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Research article

The Angiotensin Converting Enzyme (ACE) I/D Gene Polymorphism In Malaysian Well Trained Athletes.

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Abstract

*Journal of Sports Science and Physical Education 2(*1): 20–33, 2014 - The purpose of this study was to examine the effect of the ACE I/D gene polymorphism on athletic status and physical performance of well-trained Malaysian athletes. The distribution of ACE I/D gene polymorphism among 180 well trained athletes was compared with 180 sedentary controls. 20 meter Yo-Yo intermittent recovery and leg strength tests were used to measure maximal oxygen consumption (VO_2max) and leg strength value of athletes with different ACE I/D genotype, respectively. Chi-Square and one way ANOVA tests were used for data analysis. The II and DD genotype were more prevalent among the endurance athletes and the strength/ power athletes compared to the other groups, respectively (p=0.00). The VO_2max was not significantly associated with ACE genotype in athlete (p=0.828). However, athletes with the DD genotype had recorded a greater result for leg strength (113.8 ± 36.2) than those with the II (96.2 ± 28.0) and the ID (112.2 ± 33.5) genotype (p=0.047). This study supports the notion that ACE I/D gene polymorphism might be a genetic factor associated with athletic status and strength performance among the Malaysian population. Future studies with more representation of endurance athletes might able to detect the association between I allele and endurance performance.

Keywords: ACE I/D gene polymorphism; Endurance; Strength/ Power, Athletic status, Physical performance

Introduction

There is a growing evidence demonstrating the significance of Angiotensin Converting Enzyme (ACE) I/D gene polymorphism in athletic performance (Cam, Colakoglu, Colakoglu, Sekuri, & Berdeli, 2007; Goh et al., 2009; Ma et al., 2013; Shleptsova et al., 2008; Voroshin & Astratenkova, 2008a). The I allele has been shown to be over presented in elite distance runners (Alvarez et al., 2000a; Holdys, Kryściak, Stanisławski, & Gronek, 2011; Hruskovicova et al., 2006; Myerson et al., 1999; Oh, 2007), rowers (Cieszczyk, Krupecki, Maciejewska, & Sawczuk, 2009b; Gayagay et al., 1998), triathletes (Collins et al., 2004a; Holdys, et al., 2011; Shenoy, Tandon, Sandhu, & Bhanwer, 2010), long-distance swimmers (Holdys, et al., 2011;

Tsianos et al., 2004a), skiers (Holdys, et al., 2011), race walkers (Holdys, et al., 2011) and long distance cyclists (Alvarez, et al., 2000a; Paulauskas, Danileviciutė, Povilaitis, & Poderis, 2009). Contradict to the I allele, the D allele observed to be more common among those involved with power or strength-oriented sports such as short distance swimmers (Aldo Matos Costa et al., 2009b; Nazarov et al., 2001b; Woods et al., 2001b), skiers (Nazarov, et al., 2001b) and wrestlers (Paulauskas, et al., 2009).

Physiological attributes related to the improvement in endurance performance among individuals with I allele includes an increase in the delivery of oxygenated blood to the working muscles (Rigat et al., 1990), a greater cardiac output (Hagberg et al., 2002) and a higher maximal oxygen uptake (Goh, et al., 2009; Kasikcioglu et al., 2004) compared to those with the D allele. Meanwhile, the D allele is associated with a higher left ventricular mass (Hernández et al., 2003; Kasikcioglu, et al., 2004; Montgomery et al., 1997), a greater grip strength (Aldo Matos Costa et al., 2009a), muscle hypertrophy (Charbonneau, 2007) and low risk for developing muscle damage (Yamin et al., 2007). The Renin Angiotensin System (RAS) has been suggested as the underlying mechanism responsible for these physiological attributes (Ng, 2009). The plasma ACE serum in the RAS was found lowest among individuals with I alleles and highest in individuals with D alleles (Rigat, et al., 1990). Given that the level of plasma ACE serum reflects to the production of Angiotensin II (ANG II), a potent vasospressor and aldosterone stimulating peptide (Brewster & Perazella, 2004), the individual with two copies of I allele was proposed to have a greater endurance capacity compared to those with two copies of D allele due to the less production of ANG II which resulted to increase delivery of oxygenated blood to the working muscles (Rigat, et al., 1990). Conversely, the greater production of ANG II which is a growth factor necessary for the hypertrophy of skeletal muscle in the D allele carrier may confer advantage in short duration and higher intensity activity that reflects on strength/ power-oriented sport performance (Folland et al., 2000; Tsianos et al., 2004b).

