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2 **Manuscript Title:**

3 The G allele of the *IGF2* ApaI polymorphism is associated with judo status

Abstract

Previous studies have reported that the insulin-like growth factor 2 (*IGF2*) ApaI polymorphism is associated with body mass index, fat mass, and grip strength. Competitive judo requires high levels of strength and power. The purpose of this study was to investigate the association between the *IGF2* ApaI and *ACTN3* R577X polymorphisms and judo status. The subjects were 156 male judo athletes from a top-level university in Japan. They were divided into three groups based on their competitive history: international-level athletes, national-level athletes, and others. Genomic DNA was extracted from the saliva of each athlete, and the maximal isometric strength of the trunk muscles and handgrip strength were measured. Genotyping by PCR-RFLP was used to detect *IGF2* (rs680) and α-actinin-3 (*ACTN3*) (rs1815739) gene polymorphisms. The genotype frequencies of the two gene polymorphisms were compared among the three groups of judo athletes and controls.

International-level judo athletes showed a higher frequency of the GG+GA genotype of the *IGF2* gene than national-level athletes and others. There was an inverse linear correlation between the frequency of the *IGF2* AA genotype and level of judo performance ($p = 0.041$). Back muscle strength relative to height and weight was higher in subjects with the GG+GA genotype than in those with the AA genotype. Conversely, the *ACTN3* R577X polymorphism was not associated with judo status. Additionally, no differences were found in back muscle or handgrip strength among the *ACTN3* genotypes.

In conclusion, the results indicate that the *IGF2* gene polymorphism may be associated with judo status.

Key words: *IGF2* gene; gene polymorphism; judo athletes; sports performance; back muscle strength; grip strength

Introduction

Judo is a Japanese martial art and Olympic sport. Competitive judo is a combative, high-intensity intermittent sport in which the athlete attempts to throw the opponent onto his/her back or to control him/her during groundwork techniques. Therefore, judo requires high absolute and relative levels of maximal strength and muscle power, particularly in the upper body (8). Although strength training can improve muscle strength or muscle power, it is known that trainability widely differs from one person to another. This individual difference is evidently determined not only by environmental factors such as lifestyle and diet but also by genetic factors (14, 15). Genetic contributions to performance at the elite level in judo have received little attention (3, 16). Identification of genes that predispose individuals to high performance in judo may help coaches to recognize and guide those with better genetic potential to be elite athletes.

In this study, we specifically considered two genes that have previously been studied in terms of their association with athletic performance and/or muscle strength, namely, α-actinin-3 (*ACTN3*) and insulin-like growth factor 2 (*IGF2*). In particular, the *ACTN3* gene has been extensively studied for its association with athletic performance. The *ACTN3* gene encodes α-actinin-3, a sarcomeric protein that is almost exclusively expressed in the fast-twitch (type II) fibers of the skeletal muscle (11), where it is used to produce powerful contractions. A common single nucleotide polymorphism of the human *ACTN3* gene results in either an arginine (R) or a stop codon (X) at amino acid 577 of exon 16 on chromosome 11 (13). Therefore, three genotypes exist in the general population: RR, RX, and XX. The XX homozygotes are unable to produce ACTN3 protein. Recently, in a meta-analysis study, Ma et al. (2013) found strong evidence for associations between the *ACTN3* R allele and power/sprint events in Caucasian athletes

(9). In Japanese subjects, the RR+RX genotype was also found at a higher frequency in elite sprint/power athletes than in controls (10). Although some research has been conducted on the *ACTN3* genotypes of judo athletes (16), there have been no reports on the association between *ACTN3* polymorphism and status in Japanese judo athletes.

IGF2 has a role in muscle development in humans and is located on chromosome 11p15.5. Previous studies have reported significant associations between the *IGF2* gene 3′ -untranslated region ApaI polymorphism and muscle strength (18, 19). Previous studies have also demonstrated that arm and leg strength and total body fat-free mass were higher in individuals with the GG+GA genotype than in those with the AA genotype (19). The *IGF2* gene may be associated with adult skeletal muscle mass. However, to date, there have been no reports of any association between the *IGF2* ApaI polymorphism and sports performance.

