Clinical Case Report

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CHRNE compound heterozygous mutations in congenital myasthenic syndrome

A case report

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Abstract

Rationale: Congenital myasthenic syndrome (CMSs) are a group of rare genetic disorders of the neurological junction, which can result in structural or functional weakness. Here, we characterized a case of CMS in order to clarify the diagnosis and expand the understanding of it. The molecular diagnosis had implications for choice of treatment and genetic counseling.

Patient concerns: A 3-year-old male patient with CMS had ptosis and limb weakness for 2 months after birth. Clinical course and electrophysiological, imaging, and genetic findings were assessed. Protein structure/function was predicted. A novel mutation of c.295C>T (exon 4) and another known mutation of c.442T>A (exon 5) were found in *CHRNE*. Both mutations localized in conserved sequences. The c.442T>A (p.C148S) missense mutation in *CHRNE* was predicted to be damaging/deleterious. The iterative threading assembly refinement (I-TASSER) server generated vastly different 3-dimensional (3D) atomic models based on protein sequences from wide-type and novel nonsense mutation of c.295C>T (p.R99X) in *CHRNE*.

Diagnoses: The diagnosis of CMS with *CHRNE* mutations in Han Chinese was confirmed.

Interventions: The patient was given prednisone (10 mg, once daily, taken orally) and pyridostigmine (15 mg, three times a day, taken orally).

Outcomes: The patient had a moderate response to prednisone and pyridostigmine.

Lessons: We expanded the genotype and phenotype of CMS with *CHRNE* mutations in Han Chinese and provided new insights into the molecular mechanism of CMS and help to the diagnosis and treatment of CMS.

Abbreviations: 3D = 3-dimensional, ACh = acetylcholine, CMS = Congenital myasthenic syndrome, EEG = electrocardiography, EMG = electromyography, I-TASSER = iterative threading assembly refinement, MAF = minor allele frequency, NGS = next-generation sequencing, qPCR = quantitative polymerase chain reaction, SNP = single-nucleotide polymorphism.

Keywords: CHRNE, congenital myasthenic syndrome, neuromuscular junction

1. Introduction

Congenital myasthenic syndromes (CMSs) are hereditary disorders of neuromuscular junction.[1] They are highly treatable, and the appropriate pharmacotherapy depends on the underlying genetic defect. Normally, acetylcholine (ACh) is excessively released by the activated motor neurons and then binds to nicotinic acetylcholine receptor (AChR) to trigger muscle contraction, but acetylcholinesterase can terminate this effect by degrading ACh.[2,3] Mutations in around 30 genes that encode proteins involved in this signaling pathway are known to cause CMS (as shown in NCBI). Gene mutations in presynaptic, synaptic, and postsynaptic proteins have been demonstrated in patients, with more than half involving aberrations in postsynaptic AChR subunits CHRNE, CHRNA1, CHRNB1, CHRND, and CHRNG. Mutations of *CHRNA1*, *CHRNB1*, *CHRND*, or *CHRNG* are typically embryonic lethal because their products are critical to the function of fetal AChR. Thus, mutations in *CHRNE*, which encodes the AChR ε-subunit, account for almost 75% to 80% of causes of all human CMSs related to AChR deficiency.[4–6]

Herein, we describe a Han Chinese CMS patient with infant onset and a moderate clinical course, which expands the previously described phenotype of CMS. By performing next-

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The authors confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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generation sequencing (NGS), novel and another known mutations in *CHRNE* were identified.

