The relationship between *ACE* gene *ID* polymorphism and aerobic capacity in Asian rugby players

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ABSTRACT

Introduction: The aim of this study was to analyse the association between the *ACE ID* polymorphism and aerobic capacity in a homogeneous cohort of national Asian rugby players.

Methods: 17 subjects recruited during active training had their maximal oxygen uptake ($V0_{2max}$) and ventilatory threshold (VT) measured during maximal exercise testing. *ACE* genotyping was performed for all players.

Results: The likelihood of having a $V0_{2max}$ above the 80th percentile of a gender-specific reference range for a normal population was 14.3-fold greater among subjects with the *II* genotype as compared to the *ID* genotype (p-value is 0.030). Similarly, subjects with the *II* genotype were 29.4 times more likely to have a VT above the gender-specific median value compared to the *ID* genotype (p-value is 0.019). The results suggest that the *I* allele confers an advantage in aerobic capacity as measured by the $V0_{2max}$ and VT.

Conclusion: It is likely that the same physiological mechanisms mediated by the *ACE* gene are responsible for aerobic capacity in both Asians and Caucasians.

Keywords: aerobic capacity, angiotensin converting enzyme, exercise physiology, gene polymorphism

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INTRODUCTION

Rugby is a high-speed contact sport that involves aerobic and anaerobic fitness, as well as muscle strength and endurance. There is a combination of both low- and high-intensity activities, and a typical international game would involve running that covers 5–8 km with speeds of 18–20 km/h.[1] As with many sports, the assessment of aerobic fitness is important, and the ability to predict high aerobic capacity can be advantageous. Aerobic fitness is a state of optimal cardiorespiratory performance as it relates to coronary heart disease prevention; and maximal oxygen uptake ($V0_{2max}$) has been chosen by the World Health Organization as a reference standard of cardiorespiratory fitness and aerobic capacity since 1967, and has been widely used thereafter.[2,3] Another common measure of endurance capacity is the anaerobic or lactate threshold. Its surrogate marker is the ventilatory threshold (VT), which is defined as the oxygen uptake ($V0_2$) above which aerobic energy production is supplemented by anaerobic mechanisms, resulting in a significant increase in lactate production; this can be determined by gas analysis during exercise testing. It has also been proposed that VT for long-term exercise is a good predictor of maximal endurance performance.[4]

The genetic component to $V0_{2max}$ has been estimated to be between 20% and 50%.[5] One of the possible genes that have been studied is the human angiotensin converting enzyme *(ACE)* gene. It is an integral component of the renin-angiotensin-aldosterone system (RAAS), and its gene is located on chromosome 17, which consists of an insertion (*I*) allele/deletion (*D*) allele polymorphism of a 287 base pair Alu repeat sequence in intron 16. The gene has been recoded as dipeptidyl carboxypeptidase 1 (DCP1) with DCP1*D and DCP1*I as the two designated alleles. Montgomery et al were the first to show that a relationship exists between the *ACE ID* polymorphism and exercise performance in a cohort of British Army recruits.[6] The association of the *I* allele with better endurance has been demonstrated in long distance runners,[7] long distance swimmers,[8] rowers[9] and mountaineers.[6] It is likely that the *ACE ID* polymorphism is associated with endurance capacity only in the trained state and exerts its phenotypic effects through gene-environment interaction.[10] Recent data has also suggested that the improved performance associated with the *I* allele is due to a local muscle effect rather than a central cardiorespiratory response to training.[11]

Increased *ACE* activity has been shown to be associated with increased angiotensin I to angiotensin II conversion and increased bradykinin degradation.[12]

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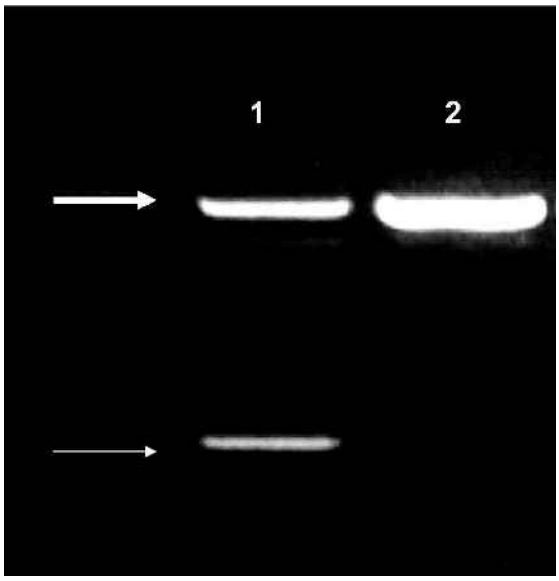

