CASE REPORT

A case report of congenital myasthenic syndrome caused by a mutation in the *CHRNE* gene in the Iranian population

Zahra FARJAMI MSc[1],

Negar KHODAENIA MSc[1],

Neshat EBRAHIMI MSc[2],

Gholamreza ZAMANI MD, PhD[3],

Amir Hosein ASHNAEI BSc[5],

Mohammad GALEHDARI PhD[6],

Mehdi MORADYAR PhD[1],

Massoud HOUSHMAND PhD[1]

1. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Laboratory of Cedars-Sinai Medical Center, Los Angeles, California, USA
3. Pediatric Neurologist, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Biotechnology, Science and Research Branch, Islamic Azad University, Tehran, Iran
5. Department of Modern Sciences and technologies; Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
6. Department of Biology, Faculty of Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran

Corresponding Author
Houshmand M. MD
National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

Email: housh62@yahoo.com

Received: 28-Jan- 2018

Accepted: 10-Apr-2019

Abstract

Congenital myasthenic syndrome (CMS) refers to a heterogeneous group of inherited disorders, characterized by defective transmission at the neuromuscular junction (NMJ). Patients with CMS showed similar muscle weakness, while other clinical manifestations are mostly dependent on genetic factors. This disease, caused by different DNA mutations, is genetically inherited. It is also associated with mutations of genes at NMJ, involving the acetylcholine receptor (AChR) subunits. Here, we present the case of a five-year-old Iranian boy with CMS, undergoing targeted sequencing of a panel of genes, associated with arthrogryposis and CMS. The patient had six affected relatives in his genetic pedigree chart. The investigations indicated a homozygous single base pair deletion at exon 12 of the *CHRNE* gene (chr17:4802186delC). This region was conserved across mammalian evolution and was not submitted to the 1000 Genomes Project database. Overall, the *CHRNE* variant may be classified as a significant variant in the etiology of CMS. It can be suggested that the Iranian CMS population carry regional pathogenic mutations, which can be detected via targeted and whole genome sequencing.

Keywords: Congenital myasthenic syndrome, CMS, *CHRNE*

Introduction

Congenital myasthenic syndrome (CMS) refers to a heterogeneous group of inherited genetic disorders, affecting neuromuscular transmission. Although most symptoms of CMS appear within several days after birth or in early childhood, in rare cases, its onset may be delayed until childhood (1). CMS affects not only the muscles moving the eyes, but also chewing and swallowing muscles, involved in abnormal physical exhaustion (2). CMS is also commonly associated with dysfunctional acetylcholine receptor (AChR) ε-subunits. Evidence shows that

Iran J Child Neurol. Autumn 2020 Vol. 14 No. 4

87

CHRNE gene mutations lead to AChR deficiency (3). The encoded AChR protein can be found in the cellular membrane of skeletal muscles at the neuromuscular junction (NMJ) (4). The sites, products, and disorders of CMS-related genes are presented in Table 1.

The severity of CMS is highly variable, ranging from minor symptoms to progressive disabling weakness. Although the prevalence of this syndrome is unknown, it has been classified as a rare disease (6). The diagnosis of CMS is established, based on clinical findings, electromyography, genetic tests, and measurement of serum AChR antibodies. All treatment protocols involve acetylcholinesterase inhibitors (9). Targeted sequencing represents a cost-effective approach to detect the variants in multiple or large genes.

In the present case report, we aimed to examine nucleotide variations in a panel of genes in an Iranian male CMS patient.

Case Presentation

The patient was a five-year-old boy with at least six CMS-affected relatives in his family pedigree (Figure 1). His parents were second cousins. According to the neurology report, he was diagnosed with ptosis at birth with diurnal variation and a high-arched palate. The muscle biopsy with hematoxylin and eosin (H&E) staining revealed a striated muscle tissue with significant variations in fiber size. The atrophic fibers were rounded and/or angular and dispersed. The ATPase reaction at pH of 9.4, 4.6, and 4.35 revealed prominent atrophy of type II fibers. Also, measurement of blood cells showed a higher white cell count (16.5 units) than the normal range (4-10).

