Press Release

Title:
Anti-MuSK autoantibodies in myasthenia gravis block binding of acetylcholinesterase (AChE)-tethered collagen Q (ColQ) to MuSK

Highlights:
- About 5-15% of patients with autoimmune myasthenia gravis (MG) have anti-MuSK autoantibody. The identity of a molecule that is blocked to bind to MuSK by the autoantibody remained unknown.
- MuSK embedded in the postsynaptic membrane binds to LRP4, and transmits a signal for acetylcholine receptor (AChR) clustering at the neuromuscular junction. LRP4 is a receptor for agrin, which is released from the nerve terminal to facilitate clustering of AChR at the motor endplate. MuSK also binds to the C-terminal domain of ColQ and tethers ColQ-tailed AChE to the synaptic basal lamia.
- The research group discovered that anti-MuSK autoantibody blocks binding of ColQ to MuSK.
- Passive transfer of anti-MuSK autoantibody to mice reduced anchoring of ColQ-tailed AChE at the neuromuscular junction, and also caused mild reduction of AChR clusters.
- Cholinesterase inhibitors are generally ineffective or even worsen myasthenic symptoms of MuSK MG patients. The present study potentially paves the way for a new therapeutic approach for MuSK MG patients.

Summary:
Yu Kawakami, BS (Medical student, 5th year) and Kinji Ohno, MD, PhD (Professor) at Neurogenetics, Nagoya University Graduate School of Medicine (Dean: Gen Sobue, MD, PhD) discovered that anti-MuSK autoantibody in MG blocks binding of ColQ to MuSK. The research results have been published in *Neurology*, the journal of the American Academy of Neurology, on Nov. 15, 2011 with an editorial by Amelia Evoli, MD at the Catholic University, Rome, Italy and Jon Lindstrom, MD, PhD. at the University of Pennsylvania, Philadelphia, Pennsylvania, USA.

1. Research Background

Myasthenia gravis (MG) is an autoimmune disease caused by autoantibody against molecules expressed at the neuromuscular junction. MG is characterized by muscle weakness, abnormal muscle fatigue, ptosis (drooping eyelids), dysphagia (difficulty in swallowing), and respiratory distress (breathing difficulty). About 80% of MG patients carry autoantibody against AChR, whereas about 5-15% carry autoantibody against MuSK, the muscle-specific receptor tyrosine kinase. In 2011, the third autoantibody against LRP4 has also been reported.

Anti-AChR autoantibodies are comprised of IgG1 and IgG3 that are capable of activating complements, which degrades AChR and the ultrastructure of the neuromuscular junction. Anti-AChR autoantibodies have been extensively studied since 1970's and specific treatments are available.

On the other hand, anti-MuSK autoantibody belongs to IgG4 subclass, which does not activate complements and is classified a blocking antibody. MuSK IgG4 antibody is therefore likely to block binding of MuSK and another molecule. The identity of the partner molecule, however, remained unknown. As in other autoimmune diseases including AChR-MG, MuSK-MG patients respond to immunosuppressive therapies, but not to cholinesterase inhibitors. Cholinesterase inhibitors block acetylcholinesterase (AChE) activity and slow down degradation of acetylcholine, which increases binding of acetylcholine to AChR. Cholinesterase inhibitors are mostly effective for AChR MG. Elucidation of detailed molecular mechanisms underlying MuSK-MG is expected to lead to development of rational therapies.

2. Research Results

Based on the observations that AChR deficiency is mild and cholinesterase inhibitors are generally ineffective in MuSK-MG patients, the research group hypothesized and proved that

anti-MuSK antibody blocks binding of ColQ to MuSK. Under the approvals of the Ethical Review Boards at the attending institutes and after written informed consents are given by the patients and control subjects, the group purified IgG antibodies from blood of four MuSK-MG patients and two control subjects and performed the following experiments.

First, purified human recombinant AChE/ColQ complex was overlaid on muscle sections of *Colq*-knockout mice that lack a gene for ColQ. The research group confirmed that human ColQ efficiently binds to the mouse neuromuscular junction. The group next proved that control IgG's do not block anchoring of ColQ to the mouse neuromuscular junction, while patient's IgG's strongly inhibit this anchoring.

Second, purified human recombinant AChE/ColQ complex was bound to purified recombinant MuSK protein on a plastic plate. Control IgG's did not block binding of ColQ and MuSK, while patient's IgG's blocked it in a dose-responsive manner.

Third, normal mice were injected with control IgG or patient's IgG in the abdominal space for two weeks to make a passive-transfer model of MuSK-MG. Control IgG had no effect on the neuromuscular junction. Patient's IgG strongly reduced anchoring of AChE/ColQ complex at the neuromuscular junction. Expression of AChR at the neuromuscular junction was also moderately affected.

