PERGAMON

Neuromuscular Disorders 17 (2007) 409–414

www.elsevier.com/locate/nmd

A synonymous *CHRNE* mutation responsible for an aberrant splicing leading to congenital myasthenic syndrome

Pascale Richard [a,b,*,1], Karen Gaudon [a,b,1], Emmanuel Fournier [c,1], Christopher Jackson [b], Stéphanie Bauché [b], Hafedh Haddad [b,d,1], Jeanine Koenig [b,f], Bernard Echenne [e], Daniel Hantaï [b], Bruno Eymard [b,d,1]

[a] *AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Unité Fonctionnelle de Cardiogénétique et Myogénétique, Service de Biochimie B, Paris, France*
[b] *Inserm, U582, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière and Université Pierre et Marie Curie Paris 6, Paris, France*
[c] *AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Fédération de Neurophysiologie Clinique, Paris, France*
[d] *AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Centre de Référence de Pathologie Neuromusculaire, Institut de Myologie, Paris, France*
[e] *Service de Neuropédiatrie, Hôpital Saint Eloi, CHU Montpellier, France*
[f] *Université Bordeaux II, Bordeaux, France*

Received 24 October 2006; received in revised form 29 December 2006; accepted 22 January 2007

Abstract

Congenital myasthenic syndromes (CMSs) are rare hereditary disorders transmitted in a recessive or dominant pattern, and are caused by mutations in the genes encoding proteins of the neuromuscular junction. They are classified in three groups depending on the origin of the molecular defect. Postsynaptic defects are the most frequent and have been reported to be partly due to abnormalities of the acetylcholine receptor, and particularly to mutations in *CHRNE*, the gene encoding the acetylcholine receptor ε-subunit. In a Portuguese patient with a mild form of recessive CMS, *CHRNE* sequencing identified an unknown homozygous transition. This variation affects the third nucleotide of the glycine 285 condon, and leads to a synonymous variant. Analysis of transcripts demonstrated that this single change creates a new splice donor site located 4 nucleotides upstream of the normal site, leading to a deletion and generating a frameshift in exon 9 followed by a premature termination codon. This paper relates the identification of a synonymous mutation in *CHRNE* that creates a new splice donor site leading to an aberrant splicing of pre-mRNAs and so to their instability. This is the first synonymous mutation in *CHRNE* known to generate a cryptic splice site, and mRNA quantification strongly suggests that it is the disease-causing mutation.

Keywords: Congenital myasthenic syndrome; Genetics; Splicing; Acetylcholine receptor; *CHRNE*

1. Introduction

Congenital myasthenic syndromes (CMSs) are rare hereditary diseases characterized by dysfunction of neuromuscular transmission resulting in fatigable muscle weakness. They form a heterogeneous group of disorders with a recessive or dominant mode of inheritance, and are classified as due to presynaptic, synaptic, or postsynaptic defects. Several genes and mutations responsible for CMS have been identified, leading to an important genetic heterogeneity of defects [1,2]. Most of the diagnosed CMSs are postsynaptic defects and are due to mutations in different genes of the postsynaptic region, particularly the genes encoding the nicotinic acetylcholine receptor (AChR) subunits

* Corresponding author. Address: UF Cardiogénétique et Myogénétique, 6 rue de la Peyronie, Groupe Hospitalier Pitié-Salpêtrière, 47 Boulevard de l'Hôpital, 75651 Paris Cedex 13, France. Tel.: +33 1 42 17 76 56; fax: +33 1 42 17 76 18.
E-mail address: pascale.richard@psl.aphp.fr (P. Richard).
[1] Members of Assistance Publique-Hôpitaux de Paris.

0960-8966/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.nmd.2007.01.018

(OMIM#100725) [3,4]. These mutations alter AChR channel kinetics but most often cause endplate (EP-) AChR deficiency [5,6], especially mutations in *CHRNE*, the gene encoding the ε-subunit of the AChR [7,8].