The purpose of this study was to investigate the association between *ACTN3* R577X or *IGF2* ApaI polymorphisms and judo status or muscle strength. We hypothesized that (1) the frequency of the RR+RX genotype in the *ACTN3* gene polymorphism may be higher in international-level judo athletes than in other athletes or controls and that (2) the *IGF2* genotype may be associated with judo performance or muscle strength. Further, we investigated the association between the *IGF2* ApaI polymorphism and judo status.

Method

Experimental Approach to the Problem

This study was designed to investigate the correlation between *ACTN3* and *IGF2* gene polymorphisms and judo status and muscle strength. To accomplish these objectives, we designed a case–control study for which 156 judo athletes were recruited. The judo athletes, who were divided into three groups based on results, were compared to Japanese controls as in previous studies.

The associations between grip strength and back muscle strength and genotypes within the group of judo athletes were evaluated using statistical analysis.

Subjects

The subjects in the present study were 156 male judo athletes belonging to the judo club of Tokai University in Japan. They were divided into three groups based on their results in national or international competitions. Sixteen athletes were classified as "international level" (winners of national championships or participants in international competitions), 37 as "national level" (in the top eight at a college competition), and 103 as "others" (members of the University Judo Club) (Table 1). Controls were individuals of known genotype from the general population as reported in previous studies [*IGF2*-Controls, n = 167 (1); *ACTN3*-Controls, n = 1191 (6, 7, 10, 17)].

【Table 1 about here】

The study was approved by the Ethics Committee of Tokai University in Japan and was conducted according to the Declaration of Helsinki. The objectives and methods of the study were explained, and written informed consent was obtained from all subjects.

Genotyping

DNA was extracted from the saliva of all subjects using the QIAamp DNA Mini Kit (QIAGEN, Milano, Italy) according to the manufacturer's protocol. *IGF2* ApaI (rs680) and *ACTN3* R577X (rs1815739) polymorphisms were determined by polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP), and the resulting PCR products were genotyped using agarose gel electrophoresis (Mupid-2plus, a submarine type electrophoresis system; ADVANCE, Kyoto, Japan). The primers used (Fasmac Co., Ltd., Kanagawa, Japan) for the *IGF2* polymorphism were forward 5′-CTTGGACTTTGAGTCAAATTGG-3′ and reverse 5′-CCTCCTTTGGTCTTACTGGG-3′, generating a fragment of 236 base pairs (bp) (2). The PCR conditions (Program Temp Control System PC-816; ASTEC Co., Ltd., Fukuoka, Japan) were as follows: initial denaturation for 5 min at 94.0°C; 35 cycles of denaturation for 35 s at 94.0°C, annealing for 40 s at 55.0°C, and synthesis for 40 s at 72.0°C; and a final extension for 10 min at 72.0°C. The *IGF2* genotypes were established by the enzymatic digestion of amplicons with ApaI (New England Biolabs Japan Inc., Tokyo, Japan). All subjects were categorized as exhibiting the GG, GA, or AA genotype.

For *ACTN3* R577X polymorphism genotyping, a fragment of 290 bp was amplified with the forward 5′-CTGGGCTGGAAGACAGGAG-3′ and reverse 5′-AGGGTGATGTAGGGATTGGTG-3′ primers (12). The PCR conditions were as follows: initial denaturation for 2 min 30 s at 94.0°C; 40 cycles of denaturation for 45 s at 94.0°C, annealing for 30 s at 59.0°C, and synthesis for 2 min at 72.0°C; and a final extension at 72.0°C for 10 min. The *ACTN3* genotypes were established using the enzymatic digestion of amplicons with DdeI (New England Biolabs Japan Inc., Tokyo, Japan). All subjects were categorized as exhibiting the RR, RX, or XX genotype. The

digested products were separated by 2% or 3% agarose (Agarose for 150–1,500 bp fragment; Nacalai Tesque, Inc., Kyoto, Japan) gel electrophoresis, stained with ethidium bromide, and visualized under ultraviolet light. All genotyping analyses were conducted blind to the subjects' identities.

Phenotype measurement

The subjects underwent anthropometric measurements (height [cm], weight [kg], and BMI [kg/m^2]). Standard anthropometric methods were used to determine body mass and height, measured to the nearest 0.1 kg and 0.1 cm, respectively. Back muscle strength was measured from the maximal isometric strength of the trunk muscles in a standing position with 30° lumbar flexion using a digital back muscle strength meter (T.K.K 5002; Takei Scientific Instruments Co., Ltd., Tokyo, Japan). Handgrip strength was measured bilaterally using a handgrip dynamometer (T.K.K 5401; Takei Scientific Instruments Co., Ltd., Tokyo, Japan). Both hands were tested twice, and the mean value was used to represent the maximum muscle strength of the subject. The measurement data for some subjects had been obtained on enrolment at the university.