The association of superior endurance and strength performance with the ACE I/D gene polymorphism were mostly reported in Caucasians (Cam, et al., 2007; Shleptsova, et al., 2008; Voroshin & Astratenkova, 2008a) with limited observation were performed among Asians (Goh, et al., 2009). Within this limited data set, a distinct difference in the frequency of ACE I/D polymorphism between Asians and Caucasians populations were observed (Jayapalan, Muniandy, & Chan, 2008; Melton et al., 1995; Movva et al., 2007) in which the prevalence of I allele is more frequent among various Asians populations (Jayapalan, et al., 2008; Melton, et al., 1995; Nitiyanant et al., 1997; Yoshida et al., 1995) compared to Caucasians (Batzer et al., 1996; Pereira, Mota, Bensenor, Lotufo, & Krieger, 2001; Salem, 2008; Sprovieri & Sens, 2005).

This raises the question of whether ACE I/D gene polymorphism among the Asians populations will provide a boost in athletic performance as previously reported among the Caucasians athletes. Hence, this study was designed to explore the association of the Angiotensin Converting Enzyme (ACE) I/D gene polymorphism on athletic status and physical performance of well-trained Malaysian athletes.

Methods and Subjects

Subjects

This study involved a case control and cross sectional study which comprised of 180 varsity athletes (148 male, 31 female) aged 20 ± 2 (mean ± standard deviation) years old and 180 sedentary healthy individuals (70

male, 110 female) aged 20 ± 2 (mean ± standard deviation) years old. All the subjects were university students from several universities in Malaysia. The In the whole cohort of athletes, 34 subjects were classified as the endurance athletes, 41 subjects as the strength/ power athletes and 101 subjects as the intermittent athletes. Given that Malaysia is a multicultural country, the proportion of subjects in athletes and controls groups has been set according to the ratio of four major ethnics in Malaysia as follow: Malay (55%), Chinese (24.7%), Bumiputera (12.9%) and India (7.4%). All subjects were defined as purely ethnic origin indicated by having non-mixed ancestry for at least three generations. The study was approved by the Universiti Sains Malaysia Human Ethnics Committee, Malaysia.

Sample collection

After obtaining written consent from the subjects, they were shortly interviewed and assessed in order to obtain their personal information, including gender, age, ethnicity and health status as well as anthropometric data such as body weight, body height, body mass index and body fat. Then, the DNA samples were obtained through buccal swab by using a sterile swab applicator (Classic Swabs by Copan Flock Tehnologies). The swabs were then placed in a sterile 1.5 ml micro centrifuge tubes and stored at -20°C until ready for DNA isolation.

Physical Test

Subsequent to DNA sampling, two physical tests were administered to athletes group; Yo-Yo intermittent recovery level 2 and leg strength tests, in order to determine their endurance and strength/ power performance.

Yo-Yo intermittent recovery level 2 test:

The athlete was started out shuttling from one end of the marked course to the other at a relatively slow pace and then quickly ramp their speed according to the pace set by the beeps. In each bout of intense running, they performed 10 seconds of active recovery and should return to the start/ finish line and await cue or beeps for the next stage. A warning was given when they do not complete a successful out and back shuttle at the allocated time. The last level and number of shuttles reached before they received a second warning or voluntarily withdraws from the test was recorded as a score for the test. The maximal oxygen consumption of the athlete was computed by converting the score to VO_2max equivalent score using a standard norm for Yo-Yo intermittent recovery level 2 tests.

Leg strength test

For the leg strength test, a Back-Leg-Chest dynamometer was used in this study. The athletes stand upright with both feet on the base of the dynamometer. The chain length was adjusted to accommodate the test. The athletes pull as hard as possible on the chain and the maximum reading indicated on the dynamometer was recorded as the score for the leg strength. They performed the test for three trials with a pause of about 10-20 seconds between each trial and the average score was taken for data analysis.

