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ORIGINAL ARTICLE

The angiotensin I-converting enzyme I/D gene polymorphism in well-trained Malaysian athletes

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Abstract
Purpose The purpose of this study was to examine the effect of the angiotensin I-converting enzyme (ACE) I/D gene polymorphism on athletic status and physical performance of well-trained Malaysian athletes.
Methods 180 well-trained athletes (34 endurance, 41 strength/power, and 105 intermittent athletes) and 180 sedentary controls involved in the study. A sample of DNA was retrieved via buccal cell from each subject and the polymorphism was then identified through Polymerase Chain Reaction. The endurance performance and leg strength of athletes were evaluated with twenty meters Yo–Yo intermittent recovery level 2 and maximal voluntary contraction tests, respectively. Chi-square test and one-way ANOVA were used for data analysis.
Results The II genotype was more prevalent among the endurance athletes (0.38) compared to the intermittent athletes (0.14), strength/power athletes (0.05), and controls (0.31) ($p = 0.00$). Conversely, the DD genotype was more prevalent among the strength/power athletes (0.66) compared to the endurance athletes (0.26), intermittent athletes (0.47), and controls (0.15). The endurance performance was not significantly associated with ACE genotype in the athletes ($p = 0.828$). However, athletes with the DD genotype had a better result for leg strength (113.8 ± 36.2) compared to those with the II (96.2 ± 28.0) and the ID (112.2 ± 33.5) genotypes ($p = 0.047$).
Conclusion This study reaffirms previous finding reported in Caucasian samples for the association of I and D alleles with endurance and strength/power performance, respectively. The finding of this study highlights the importance of genetic screening in identifying future sporting talents.

Keywords ACE · Insertion/deletion · Athletic performance · Malaysian

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Introduction

Growing evidence supports the significance of angiotensin I-converting enzyme (ACE) I/D gene polymorphism in athletic performance [1–5]. The I allele is overrepresented in elite distance runners [6–10], rowers [11, 12], triathletes [10, 13], long distance swimmers [10, 14], skiers [10], race walkers [10], and long distance cyclists [7, 15]. In contrast, the D allele is more common among those involved in power or strength-oriented sports, such as short distance swimmers [16–18], skiers [18], and wrestlers [15].

Physiological attributes related to improved endurance performance among individuals with the I allele include an increase in the delivery of oxygenated blood to the working muscles [19], greater cardiac output [20], and a higher maximal oxygen uptake [1, 21] compared to those with the D allele. The D allele is associated with a higher left ventricular mass [21–23], greater grip strength [17], muscle hypertrophy [24], and low risk for developing muscle damage [25]. The renin angiotensin system (RAS) may be the underlying mechanism responsible for these physiological attributes [26]. Rigat et al. [19] reported that the serum ACE concentration in the RAS was lowest among individuals with I alleles and highest in individuals with D alleles. Because the lower level of serum ACE decreased the production of angiotensin II (ANG II), a potent vasodepressor and aldosterone-stimulating peptide [27], an individual with two copies of the I allele may have a greater endurance capacity compared to those with two copies of the D allele and the resulting increased delivery of oxygenated blood to the working muscles [19]. Conversely, greater production of ANG II, which is a growth factor necessary for the hypertrophy of skeletal muscle, in the D allele carrier may confer an advantage for the short duration and high intensity activity that characterizes strength/power-oriented sports performance [14, 28].

To date, the association between superior endurance and strength performance and the ACE I/D gene polymorphism has mostly been reported in Caucasians [2–4], and limited observations have been made among Asians [1]. Within this limited data set, a distinct difference in the ACE I/D polymorphism between Asians and Caucasians was observed [29–31]; the prevalence of the I allele was found to be more frequent among various Asians populations [29, 30, 32] compared to Caucasians [33–36]. This finding raises the question of whether the effect of ACE I/D gene polymorphism on athletic performance that previously reported for Caucasians athletes would also appear in Malaysian athletes. Hence, this study was designed to explore the association between the ACE I/D gene polymorphism and athletic status and physical performance of well-trained Malaysian athletes.