After collecting a blood sample, DNA was extracted by a manual method. The gene panel included the following genes: MYH7, CHAT, CHRNA1, CHRNB1, CHRND, CHRNE, CHRNG, DPAGT1, GFPT1, LAMB2, MUSK, MYH3, MYH8, RAPSN, SCN4A, TNNI2, TNNT3, COLQ, DOK7, AGRN, MYBPC1, TPM2, PLEC, SLC35A3, ECEL1, GLE1, VIPAS39, VPS33B, PIEZO2, UBA1, CHST14, and FBN2. Generally, targeted gene sequencing involves targeted capture and sequencing of protein-coding regions of genomes/genes. Mutations that are identified in the exonic regions are generally considered to be more pathogenic than variations that occur in non-coding regions.

The libraries were sequenced to a mean coverage of >80-100X on an Illumina sequencing platform. The obtained sequences were aligned to the human reference genome (GRCh37/hg19) in the BWA software package (10, 11). They were also analyzed using the Picard and GATK-Lite toolkit (12, 13) to identify variants in targeted genes, associated with clinical presentations. Clinically relevant mutations were annotated, using the variants published in the literature, as well as a set of variant databases, including ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), OMIM, GWAS, HGMD, and SwissVar (http://swissvar.expasy.org/). Only non-synonymous and splice-site variants, found in the arthrogryposis and CMS panel genes, were used for clinical interpretations.

The results showed a homozygous single base pair deletion at exon 12 of the *CHRNE* gene (chr17:4802186delC), causing the frameshift and premature truncation of 64 amino acid proteins downstream of codon 443 (p.Glu443LysfsTer64; ENST00000293780). No other variants were detected that need to be reported.

88

Iran J Child Neurol. Autumn 2020 Vol. 14 No. 4

A case report of congenital myasthenic syndrome caused by a mutation in the CHRNE gene in the Iranian population

Table 1. Conjenital myasthenic syndrome (CMS)

Genes	Sites	Percentage of pathogenic variants	Products	Disorders
CHRNE	17p13.2 at the short (p) arm of chromosome 17 at position 13.2	1%	AChR epsilon subunit	1- Multiple pterygium syndrome lethal type (MUPSL) 2- Slow-channel congenital myasthenic syndrome (SCCMS) 3- Fast-channel congenital myasthenic syndrome (FCCMS)
CHRND	2q37.1 at the long (q) arm of chromosome 2 at position 37.1	50%		1- MUPSL 2- SCCMS 3- FCCMS
CHAT	10q11.23 at the long (q) arm of chromosome 10 at position 11.23	4-5%	Choline acetyltransferase	1- CMS
MUSK	9q31.3 at the long (q) arm of chromosome 9 at position 31.3	1%	Muscle-specific tyrosine kinase receptor	1- CMS
ALG14	1p21.3 at the short (p) arm of chromosome 1 at position 21.3	1%	Subunits of UDP-GlcNAc transferase	1- CMS
CACNB2	10p12.33-p12.31 at the short (p) arm of chromosome 10 between positions 12.33 and 12.31	1%	Voltage-gated calcium channel superfamily	1- Lambert-Eaton myasthenic syndrome 2- Brugada syndrome
SYT2	1q32.1 at the long (q) arm of chromosome 1 at position 32.1		Calcium sensor 1	1- CMS 2- Presynaptic CMS with or without motor neuropathy
COLQ	3p25.1 at the short (p) arm of chromosome 3 at position 25.1	10-15%	Acetylcholinesterase	

