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Is *ACTN3* R577X Genotype Associated with a Preference in Type of Exercise Such as Sprint or Endurance?

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Abstract

The purpose of this study was to determine whether genotypes in the α-actinin-3 gene are associated with preferences in the type of exercise (sprint vs. endurance) in male college students. The study enrolled 64 healthy male students aged 18 - 21 years old, excluding elite athletes. DNA was extracted from the subjects' hair samples to determine the genotypes of the α-actinin-3 gene, whereas a simple questionnaire was used to investigate preference in the type of exercise. In summary, 35.5% of subjects with the RR and RX types (R-allele group) preferred sprint, whereas 47.3% of subjects with the XX type preferred endurance. Furthermore, 53.3% of the R-allele group and 31.6% of the XX group preferred endurance and sprint, respectively. This implies that the inconsistency (or mismatch) between muscle functional properties, which are characterized by genotypes and preference in type of exercise, tends to be greater in the R-allele group than in the XX type. In conclusion, the genotypes of α-actinin-3 did not play a significant role in deciding the preference in type of exercise. It can also be suspected that the mismatch between muscle functional properties and exercise preference is influenced by the daily life of students who are not exposed to high-intensity exercise stimuli.

Keywords

α-Actinin-3, Genotype, Preference, Type of Exercise

1. Introduction

Four types of actinin, α-actinin-1 (*ACTN1*) to α-actinin-4 (*ACTN4*), exist in vertebrates. These types were considered to have diversified from only one type of actinin through evolution (Virel & Backman, 2004). Both *ACTN1* and

ACTN4 are ubiquitously expressed in every possible cell, whereas α-actinin-2 (*ACTN2*) and α-actinin-3 (*ACTN3*) are specifically expressed in skeletal muscle (North et al., 1999; Vincent et al., 2007). In *ACTN3*, genotypes are distinguished between the R-allele type (RR and RX) with the 577th amino acid of the arginine (R) and the XX type with deficient *ACTN3* due to a nonsense mutation. The RR type is known to appear in some countries such as Africa (Yang et al., 2007) and is especially present in elite sprint/power athletes (Yang et al., 2003; Yang et al., 2017; Garatachea et al., 2014; Orysiak et al., 2014). *ACTN3* is expressed in the Z zone of fast-twitch muscle fibers (Masaki et al., 1967) and is associated with the development of these fibers through physical training (RR and RX types). However, the XX type, wherein *ACTN3* is deficient, exists in approximately 1.5 billion people all over the world, which is more than the number of people with the RR type (Head et al., 2015; Houweling et al., 2018; Wyckelsma et al., 2021).

It is also known that *ACTN2* and the calcineurin activity are increased in *ACTN3*-deficient organisms (Papadimitriou et al., 2019; Garton et al., 2014). Moreover, the activity of calcineurin reduces testosterone levels and suppresses fast-twitch muscle fiber development (Ahmetov et al., 2014). This has been regarded as an alternative effect to increase the dependence of the myoglobin metabolizing system in slow muscle fibers as well as energy efficiency (Ahmetov et al., 2014). Therefore, the XX type is considered to be reprogrammed for aerobic metabolism by slow muscle fibers rather than anaerobic metabolism in fast muscle fibers (Seto et al., 2013; Garton et al., 2014). Moreover, the XX type is reportedly more resistant to coldness and nutritional starvation and less susceptible to fatigue-induced cramps. This is also advantageous in sports that require aerobic endurance. In fact, the relationship between the XX type and endurance has been reported in some literatures (MacArthur et al., 2007; MacArthur et al., 2008; Berman & North, 2010).

Elite athletes were the focus of most studies that examined the association between sprint/power or endurance and *ACTN3* genotypes. However, elite athletes capable of high-level performance are aware of their physical abilities through their athletic experiences and thus already have their preferred events. Furthermore, it remains unknown which factors induced the awareness of excellent abilities in athletes. Costill et al. (1976) reported that the track athlete's preference for strength, speed, and/or endurance events would be in part a matter of genetic endowment. Thus, determining whether the *ACTN3* genotype plays a role in this awareness would be useful for contextualizing the choice of sports events for children, adolescents, and the general public. It can also provide fundamental knowledge for suitable physical activities in individuals. Therefore, the purpose of this study was to investigate the association between the *ACTN3* gene polymorphism and the experience-based preferences for the type of exercise in general college students.

