# IS *ACTN3* R577X GENOTYPE ASSOCIATED WITH WEIGHT-BEARING INDEX IN MALE COLLEGE STUDENTS?

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The purpose of this study was to determine the association between weight-bearing index (WBI) and *ACTN3* genotype in male college students. Subjects were healthy male college students aged 18 to 21 years old. The maximal static forces of knee extension (leg strength) of the subjects were measured, and WBI was calculated by dividing the leg strength by the subjects' body weight. Genomic DNA was extracted from each subject using hair strands, and its base sequence determined genotypes in RR, RX, and XX. Welch-type ANOVA revealed that WBI in the XX type was greater than in the RR and RX types despite no significant difference in leg strength and lean body mass among genotypes. Although it is difficult to find apparent or direct evidence to explain this result, some inferences can be proposed, such as compensatory force exertion based on its muscle fibers, regulation in calcineurin via the α-actine-2 gene, or functional enhancement of myoglobin-based endurance slow muscles in XX type may increase muscular activities against gravity, making WBI greater.

**KEYWORDS:** *ACTN3*, weight-bearing index, knee extension.

**INTRODUCTION:** It is estimated that 50% of endurance capacity and muscle power as athletic performances are due to genetic factors (Ahmetov et al., 2016; Miyamoto-Mikami et al., 2017). The α-actine-3 gene (ACTN3), expressed only in the myotubes of type II fast-twitch muscle fibers, has recently focused on sprint/power athletes. Many studies have shown that the ACTN3 genotype is not expressed when a nonsense base substitution on chromosome 11q13-q14 changes the 577th amino acid from R (arginine) to X (termination codon) (XX type). In addition, athletes with the R allele (RR and RX types) are superior in sprint/power muscular exertion (Alfred, 2011; Yang et al., 2017).

However, there have been reports that the ACTN3 genotype does not affect muscle power in the general population (Alfred, 2011) or young non-athletes (Santiago, 2010). In addition, Vincent et al. (2007) reported that ACTN3 genotype did not affect muscle torque, except for a specific angular velocity. In other words, it is still unclear whether the association between muscle power and ACTN3 genotypes depends on the target or the speed of movements. More particularly, if referring to non-athletes or non-trained individuals, the relationship between the ACTN3 genotype and physical ability based on daily life should be investigated. Still, such findings have not yet been obtained.

The weight-bearing index (WBI) proposed by Kigawa (1986) is the relative muscle strength calculated by dividing the static leg extension strength by the body weight. It has been reported that it may be used to evaluate mobility in locomotion and jumping and a criterion for recovery from injury (Kigawa, 1986). Furthermore, WBI is related to physical activity among the middle-aged and the elderly (Yamashita et al., 2007). Thus, WBI can be regarded as an index that can evaluate physical power based on daily locomotor ability and mobility for trained and untrained people. Hence, there is a need to clarify the relationship between ACTN3 genotype for the general population and muscle function related to daily living, such as WBI.

Therefore, the purpose of this study was to determine the association between WBI and ACTN3 genotype in male students, excluding elite athletes.

**METHODS:** The subjects were 64 healthy male (regular) students aged 18–21 years (age: 19.1 ± 1.0 years, height: 170.6 ± 5.8 cm, weight: 61.6 ± 9.3 kg) without any orthopedic history of the lower extremities that might interfere with the measurements. Elite or high-level athletes (competing at a national level) were excluded in this study. The purpose, expected risks, and publication of the findings in this study were adequately explained to the subjects, and written consent was obtained from all of them. The Research Ethics Committee approved this study of the National Institute of Technology, Fukui College (#R2-01). The characteristics of subjects, stature, body weight, and percent of body fat (%fat, using bioelectrical impedance technique; RD-800, TANITA Corp.) were measured. In addition, the lean body mass (LBM) and fat mass (FM) of each subject were calculated from their body weight and %fat.

