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ORIGINAL PAPER

Association between the angiotensin I-converting enzyme gene insertion/deletion polymorphism and endurance running speed in Japanese runners

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Abstract We investigated the association between the angiotensin I-converting enzyme (*ACE*) gene insertion (I)/deletion (D) polymorphism and endurance running performance in Japanese elite runners, including several Olympic athletes. The frequency of the I/I genotype was not significantly higher and the frequency of the D/D genotype was not significantly lower in elite runners compared with non-athletes. However, the frequency of the I/D genotype tended to be lower in elite runners than in non-athletes. The best performance was significantly higher for runners with the D/D genotype than for those with the I/I genotype, and the average running speed was significantly higher for those with the combined D/D + I/D genotypes than for those with the I/I genotype. There were no I/I genotypes among the five fastest marathon runners. These results suggest that the D allele of the *ACE* gene I/D polymorphism is associated with a high level of human endurance.

Keywords Angiotensin I-converting enzyme · Gene polymorphism · Endurance performance · Genetic factor

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Introduction

An insertion (I) or deletion (D) polymorphism in the angiotensin I-converting enzyme (*ACE*, or kininase II) gene was the first genetic factor reported to influence human physical performance [1]. The polymorphism constitutes a 287-bp insertion/deletion of the 17q23 chromosome and has been found to strongly affect circulatory [2] and tissue [3] *ACE* activity. *ACE* is a key factor in regulating blood pressure in the renin–angiotensin system via the production of angiotensin II.

Previous studies have reported that the I allele of the *ACE* gene I/D polymorphism is present at a higher frequency in British mountaineers [1], Australian and Polish rowers [4, 5], British long distance runners [6], and South African triathletes [7] than in the general population. In addition, a higher maximal oxygen consumption (aerobic capacity), VO_2 max, has been reported in postmenopausal women carrying the I/I rather than the D/D genotype [8]. Conversely, the D allele was found to be favorable for (better) performance in power-oriented sports among Russian athletes and Caucasian swimmers [9–11]. However, several studies have reported no difference in the frequency of the I allele in elite endurance athletes in Australia and Korea [12, 13]. Nazarov et al. [9] found an

association between the *ACE* gene I/D polymorphism and sporting prowess in swimmers and track and field athletes, but not in skiers or triathletes. Based on this result, these authors suggested that the association between the *ACE* gene I/D polymorphism and sporting prowess may only emerge in studies of elite athletes recruited from a single sporting discipline and that the use of subjects from mixed sporting disciplines may account for the absence of an association observed in some studies. In an earlier study, we demonstrated that individuals with the I/I genotype exhibited a significantly higher percentage of type I skeletal muscle than those with the D/D genotype. Conversely, the proportion of type IIb skeletal muscle was higher in D/D individuals [14]. These results support the notion that the I allele is favorable for enhanced performance in endurance sports.

The influence of the *ACE* gene I/D polymorphism on human physical performance has been investigated in Caucasian populations and people of African descent. In the study reported here, our aim was to identify the effect of this gene polymorphism on endurance performance in Asian people by comparing endurance performance (i.e., running speed) between genotypes.

Materials and methods

Ethical approval

This study was approved by the Ethics committee of Fukuoka University School of Medicine, and written informed consent was obtained from all subjects prior to participation.

Subjects

Thirty-seven elite long distance (over 5,000 m) runners and 335 (non-athlete) control subjects were recruited for this study. All subjects were Japanese males. The elite runners were selected by the Japanese Amateur Athletics Federation. The runners performed at the national or international level, and the group included an Olympic medalist and world record holder.

Genotyping

Peripheral blood samples were obtained from all subjects. DNA was extracted from white blood cells. The *ACE* gene I/D polymorphism was genotyped by PCR amplification of the relevant fragment of intron 16 of the *ACE* gene. The PCR products, 490 and 290 bp, respectively, were then separated by electrophoresis in 1.5% agarose gels and visualized under UV light after ethidium bromide staining [14, 15].

Endurance running performance

The best performance of each runner were obtained from a yearbook published by the Japanese Amateur Athletics Federation. For 36 runners, there was a best performance record for a 5,000 m race, and for 12 of the 37 runners, there was also a best performance record for a marathon (one elite runner had only a marathon record). The running speeds (m s^{-1}) in the 5,000 m race and the marathon were calculated from the athletes' best performances to compare endurance performance between the different genotypes.