2. Materials and methods

2.1. Variant detection

On admission, venous blood samples were obtained from the patient as well as from his unaffected parents and then NGS was performed. Genomic DNA samples were sonicated, followed by the hybridization with the NimbleGen 2.0 probe sequence capture array of Roche, (http://www.nimblegen.com/products/seqcap/ez/v2/index.html) to enrich the exon DNA (Joy Orient, China). Libraries were first tested for enrichment by quantitative polymerase chain reaction (qPCR) and for size distribution, then concentration using the Agilent Bioanalyzer 2100 (Illumina Co., Santa Clara, CA). The samples were sequenced on the Illumina Hiseq2500. Two parallel reactions were performed for each sample. Data filtering, mapping, and variant detection were applied. Exon-enriched DNA was sequenced on the Illumina hiseq2500 platform according to the manufacturer's instructions (Illumina). Raw image files were processed using the BclToFastq (Illumina) for base calling and generating the raw data. Low-quality variations were filtered out using the quality score $\geq$20 (Q20). The sequencing reads were aligned to the NCBI human reference genome (hg19) using BWA. Samtools and Pindel were used to analyze the single-nucleotide polymorphism (SNP) and the indexing of the sequence. Lastly, data analysis was performed as follows: synonymous changes and SNPs with the minor allele frequency (MAF) higher than 5% were removed (http://www.ncbi.nlm.nih.gov/projects/SNP); Nonsynonymous changes were filtered using SIFT software (http://sift.jcvi.org); The function of mutated genes and its relationship to CMS were further analyzed.

2.2. Conserved sequence analysis

UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly was used to analyze whether the locations of mutations on both alleles were in conserved sequences.

2.3. Protein function prediction for the missense mutation

To predict the effect of amino acid substitutions, we performed in silico analysis using the SIFT/PROVEAN (http://sift.jcvi.org) and Polyphen-2 (http://genetics.bwh.harvard.edu/pph2) web software. SIFT Score ranges from 0 to 1. The amino acid substitution is predicted as damaging if the score is $\leq$0.05 and tolerated if the score is $>$ 0.05 (J. Craig Venter Institute, Rockville, MD). The variant is predicted to be deleterious if the PROVEAN score is ≤ -2.5), and neutral if the score is > -2.5 (J. Craig Venter Institute). Polyphen-2 prediction outcomes can be one of "probably damaging," "possibly damaging," or "benign."

2.4. Protein structure prediction on the I-TASSER server for the nonsense mutation

The I-TASSER Suite pipeline consists of 4 general steps: threading template identification, iterative structure assembly simulation, model selection and refinement, and structure-based function annotation. The server is available at http://zhanglab.ccmb.med.umich.edu/I-TASSER.

2.5. Ethical considerations

In order to further clarify the genetic cause and diagnosis, the consultants/registrars in the Department of Pediatric Neurology consulted the parents on the purpose of the DNA analysis. The samples from the child and his parents were studied after obtaining informed consent from his parents. This work was approved by the Ethics Committee of Shanghai Children's Hospital.

3. Results

3.1. Clinical data

A 3-year-old Han Chinese male patient was referred to our clinic from a regional hospital. He presented with limb weakness and ptosis for 2 months after birth. Reviewing medical history revealed that a test dose of prostigmine was given, showing a positive result. The brain magnetic resonance imaging (MRI) demonstrated a poorly developed corpus callosum (Fig. 1). The detections of tandem mass spectrometry, blood electrolytes, thyroid hormone, liver function, immunoglobulin, anti-nuclear antibody, and anti-AChR antibody were normal. After repetitive nerve stimulation, electromyography (EMG) of the right ulnar nerve did not show decrement; electrocardiography (EEG) was also normal. His parents refused to perform a muscle biopsy on him. Reviewing his medical history revealed that he could raise his head at 8 months of age and sit up by 18 months of age, which demonstrated delayed motor development. Then, he was given prednisone (10 mg, once daily, taken orally) and pyridostigmine (15 mg, three times a day, taken orally) following the diagnosis of myasthenia gravis in the regional hospital. The symptoms were improved to a certain extent. On referral to our clinic, he could not stand up and walk independently at 3 years of age. The speech and cognitive development was normal. Because the previous diagnosis of myasthenia gravis in the regional hospital was suspected, NGS was performed.

3.2. Variant detection

The result of NGS showed compound heterozygous mutations in *CHRNE* [c.442T>A (p.C148S), exon 5; c.295C>T (p.R99X),