2. Methods

2.1. Subjects

This study included 64 healthy college male students aged 18 - 21 years old

(mean ± SD in age: 19.1 ± 1.0 years old, stature: 170.6 ± 5.8 cm, body weight: 61.6 ± 9.3 kg) without any orthopedic history of the lower limbs that may have interfered with the measurements. They all were students at the National College of Technology in Japan, but elite athletes such as those who participate in national or international competitions were excluded. Informed written consent was collected from the subjects, who were briefed about the aim and procedure of the study and its potential for publication.

This study was approved by the Ethics Committee at the National Institute of Technology, Fukui College (reference number: R2-7), and was conducted in accordance with the Ethical Guidelines for Human Genome and Genetic Analysis Research issued by the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor and Welfare, and the Ministry of Economy, Trade, and Industry in Japan.

2.2. Analysis of *ACTN3* Genotypes

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 from the samples using a DNA extraction kit (ISOHAIR, Nippon Gene Co., Ltd.). Then, the *ACNT3* primer set was followed on the basis of the report of Mills et al. (2001) with regard to the forward (5'CTGTTGCCTGTGGTAAGTGGG3') and reverse (5'TGGTCACAGTATGCAGGAGGG3') primers. Afterwards, PCR reaction was carried out using DNA polymerase (EX Taq, TaKaRa Bio Inc.) in three steps (at 94˚C for 30 s, 60˚C for 30 s, and then 72˚C for 40 s).

The PCR products were separated via electrophoresis with 3% agarose gel, and the amplified product only for 280 base pairs was cut out from the gel. Then the PCR products were purified from the gel using a DNA purification kit (NucleoSpin Gel and PCR Clean-up, MACHEREY-NAGEL GmbH & Co. KG). The PCR product was sequenced via the Sanger method using the forward primer in the *ACTN3* primer set (Mills et al. 2001). The *ACTN3* genotypes (RR, RX, and XX) were determined by analyzing whether the 577th codon encodes arginine (R) or is a stop codon (X). The RR and XX types are defined as the 577th codon being CGA and TGA, respectively. However, when both CGA and TGA codons are seen at the 577th codon, the genotype is determined as RX, as demonstrated in **Figure 1**.

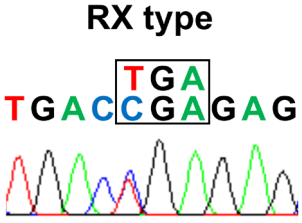

Figure 1. Four bases detected by different fluorescent peaks for determining the base sequence according to Lewis et al. (2005). In the case of the RX type, the codon 577 of the α-actinin-3 gene (inside the square) is expressed as both TGA (stop codon: X) and CGA (arginine: R).

2.3. Investigation of Preference in Type of Exercise

A simple questionnaire was adopted to investigate the subjects' preferences in type of exercise. Using a paper-based questionnaire sheet, subjects were asked to select one of the following three options: "I prefer sprint rather than distance running" (hereinafter referred to as "sprint"); "I prefer distance running rather than sprint" (hereinafter referred to as "endurance"); or "Neither."

2.4. Analysis

The percentages of each genotype for the subjects were first examined. Then, after aggregating the answers of questionnaire, the percentages of each answer were also calculated for each genotype. The answers of the RR and RX type subjects were combined under the R-allele group. The percentages were compared in each genotype.

3. Results

The aggregated results in this study are shown in Table 1. Regarding the classification of *ACTN3* genotype, 18.8% (n = 12) were RR type, 51.6% (n = 33) were RX type, and 29.7% (n = 19) were XX type. Stature and body weight in the group of RR, RX, and XX genotypes were 170.4 ± 5, 170.4 ± 6.2, and 171.1 ± 5.7 cm for stature, 64.3 ± 11.3, 62.1 ± 7.5, and 59.0 ± 10.5 kg for body weight, respectively. One-way ANOVA revealed that the differences between groups for those physical characteristics were not statistically significant (stature: F = 0.085, body weight: F = 1.364; Both $P > 0.05$). For RR and RX type subjects, 41.7% and 33.3% of respondents answered "sprint," respectively. In contrast, more than half these subjects answered "endurance," specifically 58.3% and 51.5% for the RR and RX type, respectively. When calculated together as the R-allele group, 35.6% and

Table 1. Aggregation of the questionnaire in each genotype.

Variable	*ACTN3* Genotype			
	RR	RX	R allele total	XX
Number	12	33	45	19
Preference				
Sprint	5	11	16	6
Endurance	7	17	24	9
Neither	0	5	5	4
%	18.8	51.6	70.3	29.7
Preference				
Sprint	41.7	33.3	35.6	31.6
Endurance	58.3	51.5	53.3	47.4
Neither	0.0	41.7	41.7	33.3

Total number = 64.