Fig. 1 Photograph shows the electrophoretic separation of DNA fragments on 2% gel after polymerase chain reaction and resolved under ultraviolet light. Fragments (seen as bands) of two sizes are seen: 199 base pairs (thin arrow) and 479 base pairs (thick arrow). The presence of both bands represents *ID* genotype (Lane 1) and the presence of a single band represents *II* genotype (Lane 2).

Specifically, the *D* allele is associated with higher *ACE* levels and activity in both the serum and cardiac tissue, and consequently, a higher risk of endothelial dysfunction, postulated to be due to a blunting of nitric oxide (NO) release.[13] On the other hand, the *ACE II* genotype confers a better endothelial-dependent response during exercise. This has been suggested to be due to decreased angiotensin II activity, leading to increased NO bioactivity, secondary to increased nicotinamide adenine dinucleotide/ nicotinamide adenine dinucleotide phosphate (NADH/ NADPH) oxidase activity.[14] Whether the *ACE* gene exerts its effects on aerobic capacity directly via the RAAS or through another gene(s) in linkage disequilibrium with the *ACE* locus, is still unclear.[11]

However, some other studies have either not shown an association between the *ACE ID* polymorphism and exercise performance or have yielded conflicting results. A possible reason is the inclusion of subjects who were of different fitness levels and ethnic groups,[15] or who were from different sporting disciplines.[16] There is evidence that the *D* allele in Chinese is associated with better exercise improvement in $V0_{2max}$.[17] Furthermore, an increased risk of diabetic nephropathy was associated with the *D* allele in Asians, but not in Caucasian patients.[18] Finally, the HERITAGE Family Study, which included 476 Caucasians and 248 Black subjects, showed that the *D* allele conferred better exercise improvement in $V0_{2max}$.[16] This was in contrast to earlier studies, which used only Caucasian subjects.[6,7] This raised the possibility that ethnic differences may play a part in influencing the phenotypic expression of *ACE ID* polymorphism on exercise performance. This was a pilot study in which the primary aim was to explore the relationship between the *ACE ID* polymorphism and aerobic capacity in a homogeneous cohort of national rugby players of Asian ethnicity. The secondary aim was to investigate whether ethnic differences exist in the phenotypic expression in aerobic capacity according to *ACE* genotype.

METHODS

The subjects, aged 18–35 years, were all national rugby union players who had been training for at least one year prior to the study and were in active training for an international competition during the period of study. Hence, they were at the competition stage of training and at or near peak training form. Ethnicity was determined by direct questioning. Exclusion criteria were adapted and modified from the American College of Sports Medicine Guidelines for Exercise Testing.[19] This study was funded by the National Medical Research Council and approved by the National Healthcare Group Domain Specific Institutional Review Board for Medical Research. Written informed consent was obtained from all the subjects.

All the subjects were given a pre-exercise testing screening questionnaire adapted from the American Heart Association/American College of Sports Medicine Health/Fitness Facility Pre-participation Screening Questionnaire.[20] A physical examination was performed by a registered medical doctor and an electrocardiogram was also carried out prior to maximal exercise testing. Maximal exercise testing was done with an electromagnetically braked cycle ergometer (Corival, Lode, Netherlands) with a breath-by-breath gas analyser (Quark b^2, Cosmed, Rome, Italy), which has been validated in previous studies.[21] The cycle ergometer was chosen as the exercise modality over the treadmill as it has the advantage of allowing the power output to be selected with some precision. All the subjects did predominantly running activities in their training and none cycled as a sporting activity. The predicted $V0_{2max}$ was derived from the calculations and process described by Wasserman et al, using a prediction equation: Desired work increment per minute (Watts) = (predicted $V0_{2max}$–unloaded $V0_2$)/103.[22,23]

