Apolipoprotein E3 Modulates Motor Endplate Formation and Muscle Force

Dominic Barr[1], Ryan Roberts[1], James T. Redden[1], D. Joshua Cohen[1], Zvi Schwartz[1], Michael J. McClure[1]
[1]Virginia Commonwealth University, Richmond, VA

Disclosures: Zvi Schwartz (6; MTF Biologics), Michael J. McClure (6; MTF Biologics)

INTRODUCTION: Although motor endplates represent only 0.1% of the myofiber surface area, they are densely packed with acetylcholine receptors (AChRs) that mediate muscle contraction. Each year in the U.S., approximately 250,000 cases of volumetric muscle loss (VML) occur due to gunshots, open fractures, lacerations, and soft tissue sarcomas. These injuries damage both muscle and nerves, resulting in profound weakness, reduced force generation, and functional disability. Importantly, intramuscular nerves are severed, leaving adjacent intact muscle denervated. Denervation promotes motor endplate fragmentation and paralysis of affected fibers. Our long-term goal is to regenerate functional muscle fibers and promote motor endplate formation in denervated muscle to drive reinnervation and recovery. In prior VML studies, we observed increased levels of apolipoprotein E (ApoE), suggesting a potential role in injury response. To explore whether ApoE was linked to muscle–nerve interactions, we developed a VML model, treated the injury with a regenerative biologic material (decellularized muscle matrix, DMM), and neurotized DMM. Neurotization, a surgical procedure in which donor motor axons are introduced into denervated muscle, promotes reinnervation. Using this model, we found that ApoE expression depended on the presence of motor axons. An in vitro muscle–nerve co-culture system confirmed that ApoE levels increased in response to motor nerves. These findings led us to test the hypothesis that ApoE regulates motor endplate formation and muscle function.

METHODS: In vitro, C2C12 myoblasts were seeded on Corning TCPS 24-well plates at 10,000 cells/cm² in α-MEM supplemented with 10% FBS and 1% P/S, and cultured at 37°C, 5% CO_2. At 100% confluence, cells were treated with recombinant ApoE3 or ApoE4 at 10^{-7}, 10^{-8}, or 10^{-9} M (n=6/group). RNA was isolated for analysis of myogenic and synaptic gene expression. To assess receptor involvement, cells were treated with ApoE3 (0 or 10^{-7} M) in the presence of an anti-Lrp4 antibody (0, 0.5, 0.1, or 0.01 µg/well). AChR clusters were quantified using fluorescent α-bungarotoxin staining. Gene expression was measured by RT-qPCR for myogenic markers (Myod, Myog, Myh1) and synaptic markers (Chrng, Chrne, Lrp4, MuSK, Rapsn, and ApoE). Next we tested muscle function using in vivo studies. Male ApoE3 knock-in (KI) mice were compared to male C57BL/6 controls (n=6/group) to assess differences in muscle function (force production; Aurora Scientific) and bone mineral density (µCT).

RESULTS: Treatment of myoblasts with ApoE3 or ApoE4 did not alter Myod, Myog, or Myh1 expression. However, ApoE3 significantly increased Chrng expression in a dose-dependent manner (Fig 1A), whereas ApoE4 had no effect (Fig. 1B). No changes were observed in Chrne, Lrp4, MuSK, or Rapsn. Increased Chrng expression correlated with α-bungarotoxin staining, which revealed a dose-dependent increase in dispersed AChR clusters with ApoE3 treatment (Fig. 1C). Inhibition of Lrp4 reduced ApoE3-induced clustering in a dose-dependent manner, indicating receptor involvement. In vivo, ApoE3 KI mice generated greater muscle force than wild-type controls (Fig 1D,E), while bone volume and tissue volume ratios were unchanged (Fig 1F,G).

DISCUSSION: Muscle and nerve injuries disrupt the specialized signaling between motor axons and myofibers, leading to progressive motor endplate degeneration and impaired function. Our lab previously identified ApoE as a candidate regulator in preclinical injury models. ApoE, a cholesterol transporter also localized to the neuromuscular junction, has an underexplored role in synaptic maintenance. In this study, ApoE3 (but not ApoE4) enhanced AChR expression and clustering, highlighting its potential as a therapeutic factor in neuromuscular repair. Mechanistically, ApoE3-induced clustering was partially dependent on Lrp4, suggesting interplay between ApoE and agrin–MuSK–Lrp4 signaling. Transgenic ApoE3 KI mice further demonstrated improved muscle force, supporting a functional benefit of ApoE3 at the neuromuscular junction. Notably, ApoE3 had no effect on bone mineral density, indicating its action was muscle-specific. Future work should determine whether ApoE3 alleviates motor endplate fragmentation following denervation, and whether it can be leveraged to improve outcomes in VML and other neuromuscular injuries.

SIGNIFICANCE/CLINICAL RELEVANCE: Optimizing recovery from denervation injuries requires better understanding of the molecular pathways governing motor endplate formation and preservation. Our data indicate that ApoE3 enhances AChR clustering and muscle force, positioning it as a potential therapeutic candidate to preserve neuromuscular connectivity. The correlation between increased ApoE expression, AChR density, and functional improvement in ApoE3 KI mice underscores the importance of further exploring ApoE3's role in neuromuscular regeneration.

IMAGES AND TABLES:

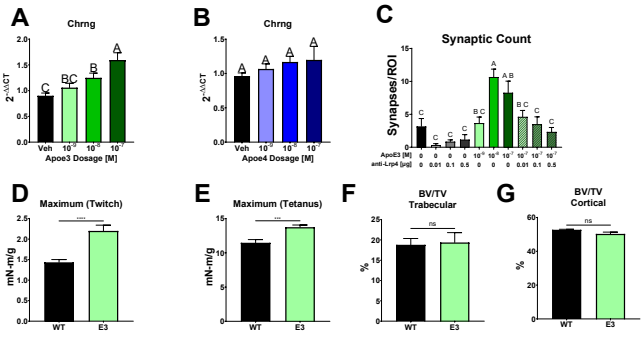

Fig. 1. ApoE3 augments AChR clustering and muscle force. *In vitro* C2C12 myoblast experiments showing that Chrng mRNA dose dependently increased with exogenous Apoe3 delivery (A), but the effect is lost dosing with exogenous Apoe4 (B). AChR clustering was measured using a-bungarotoxin staining and showed an increase in AChR clusters with increasing ApoE3 dose. These increases were lost when Lrp4 was inhibited (C). Muscle twitch force (D) and tetanic force (E) were higher in ApoE3 KI animals compared to WT. uCT showed no differences between WT and E3 trabecular BV/TV ratios (F) and no differences in cortical BV/TV (G).