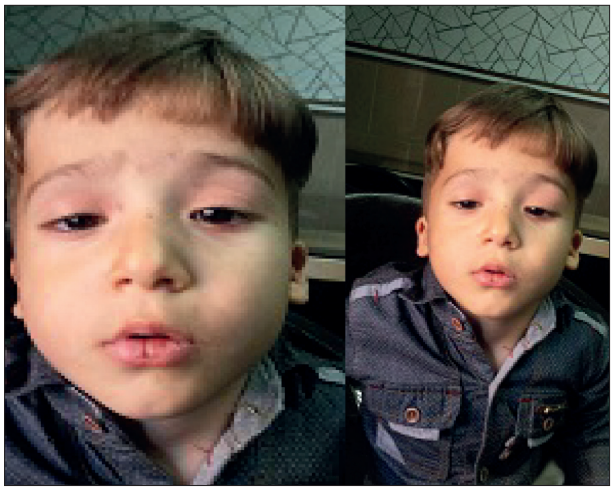

Figure 1. The photographs of the patient with bilateral ptosis (here he is 2 and a half years old).

Informed consent for the genetic studies and publication of medical information was obtained from patient's parents. Venous blood sample was obtained from the patient as well as from all his family members for segregation analysis. Molecular analysis was designed based on the mutation frequencies in genes responsible for post-synaptic CMSs. The family were screened for pathogenic variants in the *CHRNE* gene.

Genomic DNA was extracted from peripheral leukocytes using standard procedures. Polymerase chain reaction (PCR) was carried out in a final volume of 50 µL using PCR Master Mix (Ampliqon A/S, Odense, Denmark). The PCR reactions were performed in a thermal cycler (Techne® Prime; Techne, Cambridge, Cambridgeshire, UK) for 5 min. at 95 °C followed by 30 cycles of denaturation for 30 seconds at 95 °C, annealing for 30 seconds depended on the melting temperature of primers, primer extension for 1 min. at 72 °C, with a final 5 min. extension at 72 °C. The PCR product was evaluated on an 1.5% agarose gel. Then, sequencing was carried out using an ABI PRISM® 3100 capillary sequencer (Thermo Fisher, Waltham, MA, USA). Sequences data were analyzed by comparing these results with the reference wild-type sequence (GenBank CHRNE accession numbers: NM_000080.3) using the Finch TV program (version 1.4.0; Geospiza Inc., Seattle, WA, USA).

Bioinformatics Analysis. To predict the possible structural and functional effects of the newly identified mutation in the *CHRNE* gene, the PolyPhen (Polymorphism phenotyping-2) [5], MutationTaster [6] and SIFT (Sorting Intolerant from Tolerant) [7] programs were used. These are the most frequently used tools for variant effect prediction. In order to classify the mutation, we used the 2015 American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guideline as implemented in the InterVar Web-server.

RESULTS

Mutation Analysis. Direct sequencing of all 12 exons of the *CHRNE* gene, including all intron-exon boundaries, revealed the plausible presence of a novel missense mutation in a homozygous state. The mutation was found

in exon 11, and substitution of the nucleotide G>T at the position 973 of the cDNA (c.973G>T) [Figure 2(a)]. This results in a substitution of valine to leucine amino acid at position 325 (V325L) of the protein. In addition, the target sequencing of family members revealed that both parents carried the same mutation in the heterozygous state [Figure 2(b)]. This formally confirmed that this patient likely inherited the mutation from each parent. No homozygous or heterozygous mutations were detected in the patient's siblings.