In DNA analysis, samples of 2–3 strands of hair, approximately 1 cm in length, including the hair bulb, from the subjects were collected, and genomic DNAs were extracted. The DNA in the exon 16 region containing the R577X polymorphism site was amplified by a forward and a reverse primer, according to Mills et al. (2001). The amplified DNA was separated using 3% agarose gel electrophoresis and then excised and purified. The purified DNA solution was used to determine the base sequence after sequencing reaction using the Sanger method. Sequence analysis was carried out using the 3730xl DNA analyzer (Applied Biosystems®) from the obtained DNA sequence. Then whether the 577th codon in the sequence was CGA (arginine, R) or TGA (nonsense mutation; stop codon, X) was determined, and RR, RX, or XX types were discriminated (Figure 1).

On the other hand, the static maximum leg strength of the subjects was measured using a strain-gage type tension meter (KE-D300, YAGAMI Co., Ltd.). The tension meter was fixed with a non-stretchable band via a wire at one ankle while the subject was sitting in a chair and in a 90-degree knee flexion position. The maximum leg strength was measured twice, and the value of the largest one from each side was averaged as the maximum leg strength. The WBI was calculated by dividing the maximum leg strength by body weight.

Physical characteristics, maximal leg strength, and WBI were compared among three genotypes (RR, RX, and XX). Group comparisons were made using Welch-type ANOVA to determine if there were significant differences between groups; if significant, Fisher's LSD method was used as a post hoc test to identify differences between groups. The level of statistical significance was set at 5%.

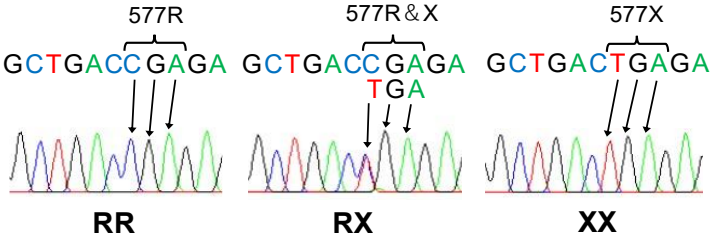


Figure 1: Determination of the *ACTN3* R577X genotype. The RR type is with the 557th codon as CGA (left). RX heterotype has both codons, which encodes arginine and stop codon as TGA (center). The XX type is with the 577th codon as TGA (right).

Table 1: Means and SDs of the variables in each group and the results of Welch-type ANOVA.

| Variables | *ACTN3* genotypes | | | Welch-type ANOVA | Post hoc |
|---|---|---|---|---|---|
| | RR | RX | XX | | |
| n (%) | 12 (18.8) | 33 (51.6) | 19 (29.7) | n.s. | |
| Age, year | 18.8 ± 1.0 | 19.4 ± 1.0 | 18.9 ± 0.8 | n.s. | |
| Stature, cm | 170.4 ± 5.3 | 169.8 ± 5.4 | 171.1 ± 5.7 | n.s. | |
| Body weight, kg | 65.3 ± 11.3 | 63.1 ± 7.5 | 60.0 ± 10.5 | n.s. | |
| %fat, % | 16.9 ± 6.5 | 16.4 ± 4.8 | 14.7 ± 5.7 | n.s. | |
| Fat mass, kg | 11.4 ± 7.2 | 10.4 ± 4.1 | 9.1 ± 5.2 | n.s. | |
| Lean body mass, kg | 52.9 ± 5.7 | 51.7 ± 4.8 | 49.8 ± 6.5 | n.s. | |
| Leg strength, kgw | 42.4 ± 7.1 | 44.4 ± 8.8 | 44.5 ± 9.5 | n.s. | |
| WBI | 0.653 ± 0.071 | 0.718 ± 0.137 | 0.765 ± 0.153 | $P < 0.05$ | RR > XX |

"n. s." means no significant.
Fisher's LSD was adopted as post hoc analysis.

**RESULTS**: The means and standard deviations of each variable for the genotype groups are shown in Table 1. The genotypes of the subjects were 18.8% (n = 12) in the RR type, 51.6% (n = 33) in the RX type, and 29.7% (n = 19) in the XX type. The ANOVA revealed no significant group differences in any of the variables, except for WBI. The difference in genotypes for WBI was significant (ANOVA, $P < 0.05$), the WBI was greater in the XX type than in the RR type (Post hoc, $P < 0.05$).

