ORIGINAL ARTICLE

Judo status is not associated with the angiotensin-converting enzyme insertion/deletion polymorphism in Japanese judo athletes

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Manuscript Preparation
E Funds Collection

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Abstract

Background & Study Aim: In high-level competitions, judo athletes perform several matches in a day and require a high endurance. The *angiotensin-converting enzyme* (*ACE*) polymorphism has been widely studied in relation with endurance and sports status. The purpose of this study was knowledge about the influence of the *ACE* insertion/deletion (*I/D*) polymorphism on the judo status and endurance of Japanese athletes.

Material & Methods: This study included 154 Japanese male judo athletes from a top-level university in Japan. They were divided into three groups based on their competitive history (international level, national level, and others). Genomic DNA was extracted from the saliva of each athlete and the distance travelled in a 5-min running test was measured. Genotyping using polymerase chain reaction was performed to detect the *ACE I/D* polymorphism, rs1799752.

Results: *ACE* genotype and allele frequency were compared between the judo athletes groups and control subjects. The *ACE I/D* polymorphism was not associated with judo status in these athletes. Furthermore, no differences were found among the *ACE* genotypes pertaining to endurance.

Conclusions: The results of the present study suggest that the *ACE* gene does not greatly influence the judo status and endurance in Japanese athletes. However, the ambiguity of these results with studies of other populations judo athletes tend to the conclusion that the precise determination of the biological determinants of talent in judo is a complex phenomenon and requires further study and taking into account the specific genetic methods.

Key words: *ACE I/D* polymorphism, endurance performance, international judo level, national judo level

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Allele – one of the alternative versions of a gene at a given location (locus) along a chromosome.

Endurance – *noun* the ability or power to bear prolonged exertion, pain or hardship endurance athlete.

Endurance athlete – noun an athlete who has a high level of aerobic fitness.

Introduction

The International Judo Federation is a global organization, with 200 affiliated countries. To be successful in international competitions, judo athletes must achieve an excellent level of physical fitness and conditioning. Little suggested that a successful judo performance depends upon the athlete having a high technical and tactical ability, power, strength, endurance and flexibility [1]. Although judo requires explosive power, judo athletes need endurance to exert this power for 5 min or more (until the end of a match). In a high-level competition, judo athletes perform several matches in a day; therefore, judo specifically requires a high endurance.

Aerobic exercises such as running and bike riding can improve the endurance. However, it is known that trainability and training effects are highly individualized. Individual differences in endurance are influenced not only by environmental but also by genetic factors [2]. To date, at least 206 nuclear genetic markers in the human genome have been found to be associated with physical performance phenotypes and physical fitness [3]. Among these, the *angiotensin-converting enzyme* (*ACE*) gene has been extensively studied for its association with endurance [4, 5].

ACE insertion/deletion (*I/D*) polymorphism was first shown to be a genetic factor that influences human physical performance and trainability in 1998. In that study, Montgomery et al. [6] reported that the *ACE I/D* polymorphism influenced endurance in elite climbers and response to training in British army recruits. Since then, many studies have demonstrated significant associations between the I allele of the *ACE I/D* polymorphism and superior endurance in elite athletes [7 ,8]. In a meta-analysis, Ma et al. [5] found strong evidence for associations between the *ACE* II genotype and endurance events in Caucasian athletes. Some studies [9, 10] have defined endurance-oriented sports as those involving more than 5 min of competition, including swimming a distance of 500 m or more and track and field events lasting for at least 5 minutes. Although judo matches can sometimes end in less than 5 min, the designated time for each match is 5 min or more. Therefore, in case of the investigation of the relationship between gene polymorphism and sports status, judo seems to involve the endurance-oriented sports. Cieszczyk et al. [11] reported that the frequency of the I allele in the *ACE* gene polymorphism was significantly higher in Polish and Lithuanian judo athletes than in other Caucasian control subjects. However, there have been no reports of any association between the *ACE I/D* polymorphism and judo status or endurance ability in Japanese judo athletes. Since the Olympics Games of Tokyo in 1964, the Japanese judo team has won 36 gold medals.