The optimal seat height for each subject was determined by allowing for a knee bend of 15° with the pedal at its lowest point. Each subject was given sufficient time for familiarisation of the cycle ergometer and face-mask. The exercise test consisted of four phases: (1) resting phase; (2) warm-up phase; (3) exercise phase; and (4) recovery phase. Baseline resting data was collected for two

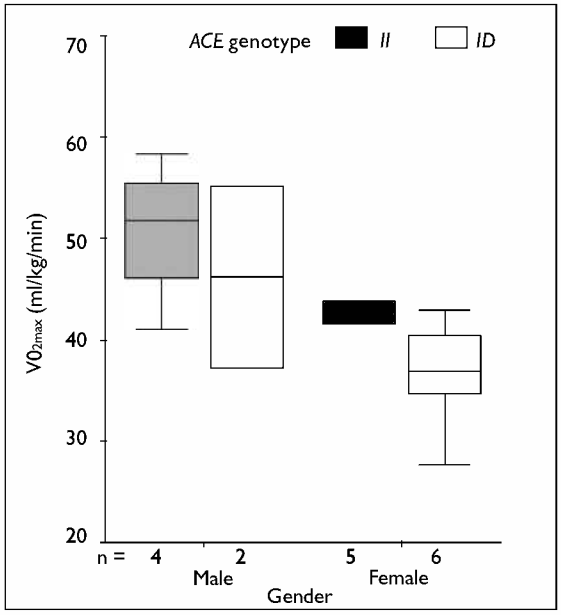

Fig. 2 Box plot shows the maximal oxygen uptake ($V0_{2max}$) between *ACE* genotypes in males and females. $V0_{2max}$ was higher for the *II* genotype in both males and females. The sample maximum, upper quartile, median, lower quartile and sample minimum are shown from top to bottom.

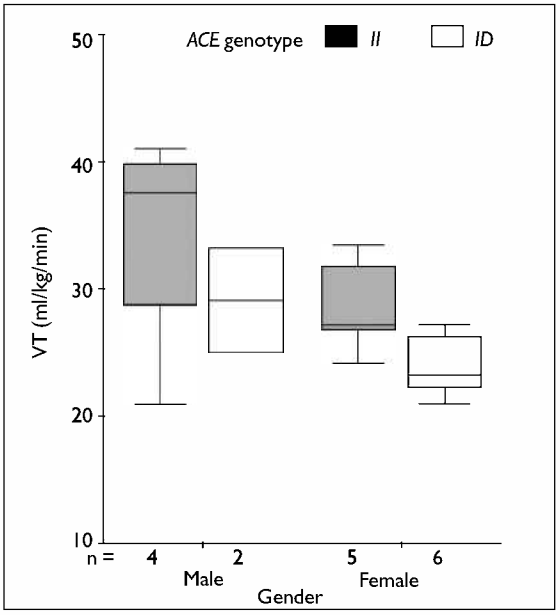

Fig. 3 Box plot shows the ventilatory threshold (VT) between *ACE* genotypes in males and females. VT was higher for the *II* genotype in both males and females. The sample maximum, upper quartile, median, lower quartile and sample minimum are shown from top to bottom.

minutes, followed by a warm-up phase of unloaded cycling for three minutes. A progressive increment in work rate was commenced immediately after the warm-up phase, and continued until the subject was unable to proceed any further due to fatigue. Each subject was instructed to maintain a constant cycling cadence of 50–80 revolutions per minute with smooth and regular breathing throughout the test. Heart rate was monitored continuously, and blood pressure every three minutes during the test. Immediately upon stopping, each subject was presented with the Borg's Rating of Perceived Exertion (RPE) for scoring. This was followed by a recovery phase which consisted of 2–4 minutes of unloaded cycling and then 6–8 minutes of rest.