Statistical analysis

The distributions of the I/I, I/D, and D/D genotypes in elite runners and non-athletes were compared using chi-square tests, and the odd ratios (OR) were calculated. Running speed was compared using Tukey–Kramer tests and unpaired *t* tests. Because the group of elite runners who had a best performance marathon record was very small, we used Scheffe's test to compare the running speed. Additionally, Spearman rank correlation coefficients were used for the trend analysis of the association between marathon running speed and the *ACE* gene I/D polymorphism. A p value of <0.05 was considered to be statistically significant.

Results

The characteristics of the subjects enrolled in the study are shown in Table 1. There was no significant age difference among the genotypes in the elite runner or the non-athlete groups. Since world records are continuously improving, the relative significance of an athlete's best record changes over time. The fact that the ages of the groups were not significantly different indicates that the athletes of each group were likely to have been around the same age when

Table 1 Characteristics of the subjects

Study cohort	Genotype				
	I/I	I/D	D/D	I/I + I/D	D/D + I/D
Elite runners					
n	19	10	8	29	18
Age (years)	42 ± 10	42 ± 7	40 ± 5	42 ± 9	41 ± 6
Non-athletes					
n	155	146	34	301	180
Age (years)	63 ± 18	63 ± 18	65 ± 17	63 ± 18	65 ± 17

n Number of subjects, *ACE* angiotensin I-converting enzyme gene, *I* insertion, *D* deletion

Values are shown as mean ± standard deviation (SD)

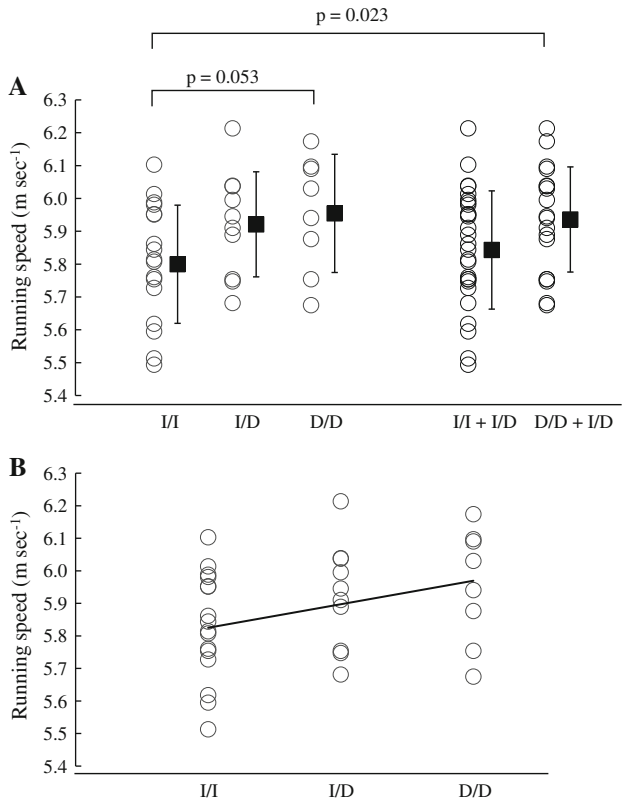

Fig. 1 Running speed, calculated on the basis of each athlete's best performance record in a 5,000 m race, was higher in individuals with the D/D or I/D genotype than in those with the I/I genotype (unpaired *t* test $p = 0.023$). The running speed of D/D individuals tended to be higher than that of I/I individuals (Tukey–Kramer $p = 0.053$). A linear trend was observed between genotype and 5,000 m running speed (Spearman rank correlation coefficients $r = 0.345$; $p = 0.042$). Data are shown as the mean and standard deviation (SD)