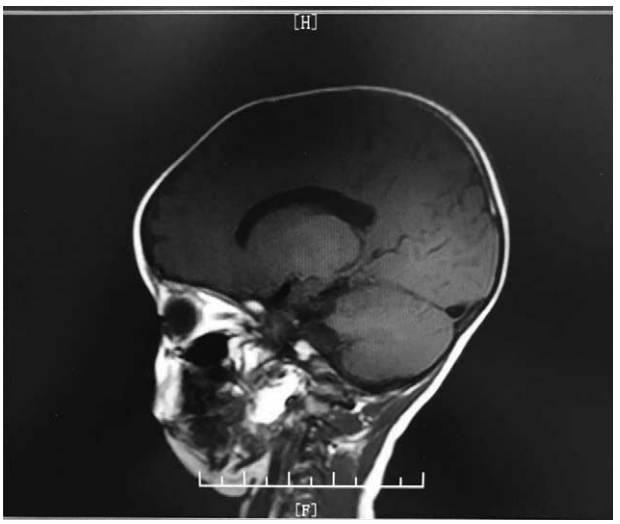

Figure 1. Brain MRI of the patient demonstrated poor development of the corpus callosum.

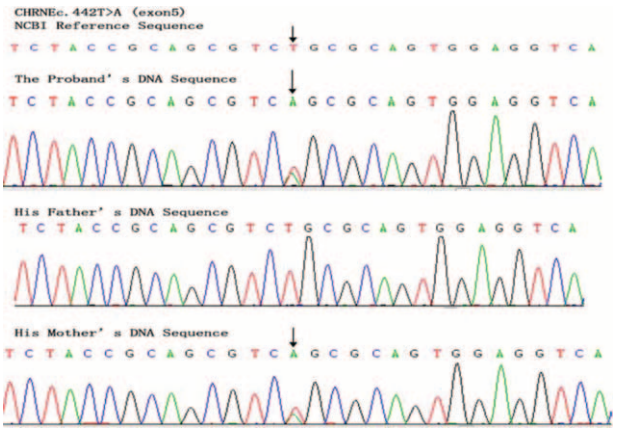

Figure 2. Electropherograms of the *CHRNE* sequence showing the mutation of c.442T>A (p.C148S) in exon 5 in the patient and the mother.

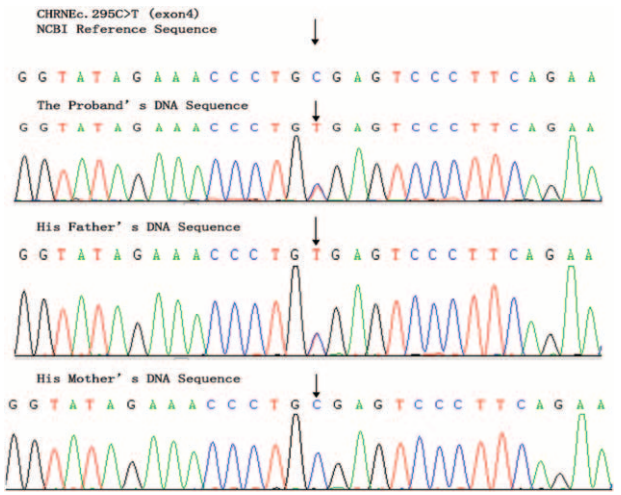

Figure 3. Electropherograms of the *CHRNE* sequence showing the mutation of c.295C>T (p.R99X) in exon 4 in the patient and the father.

exon 4, each inherited in trans from each parent] (Figs. 2 and 3), establishing the final diagnosis of CMS. The NCBI reference sequence was used: *CHRNE* (NM_000080.3). Other clinically unknown mutations identified by the NGS are summarized in Table 1.

3.3. Conserved sequence analysis

After the genetic diagnosis, analysis with the UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly showed that the locations of mutations on both alleles were in conserved sequences.

3.4. Protein function prediction for the missense mutation

The missense mutation of c.442T>A (p.C148S) in *CHRNE* was predicted to be "damaging" by the SIFT, "deleterious" by the Provean, and "probably damaging" by the Polyphen-2 web software.

3.5. Protein structure prediction on the I-TASSER server for the nonsense mutation

The nonsense mutation of c.295C>T (p.R99X) in *CHRNE* resulted in a premature stop codon at position 99 (Fig. 4). The I-TASSER server generated vastly different 3D atomic models based on protein sequences from wide-type and novel nonsense mutation of c.295C>T (p.R99X) in *CHRNE* (Figs. 5 and 6).

