

## Exploration of Therapeutic Agents for Sarcopenia Targeting Functional Improvement of Neuromuscular Junction (NMJ)

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Disclosures: nothing

**INTRODUCTION:** The development of interventions for age-related sarcopenia, characterized by a decline in muscle mass and strength, is an important challenge. Recently, one of the causes of sarcopenia has been suggested to be a dysfunction of the neuromuscular junction (NMJ), which connects motor neurons terminals with skeletal muscles. We identified and analyzed compounds that enhance NMJ function.

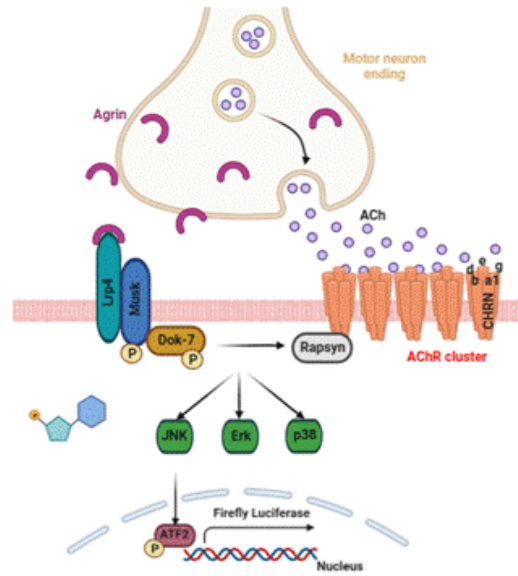
**METHODS:** From approximately 70,000 compounds held by the Institute of Transformative Bio-Molecules (ITbM) at Nagoya University, we found candidate compounds that showed effects on NMJ. Specifically, we used the following methods: (1) To enhance ATF2 activity, each compound was introduced into human embryonic kidney cells (HEK-293) through gene transfection with ATF-Luc, MuSK, LRP4, and neural agrin cDNA to enhance acetylcholine receptor (AChR) clustering. (2) To enhance ATF2 activity, ATF-Luc and each compound were introduced into mouse skeletal muscle cell line C2C12 through gene transfection to enhance AChR clustering. We confirmed the enhancement of AChR clustering in C2C12 cells when agrin and each compound were added to the culture. Furthermore, from the candidate compounds that showed particularly high activity and enhancement, (3) examined the phosphorylation of NMJ-related proteins after adding the candidate compounds using Western blotting, (4) examined the expression levels of NMJ-related genes after adding the candidate compounds using RT-PCR, and (5) investigated the toxicity of the compounds using NSC34 cells as an indicator of neurite elongation. Statistical analysis was performed using one-way analysis of variance and the Jonckheere-Terpstra trend test, considering  $p < 0.05$  as statistically significant.

**RESULTS SECTION:** Candidate compounds that promote ATF2 activity and AChR clustering were found to have similar chemical structures, and certain candidate compounds showed particularly high activity and enhancement. (1) ATF2 activity increased in a concentration-dependent manner in HEK293 cells. Furthermore, (2) in differentiated C2C12 cells, several candidate compounds ( $p < 0.05$ ) significantly increased the aggregation area of AChR by 1.3-fold. Under these conditions, (3) phosphorylation of DOK7 was observed in NMJ-related proteins. Additionally, (4) the expression levels of NMJ-related genes that form AChR subunits (Chrna1a, Chrnd, Chrne, and Chrng) increased. (5) The candidate compounds showed similar structures but exhibited varying levels of toxicity.

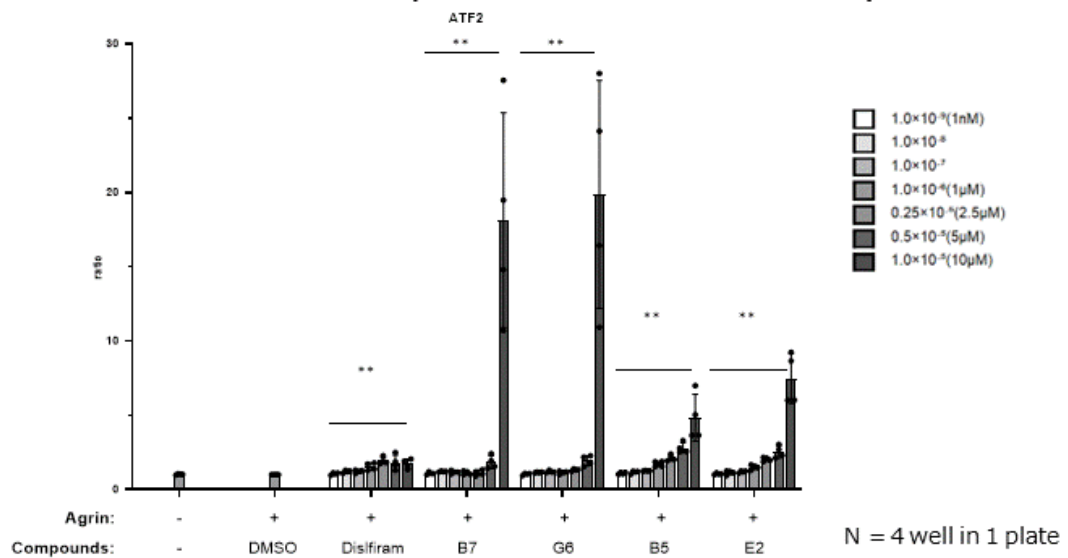
**DISCUSSION:** The candidate compounds are believed to promote NMJ formation in skeletal muscle cells through interactions with DOK7 and other NMJ-related proteins.