Methods and subjects

Subjects

This case control, cross-sectional study included 180 varsity athletes (148 male, 32 female), who represent the university in sporting competition, aged 20 ± 2 (mean ± standard deviation) years and 180 sedentary healthy individuals (70 male, 110 female) aged 20 ± 2 years. All subjects were university students from several universities in Malaysia. Within the cohort of athletes, 34 subjects were classified as endurance athletes, 41 subjects as strength/power athletes, and 105 subjects as intermittent athletes according to their sport discipline. All the subjects in the athlete and control groups were Malaysian Asians, with Malay (55 %), Chinese (24.7 %), Other Bumiputras (12.9 %), and Indian (7.4 %) descent in each group. Subjects who reported of having mixed ancestry within three generations were excluded from this study. The study was approved by the Universiti Sains Malaysia Human Ethics Committee.

Sample collection

After obtaining written consent from the subjects, they were interviewed to obtain their personal information, including gender, age, ethnicity, and health status. Subject's body height was measured using a portable stadiometer (Seca 213, Seca Corporation, USA). Meanwhile, subject's body weight, body mass index, and body fat, were measured using an Omron KARADA Scan Body Composition & Scale (HBF-362, Omron Corporation, Japan). DNA samples were obtained through buccal swab using a sterile swab applicator (Classic Swabs by Copan Flock Technologies, Italy). The swabs were placed in sterile 1.5-ml microcentrifuge tubes and stored at −20 °C until used for DNA isolation.

Physical tests

Subsequent to DNA sampling, two physical tests were administered to participants in the athlete group (Yo–Yo intermittent recovery level 2 and leg strength tests) to determine their endurance and strength/power performance. The reliability of Yo–Yo intermittent recovery level 2 test was validated via maximal test-modified Bruce protocol test. Meanwhile, the isometric leg strength test was validated via one-repetition maximum (1-RM) leg extension test.

Yo–Yo intermittent recovery level 2 test

In this test, the athletes started out shuttling from one end of the marked course to the other at a relatively slow pace and then quickly ramped up their speed according to the pace set by the recorded beeps. In each bout of intense running, they performed 10 s of active recovery and then returned to the start/finish line to await the cue for the next stage. A warning was given when they did not complete a successful out and back shuttle within the allocated time. The last speed level and number of shuttles reached before they received a second warning or voluntarily withdrew from the test was recorded as the score for the test. The endurance capacity of the athlete was computed by converting the score to the total distance covered using standard norm for Yo–Yo intermittent recovery level 2 test [37].

Leg strength test

Isometric leg strength was measured using a back-leg-chest dynamometer (Takei A5402, Takei Scientific Instruments

Co. Ltd., Japan). The dynamometer was calibrated according to manufacturer's instruction. Prior to starting test, the dial on the dynamometer was reset to zero. The athletes stood upright with both feet on the base of the dynamometer. The chain length was adjusted until the athlete's knees bent around 110°. In this position, the athletes pulled the handle bar as hard as possible for 5 s, and the maximum reading indicated on the dynamometer was recorded as the score for isometric leg strength. Each athlete performed the test three times with a pause of about 10–20 s between each trial and the average score was used for data analysis.

Genotype determination

Genomic DNA was isolated from the swab samples using the GeneAllRExgeneTM Cell SV kit following the manufacturer's protocol (GeneAll Biotechnology Co. Ltd, Korea). Polymerase chain reaction (PCR) was carried out in a final volume of 25 µl consisting of 2.5 µl of 10X standard reaction buffer (GeneAll Biotechnology) (25 mM Mg^{2+}, 50 mM Tris–HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 50 % glycerol), 2.0 µl of dNTP mix (200 µM each dNTP (dATP, dCTP, dGTP, dDTP)), 0.8 µM of each primer (forward primer: 5′-CTGGAGACCACTC CCATCCTTTCT-3′: reverse primer: 5′-CTGGAGACCA CTCCCATCCTTTCT-3′), 0.5 units of *Taq* DNA polymerase, 2.5 µl of dimethylsulfoxide, 10.8 µl of sterilize distilled water, and 5 µl of genomic DNA. The target fragment bearing the ACE I/D polymorphism was amplified under the following conditions; 7 min at 95 °C followed by 25 cycles of 30 s at 95 °C, 30 s at 62 °C, and 1 min at 72 °C, with a final step of 7 min at 72 °C. The amplified products were electrophoresed on a 1.5 % agarose gel that was prestained with ethidium bromide at 70 volts for 1 h. The presence of 490 and 190 base pair bands indicated the ACE insertion (I) and deletion (D) alleles, respectively.