The $V0_{2max}$ was determined as the point during the course of the incremental maximal exercise test when the $V0_2$ was at its peak and plateaued.[23] This plateau was observed in all subjects and the respiratory quotient (R) was ≥ 1.1 in all subjects during the attainment of $V0_{2max}$ except for one *ID* genotype female subject (R 1.04). From gas analysis and using the criteria by Wasserman et al, the VT was identified as the $V0_2$ at which minute ventilation increased disproportionately in relation to $V0_2$ due to a relative increase in $VC0_2$.[24]

Using a salt precipitation protocol, as described by Miller et al, genomic DNA was extracted from peripheral blood, which was obtained by venepuncture in a standardised manner at the start of the study.[25] The genomic DNA fragment on intron 16 was amplified by polymerase chain reaction (PCR) using flanking primers, as previously described.[26] PCR amplification was carried out in 20 µL reactions (40 ng genomic DNA, 10.0 uM each of forward and reverse primers, 50 uM each of deoxyATP, GTP, CTP and TTP, 1.5 mM $MgCl_2$, 1 U Taq DNA Polymerase, [Promega, Madison, WI, USA] and Taq Buffer [Promega, Madison, WI, USA]), with five minutes denaturation at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 67°C, 30 s at 72°C, and final extension at 72°C for seven minutes in a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems Inc, Foster City, CA, USA). The products of 199 bp and 479 bp for *D* and *I* alleles, respectively, were resolved electrophoretically on 2% agarose gel containing ethidium bromide and visualised under ultraviolet light (Fig. 1). Assignment of genotypes was carried out by an investigator who has been trained in molecular studies and blinded to the subjects' exercise performance status.

All analyses were done using the Statistical Package for Social Sciences version 11.5.1 (SPSS Inc, Chicago, IL, USA), and results were tabulated with the median and standard deviation (SD). Multivariate logistic regression analysis was used to estimate the likelihood of high aerobic fitness according to *ACE* genotype, after controlling for body mass index (BMI) and age as possible confounding factors. A p-value of < 0.05 was considered to be statistically significant.

RESULTS

A total of 17 subjects of Southeast Asian descent were recruited (11 females and six males), and all the subjects

Table I. Baseline characteristics of subjects.

Baseline characteristic	Mean	Median	Standard deviation	Range
Male (n = 6)				
Age (years)	21.2	20.0	2.40	20–26
Height (m)	1.76	1.75	0.06	
Body mass index (kg/m^2)	24.2	24.0	2.65	
Predicted $V0_{2max}$ (ml/kg/min)	45.95	46.55	4.033	
Female (n = 11)				
Age (years)	26.3	26.0	2.76	21–30
Height (m)	1.61	1.62	0.05	
Body mass index (kg/m^2)	21.5	21.3	2.53	
Predicted $V0_{2max}$ (ml/kg/min)	35.66	34.78	3.851	

participated in the study to completion. The median age and SD for the male and female subjects were 20.0 years ± 2.4 and 26.0 years ± 2.8 years, respectively. Among the male subjects, the median and SD BMI and predicted $V0_{2max}$ were 24.0 ± 2.7 kg/m^2 and 46.6 ± 4.0 ml/kg/min, respectively. Among the female subjects, the median and SD BMI and predicted $V0_{2max}$ were 21.3 ± 2.5 kg/m^2 and 34.8 ± 3.9 ml/kg/min, respectively (Table I). Table II presents the characteristics of the subjects according to the *ACE* genotype. The subjects of both genotypes were well-matched, with no significant differences in genotype frequencies detected by gender, age, BMI and predicted $V0_{2max}$. Overall, 9 (52.9%) of the subjects were homozygous for the *I* allele and 8 (47.1%) had the *ID* genotype. The allele frequencies were 26 (76.5%) and 8 (23.5%) for the *I* and *D* allele, respectively, and were in Hardy-Weinberg equilibrium. There were no subjects who were homozygous for the *D* allele.

All the subjects completed the test protocol and none had the test terminated for reasons other than failure to continue cycling due to fatigue. In all the subjects, there was an expected progressive increase in the heart rate proportional to the increase in $V0_2$ and an RPE ≥ 15 was achieved. As expected, the median $V0_{2max}$ for males was higher compared to that for females (51.9 vs. 40.4 ml/kg/min), and this was statistically significant when using the Mann-Whitney non-parametric analysis (p = 0.037). Figs. 2 and 3 show the comparison between the *ACE* genotype and the $V0_{2max}$ and VT respectively. Both indicators of aerobic capacity were higher for the homozygous *II* subjects in both genders.