4. Discussion

CMS is a rare disease and *CHRNE* mutants account for over 50% of human CMSs related to AChR deficiency.[7,8] CMS with severe EP AChR deficiency results from different types of homozygous or more frequently heterozygous recessive mutations in the *AChRα* (CHRNA1), *β* (CHRNB1), *δ* (CHRND), and *ε* (CHRNE) subunit genes. The mutations are mainly found in the *ε* (CHRNE) subunit gene, which may be explained as that the

Table 1

Other clinically unknown mutations identified by next-generation sequencing.

Genes	Chromosomes	Mutation sites	Heterozygote/ homozygote	Related phenotypes
MUSK	Chr9: 113431116	c.-69G>T (noncoding area)	Heterozygote	CMS, AchR deficiency, AR
CHRND	chr2: 233391303	c.117C>G (p.N39K)	Heterozygote	CMS, 3B, fast channel type, AD, AR; CMS, 3A, slow channel type, AD, AR
STIM1	chr11: 4080543	c.530C>T (p.T177I)	Heterozygote	Tubular aggregate myopathy, AD
NEB	chr2: 152447903	c.14893G>A (p.G4965R)	Heterozygote	Nemaline myopathy, type II, AR
SHANK3	chr22: 51135982- 51135983	c.1303–3_c.1303–2 ins C(splicing site)	Homozygote	Phelan–McDermid syndrome; Chromosome 22q13.3 deletion syndrome
	chr22: 51135985- 51135991	c.1303_c.1309 del CTCGGTT (p.L435Pfs*38)	Homozygote	
DNM2	chr19: 10893735	c.788C>T (p.P263L)	Heterozygote	Centronuclear myopathy, type I, AD; Lethal congenital contracture syndrome, type V, AR
	chr19: 10940823	c.2312C>T (p.P771L)	Heterozygote	
SYNE1	chr6: 152652184	c.13636G>A (p.V4546I)	Heterozygote	Emery–Dreifuss myodystrophy, type IV, AD

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Wild type   MARAPLGVLLLLGLLGRGVGKNEELRLYHHLFNNYDPGSRPVREPEDTVTISLKVTLTNL
P. R99X     MARAPLGVLLLLGLLGRGVGKNEELRLYHHLFNNYDPGSRPVREPEDTVTISLKVTLTNL

                                                  99
Wild type   ISLNEKEETLTTSVWIGIDWQDYRLNYSKDDFGGIETLRVPSELVWLPEIVLENNIDGQF
P. R99X     ISLNEKEETLTTSVWIGIDWQDYRLNYSKDDFGGIETL*

Wild type   GVAYDANVLVYEGGSVTWLPPAIYRSVCAVEVTYFPFDWQNCSLIFRSQTYNAEEVEFTF
P. R99X

Wild type   AVDNDGKTINKIDIDTEAYTENGEWAIDFCPGVIRRHHGGATDGPGETDVIYSLIIRRKP
P. R99X

Wild type   LFYVINIIVPCVLISGLVLLAYFLPAQAGGQKCTVSINVLLAQTVFLFLIAQKIPETSLS
P. R99X

Wild type   VPLLGRFLIFVMVVATLIVMNCVIVLNVSQRTPTTHAMSPRLRHVLLELLPRLLGSPPPP
P. R99X

Wild type   EAPRAASPPRRASSVGLLLRAEELILKKPRSELVFEGQRHRQGTWTAAFCQSLGAAAPEV
P. R99X

Wild type   RCCVDAVNFVAESTRDQEATGEEVSDWVRMGNALDNICFWAALVLFSVGSSLIFLGAYFN
P. R99X

Wild type   RVPDLPYAPCIQP
P. R99X
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Figure 4. Amino acid alignment of wild-type and mutation of c.295C>T (p.R99X) in *CHRNE*.

Figure 6. The 3-dimensional atomic model based on protein sequence from the novel nonsense mutation of c.295C>T (p.R99X) in *CHRNE* generated by the iterative threading assembly refinement server.

