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 = electroencephalography, 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.\cite{1,2,3} 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.\cite{4,5} 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 CHRNA1, CHRNB1, CHRN1, CHRN5, and CHRNA. Mutations of CHRNA1, CHRNB1, CHRN1, or CHRNA5 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.\cite{6,7}

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-
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/ezv2/index.html) to enrich the exon DNA (Joy Orient, 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 \( Q20 \). 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 \( \leq -2.5 \), 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 symptoms of limb weakness and ptosis 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), [c.442T>A (p.C148S), exon 5; c.295C>T (p.R99X),]
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: \textit{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 \textit{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 \textit{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 \textit{CHRNE} (Figs. 5 and 6).

### 4. Discussion

CMS is a rare disease and \textit{CHRNE} mutants account for over 50% of human CMSs related to AChR deficiency.\cite{7,8} CMS with severe EP AChR deficiency results from different types of homozygous or more frequently heterozygous recessive mutations in the \textit{AChR} (CHRNA1), \textit{b} (CHRNB1), \textit{d} (CHRND), and \textit{e} (CHRNE) subunit genes. The mutations are mainly found in the \textit{e} (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 |
| CHRNA4 | 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.T177V) | Heterozygote | Tubular aggregate myopathy, AD |
| CHRND | chr2: 152447903 | c.1493G>A (p.Q496X) | 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 |
| DNM2 | chr22: 51135985-51135991 | c.1303_c.1309delCTGGTT (p.L435Pfs*38) | Homozygote | Centronuclear myopathy, type I, AD; Lethal congenital contracture syndrome, type V, AR |
| CHRNA1 | chr19: 10893735 | c.788C>T (p.P263L) | Heterozygote | Emery–Dreifuss myodystrophy, type IV, AD |
| SYN1 | chr6: 152652184 | c.2312C>T (p.P771L) | Heterozygote | |

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Figure 2. Electropherograms of the \textit{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 \textit{CHRNE} sequence showing the mutation of c.295C>T (p.R99X) in exon 4 in the patient and the father.
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.broad institute.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.

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.\(^\text{[19,20]}\) 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.\(^\text{[21]}\) 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.\(^\text{[22,23]}\)

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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