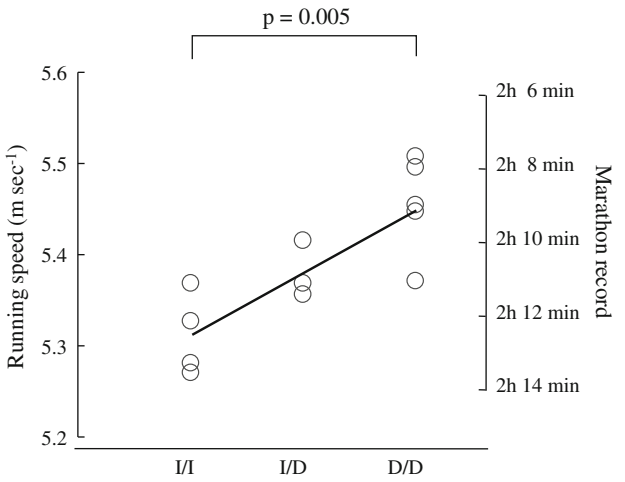

Fig. 2 The marathon running speed (m s^{-1}) for each genotype was 5.31 (0.08) (median and interquartile range), 5.37 (0.05), and 5.46 (0.07) m s^{-1} for the I/I, I/D, and D/D genotypes, respectively. Individuals with the D/D genotype had a significantly faster running speed than those with the I/I genotype (Scheffe's test $p = 0.005$). A linear trend was observed between the genotype and marathon running speed (Spearman rank correlation coefficients $r = 0.833$, $p = 0.006$). The five marathon runners who ran faster than 5.41 m s^{-1} (i.e., completing the marathon within 2 h 10 min) all exhibited the D/D or I/D genotype (D/D, 4; I/D, 1)

they set their records. The distribution of the *ACE* gene I/D polymorphism was in Hardy–Weinberg equilibrium in non-athletes [the expected genotype frequencies were: I/I, 0.46; I/D, 0.44; D/D, 0.10; $\chi^2 = 0.01$, $df = 2$, not significant (NS)], but not in elite runners (expected genotype frequencies were: I/I, 0.42; I/D, 0.46; D/D, 0.12; $\chi^2 = 16.57$, $df = 2$, $p < 0.001$).

The distribution of genotypes of the *ACE* gene I/D polymorphism was significantly different between elite runners and non-athletes (elite runners: I/I, 0.51; I/D, 0.27; D/D, 0.22; male non-athletes: I/I, 0.46; I/D, 0.44; D/D, 0.10; $\chi^2 = 19.81$, $df = 2$, $p < 0.001$). The allelic frequency was similar in both groups (elite runners: I/I + I/D, 0.65; D/D + I/D, 0.35; non-athletes: I/I + I/D, 0.68; D/D + I/D, 0.32; $\chi^2 = 0.003$, $df = 1$, NS). The frequency of the I/I genotype was not significantly higher (OR 1.23, 95% CI 0.62–2.42, NS), and the frequency of the D/D genotype was not significantly lower (OR 1.63, 95% CI 0.69–3.87, NS) in elite runners than in non-athletes. In contrast, the frequency of the I/D genotype tended to be lower in elite runners than in the non-athletes (OR 0.48, 95% CI 0.23–1.02, $p = 0.055$).

We observed a trend towards a faster 5,000 m running speed in individuals with the D/D genotype than in those with the I/I genotype, although the difference was not significant ($p = 0.053$). However, combined D/D + I/D individuals ran, on average, significantly faster than I/I individuals ($p = 0.023$; Fig. 1a), and a significant linear trend was observed for this genotype and the 5,000 m running speed ($r = 0.345$, $p = 0.042$; Fig. 1b). The marathon running speed (m s^{-1}) for each genotype was calculated to be 5.31 (0.08) (median and interquartile range), 5.37 (0.05), and 5.46 (0.07) m s^{-1} for the I/I, I/D, and D/D genotypes, respectively (Fig. 2). Individuals with the D/D genotype had a significantly faster running speed than those with the I/I genotype ($p = 0.005$). A linear relationship was also observed between genotype and marathon running speed ($r = 0.833$, $p = 0.006$). The five marathon runners who completed the marathon within 2 h 10 min all exhibited either the D/D or I/D genotype (D/D, 4; I/D, 1; Fig. 2).

Discussion

The study reported here is, to the best of our knowledge, the first investigation on whether the *ACE* gene I/D polymorphism influences endurance running performance in a Japanese population. We also compared running performance among the

genotypes, and our analysis of the results revealed that the D allele was significantly associated with higher endurance performance.

A number of studies have found a disproportionately high frequency of the I allele of the *ACE* gene I/D polymorphism among elite mountaineers [1] and endurance athletes [1, 4–7, 16, 17] compared with the general population. In contrast, no association between the *ACE* gene I/D polymorphism and human physical performance was found in a number of large cohort studies [12, 18, 19]. Nazarov et al. [9] suggested that the inclusion of subjects from multiple sporting disciplines within a single study design may remove the association between the *ACE* gene I/D polymorphism and physical performance. Consequently, in our study of a Japanese sample of elite athletes, we recruited only endurance runners (and controls) to enable a simple comparison of endurance performance.