Gene	Location	Frequency	Function	Type
DOK7	4p16.3 at the short (p) arm of chromosome 4 at position 16.3	10-15%	Connection between nerve cells and muscle cells	CMS
RAPSN	11p11.2 at the short (p) arm of chromosome 11 at position 11.2	1%	AChR	CMS
CHRNB1	17p13.1 at the short (p) arm of chromosome 17 at position 13.1	1%	AChR beta subunit	SCCMS
AGRN	1p36.33 at the short (p) arm of chromosome 1 at position 36.33	1%	1- Laminin G 2- Kazal-type serine protease inhibitor 3- Epidermal growth factor domains	CMS
SCN4A	17q23.3 at the long (q) arm of chromosome 17 at position 23.3	1%		Sodium channel alpha subunit
B3GLCT	13q12.3 at the long (q) arm of chromosome 13 at position 12.3	10-15%	Beta 3-glucosyltransferase (B3Glc-T)	CMS

Table 2. Arthrogryposis multiplex congenita

Genes	Sites	Products	Disorders
ECEL1	2q37.1 at the long (q) arm of chromosome 2 at position 37.1	Endopeptidases (M13) and zinc-containing type II integral-membrane proteins	1- Arthrogryposis multiplex congenita 2- Autosomal recessive distal arthrogryposis (type 5D)
TPM2	9p13.3 at the short (p) arm of chromosome 9 at position 13.3	β-tropomyosin (part of tropomyosin)	1- Cap myopathy 2- Congenital fiber-type disproportion 3- Distal arthrogryposis type 1 4- Nemaline myopathy 5- Sheldon-Hall syndrome 6- Arthrogryposis multiplex congenita
MYBPC1	12q23.2 at the long (q) arm of chromosome 12 at position 23.2	Myosin-binding protein C (a slow skeletal muscle isoform)	1- Arthrogryposis multiplex congenita
ADCY6	12q13.12 at the long (q) arm of chromosome 12 at position 13.12	Adenylyl cyclase	1- Arthrogryposis multiplex congenita
KRT17	17q21.2 at the long (q) arm of chromosome 17 at position 21.2	Keratin 17 (K17)	1- Arthrogryposis multiplex congenita
KRT16	17q21.2 at the long (q) arm of chromosome 17 at position 21.2	Keratin 16 (K16)	1- Arthrogryposis multiplex congenita
RBPJ	4p15.2 at the short (p) arm of chromosome 4 at position 15.2	RBP-J	1- Arthrogryposis multiplex congenita
VIPAS39	14q24.3 at the long (q) arm of chromosome 14 at position 24.3	Sorting of lysosomal proteins	1- Arthrogryposis multiplex congenita 2- Renal dysfunction 3- Cholestasis type 2
NR0B1	Xp21.2 at the short (p) arm of the X chromosome at position 21.2	DAX1	1- Arthrogryposis multiplex congenita

