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Congenital myasthenic syndrome in Japan: Ethnically unique mutations in muscle nicotinic acetylcholine receptor subunits

Yoshiteru Azuma [a,b], Tomohiko Nakata [a,b], Motoki Tanaka [c], Xin-Ming Shen [d], Mikako Ito [a], Satoshi Iwata [a], Tatsuya Okuno [a], Yoshiko Nomura [e], Naoki Ando [f], Keiko Ishigaki [g], Bisei Ohkawara [a], Akio Masuda [a], Jun Natsume [b], Seiji Kojima [b], Masahiro Sokabe [c], Kinji Ohno [a,*]

[a] *Division of Neurogenetics, Center for Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[b] *Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[c] *Department of Physiology, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[d] *Department of Neurology, Mayo Clinic, Rochester, MN, USA*
[e] *Segawa Neurological Clinic for Children, Tokyo, Japan*
[f] *Department of Pediatrics, Nagoya City University Graduate School of Medicine, Nagoya, Japan*
[g] *Department of Pediatrics, Tokyo Women's Medical University, Tokyo, Japan*

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Abstract

Congenital myasthenic syndromes (CMS) are caused by mutations in genes expressed at the neuromuscular junction. Most CMS patients have been reported in Western and Middle Eastern countries, and only four patients with *COLQ* mutations have been reported in Japan. We here report six mutations in acetylcholine receptor (AChR) subunit genes in five Japanese patients. Five mutations are novel, and one mutation is shared with a European American patient but with a different haplotype. Among the observed mutations, p.Thr284Pro (p.Thr264Pro according to the legacy annotation) in the epsilon subunit causes a slow-channel CMS. Five other mutations in the delta and epsilon subunits are splice site, frameshift, null, or missense mutations causing endplate AChR deficiency. We also found a heteroallelic p.Met465Thr in the beta subunit in another patient. p.Met465Thr, however, was likely to be polymorphism, because single channel recordings showed mild shortening of channel openings without affecting cell surface expression of AChR, and the minor allelic frequency of p.Met465Thr was 5.1% in the Japanese population. Lack of shared mutant alleles between the Japanese and the other patients suggests that most mutations described here are ethnically unique or *de novo* in each family.

Keywords: Congenital myasthenic syndromes; Acetylcholine receptor; Slow channel syndrome; Fast channel syndrome; Endplate acetylcholine receptor deficiency

1. Introduction

Acetylcholine released from the nerve terminal binds to muscle nicotinic acetylcholine receptor (AChR) at the motor endplate. AChR is clustered at the neuromuscular junction (NMJ) by binding to rapsyn with a stoichiometry of rapsyn to AChR of 1:1 to 2:1 [1]. AChR clustering is mediated by neural agrin that is released from the nerve terminal [2]. In early embryonic development, AChR clustering is also mediated by Wnt ligands [3,4]. Embryonic AChR is composed of α, β, δ, and γ subunits with a stoichiometry of $\alpha_2\beta\delta\gamma$. After birth, the ε subunit is substituted for the γ subunit, generating $\alpha_2\beta\delta\varepsilon$-AChR.

Congenital myasthenic syndromes (CMS) are heterogeneous disorders caused by mutations in genes expressed at the NMJ [5]. They are characterized by fatigable muscle weakness, variable muscle atrophy, and sometimes dysmorphic features. CMS mutations have been reported in 19 genes, with most mutations in *CHRNA1*, *CHRNB1*, *CHNRD*, and *CHNRE* encoding the AChR α, β, δ, and ε subunits, respectively. These mutations fall into three subsets: i) slow-channel CMS (SCCMS), in which the open time of AChR is abnormally prolonged; ii) fast-channel CMS (FCCMS), in which the open time of AChR is abnormally brief; and iii) endplate AChR deficiency. SCCMS is caused by a gain-of-function mutation and is dominantly inherited with variable penetrance [6]. In contrast, FCCMS and endplate AChR deficiency are caused by loss-of-function mutations on both alleles, and are recessively

* Corresponding author. Division of Neurogenetics, Center for Neurological Disease and Cancer, Nagoya University Graduate School of Medicine, 65 Tsurumai, Showa-ku, Nagoya 466-8550, Japan. Tel.: +81 52 744 2446; fax: +81 52 744 2449.
E-mail address: ohnok@med.nagoya-u.ac.jp (K. Ohno).

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inherited. Low-expressor mutations of the AChR ε subunit are partly compensated for by expression of the embryonic AChR γ subunit, whereas the other AChR subunits have no substituting subunits. Accordingly, null and frameshift mutations are frequently detected in *CHRNE*, but not in the other subunit genes.

More than 500 patients with CMS have been reported in Western and Middle Eastern countries, whereas only four Japanese CMS patients carrying five mutations in *COLQ* encoding collagen Q that anchors acetylcholinesterase (AChE) at the NMJ have been reported by us [7,8]. Among the more than 450 CMS mutations in 19 disease genes registered in the Human Gene Mutation Database (http://www.hgmd.org), two likely have founder effects: p.Asn88Lys in *RAPSN* [9–11] and c.1124_1127dupTGCC in *DOK7* [12], whereas the others are private mutations occurring in a single or a small number of unrelated families. We here report five Japanese CMS patients with six mutations in the AChR subunit genes. We show that all the ten mutations in *COLQ*, *CHRND*, and *CHRNE* in Japanese patients are ethnically unique, which indicates that most CMS mutations arose *de novo* in recent human history or in each family.