**SIGNIFICANCE/CLINICAL RELEVANCE:** The candidate compounds are expected to improve NMJ function and have the potential to become candidate drugs for sarcopenia treatment in the future.

**IMAGES AND TABLES:**

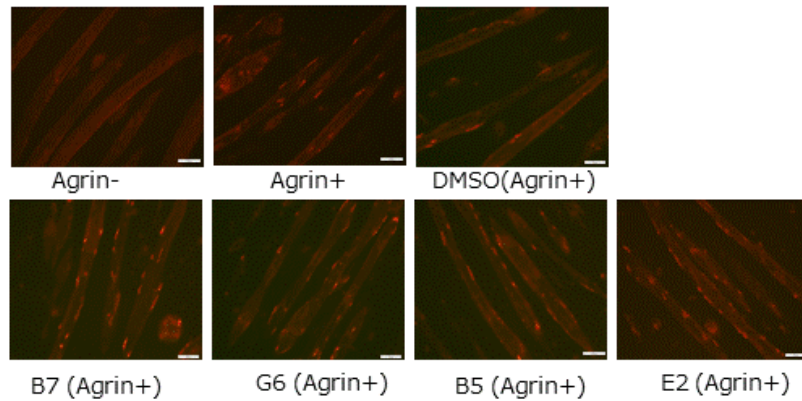


## Candidate compounds with 1nM-10 μM

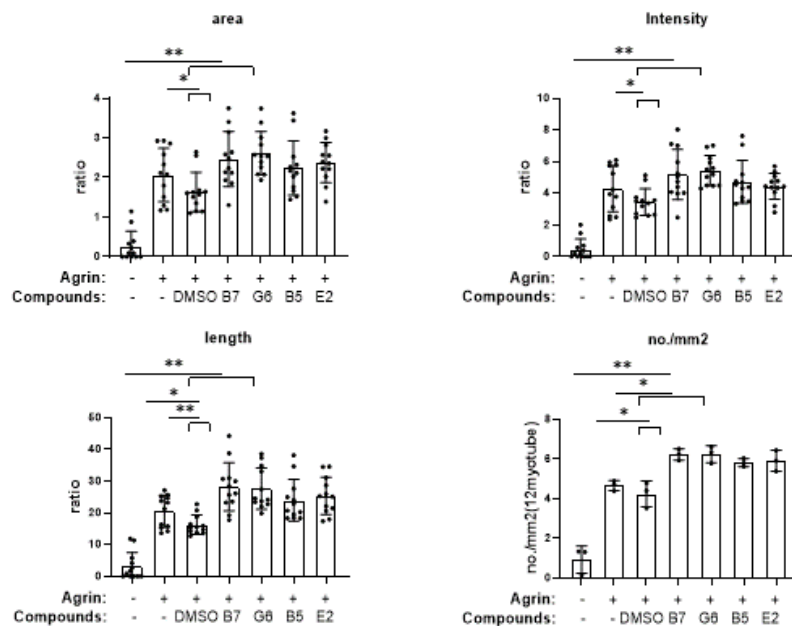


\*\*  $p < 0.001$  by Jonckheere-Terpstra trend test

## Protocol of culturing and differentiating C2C12 Cells

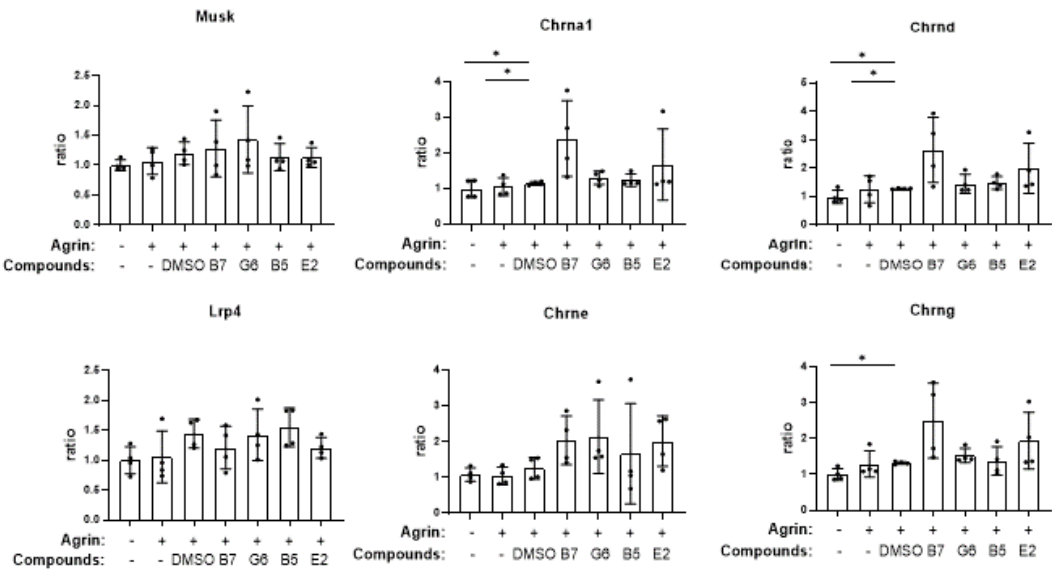


## AchR clustering (C2C12) (quantitative analysis)



\*p < 0.05, \*\* p < 0.001 by one-way ANOVA followed by Tukey HSD.

RT-PCR(C2C12 single)



N=4

\*p < 0.05 by one-way ANOVA followed by Tukey HSD.

Western blotting (C2C12 single)

