学 位 論 文 の 要 旨

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学 位 論 文 名　A Genome-wide Association Study Identifies a Locus Associated With Knee Extension Strength in Older Japanese Individuals

発 表 雑 誌 名（巻，初頁～終頁，年）　Communications Biology (7(1), 513, 2024)

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論 文 内 容 の 要 旨

INTRODUCTION

Sarcopenia is a common skeletal muscle disease in older people, which leads to unfavorable outcomes such as falls, fractures and death. The loss of muscle mass has mainly been used as an indicator of sarcopenia; however, recent studies suggest that muscle strength is a better indicator that reflects adverse health outcomes of sarcopenia. As an indicator for muscle strength, handgrip strength is commonly used; however, it only reflects the strength of the upper extremities, not that of lower extremities. Recent studies reported that knee extension strength, a proxy of lower limb strength, is more strongly associated with performance-based sarcopenia compared to handgrip strength. Thus, knee extension strength would be a more appropriate indicator of sarcopenia than handgrip strength. Muscle strength has been known to be heritable. Genome-wide association studies (GWASes) of handgrip strength suggested the heritability was 13–24%. Previous GWASes have discovered 170 variants associated with muscle strength. However, there is only one GWAS of lower limb muscle strength, which did not identify any significant loci due to a lack of statistical power. GWASes of lower limb muscle strength with adequate sample size would add good information for understanding of the genetic architecture

of sarcopenia.

In the present study, we performed a GWAS of knee extension strength using 3452 participants aged 60 years or older from two independent cohorts. We identified a locus with genome-wide significance which has not been identified in previous GWASes of muscle strength. In the locus, we identified a candidate susceptibility gene, *TACC2* (transforming acidic coiled-coil-containing 2) which is highly expressed in skeletal muscle.

MATERIALS AND METHODS

Three data sets of samples from two independent cohorts (Shimane CoHRE Study and Bunkyo Health Study) were enrolled in this study, which consisted of a total of 3478 participants aged 60 years or older. Shimane CoHRE study is a cross-sectional study which is a part of the cohort study conducted by the Center for Community-based Healthcare Research and Education in Shimane University. The study is ongoing health examination for the community-dwelling people in Shimane prefecture, Japan. The study protocol was approved by the Research Ethics Committee of Shimane University. In Shimane CoHRE Study, knee extension strength was measured by using the Quadriceps Training Machine (QTM) (QTM-05F, Alcare, Tokyo, Japan). The device has a knee holding part corresponding to the knee joint with approximately 30° flexion. Bunkyo Health Study prospective cohort study of over 10 years, which recruited older subjects aged 65–84 years living in Bunkyo-Ku, an urban area in Tokyo, Japan. In Bunkyo Health Study, knee extension strength was measured by using the BIODEX system 4 (Biodex Medical Systems, Upton, New York, USA), which measures isokinetic knee muscle strength. We genotyped samples with the Illumina HumanOmniExpressExome BeadChip and the Illumina Asian Screening Array. After quality controls for samples and variants, 1007, 841 and 1623 samples remained in three data sets, respectively. Then, we conducted GWAS using linear mixed model package and used the top three principal components as covariates. We performed a meta-analysis of three GWASes in a total of 3452 samples by using the fixed-effect inverse-variance weighted method. We investigated if 140 variants identified in the previous GWAS of handgrip strength had the same direction of effect in our GWAS.

RESULTS AND DISCUSSION

We identified a novel locus significantly associated with knee extension strength. The lead variant is rs10749438, an intronic variant in *TACC2* (transforming acidic coiled-coil-containing 2) located at 10q26 (Beta =−0.15, $P = 4.2\times10^{-8}$). We observed consistent associations of this variant across the three data sets and no heterogeneity of association results was observed. To the best of our knowledge, the present study is the first GWAS for lower limb muscle strength that identified a significant locus. In line with our results, a previous GWAS of muscle weakness based on handgrip strength in European elderly showed the consistent association between rs10749438 and muscle weakness with nominal statistical significance ($P = 0.037$). We also

investigated if rs10749438 is associated with other sarcopenia-related traits such as lean body mass, frailty, walking pace, fatigue, testosterone and IGF1 in the UK Biobank, which did not show any nominal significant association. rs10749438 was positioned at enhancer-like histone marks, H3K27ac in skeletal muscle according to the ENCODE database and HaploReg (v4.1). *TACC2* encodes a cytoskeleton-related protein that concentrates at centrosomes throughout the cell cycle and is reported as a target of myotonic dystrophy 1-associated splicing alterations. Skeletal muscle showed high *TACC2* expression according to Genotype-Tissue Expression project version 8 (GTEx v8). Regarding a responsible gene in this association, using cell cultures from human embryonic muscle, myotonic dystrophy 1-associated splicing alterations were significantly enriched in *TACC2* which is one of cytoskeleton-related genes. Although the variants are not an expression quantitative trait locus (eQTL) for *TACC2* according to GTEx v8, there is a possibility that the variant's functional effect is more context dependent. In fact, the sampling site of muscle in GTEx is not quadriceps femoris muscle but gastrocnemius muscle. These findings suggest that *TACC2* is a good candidate gene for muscle strength and further experimental validation using animal models will be needed.

We further tested whether the variants associated with handgrip strength showed associations in our dataset. Out of 170 variants associated with three GWASes of handgrip strength, 132 were included in our dataset, and 18 proxy variants were selected for the test based on the linkage disequilibrium of Europeans. Among the 150 variants, 87 showed the same direction of effect and a binomial test p-value was 0.03. Among the 87 variants, five showed an association of nominal statistical significance (expected number: 4.35). These findings suggest that muscle strength of upper and lower limbs may share a small part of genetic architecture.

We also identified 17 suggestive loci including candidate causal variants. Among these loci, rs1718074 in an intron of the dystrophin gene (*DMD*) located at Xp21.2-p21.1 is the most noteworthy (Beta = 0.14, P = 2.9×10−6). *DMD* is the disease gene for Duchenne muscular dystrophy and Becker muscular dystrophy, both of which show progressive deterioration of muscle tissue and resultant weakness. The effect size and the effect direction of rs1718074 were consistent between males and females. We investigated if those suggestive variants are associated with sarcopenia-related traits such as lean body mass, frailty, walking pace fatigue, testosterone and IGF1 in the UK Biobank. We did not find very consistent patterns of associations, suggesting that ancestry matching for GWAS and further expansion of sample size for muscle strength is necessary.

CONCLUSION

We identified a novel locus associated with knee extension strength. This finding provides insights into the genetic architectures underlying muscle strength in lower limbs. It would be interesting to integrate the current results with studies of sarcopenia in the future.