Statistical analysis

The descriptive data are presented as mean ± standard deviation (SD). ACE I/D allele frequency was determined by direct counting. A Chi-square (χ^2) test was used to confirm that the observed ACE I/D genotype frequency was in Hardy–Weinberg equilibrium for the athlete and controls groups. The χ^2 test was also used to examine the difference in the ACE I/D allele and genotype frequencies between the whole cohort of athletes and controls as well as between different groups of athletes and controls. The mean of total distance covered during the Yo–Yo intermittent recovery level 2 test and leg strength scores were compared between the ACE genotype groups by one-way analysis of variance (ANOVA) and followed by Bonferroni post hoc test when appropriate. All statistical evaluations were performed using the IBM SPSS statistical version 20.0, United States, with the level of significance set at $p < 0.05$.

Results

Physical characteristics of subjects

Table 1 shows the descriptive statistics for the subjects' physical characteristics. Athletes and controls were similar in age and resting heart rate ($p > 0.05$). Nevertheless, there were significant differences in other variables between the groups, with controls having lower mean values for body height, body weight, and body mass index compared to athletes ($p < 0.05$). Conversely, the mean value of body fat was higher in controls than in athletes.

Yo–Yo intermittent recovery level 2 performance differed significantly among athletes from the three different sporting disciplines. The endurance athletes had higher performance scores than the strength/power and intermittent athletes, whereas no significant difference was observed for the leg strength value among the sporting groups (Table 2).

Prevalence of ACE I/D gene polymorphism

There was a significant difference in allele ($\chi^2 = 18.776$, $df = 1$, $p < 0.05$) and genotype ($\chi^2 = 44.070$, $df = 2$, $p = 0.000$) frequencies for ACE I/D gene polymorphism between athletes and controls. We observed a higher

Table 1 Physical characteristics of the study participant

	Athletes ($n = 180$)	Controls ($n = 180$)	p value
Age (years)	20 ± 2	20 ± 2	0.309
Height (cm)	169 ± 9	161 ± 9	0.000*
Body weight (kg)	67.1 ± 13.7	56.3 ± 12.4	0.000*
Body mass index (kg/m^2)	23.4 ± 4.0	21.8 ± 3.8	0.000*
Body fat (%)	18.7 ± 6.4	21.9 ± 7.8	0.000*

Variables are expressed as mean ± SD

* Significantly different compared to controls ($p < 0.05$)

Table 2 Yo–Yo intermittent recovery level 2 performance and leg strength value in athletes from different sporting disciplines

	Endurance ($n = 34$)	Strength/power ($n = 41$)	Intermittent ($n = 105$)	p value
Yo–Yo intermittent recovery level 2 performance (m)	429.4 ± 203.8	320.8 ± 197.5	292.2 ± 303.9*	0.035
Leg strength (kg)	105.5 ± 28.2	108.9 ± 41.2	112.3 ± 33.5	0.663

Variables are expressed as mean ± SD

* Significantly different compared to the endurance athletes ($p = 0.029$)