Iran J Child Neurol. Autumn 2020 Vol. 14 No. 4

91

TINF2	14q12 at the long (q) arm of chromosome 14 at position 12	Telomere protein protectors	1- Arthrogryposis multiplex congenita
CLCN1	7q34 at the long (q) arm of chromosome 7 at position 34	Chloride channels	1- Arthrogryposis multiplex congenita
RBPJ	4p15.2 at the short (p) arm of chromosome 4 at position 15.2	RBP-J (part of the Notch signaling pathway)	1- Arthrogryposis multiplex congenita
ARHGAP31	3q13.32-q13.33 at the long (q) arm of chromosome 3 between positions 13.32 and 13.33	GTPase-activating protein (GAP)	1- Arthrogryposis multiplex congenita
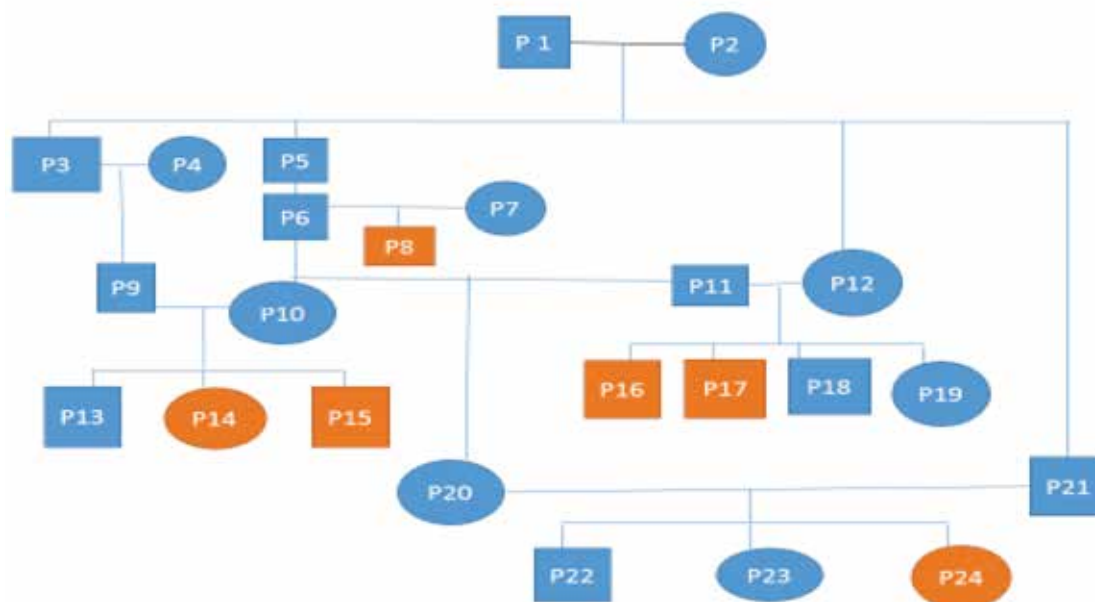
Figure 1. The patient's pedigree chart

Discussion

The muscle AChR contains five subunits of four different types (two alpha, one beta, one gamma, and one delta). Mutations in the AChR ε-subunit are more frequent than mutations of alpha, beta, and delta subunits. Mutations in these subunits are frequently related to a severe phenotype. The CHRND gene mutations should be considered in severe and early cases of the disease. They clinically resemble rapsyn phenotypes with recurrent episodic apneas (16). In this regard, Hoffmann et al. and Morgan et al. showed that mutations in the CHRNG gene resulted in Escobar syndrome (EVMPS; 265000), as well as lethal multiple pterygium syndrome (LMPS; 253290).

92

Iran J Child Neurol. Autumn 2020 Vol. 14 No. 4

The CHRNG gene encodes the AChR gamma subunit and is expressed before week 33 of pregnancy in humans, but is replaced by the ε-subunit (100725) in the perinatal period. Therefore, the gamma subunit not only contributes to neuromuscular signal transduction, but is also important for neuromuscular organogenesis. Mutations in the CHRNA gene may cause disorders, such as LMPS and CMS (slow- and fast-channel CMS). Also, mutations in the CHRNB gene may lead to slow-channel CMS. Homozygous or compound heterozygous mutations in the CHRNE gene (OMIM entry #100725) have been implicated in CMS4C, associated with AChR deficiency and fast-channel CMS4B (OMIM entry #616324). This CHRNE variant has been previously identified in five CMS patients with AChR deficiency in Southeast of Iran (Khuzestan) (14, 15). It has not been submitted to the 1000 Genome Project database and is conserved across mammals.

Based on the abovementioned findings, this CHRNE variant can be classified as a pathogenically significant variant that may be included in genetic screenings. Sequencing of this variant in the parents (an in vitro strategy) and other affected and non-affected members of the family is recommended to confirm its significance. It seems that mutations in CHRNE genes are common in the Iranian population, which may contribute to the pathogenicity of CMS. Such variants may be detected via further targeted and whole genome sequencing.