The purpose of this study was knowledge about the influence of the *ACE* insertion/deletion (*I/D*) polymorphism on the judo status and endurance of Japanese athletes.

We hypothesized that: (1) the frequency of the I allele in the *ACE* gene polymorphism may be higher in international-level judo athletes than in other judo athletes or controls; (2) the *ACE* genotype may be associated with endurance in judo athletes.

Materials and methods

Participants

The study included 154 Japanese male judo athletes belonging to the judo club of Tokai University, a club that has produced many international-level judo athletes. The athletes were divided into three groups based on their results (sports level of judo) in international- and national-level competitions (Table 1). Sixteen athletes were classified as "international level" (winners of national championships or participants in international competitions), 36 as "national level" (athletes ranking among the top eight in a college-level competition), and 102 as "others" (members of the University Judo Club). They were also classified into three cumulative weight categories: light (60 kg and 66 kg), medium (71 kg, 73 kg, 81 kg, and 90 kg), and heavy (100 kg and over 100 kg). The controls were individuals of known genotype from the general Japanese population, as reported in previous studies: *ACE*-Controls, n = 5,679 [12-21].

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 the subjects, and written informed consent was obtained from each of them.

Genotyping

DNA was extracted from the saliva of all subjects using the QIAamp DNA Mini Kit (Qiagen, Italy)

Table 1. Characteristic of judo male athletes (n = 154).

Sports level of judo	N	Cumulative weight categories		
		Light (-60, -66kg)	Middle (-71*, -73, -81, -90kg)	Heavy (-100, +100kg)
International	16	3	7	6
National	36	9	14	13
Others	102	22	54	26
All judo athletes	154	34	75	45

*71 kg (according to the old regulations of sport judo)

according to the manufacturer's protocol. The *ACE I/D* (rs1799752) polymorphism was determined using polymerase chain reaction (PCR), and the resulting PCR products were genotyped using agarose gel electrophoresis (Mupid-2plus, a submarine type electrophoresis system, Advance, Japan). The primers (Fasmac Co. Ltd., Japan) used for the *ACE I/D* polymorphism were as follows: forward 5′-GCCCTGCAGGTGTCTGCAGCATGT-3′ and reverse 5′-GGATGGCTCTCCCCGCCTTGTCTC-3′, generating a fragment of 597 base pairs (bp) [22].

DNA amplification was performed using a thermal cycler Program Temp Control System PC-816 (Astec Co. Ltd., Japan). The PCR conditions were as follows: initial denaturation for 2 min 30 s at 94.0°C; 35 cycles of denaturation for 30 s at 94.0°C, annealing for 45 s at 56.0°C, synthesis for 2 min at 72.0°C, and final extension for 10 min at 72.0°C. Homozygotes produced either a single 597 bp band (II) or a 319 bp band (DD); heterozygotes (ID) produced both bands. All subjects were categorized according to whether they exhibited II, ID, or DD genotypes.

To avoid the misclassification of ID heterozygotes as DD homozygotes, a second PCR was performed for the ID and DD genotype samples. The insertion-specific primers used for the *ACE* I band polymorphism were as follows: forward 5′-TGGGACCACAGCGCCCGCCACTAC-3′ and reverse 5′-TCGCCAGCCCTCCCATGCCCATAA-3′, generating a fragment of 335 bp [22].

The PCR conditions were as follows: initial denaturation for 1 min at 94.0°C, 35 cycles of denaturation for 30 s at 94.0°C, annealing for 45 s at 67.0°C, and synthesis for 2 min at 72.0°C. The reaction yielded a 335 bp amplicon only in the presence of the I allele, whereas no product was generated in samples that were homozygous for DD. The digested products were separated using 2% agarose (Agarose for 150–1,500 bp fragments, Nacalai Tesque Inc., Japan) gel electrophoresis, stained with ethidium bromide, and visualized under ultraviolet light. All genotyping analyses were conducted blind to the subjects' identities.