The distributions of the *ACE* gene genotypes were not in Hardy–Weinberg equilibrium in the elite runners enrolled in our study; however, the allelic frequency was similar to that in the control group of non-athletes. In addition, the frequency of the I/D genotype tended to be lower in the elite runners than in the non-athletes (OR 0.48, 95% CI 0.23–1.02, $p = 0.055$). These results demonstrate that the frequency of the *ACE* gene I/D polymorphism in elite runners was bimodally distributed in our sample. As such, we suggest that the D/D is also favorable to human endurance performance.

Surprisingly, we found that endurance performance was better in D/D + I/D individuals than in I/I individuals. Furthermore, all elite runners who had completed marathons within 2 h 10 min were either D/D or I/D. Only about 50 Japanese runners before 2009 and 15 runners in the last 5 years have completed marathons within 2 h and 10 min. In addition, only one Japanese runner has run a marathon within this time in 2009. The result that the most outstanding runners in our sample were D/D or I/D suggests that the D allele is favorable for human endurance performance.

Interestingly, Amir et al. [20] also suggested that the D allele was more prevalent in Israeli elite endurance athletes, although Zoossmann-Diskin [21] subsequently proposed that the Jewish populations of Israel come from many countries and that the relatively distinct gene pools may have affected the results of Amir et al.'s study. Because all participants in our study were Japanese, our results were not affected by ethnicity bias. It remains unclear just why the D allele may be associated with higher endurance performance, but our results suggest that this allele may confer some advantage for endurance running. Consequently, we present several speculative possibilities.

One possibility is that the D allele is related to better cardiac function during exercise. Previous studies have suggested that the D allele is strongly associated with exercise-induced left ventricular (LV) growth [22–25]. Greater LV wall thickness and LV mass may contribute to high endurance running performance due to higher cardiac function during exercise. In accordance with this possibility, the results of our previous study demonstrated that exercise-induced improvement in cardiac function was greater in individuals with the D/D or I/D genotype than in those with the I/I genotype [26].

Second, it may be that the D/D genotype confers less stress during exercise than the I/I genotype. In a previous study, we examined the I/D polymorphism in relation to the double product (DP) [27], which is the product of heart rate and systolic blood pressure and a commonly used index of myocardial oxygen consumption during exercise [28]. We found that at the lactate threshold, the DP was lower in D/D individuals [27]. Rankinen et al. [18] showed that D/D homozygotes have a lower blood lactate level during exercise at 60 and 80% maximum oxygen consumption than I/I homozygotes. Based on the results of our previous study, we were also able to determine that the blood lactate concentration during exercise tended to be lower in D/D individuals than in I/I individuals [29]. High blood lactic acid levels have been reported to cause a decreased power output. The D/D genotype may therefore prevent a decrease in power output due to lower blood lactic acid concentration during exercise. In this way, D/D individuals may be able to perform exercise with lower stress than I/I individuals.

Third, the D allele may reduce body weight gain caused by physical training. The I allele is associated with greater fat storage during physical training [30]. Fat is an important energy source for skeletal muscle; however, the majority of elite long distance runners have a low percentage of body fat mass. A heavier body mass consumes more energy with movement, so a low body fat mass is efficient for endurance running.

Finally, *ACE* may affect energy supply and fatigue. Bradykinin is one of the mediators that increases glucose uptake in skeletal muscle due to GLUT4 translocation [31]. On the other hand, bradykinin is inactivated by *ACE*. Long distance running, such as completing a marathon, requires a great deal of glucose in the skeletal muscles and brain, particularly in the latter since glucose is the only energy source for the brain. Hypoglycemia due to the depletion of glucose causes central fatigue and a decreased power output [32]. The D allele, which is associated with higher *ACE* activity, may not facilitate glucose uptake in skeletal muscle as well as the I allele, which would be advantageous in conserving glucose and preventing central fatigue due to hypoglycemia.

The main limitation of the current study is the small number of elite runners. Although these athletes were

reliable elite-level runners, as attested by their running performance, the statistical power of the study is relatively low.

In conclusion, our results suggest that the I allele of the *ACE* gene I/D polymorphism is not a strong genetic factor affecting human endurance performance in Japanese elite runners. However, the D allele of the *ACE* gene I/D polymorphism appears to be associated with enhanced performance in endurance running. Further studies are required to understand the mechanism of action involved. Because this study focused only on Japanese elite runners, future studies are also required to test whether this association is also present in non-athletes.

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