Table III shows the results of the $V0_{2max}$ and VT by *ACE* genotype. As the cohort consisted of subjects from both genders, subsequent analysis took into account gender differences by using gender-specific cut-offs for defining high aerobic capacity. Using reference standards from a well-established published data of a normal population, we defined high aerobic capacity as subjects with a $V0_{2max}$ above the 80th percentile of the population,

Table II. Subject characteristics by *ACE* genotype.

Characteristic	*II*	*ID*	p-value
Gender, no. (%)			0.62
Male	4 (67)	2 (33)	
Female	5 (45)	6 (55)	
Mean age and SD (years)	23.9 (3.1)	25.1 (4.2)	0.50
Mean BMI and SD (kg/m^2)	22.8 (3.6)	22.0 (1.7)	0.61
Mean predicted $V0_{2max}$ and SD (ml/kg/min)	40.0 (6.8)	38.5 (6.1)	0.63

SD: standard deviation; BMI: body mass index; $V0_{2max}$: maximal oxygen uptake

which was above 48.2 ml/kg/min and 41.0 ml/kg/min for males and females, respectively.[19] Unlike $V0_{2max}$, there was no good epidemiological data establishing normal population reference ranges for VT. Hence, in defining subjects with high aerobic capacity using VT, we elected to use the median values obtained in this study as cut-offs, which was 34.9 ml/kg/min and 26.3 ml/kg/min for males and females, respectively.

Multivariate logistic regression analysis was performed with the following covariates: *ACE* genotype, age and BMI. Gender was not included in the final analysis as gender-specific $V0_{2max}$ cut-offs had already been used, and the initial analysis, which included gender as a covariate, showed multi-collinearity with a SD of 2.6. The results are presented in Table IV. After adjusting for age and BMI, the likelihood of having a high aerobic capacity was 14.3-fold greater among subjects with the *II* genotype as compared to the *ID* genotype (p = 0.030). A separate multivariate logistic regression analysis for VT above the gender-specific median values was performed with the same covariates. The results are shown in Table V. After correcting for age and BMI, subjects with the *II* genotype were 29.4 times more likely to have high aerobic capacity compared to the *ID* genotype (p = 0.019).

DISCUSSION

Mean $V0_{2max}$ ranging from 55.4 to 67.5 ml/kg/min for

Table III. Results of maximal oxygen uptake and ventilatory threshold by *ACE* genotype.

Gender/*ACE* genotype	No. of subjects	Median $V0_{2max}$ ± SD (ml/kg/min)	Median VT ± SD (ml/kg/min)
Male			
II	4	51.92 ± 7.16	37.59 ± 9.08
ID	2	46.21 ± 12.69	29.11 ± 5.71
Total	6	51.92 ± 8.28	34.86 ± 7.95
Female			
II	5	42.73 ± 5.60	27.15 ± 3.79
ID	6	36.92 ± 5.31	23.29 ± 2.40
Total	11	40.39 ± 5.82	26.25 ± 3.86

$V0_{2max}$: maximal oxygen uptake; VT: ventilatory threshold; SD: standard deviation

Table IV. Multivariate analysis of covariates for maximal oxygen uptake above the 80th percentile.

Covariates	Standard error	p-value	Odds ratio	95% confidence interval
ACE (*II* as reference)	1.22	0.030	14.27	1.30–156.75
Body mass index (kg/m^2)	0.141	0.814	0.97	0.73–1.28
Age (years)	0.125	0.829	0.97	0.76–1.24

Table V. Multivariate analysis of covariates for ventilatory threshold above the gender-specific median.