3. Research Summary and Future Perspectives

The research group disclosed that anti-MuSK autoantibody blocks binding of ColQ to MuSK. This causes reduction of AChE/ColQ at the neuromuscular junction. Their observations underscore clinical observations that MuSK-MG patients do not respond well to cholinesterase inhibitors. In biopsied intercostal muscles of MuSK-MG patients, however, AChR deficiency and AChE deficiency are mild in all the three examined patients. Although the discrepancy between our observations and findings in the patients' muscles needs to be elucidated in the future, it is expected that the currently disclosed mechanisms pave the way for a new therapeutic approach for MuSK-MG patients.

Investigators

Yu Kawakami, BS
Medical Student, 5th year
Neurogenetics, Nagoya University Graduate School of Medicine

Kinji Ohno, MD, PhD
Professor of Neurogenetics, Nagoya University Graduate School of Medicine

Correspondence to

Kinji Ohno, MD, PhD
Professor of Neurogenetics, Nagoya University Graduate School of Medicine
e-mail: ohnok@med.nagoya-u.ac.jp
Phone: +81-52-744-2446

Office of Public Affairs

Soumukikaku, Nagoya University Graduate School of Medicine
Phone: +81-52-744-2228
Fax: +81-52-744-2785
e-mail: iga-souk@post.jimu.nagoya-u.ac.jp

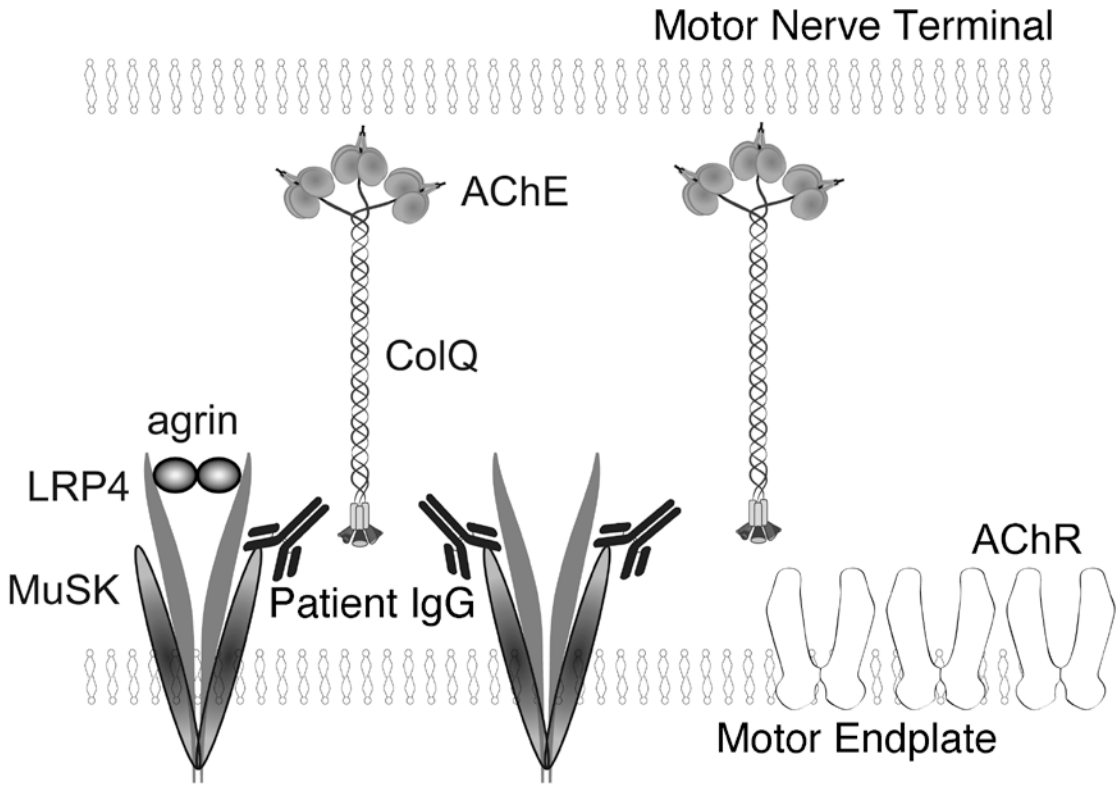

Figure
Anti-MuSK autoantibody in patients with myasthenia gravis blocks binding of MuSK and AChE/ColQ complex. Two units of MuSK (MuSK dimer) make a transmembrane complex with two units of LRP4 (LRP4 dimer). Agrin released from the motor nerve terminal binds to LRP4 and activates MuSK, which then facilitates clustering of AChR. AChE degrade acetylcholine released from the nerve terminal. Twelve molecules of AChE bind to triple helical ColQ and make a large AChE/ColQ complex. ColQ is anchored to synaptic basal lamina by binding to MuSK.

Glossary
AChE, acetylcholinesterase; AChR, acetylcholine receptor; agrin; ColQ, Collagen Q; LRP4, low-density lipoprotein receptor-related protein 4; MuSK, muscle specific receptor tyrosine kinase; IgG, immunoglobulin G