The 5-min running test

Considering the ideal duration of a judo match, a 5-min running test was conducted with each athlete as an index of their endurance ability. We measured the total distance traveled in the 5-min running [23]. This test involved three trials at 5 min intervals, and was conducted in a 400 m athletic field in the morning.

Statistical analysis

The genotype distribution was evaluated for conformity with the Hardy–Weinberg equilibrium using a chi-square test with two degrees of freedom. The genotype distribution and allele frequency were compared between the groups of judo athletes and *ACE*-controls [12-21]. using the chi-square test. Analysis of variance (ANOVA) was used to compare the performance of the different genotype groups in each test. The level of significance was set at $p<0.05$, and all the statistical analyses were conducted using the Statistical Package for the Ekuseru-Toukei 2012 (Social Survey Research Information Co. Ltd., Japan).

Results

The genotype frequencies for the *ACE I/D* polymorphism (II = 36.4%, ID = 46.1%, DD = 17.5%) were as per the Hardy–Weinberg equilibrium (p>0.05). The *ACE* genotype frequencies of the *ACE*-control subjects have been previously published. The frequencies of the *ACE I/D* polymorphism in the controls were 41.1% for II, 46.1% for ID, and 12.8% for DD. There was no significant difference between each group and the control subjects in the *ACE* genotype and allele frequency (Table 2).

There was no significant difference between each group and the control subjects in the *ACE* genotype and allele frequency (Table 3). However, the higher frequency of the D allele in the heavy categories group (45.6%) compared with the *ACE*-controls (35.8%) was close to being statistically significant (p = 0.06).

Of the 154 judo athletes, 75 undertook the 5-min running test. These included 28 with II, 30 with ID, and 17 with DD genotypes (with 2 international-level, 23 national-level, and 50 other athletes).

One-way ANOVA indicated no significant differences between the genotype groups. Moreover, one-way ANOVA indicated no significant differences between the genotype groups in the total distance covered or decrease in distance between the first and third trials (Figure 1). There were no significant differences in the distance travelled in the 5-min running of first trials among genotype groups.

Discussion

In this study, we found no association between the *ACE I/D* polymorphism and judo status or endurance in Japanese athletes. ACE is a key enzyme in the renin–angiotensin system *(RAS)* that regulates blood pressure via the production of angiotensin II and converts angiotensin I to angiotensin II. An *I/D* polymorphism in the *ACE* gene has been found to have a strong effect on circulatory and tissue ACE activity [24, 25]. The *ACE* gene, located on chromosome 17 comprises a 287 bp *I/D* polymorphism in intron 16, which results in three genotypes (II, ID, and DD) [26]. Homozygotes for the I allele (II) have significantly lower ACE activity than heterozygotes (ID), and heterozygotes have lower ACE activity than homozygotes (DD) [24]. Low ACE activity (the II genotype) results in vasodilatation. Therefore, it is believed to enhance endurance performance, and the *ACE* II genotype has been found to be predominant in elite athletes that require a high endurance, including long-distance runners [4, 7], long-distance swimmers [4], and ironman triathletes [27]. Therefore, we hypothesized that the *ACE* II genotype was associated with endurance ability in judo athletes. However, in this study, no differences were found among the *ACE* genotypes pertaining to endurance in Japanese judo athletes.

A meta-analysis study provides evidence for the strong association between the *ACE* II genotype and endurance events in Caucasian [5]. However, previous studies have reported ethnic differences of phenotype (endurance) in the role of the *ACE I/D* polymorphism between Caucasians and

Table 2. The genotype and allele frequency of the *ACE* I/D polymorphism in the different judo status groups.