Statistical analysis

The genotype distribution was evaluated for conformity with the Hardy–Weinberg equilibrium using a chi-square test with two degrees of freedom. Genotype distribution and allele frequencies among the three groups of judo athletes and controls were compared using chi-square tests or Fisher's exact test. The Jonckheere–Terpstra trend test was used to examine the relationship between the frequency of the gene type and the level of judo status.

Analysis of variance (ANOVA) was used to compare performance by the

different genotype groups in each test. The data are presented as the mean ± standard deviation (SD). Levels of significance were set at $p < 0.05$, and all statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) ver. 18.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results

The genotype frequencies for the *IGF2* ApaI polymorphism (GG = 35.9%, GA = 51.3%, AA = 12.8%) and *ACTN3* R577X polymorphism (RR = 19.2%, RX = 47.4%, XX = 33.3%) were in Hardy–Weinberg equilibrium ($p > 0.05$). The *IGF2* and *ACTN3* genotype frequencies of the Japanese control subjects have been previously published (1, 6, 7, 10, 17). The distributions of the *IGF2* ApaI and *ACTN3* R577X polymorphisms in the 156 judo athletes and controls were compared.

Table 2A shows the genotype frequency of the *IGF2* ApaI polymorphism in the different groups. The frequencies of the *IGF2* genotypes in the Japanese controls were 37.1% for GG, 44.3% for GA, and 18.6% for AA (1). There was no significant difference in the *IGF2* genotype (GG+GA genotype vs. AA genotype) frequency of the judo athletes as a whole compared with that of the Japanese controls ($\chi^2 = 2.00$, $p = 0.16$). However, there was a trend toward a higher frequency of the GG+GA genotype in the international-level athletes (GG+GA genotype 100.0% vs. AA genotype 0.0%) than that in the controls (GG+GA genotype 81.4% vs. AA genotype 18.6%) ($p = 0.08$). All the international-level athletes carried the G allele of the *IGF2* gene. Furthermore, there was a trend toward a higher frequency of the GG+GA genotype in the international- and national-level athletes (GG+GA genotype 92.5% vs. AA genotype 7.5%) than that in the controls ($p = 0.08$). The AA genotype frequency was 18.6% in the controls and 0.0%, 10.8%, and 15.5% for the international-level athletes, national-level athletes, and others, respectively (Figure 1). There was an inverse linear correlation between the frequency of the *IGF2* AA genotype and the level of judo status ($p = 0.041$ for linear trend).

【Table 2A and 2B about here】

【Figure 1 about here】

Table 2B shows the genotype frequency of the *ACTN3* R577X polymorphism

in the different groups. The frequencies of the *ACTN3* genotypes in the Japanese controls were 21.7% for RR, 52.0% for RX, and 26.3% for XX (6, 7, 10, 17). The genotype distribution was significantly different between the others group and the controls ($\chi^2 = 5.40$, $p = 0.02$). The RR+RX genotype was found at a lower frequency in the others group than that in the controls. Furthermore, there was a trend toward a lower frequency of the RR+RX genotype in all the judo athletes than that in the controls (RR+RX genotype 73.7%) ($\chi^2 = 3.47$, $p = 0.06$). However, there was no significant difference in the frequency of the *ACTN3* genotype (RR+RX genotype vs. XX genotype) in the controls compared with that of the international-level athletes ($p = 1.00$) and the international- and national-level athletes ($\chi^2 = 0.001$, $p = 0.98$). Therefore, the *ACTN3* R577X polymorphism was not associated with judo status.

Table 3 shows the characteristics and measurement of muscle strength. In relation to height and weight, there were no significant differences in the *IGF2* or *ACTN3* genotypes. One-way ANOVA indicated no significant difference in the phenotype measurements among the genotype groups. For the *IGF2* genotypes, there was a trend toward higher back muscle strength in the GG+GA genotype than that in the AA genotype ($p = 0.06$) (Table 3). Back muscle strength relative to weight and height was significantly higher in the GG+GA genotype than that in the AA genotype (Table 3). However, there was no significant difference in handgrip strength. In contrast, there were no significant differences in the *ACTN3* genotype related to back muscle or handgrip strength (Table 3).