In Conclussion

Based on the above mentioned findings, this CHRNE variantcan be classified as a pathogenically significant variant that maybe included ingeneticscreenings. Sequencing of this variant in theparents(an in vitrostrategy) and other affected and non-affected members of the family is recommended to confirm its significance.It seems that mutations inCHRNEgenes are common in the Iranian population,which may contribute to thepathogenicity of CMS. Such variants maybe detected via further targeted and wholegenome sequencing.

Conflict of Interest

Author who involved in this project declare non conflict of interest.

References

1. Palace J, Beeson D. The congenital myasthenic syndromes. Journal of neuroimmunology. 2008;201:2-5.

2. Hantaï D, Richard P, Koenig J, Eymard B. Congenital myasthenic syndromes. Current opinion in neurology. 2004;17(5):539-51.

3. Ohno K, Engel AG, Shen X-M, Selcen D, Brengman J, Harper CM, et al. Rapsyn mutations in humans cause endplate acetylcholine-receptor deficiency and myasthenic syndrome. The American Journal of Human Genetics. 2002;70(4):875-85.

4. Nichols P, Croxen R, Vincent A, Rutter R, Hutchinson M, Newsom-Davis J, et al. Mutation of the acetylcholine receptor ε-subunit promoter in congenital myasthenic syndrome. Annals of neurology. 1999;45(4):439-43.

5. Engel AG, Shen X-M, Selcen D, Sine SM. Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. The Lancet Neurology. 2015;14(4):420-34.

6. Schara U, Della Marina A, Abicht A. Congenital myasthenic syndromes: current diagnostic and therapeutic approaches. Neuropediatrics. 2012;43(04):184-93.

7. Müller JS, Mihaylova V, Abicht A, Lochmüller H. Congenital myasthenic syndromes: spotlight on genetic defects of neuromuscular transmission. Expert reviews in molecular medicine. 2007;9(22):1-20.

8. Nicole S, Chaouch A, Torbergsen T, Bauché S, de Bruyckere E, Fontenille M-J, et al. Agrin mutations lead to a congenital myasthenic syndrome with distal muscle weakness and atrophy. Brain. 2014;137(9):2429-43.

9. Dilena R, Abicht A, Sergi P, Comi GP, Di Fonzo A, Chidini G, et al. Congenital Myasthenic Syndrome Due to Choline Acetyltransferase Mutations in Infants Clinical Suspicion and Comprehensive Electrophysiological Assessment Are Important for Early Diagnosis. Journal of child neurology. 2014;29(3):389-93.

10. Li H, Homer N. A survey of sequence alignment algorithms for next-generation sequencing. Briefings in bioinformatics. 2010;11(5):473-83.

11. Meyer LR, Zweig AS, Hinrichs AS, Karolchik D, Kuhn RM, Wong M, et al. The UCSC Genome Browser database: extensions and updates 2013. Nucleic acids research. 2013;41(D1):D64-D9.

12. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome research. 2010;20(9):1297-303.

13. Li H, Durbin R. Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics. 2010;26(5):589-95.

14. Engel AG. Current status of the congenital myasthenic syndromes. Neuromuscular Disorders. 2012;22(2):99-111.

15. Abicht A, Dusl M, Gallenmüller C, Guerguelt cheva V, Schara U, Della Marina A, et al. Congenital myasthenic syndromes: Achievements and limitations of phenotype-guided gene-after-gene sequencing in diagnostic practice: A study of 680 patients. Human mutation. 2012;33(10):1474-84.

16. Müller JS, Baumeister SK, Schara U, Cossins J, Krause S, Hagen Mvd, et al. CHRND mutation causes a congenital myasthenic syndrome by impairing co-clustering of the acetylcholine receptor with rapsyn. Brain. 2006;129(10):2784-93.

94

Iran J Child Neurol. Autumn 2020 Vol. 14 No. 4