Level of judo	N	Genotype frequency n (%)			p-value	Allele frequency n (%)		p-value
		II	ID	DD		I allele	D allele	
All judo athletes	154	**56** (36.4)	**71** (46.1)	**27** (17.5)	0.18	**183** (59.4)	**125** (40.6)	0.09
International + national	52	**18** (34.6)	**26** (50.0)	**8** (15.4)	0.61	**62** (59.6)	**42** (40.4)	0.33
International	16	7 (43.8)	**5** (31.3)	**4** (25.0)	0.27	**19** (59.4)	**13** (40.6)	0.57
National	36	**11** (30.6)	**21** (58.3)	**4** (11.1)	0.33	**43** (59.7)	**29** (40.3)	0.43
Others	102	**38** (37.3)	**45** (44.1)	**19** (18.6)	0.22	**121** (59.3)	**83** (40.7)	0.15
Comparison with controls was by chi-square test. Control data were made by the published study in cited reference [12-21].								
ACE-Controls	5,679	**2,336** (41.1)	2,617 (46.1)	**726** (12.8)	-	**7,289** (64.2)	4,069 (35.8)	-

Table 3. The genotype and allele frequency of the ACE I/D polymorphism in the different weight class groups.

Cumulative weight categories	N	Genotype frequency n (%)			p-value	Allele frequency n (%)		p-value
		II	ID	DD		I allele	D allele	
Light (-60, -66kg)	34	**11** (32.4)	**15** (44.1)	**8** (23.5)	0.16	**37** (54.4)	**31** (45.6)	0.09
Middle (-71*, -73, -81, -90kg)	75	**31** (41.3)	**34** (45.3)	**10** (13.3)	0.99	96 (64.0)	**54** (36.0)	0.96
Heavy (-100, +100kg)	45	**13** (28.9)	**23** (51.1)	**9** (20.0)	0.16	**49** (54.4)	**41** (45.6)	0.06
Comparison with controls was by chi-square test. Control data were made by the published study in cited reference [12-21]								
ACE-Controls	5,679	**2,336** (41.1)	**2,617** (46.1)	**726** (12.8)		**7,289** (64.2)	4,069 (35.8)	

*71 kg (according to the old regulations of sport judo)