Genotype determination

The genomic DNA was isolated from the swab samples using the GeneAllR ExgeneTM Cell SV kit following the manufacturer's protocol (GeneAll Biotechnology Co.Ltd). Polymerase chain reaction (PCR) was carried out in a final volume of 25 µl consisting of 2.5 ul of 10 X Standard Reaction Buffer (GeneAll Biotechnology Co.Ltd) (25 mM Mg2+, 50 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 50% glycerol),

2.0 ul of dNTP mix (200 µM each dNTPs (dATP, dCTP, dGTP, dDTP)), 0.8 µM of each primer (Forward primer: 5'-CTGGAGACCACTCCCATCCTTTCT-3': Reverse Primer: 5'-CTGGAGACCACTCCCATCCTTTCT-3'), 0.5 units of *Taq* DNA Polymerase, 10% Dimethylsulfoxide (DMSO), 10.8 ul 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 minutes at 95°C followed by 25 cycles of 30 seconds at 95°C, 30 seconds at 62°C and 1 minute at 72°C, with a final step of 7 minutes at 72°C. The amplified products were electrophoresed on a 1.5% agarose gel that was pre-staining with ethidium bromide at 70 volts for 1 hour. The presence of a 490-bp and 190-bp bands indicated the ACE insertion (I) and deletion (D) alleles, respectively.

Statistical analysis

The descriptive data was performed as mean ± standard deviation (S.D). ACE I/D allele frequency was determined by direct counting. A chi square (X^2) test was used to confirm the observed ACE I/D genotype frequency is in Hardy-Weinberg equilibrium for athletes and controls groups. The X^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. One way ANOVA test was used to compare means of VO_2max and leg strength score among the ACE genotype groups. All statistical evaluations were done by using the IBM SPSS statistical version 20.0 with the levels of significance was set at $p<0.05$.

Results

Physical characteristics of subjects

Table 1 shows the descriptive statistics of subject's physical characteristics. Athletes and controls were observed similar in age and resting heart rate ($p>0.05$). Nevertheless, there were significant differences in other variables among groups with controls have lower mean value 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 rather than athletes.

The maximal oxygen consumption (VO_2max) value was differed significantly between athletes from three different sporting disciplines with the endurance athletes having the highest VO_2max score than the strength/ power and the intermittent athletes, whereas no significant difference was observed for leg strength value among the sporting groups (Table 2).

Table 1: Descriptive statistic of subject's physical characteristics

	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
Resting heart rate (/min)	79 ± 14	81 ± 15	0.123

Notes

Variables are expressed as mean ± SD

*Significantly different at $p<0.05$ compared to controls

Table 2: Maximal oxygen consumption (VO_2max) and leg strength value in athletes with different sporting disciplines.

	Endurance (n=34)	**Strength & Power (n=41)**	**Intermittent (n=105)**	**p value**
VO_2max (ml/kg/min)	51.1 ± 2.8	49.7 ± 2.7	49.3 ± 4.1	*0.035
Leg strength (kg)	105.5 ± 28.2	108.9 ± 41.22	112.3 ± 33.5	0.663