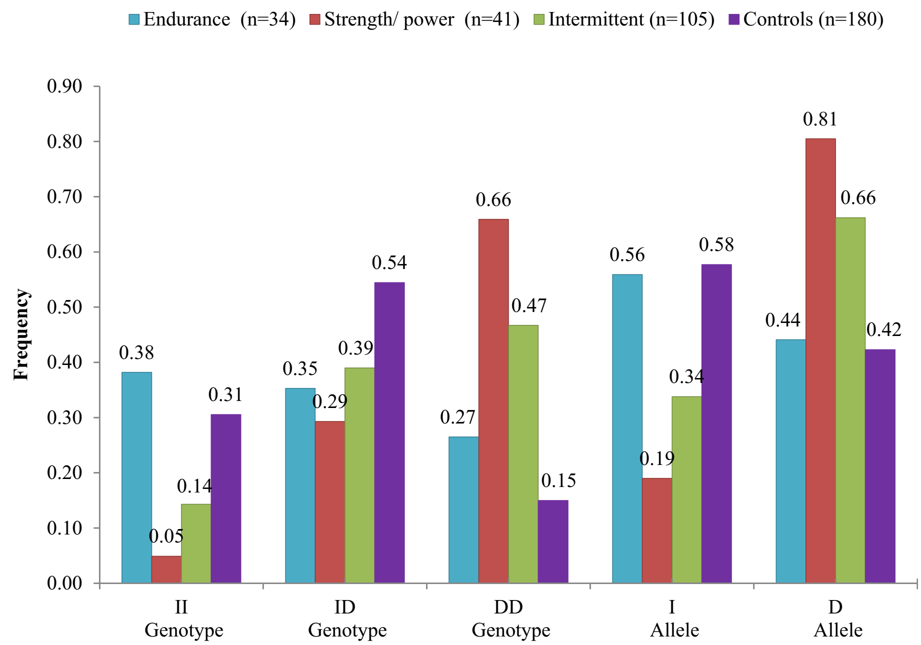

Fig. 1 Allele and genotype frequencies of ACE I/D gene polymorphism among the endurance, strength/power, intermittent, and controls groups

frequency of the I allele in the control group (0.58) compared to the athlete group (0.35). Conversely, the frequency of the D allele was higher in athletes (0.65) than in controls (0.42). A similar observation was noted in the ACE I/D genotype distribution, as the II and ID genotypes were more prevalent in the controls (II = 0.31; ID = 0.54) compared to the athletes (II = 0.17; ID = 0.36), whereas the DD genotype was more common in athletes (0.47) compared to controls (0.15). The ACE I/D genotype distribution was in Hardy–Weinberg equilibrium (Athletes: $\chi^2 = 0.8037$, $df = 1$, $p = 0.3699$; Controls: $\chi^2 = 2.4175$, $df = 1$, $p = 0.1199$).

Considering the opposing effects of the I and D alleles on particular sporting disciplines, we separated the athletes into three groups according to their sport disciplines: endurance, strength/power, and intermittent. We compared the ACE I/D allele and genotype frequencies among the three athlete groups and the controls. The ACE I/D allele ($\chi^2 = 28.71$, $df = 3$, $p < 0.0001$) and genotype ($\chi^2 = 63.4$, $df = 6$, $p < 0.0001$) frequencies differed significantly across the four groups. The endurance and strength/power groups displayed the highest prevalence of the II and DD genotypes, respectively (Fig. 1). The prevalence of the I allele was higher in the control group, and the D allele frequency was overrepresented in the strength/power group compared to other groups.

Association of ACE I/D gene polymorphism with Yo–Yo intermittent recovery level 2 performance

The Yo–Yo intermittent recovery level 2 performance was used to predict endurance performance of athletes. The performance of Yo–Yo intermittent recovery level 2 was similar among athletes with different ACE I/D genotype ($p = 0.828$).

Association of ACEI/D gene polymorphism with leg strength value

In agreement with our hypothesis that the D allele was associated with superior strength/power performance, Fig. 2 shows the significant association of leg strength performance with the ACE I/D gene polymorphism ($p = 0.047$) with the leg strength value higher in the DD

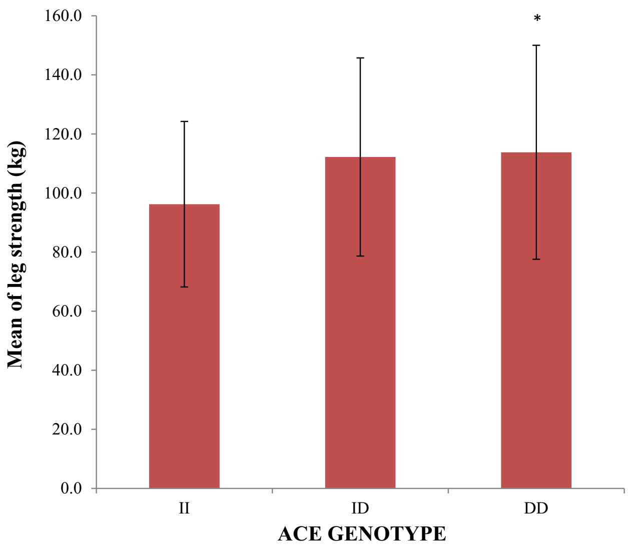

Fig. 2 Leg strength values in athletes with different ACE I/D genotypes. Data shown as mean ± SD. *II < DD, $p = 0.047$

genotype group (113.8 ± 36.2) compared with the II (96.2 ± 28.0) and the ID (112.2 ± 33.5) genotype groups.