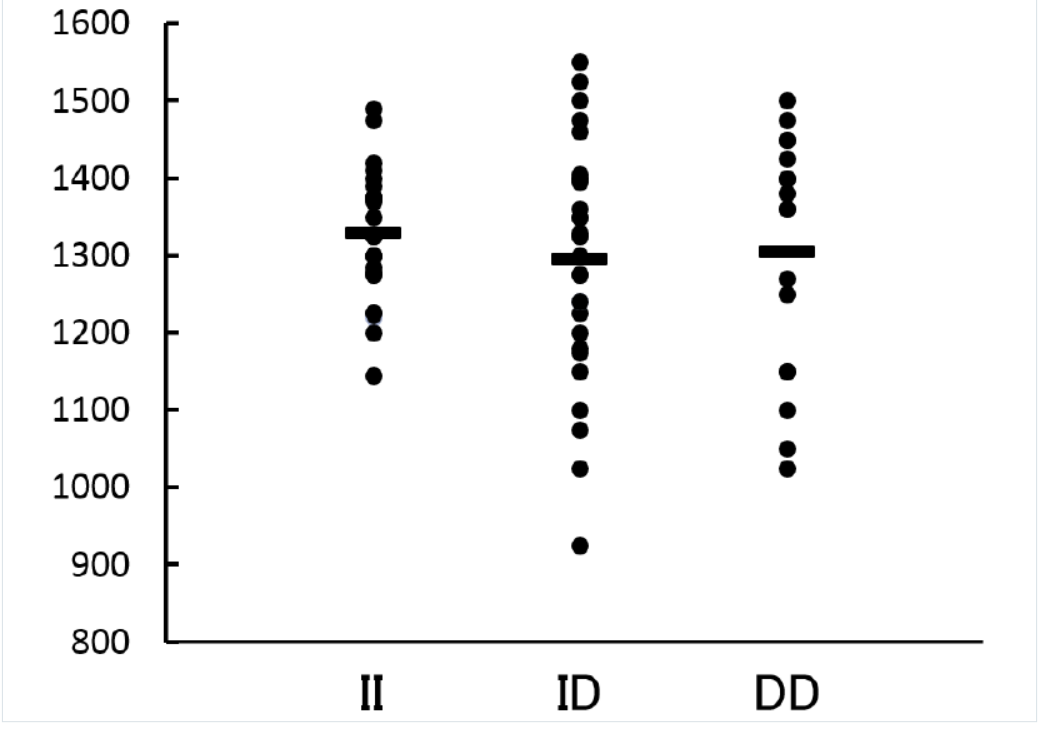

Figure 1. The distance (meters) travelled in the 5-min running of first trials according to the *ACE* genotype.

Asians [28, 29]. Tobina et al. [29] did not find any association between the *ACE* II genotype and endurance performance in Japanese elite runners. Several genetic polymorphisms have been reported to be predictive of endurance performance in elite athletes [3]. In other words, our results suggest that *ACE* polymorphism is not a genetic factor affecting endurance performance in judo athletes.

Only one previous study conducted in Poland and Lithuania reported that the *ACE* gene was associated with judo status [11]. On the contrary, our results indicated that *ACE* polymorphism was not associated with judo status. Cieszczyk et al. [11] found that elite Polish and Lithuanian male judo athletes showed a higher frequency of the I allele of the *ACE* gene than Caucasian controls (60.7% vs. 44.3%), suggesting that the I allele may be an advantage for performance in judo. In contrast, our data showed that there was no significant difference in the frequency of the *ACE I/D* polymorphism in Japanese controls compared with that of the all judo athletes. I allele frequencies of the *ACE* gene in Japanese control and all judo athletes were 64.2% and 59.4%, respectively. The

frequency of the I allele in Polish and Lithuania judo athletes (60.7%) was similar to that in the Japanese controls (64.2%) and Japanese judo athletes (59.4%). Therefore, ethnic differences between Japanese and Caucasians controls in the *ACE I/D* polymorphism may account for the discrepancy between the two results. Hence, the *ACE I/D* polymorphism could be used to predict judo status in Polish and Lithuanian, but not Japanese, judo athletes.

There was a near-significant result for the higher frequency of the D allele in the heavy weight categories athletes compared with the *ACE*-controls. To date, no study has investigated the effect of genetic polymorphisms on weight categories in sports. It is possible that the gene polymorphism may have an influence on the weight class of judo, but the mechanism responsible for this phenomenon of the *ACE I/D* polymorphism is unknown. Further studies are required to examine the association between *ACE I/D* polymorphism and body mass in athletes.

The present study had some limitations. First, the results of two of our judo status groups (international and national level) were probably limited by the relatively small sample size of each group and low statistical power. A sufficient sample size of international-level athletes would be necessary to attain robust conclusions regarding the association between genetics and sports performance. However, although the sample size of the international judo athletes in the study was small, 16 athletes in this group were winners of National Championships and the group included Olympic champions and winners of World Championships. Second, we were unable to measure general endurance in terms of maximum oxygen uptake.

Conclusions

The findings of the present study suggest that the *ACE I/D* polymorphism was not significantly associated with judo status and endurance in Japanese judo athletes. In conclusion, the *ACE I/D* polymorphism does not greatly influence the performance of Japanese judo athletes. However, the ambiguity of these results with studies of other populations judo athletes tend to the conclusion that the precise determination of the biological determinants of talent in judo is a complex phenomenon and requires further study and taking into account the specific genetic methods.

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