2. Materials and methods

2.1. Ethical approval

All the human studies were approved by the institutional review boards of Nagoya University Graduate School of Medicine, Mayo Clinic, Segawa Neurological Clinic for Children, Nagoya City University, and Tokyo Women's Medical University. Appropriate written informed consent was obtained from all the patients and family members.

2.2. Mutation analysis and splicing analysis

Genomic DNA was isolated from peripheral blood with QIAamp Blood Kit (QIAGEN). We directly sequenced all exons with their flanking noncoding regions of *CHRNE*, *CHRNA1*, *CHRNB1*, and *CHRND* in this order with CEQ 8000 (Beckman Coulter). To look for large-scale DNA rearrangements in patient (Pt.) 4, we performed mate-pair sequencing of the whole genome using SOLiD4 (Life Technologies). The mate-pair library was made to span ~2 kb genomic segments according to the manufacturer's protocols. A total of 14.9 Gb of reads were mapped to human genome GRCh37/hg19 with the mapping efficiency of 89% using CLC Genomics Workbench (CLC Bio). All the reads mapped to *CHRNE* were visually scrutinized using Integrative Genome Browser (Broad Institute). Total RNA was isolated from biopsied muscle that was obtained for histopathological diagnostic purposes using RNeasy mini kit (QIAGEN). cDNA was synthesized with ReverTra Ace (Toyobo) and Oligo(dT) Primer (Life Technologies).

2.3. Expression of AChR subunit genes in HEK293 cells

Human α, β, δ, and ε subunit cDNAs were cloned into the CMV-based vector pRBG4 for expression in HEK293 cells [13]. The identified mutations were engineered into wild-type AChR subunit cDNAs in pRBG4 using the QuikChange site-directed mutagenesis kit (Stratagene). Presence of each mutation and absence of unwanted artifacts were confirmed by sequencing the entire inserts. HEK293 cells were transfected with pRBG4-α, -β, -δ, -ε, and pcDNA3.1-EGFP at a ratio of 2:1:1:1:1 using FuGENE 6 transfection reagent (Promega). After 48 hrs, cells were incubated with α-bungarotoxin Alexa Flour 647 (Life Technologies) (1:200) in PBS for 1 hr. Signals were observed under an Olympus BX60 fluorescence microscope. The cells were trypsinized, washed with PBS, and resuspended in PBS. The total number of α-bungarotoxin-binding sites on the cell surface and EGFP was determined by the FACSCalibur system (BD Biosciences).

2.4. Single channel recordings

HEK293 cells were transfected with pRBG4-α, -β, -δ, and -ε, and pEGFP-N1 at a ratio of 2:1:1:1:1, using FuGENE 6. Recordings were obtained at 24 hrs after transfection in the cell-attached configuration at a membrane potential of –80 mV at 22 °C and with bath and pipette solutions containing (in mM): KCl, 142; NaCl, 5.4; CaCl2, 1.8; MgCl2, 1.7; HEPES, 10, pH 7.4. Single-channel currents were recorded using an Axopatch 200B amplifier (Axon Instruments) at a bandwidth of 50 kHz, digitized at 5-μs intervals using Digidata 1322A (Axon Instruments) and recorded to a hard disk using the program Clampex 8.2 (Axon Instruments). Recordings obtained with ACh at 1 μM or less were analyzed at a uniform bandwidth of 10–11.7 kHz with dead time of 15.3–17.9 μs imposed. Recordings obtained with ACh at 10 μM or more were analyzed with dead time at 25 μs at 10 kHz with TAC software (Ver. x4.0.9, Bruxton). Dwell-time histograms were plotted on a logarithmic abscissa and fitted by the sum of exponentials by maximum likelihood, as previously reported [14].

3. Results

3.1. Clinical features

All Pts. had an abnormal decremental response to repetitive nerve stimulation, and no anti-AChR and anti-MuSK antibodies. Clinical features and repetitive nerve stimulation results are summarized in Table 1.

Pt. 1 (13 y.o., male) had eyelid ptosis since age six months and a positive edrophonium test. Clinical features were previously reported in a local journal [15]. Steroid pulse therapy at ages four and five years and thymectomy at age six years had no effect. Combined use of distigmine 3 mg/day and pyridostigmine 180 mg/day enabled him to sit in a chair without assistance at age 13 years. Biopsy of deltoid muscle at age eleven years showed marked AChR deficiency by fluorescent staining with α-bungarotoxin and simplified endplates by electron microscopy.

Pt. 2 (26 y.o., female) had nasal obstruction since birth and eyelid ptosis since age one month. She had a positive edrophonium test and was thought to have myasthenia gravis. Cholinesterase inhibitors were mildly effective. She has ophthamoparesis, and is able to walk but is unable to run.