The Combination of Insulin-Like Growth Factor 2, Alpha-Actinin-3, and Angiotensin-Converting Enzyme Gene Polymorphisms in Judo Athletes: A Pilot Study

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Objective: The purpose of this study was to investigate the association between the combination of insulin-like growth factor 2 (*IGF2*), alpha-actinin-3 (*ACTN3*), and angiotensin-converting enzyme (*ACE*) gene polymorphisms and judo status.

Methods: We recruited 156 male judo athletes from a top-level university in Japan. These athletes were divided into two groups based on their competitive history: international-level athletes and others. Genomic DNA was extracted from the saliva of each athlete. Genotyping by PCR or PCR-RFLP was used to detect *IGF2* G/A (rs680), *ACTN3* R577X (rs1815739), and *ACE* I/D (rs1799752) polymorphisms. The combined frequencies of the three gene polymorphisms were compared between all judo athletes and controls and between international-level athletes and others.

Results: There was a higher frequency of the *IGF2* GG, *ACTN3* RX, and *ACE* II genotype in all judo athletes (10.3%) compared to that of this genotype in the controls (1.2%). Moreover, there was a lower frequency of the *IGF2* AA, *ACTN3* RX, and *ACE* ID genotype in all judo athletes (1.9%) compared to that of this genotype in the controls (8.9%). Two of the 156 judo athletes exhibited the *IGF2* GG, *ACTN3* RX, and *ACE* DD genotype; both of them were international-level athletes.

Conclusions: The combination of *IGF2*, *ACTN3*, and *ACE* gene polymorphisms may be associated with the judo status.

Key words: genetic polymorphism, judo status, *IGF2*, *ACTN3*, *ACE*

Introduction

Judo is one of the major sports originating from Japan and requires high absolute and relative levels of maximal strength and muscle power¹⁾. Judo athletes have to exert a significant amount of muscle strength throughout each match. Over recent years, the number of Japanese competitors who receive winning medals in the Olympic Games and World Championship competitions has markedly declined. To be successful in international competitions, judo athletes must achieve an excellent level of physical fitness and condition through

training. However, success in sports also depends upon genetic factors, with 66% heritability for athlete status²⁾.

To date, at least 206 nuclear genetic markers in the human genome have been associated with physical performance phenotypes as well as physical fitness³⁾. Of this group of genes, we specifically focused upon the insulin-like growth factor 2 (*IGF2*) gene, which has been associated with muscle strength⁴⁾. The *IGF2* gene is located on chromosome 11p15.5⁵⁾ and plays a role in human muscle development⁶⁾. *IGF2* ApaI polymorphism has resulted in the classification of individuals into

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the GG, GA, or AA genotype. Previous studies have demonstrated that both arm and leg strength and total body fat-free mass were higher in individuals with the GG+GA genotype compared to those with the AA genotype⁴⁾. Recently, we determined that the *IGF2* ApaI polymorphism may be associated with athlete status in judo⁷⁾. In other words, the *IGF2* ApaI polymorphism may represent a genetic marker associated with a predisposition for judo status. However, there have been no other reports associating the *IGF2* ApaI polymorphism with athlete status.

Previous studies have investigated the relationship between athlete status and alpha-actinin-3 (*ACTN3*) and angiotensin-converting enzyme (*ACE*) polymorphisms. The *ACTN3* gene encodes the ACTN3 protein, a sarcomeric protein that is almost exclusively expressed in the fast-twitch (type II) fibers of skeletal muscle and is located on chromosome 11q13.1. A cytosine (C) to thymine (T) transition within exon 16 of the *ACTN3* gene leads to a stop codon, which results in the muscle fibers being devoid of the ACTN3 protein⁸⁾. The homozygosity of the X allele (in other words, an XX genotype) also results in the absence of ACTN3 expression. The XX genotype appears to be far less frequent in elite power athletes compared to endurance athletes or controls⁹⁾. This data suggests that the XX genotype exerts an influence upon certain type of muscle performances. The *ACE* gene is located on chromosome 17q23.3 and contains a polymorphism based on its presence (insertion, I) or absence (deletion, D) within intron 16 of a 287-bp nonsense DNA domain, resulting in three different genotypes (II, ID, and DD). ACE is a key factor in regulating blood pressure in the renin-angiotensin system and acts via the production of angiotensin II. ACE activity is determined by the *ACE* I/D polymorphism. Individuals who are homozygous for the deletion allele (DD genotype) have ACE levels in the serum that are higher than the individuals who are homozygous for the insertion allele (II)¹⁰⁾. Previous studies have reported associations between the I allele of the *ACE* I/D polymorphism and superior endurance ability of elite athletes¹¹⁾.