Discussion

This study was designed to determine if the association between ACE I/D gene polymorphism and athletic performance reported in the Caucasian population is present in the Asian population. To the best of our knowledge, this is the first study to evaluate the association of ACE I/D gene polymorphism with athletic performance in Malaysia. This study revealed that the ACE I/D gene polymorphism is associated with Malaysian athletic performance as reported for the Caucasian population. The I allele and II genotype were more frequent in the endurance group compared to the strength/power and intermittent groups. Consistent with previous reports for other Asian [13, 38] and Caucasian [8, 14, 15, 39–41] populations, this finding suggests that the presence of the I allele might confer an advantage for endurance-based activities among athletes.

Our data also revealed a greater proportion of the D allele among strength/power athletes compared with the other athlete groups. This observation is notably similar to that previously reported in other Asian [42] and Caucasian [14–16, 43] samples. Moreover, we found that the D allele was significantly related to greater muscular strength, as athletes with the DD genotype exhibited greater leg strength than those with the II and the ID genotypes. Our results are in line with previous studies that found a relationship between the D allele and other muscular strength parameters such as hand grip strength [43], isometric and isokinetic quadriceps muscle strength [44], and knee extensor strength [45]. Taken together, all of these positive findings indicate that the D allele might have an advantageous effect on short duration and high intensity activities, as previously suggested [14, 28].

While this study demonstrated the occurrence of D allele among the whole cohort of athletes compared to controls, we found that the I allele and II genotype were more prevalent among controls. In contrast to previous studies reporting an excess of the I allele and II genotype in athletes compared with controls [7, 13, 46], we speculate that our cohort of athletes including athletes from different sports disciplines which is characterized with the small number of participants from endurance-oriented sports disciplines may have contributed to the failure to detect a positive association between ACE I/D variation and athletic performance. Other studies that assessed the association of ACE I/D gene variation with athletic performance in a cohort of mixed athletes [18, 47–49] reported similar findings. Given that ACE I/D gene variation might only relate to a single sporting discipline rather than to overall human performance as suggested by Nazarov et al. [18], a positive association between ACE I/D gene polymorphism and athletic performance might be detected in a homogenous range of sports disciplines.

However, interpreting our data is complicated, as no significant difference in the performance of Yo–Yo intermittent recovery level 2 was observed between the three ACE I/D genotype groups, and this parameter was used as the marker for evaluating endurance performance in athletes. Thus, we failed to replicate the finding from previous studies [1, 21] that individuals with the II genotype have better endurance performance compared to those with other ACE genotypes. We propose that this conflicting result may reflect the heterogeneity of the cohort of athletes in our study and the small number of participants from endurance-oriented sports disciplines.

The level of ANG II appears to be the most likely mechanism relating the I and D alleles with improvement in endurance and strength/power performance, respectively. The lower production of ANG II that results from ACE inhibition coded by the I allele results in less vasoconstriction, which in turn leads to increased delivery of oxygenated blood to the working muscles [50]. In contrast, the greater production of ANG II in D allele carriers likely assists with muscle contraction for maximal power [51]. Because our study was observational, future experimental studies are needed to elucidate the biological mechanism responsible for the association between ACE I/D gene polymorphism and improved athletic performance. In addition, further studies using a large cohort of the homogenous Asian population are needed to address the possible influence of genetic factors on athletic performance and to bridge the gap in the literature.

Conclusion

The results of this study support the notion that ACE I/D gene polymorphism is relevant to an athlete's status and physical performance. These preliminary data illustrate the importance of understanding the genetic makeup of Malaysian athletes and the relationship between genetics and performance.

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Conflict of interest There is no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the Human Research Ethics Committee, Universiti Sains Malaysia.

Informed consent Informed consent was obtained from all individual participants included in the study.

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