**DISCUSSION:** Results of the ANOVA showed no significant differences between the genotype groups for all variables except WBI. However, FM, LBM, and bodyweight of the XX type tended to be smaller than those of the RR and RX types, and leg strength in RR tended to be greater than in the RX and XX types. Because WBI was calculated by dividing the leg strength by body weight, the significantly greater WBI in the XX type than in the RR type might result from such trends. α-actinin-3 is a protein that is specifically expressed in fast-twitch muscle fibers (Type II), and the RR is frequently observed in sprint/power athletes (Alfred, 2011; Yang et al., 2017). It is known that the α-actinin-3 regulates skeletal muscle differentiation and hypertrophy through interaction with calcineurin, a signaling protein, and enhances fast-twitch fibers in RR (Ahmetov et al., 2014). It has been reported that RR types have a higher ratio of fast-twitch muscle fibers and a larger muscle cross-sectional area in the vastus lateralis muscle than those in the XX type (Vincent et al., 2007). Although the ratio of muscle fibers (fast-twitch fibers/slow-twitch fibers) could not be identified in this study, there was no difference in LBM among groups, and the enhancement of fast-twitch fibers, as observed in athletes with the RR type, is not expected in our subjects who were regular students.

In addition, no difference in sprint/power-type force exertion among genotypes in the general or untrained individuals has been reported (Alfred, 2011, Santiago, 2010), and the property of the RR type seems to be expressed in speed (rather than force) in the sprint/power-type force exertion (Vincent et al., 2007; Orysiak et al., 2015). Furthermore, Orysiak et al. (2015) reported no significant difference among genotypes in the static force exertion. In this study, the subjects were regular students who were not expected to have a greater ratio of fast-twitch muscle fibers. Moreover, the leg strength measured was an isometric strength, which was not accompanied by the speed in the movement.

However, there is no evidence to explain why WBI (a relative leg strength) in the XX type was more significant than in the RR type, and some inferences were required. Referring to the study by Clarkson et al. (1980), power athletes with a high percentage of slow-twitch fibers showed a greater static knee extension force than athletes with a high percentage of fast-twitch fibers. This implies that the slow-twitch fibers may compensate and exert force more than the fast-twitch fibers, depending on the muscle. As Vincent et al. (2007) reported, the XX type had more slow-twitch fibers; if the XX type in our study was assumed to have a greater ratio of slow-twitch fibers than in the RR type, it was possible that the compensatory force exertion of slow-twitch fibers upon knee extension would be greater than in the RR and RX types as shown in the report of Clarkson et al. (1980). Additionally, it is known that α-actinin-3 deficiency in the XX type may lead to the regulation of calcineurin signaling via (compensatory expressed) α-actine-2 gene, and it compensates for muscle strength (Seto et al., 2013).

Furthermore, the WBI is a metric to evaluate the static leg strength per unit body weight exerted by the quadriceps femoris muscle. It can be regarded as one of the functional evaluations for the antigravity muscles supporting upright and bipedal walking. The low-intensity locomotion and standing posture require sustained and slow muscular activities to support body weight and overcome gravity. This may be related to the functional enhancement of myoglobin-based muscles (endurance muscles) in the XX type (Seto et al., 2013). From the above-mentioned, it could be inferred that the XX type in general students (excluding elite athletes) might lead to greater WBI due to (1) superior compensatory force exertion based on slow-twitch fibers, (2) regulation in calcineurin via α-actinin gene, (3) functional enhancement of (endurance-type) slow muscles. *ACTN3* has been reported as advantageous for sprint/power athletes, while this study employing the general population revealed the specificity of anti-gravity like (compensatory) muscular exertion in XX type or subjects without *ACTN3*.

**CONCLUSION:** In this study, the association between WBI and the *ACTN3* R577X genotype was investigated in male college students, excluding elite athletes. The results showed no difference in LBM, which is a physical resource for muscle strength, among the RR, RX, and XX types, although WBI was significantly greater in the XX type than in the RR and RX types. As an inference, if the XX type was assumed to have more slow-twitch muscle fibers, compensatory force exertion based on slow-twitch muscle fibers, regulation in calcineurin via α-actinin gene, or functional enhancement of myoglobin-based endurance slow muscles may increase muscle activities against gravity, making WBI greater. Hence, this study employing subjects who were not excellent athletes revealed the muscle function specificity mentioned above rather in the group without *ACTN3*.

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