expression of fetal type γ (CHRNG) subunit, although at a low level, partially compensates for the absence of ε (CHRNE) subunit, whereas patients harboring low-expressor or null mutations in subunits other than ε (CHRNE) might not survive due to the lack of a substituting subunit.[9] Currently, there are 117 *CHRNE* mutation entries reported in the human gene mutation database (http://www.hgmd.cf.ac.uk), including 57 missense/nonsense mutations. Our patient had compound heterozygous mutations of c.442T>A (p.C148S) and c.295C>T (p.R99X), which were different from the most common mutation of c.1267delG in *CHRNE*.[10] One was the substitution of cysteine by serine as missense mutation. Milone et al[11] first reported the mutation in 1998; however, it was just with a different nomenclature. Protein function prediction showed this mutation was deleterious/damaging. The other mutation of c.295C>T (p.R99X) was a nonsense mutation that had not yet been reported, but it was found in the GnomAD database of unaffected individuals, just one time (as a heterozygote, never as a homozygote) (http://gnomad.broadinstitute.org/variant/17–4805561-G-A). The nonsense mutation may lead to premature termination of protein translation. The I-TASSER server generated vastly different 3D atomic models based on protein sequences from wide-type and novel nonsense mutation. Both mutations were found in conserved sequences and pathogenic.

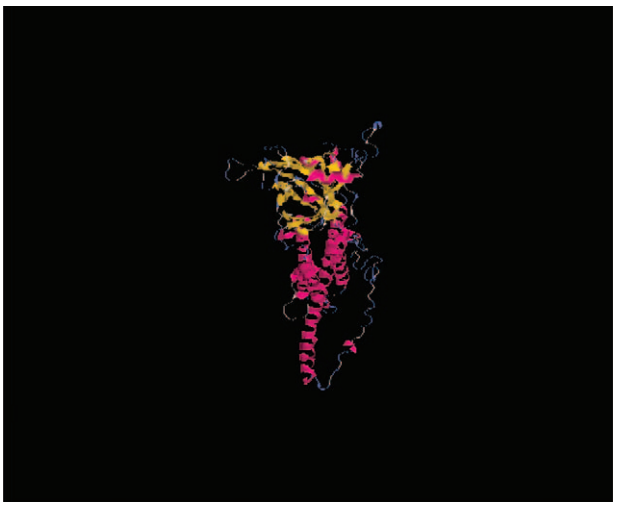

Figure 5. The 3-dimensional atomic model based on protein sequence from wide-type generated by iterative threading assembly refinement server.

The diagnosis of CMS is based on clinical symptomatology, absence of AChR antibodies, and at least one of following manifestations: EMG evidence of neuromuscular transmission defect, response to pyridostigmine, and molecular genetic confirmation.[12] Molecular diagnosis is critical because incorrect treatment in CMS will be life-threatening.[13–15] In our case, the diagnosis of CMS was finally confirmed by molecular genetic analysis. It has been reported that ptosis was present in nearly all patients with *CHRNE* mutations,[9] which was consistent with the finding in our case.

In our patient, the clinical course was similar to that in other patients with mutations in *CHRNE*. Symptoms included ptosis and limb weakness for 2 months after birth, delayed motor milestones, a positive prostigmine test, and response to pyridostigmine.[16] The findings of our case also expanded the phenotypic spectrum with a moderate infant onset, poor development of the corpus callosum, and compound heterozygous mutations in *CHRNE* (1 mutation was novel and both were in conserved sequences). We speculate that *CHRNE* mutations may be considered if the patient with the above characteristics is clinically suspected for CMS.

A recent study in the UK has shown that the detected prevalence of genetically confirmed CMS is approximately 9.2 cases per million children.[17] It is likely to increase in the future, as a significant proportion of clinically diagnosed cases have not yet been confirmed by genetic analysis.[18] It seems that the prevalence of CMS in Han Chinese is similar to that in other countries. Recognition that there are differing features of CMS

and given the limitations of clinical tools and the variable phenotypic characteristics of CMS, a genetic diagnosis is the most accurate method to confirm the CMS subtype and select the most appropriate treatment.[19,20] A direct and efficient approach is the use of microarrays specifically designed for the screening of multiple candidate disease loci in known CMS genes, but it will miss novel mutations in these genes. A novel approach to mutation discovery is the whole exome sequencing that may identify mutations in exons, although it is relatively expensive.[21] However, large deletion or duplication mutations may be missed both by Sanger sequencing and whole exome sequencing. Although rare, they can be identified by array-based comparative genomic hybridization.[22,23]

5. Conclusion

We expanded the genotype and phenotype of CMS with *CHRNE* mutations in Han Chinese and provided new insights into the molecular mechanism of CMS and help to the diagnosis and treatment of CMS.

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