CHRNE is the gene most frequently involved in postsynaptic CMSs. More than 75 different mutations have been reported by Ohno and Engel [9], and are listed on the web site www.HGMD.org. These mutations can disrupt the reading frame, as with small insertions, deletions, and splice site mutations, and are unambiguously classified as pathogenic [10,11]. However, some missense mutations were also described in the trans-membrane domains of the protein and the functional relevance of these base pair substitutions is often difficult to predict. Despite the numerous disease-causing genes and mutations described currently, a large number of CMSs as yet have no identified genetic defect. This suggests that these mutations could be located in unanalyzed regions (5′ UTR, intronic sequences, or 3′ UTR) of the known genes, or, more likely, that other disease-causing genes remain to be found.

In several diseases, exonic or intronic mutations in pathogenic genes have been described to generate cryptic consensus splicing sites when the nucleotide environment is favorable [12–14]. We report hereafter the identification of a new mutation in *CHRNE* in a young patient with recessive CMS, and we demonstrate by analysis of *CHRNE* mRNAs that this variant c.855 C>T is functionally relevant and is therefore pathogenic.

2. Patient and methods

2.1. Patient

The patient and her parents were clinically examined by one of us (BE). The myasthenic score was recorded [15], and the neostigmine test was performed. Anti-AChR and anti-MuSK antibody levels were tested. Informed written consent was obtained in accordance with a study protocol approved by the Ethics Committee of Pitié-Salpêtrière Hospital (CCPPRB #93-02).

2.2. Electromyography

Neuromuscular transmission was tested by 3 Hz repetitive nerve stimulation studies, using standard electrodiagnostic methods [16,17], with skin recording electrodes positioned to maximize CMAP amplitude. Supramaximal stimulation of the appropriate nerve (0.3 ms duration and 20–30% greater intensity than that needed for maximum CMAP amplitude) was obtained using a bipolar bar electrode. A reproducible decrement greater than 7% in both CMAP amplitude and area was considered significant.

2.3. Morphological analyses

A muscle biopsy was taken from the deltoid muscle by open biopsy. The neuromuscular junction (NMJ) zone was determined by the small twitch provoked by the tip of the scalpel on the surface of the muscle fascicles. The presence of NMJs was confirmed on a longitudinal strip of the biopsy by revealing cholinesterase activity using the classical method of Koelle and Friedenwald [18]. Whole mounts of specimens fixed with 4% paraformaldehyde in PBS were stained for AChR with TRITC-labeled α-bungarotoxin (α-BGT; Molecular Probes, Leiden, The Netherlands), for acetylcholinesterase with FITC-labeled fasciculin (a gift from Dr. Eric Krejci), and for neurofilament with an anti-200 kDa neurofilament antibody (Chemicon International, Temecula, CA), followed by the appropriate FITC-labeled secondary antibody. Whole-mount specimens were then observed by confocal microscopy (LEICA TCS40, Heidelberg, Germany).

2.4. Molecular analyses

Mutation screening in *CHRNE* was performed on extracted leukocyte genomic DNA. All 12 exons and flanking intronic sequences were amplified by polymerase chain reaction (PCR), using a procedure designed for each exon. The resultant DNA fragments were purified and directly sequenced using the BigDyeTerminator™ sequencing protocol in an automated 3100 ABI Prism® Genetic Analyzer (Applied Biosystems, Foster City, CA). Data were extracted and analyzed using Sequencing Analysis software (Applied Biosystems, Foster City, CA). The primers for amplification and sequencing of exon 8 were 5′-AGCAGTGGCCCCG AACCTAC-3′ (forward) and 5′-CACCCTTCACACT GGCCACA-3′ (reverse).

Total RNAs from the index patient were extracted from immortalized lymphocytes with the RNA-PLUS™ (Q-Biogene) kit. Reverse transcription was performed on total RNAs with the Superscript™ III First-Strand Synthesis System for RT-PCR (Invitrogen) according to the manufacturer's instructions. Then, cDNA was amplified by PCR with a forward primer located in exon 7 (5′-TAACATCATCGTGCCCTGTG-3′) and a reverse primer located in exon 10 (5′-CAATAAGCCCA CCGACGAC-3′) of the cDNA sequence (RefSeq DNA: NM_000080). The resulting PCR product of 400 base pairs was sequenced on both strands.

3. Results

3.1. Patient history and clinical examination

The patient was a 14-year-old Portuguese girl born to healthy, 2nd degree consanguineous parents. She had a