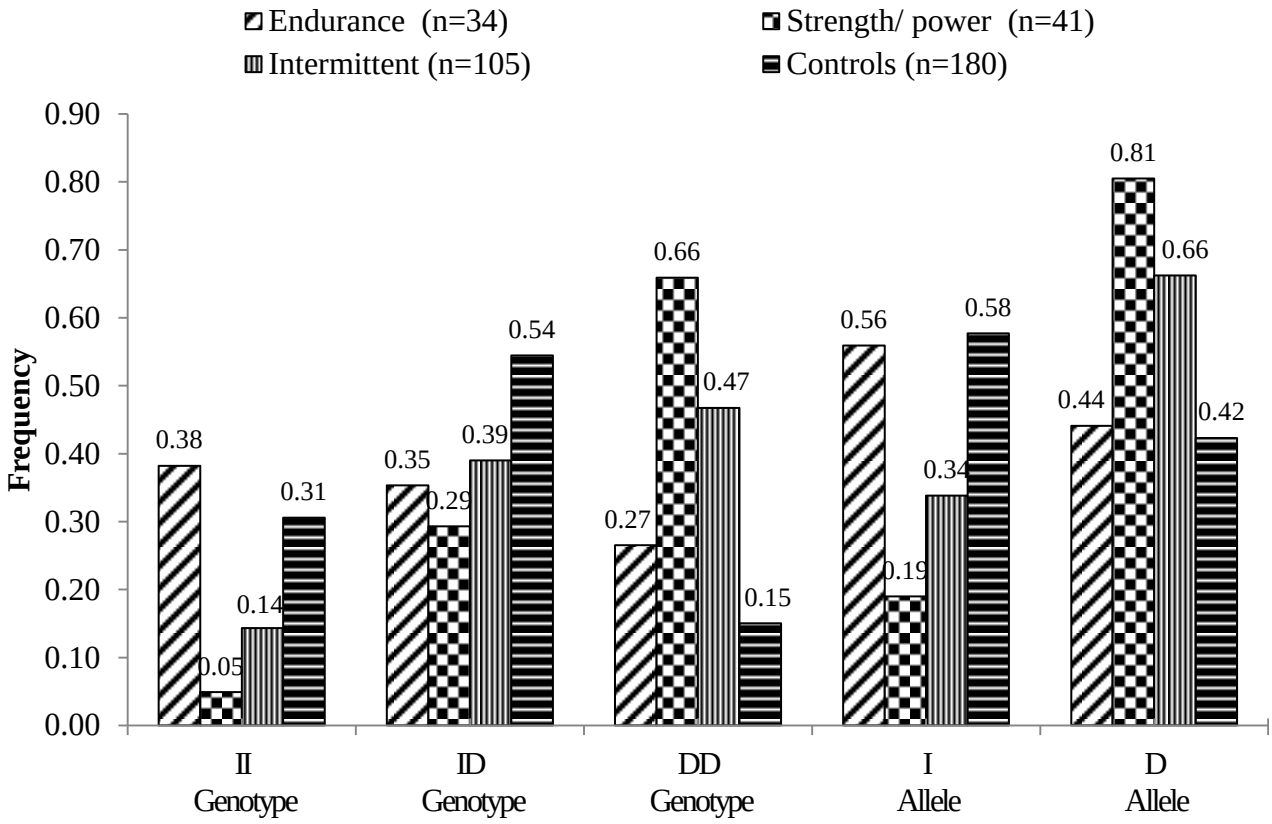
Notes
Variables are expressed as mean ± SD
*Significantly different at $p<0.05$ compared to the endurance athletes

Prevalence of ACE I/D gene polymorphism
There was a significant difference in allele ($X^2(1)=17.87$, $p<0.0001$) and genotype ($X^2(2)=44.07$, $p<0.0001$) frequencies of ACE I/D gene polymorphism between athletes and controls. However, we observed a higher frequency of I allele in the control group (0.58) than the whole cohort of athletes (0.35).

Conversely, the frequency of D allele was found higher in athletes (0.65) and lower in controls (0.42). The similar observation was noted in the ACE I/D genotype distribution as the II and ID genotypes were found more prevalent in the controls (II=0.31; ID=0.54) compared to the athletes (II=0.17; ID=0.36), while the DD genotype was shown to be more common in athletes (0.47) with less frequency in controls (0.15). The ACE I/D genotype distribution was in Hardy-Weinberg equilibrium (Athletes: $X^2(1)=0.8037$, $p=0.3699$; Controls: $X^2(1)=2.4175$, $p=0.1199$).

Considering the opposing effect of I and D allele on particular sporting disciplines, we separated the athletes into three groups according to their sport disciplines; endurance, strength/ power and intermittent, and compared the prevalence of ACE I/D allele and genotype between the three athletes groups with the controls. The ACE I/D allele ($X^2(3)=28.71$, $p<0.0001$) and genotype ($X^2(6)=63.4$, $p<0.0001$) frequency did significantly differed across the four groups. As shown in Figure 1, the endurance and the strength/ power group displayed the highest prevalence for the II and the DD genotype, respectively. Meanwhile, the prevalence of I allele was noted, remains higher in the controls group with the D allele frequency was overrepresented in the strength/ power group than other groups. The apparent skew in the ACE I/D allele distribution among the groups was noticeably reflected to a non-equivalent proportion of athletes in each sporting discipline which demonstrating low participation of endurance athletes in this study.