【Table 3 about here】

Discussion

In this study, we demonstrated the association between the *IGF2* gene polymorphism and back muscle strength as well as judo status. Previous studies have shown the association between the *IGF2* ApaI polymorphism and muscle mass (19) and birth weight (18). Our study indicated that the *IGF2* ApaI polymorphism was associated with the strength of the back muscle, which is the largest muscle in the human body. It is possible that body size could be determined by the *IGF2* ApaI polymorphism (5). When athletes and coaches consider weight class selection, the *IGF2* gene polymorphism may be a helpful genetic factor. The results of the present study suggest that the *IGF2* ApaI polymorphism was associated with back muscle strength but not handgrip strength. Our results are in agreement with the findings of Franchini et al. (2005) (4), who reported that handgrip strength was similar in elite and non-elite judo athletes in Brazil. Although handgrip strength is an important component of participation in judo, it is expected that judo athletes' absolute handgrip strength is substantially higher than that of the average person.

Several genetic polymorphisms have been reported to be predictive of some aspects of an individual's potential for becoming an elite athlete, and thus, provide useful information for strength and conditioning coaches. In the present study, there was an inverse linear correlation between the frequency of the *IGF2* AA genotype and level of athletic status ($p = 0.041$ for linear trend). This is the first study to demonstrate an association between the *IGF2* ApaI polymorphism and sports status. Although further studies are required to clarify its possible role, the *IGF2* ApaI polymorphism, but not the *ACTN3* R577X polymorphism, may be a candidate gene associated with judo status.

Many previous studies have reported associations between the *ACTN3* gene polymorphism and sports performance, sprint, muscle strength, and power (20). Our

hypothesis was that the frequency of the RR+RX genotype in the *ACTN3* gene polymorphism may be higher in international-level judo athletes than in other athletes or controls. However, we found no association between the *ACTN3* R577X polymorphism and international-level athletes, national-level athletes, or international- and national-level athletes. Rodríguez-Romo et al. (2013) also found no association between the *ACTN3* R577X polymorphism and elite judo athlete status in a Spanish population (16). Our results are in agreement with the findings of Rodríguez-Romo et al. (2013) (16). In this study, the XX genotype was found at a higher frequency in the others group than the controls. These data suggest that the XX genotype may be a disadvantage for performance in judo, calling for muscle power. The results of the present study suggest that the *ACTN3* R577X polymorphism was not associated with judo status.

The present study has some limitations. First, the results from our three groups were probably limited by the relatively small sample size of the individual groups and low statistical power. A sufficient sample size of international-level athletes was probably necessary for solid conclusions to be reached in terms of the association between genetics and sports performance. Although the sample size of the international judo athletes was small, 16 athletes were classified as "international level," being at least winners of the National Championships and including Olympic champions and winners of World Championships. Second, in this study, there is no evidence that the *IGF2* ApaI polymorphism has an influence on gene/protein function. In future, genomic analysis with larger sample sizes or replication across different samples are warranted.

In conclusion, the findings of the present study indicate that the *IGF2* ApaI polymorphism, but not the *ACTN3* R577X polymorphism, may be a candidate gene associated with some types of muscle strength as well as judo status.

Practical Applications

First, we suggest that *IGF2* GG+GA individuals present higher back muscle strength than *IGF2* AA individuals. The present study shows that the *IGF2* ApaI polymorphism may be an important genetic marker associated with predisposition to higher muscle strength of both the general population and athletes.

Furthermore, we have shown that *IGF2* gene polymorphism was associated with judo status; the *IGF2* ApaI polymorphism has the potential to be used to develop genetic tests for identifying athletic talent. Although more studies are needed, this polymorphism could be used to predict the status of athletes participating in various sports that require muscle strength.

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Figure legends

Figure 1. *IGF2* AA genotype frequency among judo athletes categorized in terms of performance level and controls. The frequency of the AA genotype was 18.6% in controls and 0.0%, 10.8%, and 15.5% for international-level athletes, national-level athletes, and others, respectively ($p = 0.041$ for linear trend).