Many previous studies have shown an association between the combination of *ACTN3* and *ACE* gene polymorphisms and athlete status. We hypothe-

sized that the combination of three gene polymorphisms is more effective than that of two gene polymorphisms. In the present study, we specifically considered three genes that have previously been associated with athlete status and/or muscle strength: *IGF2*, *ACTN3*, and *ACE*. Because the *IGF2* gene polymorphism has been related to judo status, it is plausible that the combination of three gene polymorphisms (*IGF2*, *ACTN3*, and *ACE*) may reveal the judo status. Therefore, the purpose of this study was to investigate the association between the combination of *IGF2*, *ACTN3*, and *ACE* gene polymorphisms and judo status.

Methods

1. Subjects

We investigated 156 male judo athletes belonging to the Tokai University Judo Club in Japan. Athletes 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) and the remaining 140 were classified as “others” (members of the University Judo Club). Our controls were individuals of a known genotype from the general population, as reported in previous studies [*IGF2*-Controls, $n = 167$ ¹²⁾; *ACTN3*-Controls, $n = 1,191$ ¹³⁾⁻¹⁶⁾; *ACE*-Controls, $n = 5,679$ ¹⁷⁾⁻²⁶⁾].

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 to all participants, and written informed consent was obtained.

2. Genotyping

DNA was extracted from the saliva of all subjects using the QIAamp DNA Mini Kit (QIAGEN, Milano, Italy) in accordance with the manufacturer's protocol. *ACTN3* R577X (rs1815739), *ACE* I/D (rs1799752), and *IGF2* G/A (rs680) polymorphisms were determined by polymerase chain reaction (PCR) or PCR-restriction fragment length polymorphism (RFLP)²⁷⁾⁻²⁹⁾.

3. Statistical analysis

The distribution of genotypes was evaluated for

conformity by the Hardy-Weinberg equilibrium using the chi-square test with two degrees of freedom. Levels of significance were set at $p < 0.05$. The frequency of the combination of three gene polymorphisms was calculated by extrapolating control data from previous studies.

Results

Genotype frequencies for the *IGF2* ApaI, *ACTN3* R577X, and *ACE* I/D polymorphisms were within

the Hardy-Weinberg equilibrium ($p > 0.05$). The genotype frequencies of *IGF2*, *ACTN3*, and *ACE* in Japanese control subjects have been previously published⁽¹²⁾⁻⁽²⁶⁾.

Figure-1 shows the combined frequencies of three gene polymorphisms (*IGF2*, *ACTN3*, and *ACE*) in all judo athletes and controls. There was a higher frequency of the *IGF2* GG, *ACTN3* RX, and *ACE* II genotype in all judo athletes (10.3%) compared to that of this genotype in the controls (1.2%). Furthermore, there was a lower frequency

Figure-1 The frequencies of combination of three gene polymorphisms (*IGF2*, *ACTN3*, and *ACE*) in all judo athletes and controls

Figure-2 The number of international-level and other judo athletes exhibiting the combination of three gene polymorphisms (*IGF2*, *ACTN3*, and *ACE*)

of the *IGF2* AA, *ACTN3* RX, and *ACE* ID genotype in all judo athletes (1.9%) compared to that of this genotype in the controls (8.9%). It was also evident (Figure-1) that there were differences between the frequencies of judo athletes and controls.

Figure-2 shows the number of athletes exhibiting the combination of three gene polymorphisms among all the judo athletes (international-level athletes and others). Two of the 156 judo athletes exhibited the *IGF2* GG, *ACTN3* RX, and *ACE* DD genotype; both of them were international-level athletes. Figure-2 also shows the characteristics of international-level judo athletes.

Discussion

In the present study, we investigated an association between the combination of three gene polymorphisms (*IGF2*, *ACTN3*, and *ACE*) and judo status. Many previous studies have revealed an association between the combination of two gene polymorphisms (*ACTN3* and *ACE*) and athlete status. However, to date, there have been no reports of any association between the combination of three gene polymorphisms and athlete status.

Our data suggested that the combination of three genes may have an influence on judo athletes. Moreover, two of the 156 judo athletes exhibited the *IGF2* GG, *ACTN3* RX, and *ACE* DD genotype; both of them were international-level athletes. Therefore, the *IGF2* GG, *ACTN3* RX, and *ACE* DD genotype may confer an advantage pertaining to the judo status. Kikuchi *et al.* (2012) reported higher frequencies of the *ACTN3* R allele + *ACE* DD genotype in elite Japanese wrestlers¹⁴⁾. These earlier results were supported, at least in part, by our current findings, which showed the combination of the *IGF2*, *ACTN3*, and *ACE* genes only in international-level judo athletes. This result shows that the combination of three genes may be used to develop genetic tests for identifying athletic talent.

An important limitation of the present study is that no statistical analysis was performed and it included a small sample size of international-level athletes. Sports performance is a multifactorial phenotype, influenced by both environmental and genetic factors. However, to our knowledge, this study is the first to investigate the association between the combination of three gene polymor-

phisms and judo status. Consequently, this research could shed new insight into the selection of international judo athletes.

In conclusion, the findings of the present study indicate that the combination of three genes (*IGF2*, *ACTN3*, and *ACE*) may be associated with judo status.

Conflict of interest

No conflict of interest was declared for this study.

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