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



There were significant differences in ACE I/D allele ($X^2(3)=28.71$, $p<0.0001$) and genotype ($X^2(6)=63.4$, $p<0.0001$) frequency between groups.

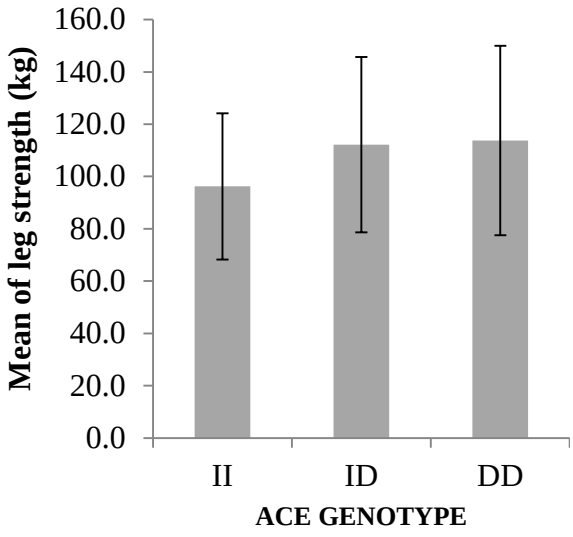
Association of ACE I/D gene polymorphism with VO_2max value and VO_2max level

The value and level of VO_2max were used to predict endurance performance of athletes. Mean of VO_2max value was similar across the ACE I/D genotype group ($p=0.828$). There was also no significant difference observed in the distribution of ACE I/D genotype among athletes with different level of VO_2max ($p=0.387$).

Association of ACE I/D gene polymorphism with leg strength value and leg strength level

Figure 2 presents the significant association of leg strength performance with the ACE I/D gene polymorphism ($p=0.047$). In concordance with our hypothesis that the D allele was associated with the superior strength/ power performance, the leg strength value was recorded higher in the DD genotype group (113.8±36.2) compared with the II (96.2±28.0) and the ID (112.2±33.5) genotype group. However, ACE I/D gene polymorphism was not associated with the level of leg strength ($p=0.173$).

Figure 2: Leg strength values in athletes with different ACE I/D genotype. Data shown as mean ± standard deviation. *II < DD, p=0.048



Discussion

As such suggested by a meta-analysis study on the association of athletic performance with ACE I/D gene polymorphism, more studies in Asians population are warranted to verify the current finding observed in the Caucasians population (Ma, et al., 2013). Our study, which includes a cohort of Malaysian athletes and controls, was designed to provide an additional evidence for the reported association of the ACE I/D gene polymorphism with athletic performance in Caucasian population. To the best of our knowledge, this is the first study reporting the association of ACE I/D gene polymorphism with athletic performance in Malaysia.

In general, this study has yielded mixed results on the association of ACE I/D gene polymorphism with athletic performance. Inconsistent with previous studies which observed the excess of I allele and II genotype amongst athletes compared with the controls (Alvarez et al., 2000b; Gineviciene, Pranculis, Jakaitiene, Milasius, & Kucinskas, 2011; Shenoy, et al., 2010), our study demonstrated that the I allele and II genotype were more prevalent among controls as compared to the whole cohort of athletes. We note that our cohort athletes that includes mixed athletes from various sports disciplines may contribute in a failure to demonstrate a positive association between ACE I/D variation and athletic performance. In accordance with other studies assessing the association of ACE I/D gene variation in a cohort of mixed athletes, (Karjalainen et al., 1999; Nazarov et al., 2001a; Rankinen et al., 2000; Taylor, Mamotte, Fallon, & van Bockxmeer, 1999), our finding confirmed that it was less likely to detect such association amongst a heterogeneous cohort of mixed athletes.