53.3% of subjects answered "sprint" and "endurance," respectively. On the other hand, for the XX type, 31.6% and 47.4% of subjects answered "sprint" and "endurance," respectively.

4. Discussion

The ratio of *ACTN3* genotype determined in this study was quite similar to that in the study of Mikami et al. (2014), which investigated 649 Japanese subjects from the general public as a control group; RR, RX, and XX types were 20.3%, 53.3%, and 26.3%, respectively. This implies that the subjects in this study can be considered as a general group of Japanese subjects who were not biased by experience with sport events or athletic level. On the basis of global data, the XX type is much more prevalent than the RR type (Head et al., 2015; Houweling et al., 2018; Wyckelsma et al., 2021); 80% of humans have the X-type nonsense variant of *ACTN3* (North et al., 1999). Thus, the ratio of *ACTN3* genotypes in this study is considered to correspond not only with global trends but also with Japanese characteristics.

Athletes who possess the R-allele are reported to have superior sprint/power (Yang et al., 2003; Yang et al., 2017; Garatachea et al., 2014; Orysiak et al., 2014). However, there are several studies stating that XX type subjects have higher energy efficiency in slow muscle fibers, thus having superior endurance (MacArthur et al., 2007; MacArthur et al., 2008; Berman & North, 2010). If it is assumed that functional properties in skeletal muscle can be characterized into sprint or endurance depending on *ACTN3* genotypes (sprint for R-allele vs. endurance for XX type), less than half of the subjects answered their corresponding muscle functional properties (35.6% in R-allele group and 47.4% in XX type). This suggests that exercise experience in childhood or adolescence would not lead to consistent preferences in type of exercise that correspond to the muscle functional properties expected from their genotypes.

Furthermore, 53.3% in the R-allele group and 31.6% with XX type preferred the type of exercise that did not correspond to muscle functional properties. In other words, their exercise preferences differed from the expected muscle functional properties. In the R-allele group, although superior sprint/power is expected because of the development of fast-twitch muscle fibers due to α-actinin-3 expression (Yang et al., 2003; Yang et al., 2017; Garatachea et al., 2014), this can only be achieved through high-intensity physical training. For ordinary students who do not undergo high-level athletic training or join competitions, there are fewer opportunities to strengthen fast-twitch muscle fibers and improve sprint/power performance. Furthermore, they may have been selectively undergoing moderate exercise stimuli rather than high-intensity sprints whenever they engage in physical activity. These factors may explain why more than half of the R-allele group chose "endurance" as their preference in type of exercise. Consequently, the ratio of inconsistency or mismatch with muscle functional properties in the R-allele group might be larger than that of XX type.

On the other hand, it is known that the energy efficiency characteristic of the XX type depends on the myoglobin metabolism in slow muscle fibers (MacArthur et al., 2007; MacArthur et al., 2008; Berman & North, 2010). Instead of anaerobic metabolism in fast-twitch muscle fibers, the reprogramming to oxygenated metabolism in red/slow-twitch muscle fibers makes fatigue-induced cramps less likely. Thus, XX type subjects theoretically have greater advantage in sports that require endurance (Seto et al., 2013; Garton et al., 2014; Head et al., 2015). Most activities of daily living done against gravity, such as standing and walking, are considered as low-intensity or low aerobic level activities. The ratio of consistency with muscle functional properties was greater in the XX type (47.4%) than in the R-allele group (35.6%), and the ratio of inconsistency (31.6%) was smaller than that of the R-allele group (53.3%) as well. In other words, the preferences in the type of exercise may have been influenced by 1) the avoidance of high-intensity exercise stimuli in daily life in general students and 2) the awareness of being poor at sprinting in the XX type who have muscle functional properties more attuned for endurance.

5. Conclusion

This study assessed the association between *ACTN3* genotypes and preference in type of exercise (sprint vs. endurance) in ordinary students who were not exposed to high-intensity exercise stimuli. The results showed that 35.5% in R-allele group (combined RR and RX types) preferred sprint, whereas 47.3% in XX type subjects preferred endurance. In contrast, 53.3% in the R-allele group and 31.6% in XX type subjects preferred endurance and sprint, respectively. Greater inconsistency/mismatch in preference and muscle functional properties was observed in the R-allele group than in the XX type group. It was suggested that exercise experience in non-trained individuals and low exercise stimuli in daily life are factors that affect the consistency of muscle functional properties expected from *ACTN3* genotypes. Hence, genotype analysis (*ACTN3*) might optimize selecting sport events and lead higher physical performances in young boys and girls without adequate exercise experiences.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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