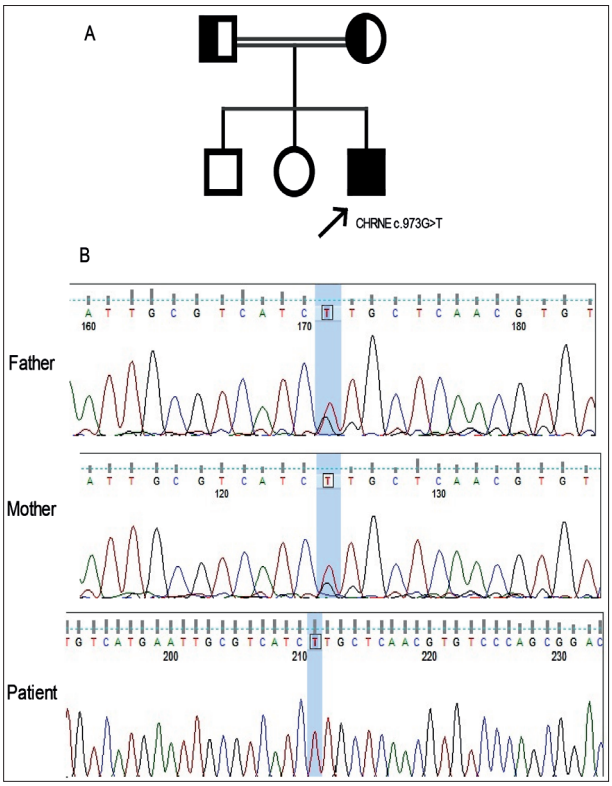

Figure 2. (a) Pedigree of the family, electropherogram of sequences from exon 12 of *CHRNE*, (b) carrier parents; the case illustrating the c.973G>T of the *CHRNE* gene mutation.

Pathogenicity of a Novel Mutation. We predicted the functional consequences of the newly identified mutation in the *CHRNE* gene through three *in silico* prediction tools. Here, the PolyPhen 2 result revealed that the c.973 G>T mutation probably damaged the structure and function of protein with a score of 0.999. According to the MutationTaster, the V325L mutation was predicted to be the causal agent for disease. Moreover, SIFT analysis showed the mutation is deleterious with the score of 0.01. The variant was not annotated in the National Center for Biotechnology Information (NCBI), database for single nucleotide polymorphism (dbSNP) [8], 1000 Genomes Project [9] Exome Variant Server (http://evs.gs.washington.edu/EVS) and was also not listed in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/). Furthermore, the identified variant was recognized as "likely pathogenic" according to ACMG/AMP recommendations [10].

DISCUSSION

We present the clinical and molecular findings of a patient with CMS due to a *CHRNE* gene mutation. Analysis of the whole ε-subunit gene by PCR-sequencing was likely purported to be the novel missense mutation NM_000080.3 (CHRNE): p.Val325Leu in an Iranian family. The possible impact of the mutation on protein structure or function has been supported by three different *in silico* prediction programs. Segregation analysis from the parents also validated the pathogenicity of the mutation. This mutation has not been detected in CMS patients of any ethnic group before.

We know that *CHRNE* gene mutations caused by postsynaptic defects represent the most common forms of CMS. Postsynaptic defects tend to be less severe in comparison with other forms of CMS. Two major abnormalities of post synaptic form are slow-channel form and fast-channel form. The slow-channel form of autosomal dominant type is a late onset form and the clinical manifestation of this form are more typical than the other types of CMS. But the clinical onset of this syndrome varies ranging from infancy to adulthood. On the other hand, fast-channel syndromes are related to autosomal recessive forms of mutations. Patients become symptomatic during early infancy. Common disorders of these post synaptic syndromes are brief channel activation episodes and reduced channel opening in response to AChRs [11,12].

Fluoxetine that normalizes abnormality of prolonged AChR activation episodes in cell cultures expressing mutated receptors can improve the clinical and electrophysiological disorders. Therefore, we used fluoxetine and we had good response in this patient after starting Prozac syrup. The patient showed improvement in his neurodevelopmental milestones and ptosis. When he was 11 months old, he could crawl and sit independently. At the last patient visit, he was 2 and a half years old and had normal developmental milestones with bilateral ptosis.

The AChR ε-subunit deficiency showed as first cause of CMS worldwide, which were inherited mostly in an autosomal recessive form [4]. The mutated *CHRNE* gene was previously reported in an Iranian family with CMS [13]. Our findings highlight the contribution of the *CHRNE* gene in pathogenesis of CMS. This implies that genetic assessment of CMS cases may lead to the discovery of new *CHRNE* variations that could expand the clinical management of the disease. It is noteworthy that mutations in the

CHRNE gene tend to be in different exons or introns or arising as a founder effect in particular geographic regions. For instance, the 1267delG frameshift mutation occurs on at least one allele of 60.0% of patients [13,4]. This mutation has been repeatedly identified in a series of Roma families in which the disease course was progressive [14]. Moreover, 1293insG was a common founder mutation in patients originating from North Africa [15].

In conclusion, we report an Iranian family carrying a *CHRNE* gene mutation. As expected, screening for *CHRNE* variants could be an effective approach to identify genetic lesion in patients with CMS. Thus, whenever clinical data are suggestive for CMS, screening the appropriate gene may help establish a significant genetic diagnosis. For further studies, the clinical and functional impacts of different *CHRNE* mutations may provide important insights into the roles of this gene in development of CMSs treatment.

ACKNOWLEDGMENTS

We thank Dominic Donkor of Concordia University, Montreal, Canada for been a part of the review process of the manuscript. We would like to express our gratitude to the patient and his parents for their cooperation.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding. This study was supported by Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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