Considering that ACE I/D gene variation might only relate to a single sporting discipline not to whole human performance as suggested by (Nazarov, et al., 2001a), the whole cohort of athletes was then stratified into three groups according to their sports disciplines; endurance, strength/ power and intermittent groups. Interestingly, we then able to demonstrate a positive association between ACE I/D gene polymorphism and athletic performance with both I allele and II genotype were found to be more frequent in the endurance group compared to the strength/ power and the intermittent groups. Consistent with previous reports in other Asian (Min et al., 2009; Shenoy, et al., 2010) and Caucasian (Ahmetov et al., 2009; Cieszczyk, Krupecki, Maciejewska, & Sawczuk, 2009a; Collins et al., 2004b; Hruskovicova, et al., 2006; Mayne, 2006; Paulauskas, et al., 2009; Tsianos, et al., 2004b) populations, this finding raises the possibility that the presence of I allele might confer an advantage for endurance-based activities among athletes as previously suggested for Caucasians athletes (Thompson et al., 2006; Voroshin & Astratenkova, 2008b). However,

the interpretation of our data on the association between ACE I/D gene polymorphism with athletic performance is further complicated as no significant difference was observed between the three ACE I/D genotype groups with VO_2max parameter that was used as the marker for evaluating endurance performance in athletes. We failed to replicate the finding from previous studies (Goh, et al., 2009; Kasikcioglu, et al., 2004) that demonstrate individual with the II genotype would have high levels of VO_2max to indicate a greater endurance capacity than those with others ACE genotype. We assumed that this conflicting result may reflect to the heterogeneous mixed cohort of athletes in our study that comprises of an imbalance number of athletes from each sport discipline with a small participation from endurance-oriented event athletes.

Conversely, our data demonstrate a significant association between the D allele and strength/ power athletes' status indicating by a greater proportion of D allele among strength/ power athletes when compared with other athletic groups. This observation is notably similar to that previously reported in other Asian (Kikuchi, Min, Ueda, Igawa, & Nakazato, 2012) and Caucasian (A. M. Costa et al., 2012; Paulauskas, et al., 2009; Tsianos, et al., 2004b; Woods et al., 2001a) samples. Moreover, we found that the D allele is significantly related with a higher muscular strength as athletes with the DD genotype had recorded greater leg strength than those athletes with the II and the ID genotypes. While some studies reported no association between the D allele and muscular strength which mostly reflect to the lack of statistical power (Charbonneau, 2007; Folland, et al., 2000), our result supports the previous findings that found a relationship between the D allele and other muscular strength parameter which includes hand grip strength (A. M. Costa, et al., 2012), isometric and isokinetic quadriceps muscle strength (Williams et al., 2005) and knee extensor strength (Giaccaglia et al., 2008). Taken together, positive findings from the previous studies and the present study indicate the possibility advantageous effect of the D allele that might favor to short duration and high intensity activity as previously suggested by (Tsianos, et al., 2004b) and (Folland, et al., 2000).

Although we did not measure the mechanism underlying the association between ACE I/D gene polymorphism and athletic performance, the level of ANG II may appear as the most likely mechanism that relates I and D allele with the improvement in endurance and strength/ power performance, respectively. The less production of ANG II as the results from ACE inhibition coded by the I allele results in less vasoconstriction that lead to an increased delivery of oxygenated blood to the working muscles (Sonna et al., 2001). In contrast, the greater production of Angiotensin II in D allele carrier was assumed to assist muscle contraction for maximal power (Rattigan, Dora, Tong, & Clark, 1996). Since our study is the observational study, future experimental studies are warranted to elucidate the underlying biological mechanism of the association between ACE I/D gene polymorphism and improvement in athletic performance.

Taken all the results observed in this study, we suggest the necessity of further studies; perform in a large cohort of the homogenous Asian population to completely address the possible influence of genetic factor on athletic performance as well as to bridge the gap in the literature. It would also certainly valuable to examine and determine the potential biological mechanisms that underlying the existing association highlighted in this study between ACE I/D

gene polymorphism and athletic performance.

Conclusion/ Practical Application

Generally, this study provides some support into the notion of genetic involvement; particularly to the ACE I/D gene polymorphism as one of the potential factors that relevance with athlete's status and physical performance. This preliminary data may serve as of important mark on the importance of understanding the genetic makeup of Malaysian athletes and its relation to their performance. The present data suggest the potential of genetic screening among athletes in Malaysia to ensure that they suited to play in a particular sport based on their genetic profile that then will help speed identify the potential talent relevant to sports.

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Conflict of interest

There is no conflict of interest

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