Covariate	Standard error	p-value	Odds ratio	95% confidence interval
ACE (*II* as reference)	1.44	0.019	29.36	1.74–494.79
Body mass index (kg/m^2)	0.165	0.447	0.88	0.64–1.22
Age (years)	0.141	0.815	1.03	0.78–1.36

rugby players have been previously reported.[27] Although the mean $V0_{2max}$ recorded in this study was lower, this is not unexpected as we had elected to use the cycle ergometer instead of a treadmill, to reduce the confounding effect of training specificity. This current study has several strengths. To the best of our knowledge, this is the first study in the scientific literature examining the relationship between the *ACE ID* polymorphism and aerobic capacity in a clearly-defined, homogenous cohort of national Asian rugby players, which adds to the increasing evidence that ethnic differences exist in the frequencies of this genetic polymorphism. Firstly, this study showed that the frequency of the *D* allele of the *ACE* gene was 24.5%. This is close to the 29.3% frequency reported in the normal controls of a Taiwanese population.[26] Both results are markedly lower than the reported frequency of 50% in Caucasian European subjects. Previous studies on other ethnic groups have shown that there was a tendency towards a higher frequency of the *D* allele in Nigerians, whereas Samoans and the Yanomami Indians displayed a much higher frequency of the *I* allele, compared to the European population.[28] This is further evidence that allelic frequency differs according to ethnicity and populations.

Although the effect of the *ACE ID* polymorphism on exercise performance has been the subject of much research, most studies do not include Asians, who may demonstrate a different phenotypic response to exercise stimulation. In fact, the question regarding whether ethnic differences affect the phenotypic expression of the *ACE* genotype has not been the subject of much research. The results of the HERITAGE Family Study already suggest that the *D* allele was associated with better exercise improvement in $V0_{2max}$. This was replicated in another study, which showed that untrained Chinese male subjects with the *DD* genotype have higher levels of $V0_{2max}$, again in contrast to other studies involving Caucasian subjects.[17] The results of one study, which reported no association between *ACE* genotype and aerobic performance status, may be due to the inclusion of athletes from different countries in addition to different sporting disciplines.[16] However, the most compelling evidence that ethnicity influences the phenotypic response of the *ACE ID* polymorphism is probably in the area of diabetes mellitus, where Kunz et al reviewed 19 studies in a meta-analysis and showed that the risk of diabetic nephropathy was likely to be increased in the presence of the *D* allele in Asians, but not in Caucasian patients (odds ratio 1.88 vs. 1.10).[18] Hence, our results replicate and support the findings of previous studies, which indicate

that the *I* allele confers an advantage in terms of endurance capacity. Secondly, despite the small sample size and the introduction of multiple covariates like *ACE* genotype, age and BMI in the regression analysis, we were also able to show statistical significance in the same direction when the $V0_{2max}$ and VT were analysed separately. Hence, we reject the hypothesis that Asian ethnicity influences the aerobic capacity according to *ACE ID* polymorphism differently from Caucasians.

The limitations of the study should be noted. Firstly, although the sample size was not large, it does follow recent recommendations on genomic research and exercise, that studies should focus on using a homogenous cohort of trained athletes within the same sporting discipline. This is to limit and control the effect of variation in the gene-environment interaction of *ACE ID* polymorphism on $V0_{2max}$. Despite the challenge that this approach presents, it is felt that this increased environmental homogeneity may mean that a smaller sample size would be adequate to show up a difference, if any.[10,11,29] Secondly, it has been shown that there are differences in $V0_{2max}$ in rugby players from different playing positions (forwards vs. backs). However, these values have not been shown to be of statistical significance.[27] Moreover, stratifying the players in our sample any further will decrease the power of the study. Despite these potential limitations, the results derived from this study support those of previous reports, especially those which used trained athletes within the same sporting discipline.[6,7,9] We look forward to further studies on the effect of genetic polymorphism on exercise-related traits in different ethnic groups.

We conclude that in our cohort of national Asian rugby players, subjects with the *ACE II* genotype are more likely to have a higher aerobic capacity, as measured by the $V0_{2max}$ and VT, compared to those with the *ID* genotype. This result is consistent with previous results involving Caucasian athletes, suggesting that the same physiological mechanisms mediated by the *ACE* gene may be responsible for aerobic capacity in these two ethnic populations. As genetic influence has been previously shown to contribute significantly to aerobic performance, the results of this study help further our understanding on the role played by the *ACE* gene polymorphism on aerobic capacity, and in particular, whether ethnic differences exist, as suggested by previous studies.

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