No association between Angiotensin Converting Enzyme (ACE) gene variation and endurance athlete status in Kenyans


aInternational Centre for East African Running Science (ICEARS), Institute of Biomedical and Life Sciences, West Medical Building, University of Glasgow, Glasgow, G12 8QQ, UK
bDepartment of Forensic and Investigative Science, University of Central Lancashire, Preston, PR1 2HE, UK
cDepartment of Exercise and Sports Science, Kenyatta University, P. O. Box 43844, Nairobi, 00100, Kenya
dInstitute of Pharmacology, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
eBritish Heart Foundation Laboratories, Centre for Cardiovascular Genetics, Royal Free and University College Medical School, Rayne Building, 5 University Street, London, WC1E 6JF, UK

Received 20 December 2004; received in revised form 30 April 2005; accepted 3 May 2005

Available online 13 June 2005

Abstract

East African runners are continually successful in international distance running. The extent to which genetic factors influence this phenomenon is unknown. The insertion (I) rather than deletion (D) of a 287 bp fragment in the human angiotensin converting enzyme (ACE) gene is associated with lower circulating and tissue ACE activity and with endurance performance amongst Caucasians. To assess the association between ACE gene variation and elite endurance athlete status in an African population successful in distance running, DNA samples were obtained from 221 national Kenyan athletes (N), 70 international Kenyan athletes (I), and 85 members of the general Kenyan population (C). Blood samples were obtained from C and assayed for circulating ACE activity. ACE I/D (rs4343—first time poly mentioned) genotype was determined, as was genotype at A22982G (rs7656162—first time poly mentioned) which has been shown to associate more closely with ACE levels in African subjects than the I/D polymorphism. ACE I/D and A22982G genotypes explained 13 and 24% of variation in circulating ACE activity levels (P = 0.034 and <0.001 respectively). I/D genotype was not associated with elite endurance athlete status (df=4, χ²=4.1, P = 0.39). In addition, genotype at 22982 was not associated with elite endurance athlete status (df=4, χ²=5.7, P = 0.23). Nor was the A allele at 22982, which is associated with lower ACE activity, more prevalent in N (0.52) or I (0.41) relative to C (0.53). We conclude that ACE I/D and A22982G polymorphisms are not strongly associated with elite endurance athlete status amongst Kenyans.

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Keywords: ACE genotype; I/D; Endurance; Genetics; ACE activity; Kenyan; African; Athletes

1. Introduction

There is much debate as to the predictors of athletic success, with the question of ‘nature’ versus ‘nurture’ at the fore. Despite the beliefs that genetic endowment plays a role in the determination of athletic success, the extent to which this is true remains largely unknown. As well as influencing some of the inter-individual differences in athletic performance, genetic endowment may also influence some of the inter-population differences, such as the continued success of athletes of African origin in running events: currently, 31 of the top 50 male road runners in the world are Kenyan (IAAF rankings, 2004).

Early familial studies suggested that there was a genetic component to endurance performance, through findings of high heritability in physiological indices of performance such as maximal oxygen uptake (V̇O₂max) (Lesage et al., 1985; Fagard et al., 1991). More recently, advances in
molecular technology have allowed a number of potential candidate genes for endurance performance to be identified. A yearly review of candidate genes for performance-related phenotypes has now identified in excess of 100 gene variants which may contribute to the wide variation in human physical performance (Rankinen et al., 2004).

Perhaps the most studied of the potential ‘performance genes’ is the Angiotensin-I-Converting Enzyme (ACE) gene. Angiotensin-I-converting enzyme influences blood pressure through the generation of the vasoconstrictor Angiotensin II, and inactivation of the vasodilator bradykinin. The most studied of the ACE polymorphisms is the I/D polymorphism, characterized by the presence (I, Insertion) or absence (D, Deletion) of a 287 bp fragment in an Alu sequence in intron 16 of the ACE gene. The I/D polymorphism has been associated with a wide variety of physical fitness phenotypes. Although associations with physical performance have been far from unequivocal (Jones et al., 2002), in general, the ACE D allele has been associated with strength- and power-related performance (Folland et al., 2000; Nazarov et al., 2001; Woods et al., 2001), and the ACE I allele with endurance performance (Hagberg et al., 2001; Jones et al., 2002). For example, a study of Australian rowers found a higher frequency of the I allele relative to controls (Gayagay et al., 1998), while there was an over-representation of the I allele in British Olympic standard runners competing in distances of over 5000 m (Myerson et al., 1999). More recently, a study of South African triathlon competitors has shown an excess of the I allele in the fastest 100 finishers relative to controls (Collins et al., 2004). The I allele has also been associated with an increase in type I muscle fibres (Zhang et al., 2003), and the II genotype with higher maximal oxygen uptake (V\textsubscript{O\textsubscript{2max}}) in studies of post-menopausal women (Hagberg et al., 1998, 2002). Not all studies, however, support an association between the I allele and endurance performance; some have found no association with endurance athlete status amongst groups of mixed sporting discipline and race (Rankinen et al., 2000b). Others however, have even shown the D allele to be associated with training-related gains in (V\textsubscript{O\textsubscript{2max}}) or with V\textsubscript{O\textsubscript{2max}} itself (Rankinen et al., 2000a; Zhao et al., 2003). Such contrasting results have been attributed to small subject numbers (Rankinen et al., 2000a) and to the inadequate stratification of subjects (Woods et al., 2000). Given the disparity in results to date, it is clear that any association may be found only in homogenous cohorts of athletes stratified by their endurance status and perhaps by level of performance.

The I/D polymorphism has been estimated to explain up to 47% of the variance in circulating ACE levels in a Caucasian population (Rigat et al., 1990), with the I allele being associated with lower plasma and tissue ACE levels than the D allele (Rigat et al., 1990; Danser et al., 1995; Alvarez et al., 2000; Woods et al., 2004). Given that the I/D polymorphism is an intronic Alu sequence, a direct influence on circulating ACE levels is not readily apparent. The I/D polymorphism is therefore considered to be a genetic marker in linkage disequilibrium with a functional variant influencing ACE levels. The association between I/D and ACE levels has been described in Caucasians, but more recent studies in African populations have shown that other variants of the ACE gene are more closely associated with circulating ACE levels than the I/D polymorphism (Zhu et al., 2000, 2001; Cox et al., 2002). A transition at nt (or nucleotide) 22982, in the sequence AF118569 as in Rieder et al. (1999), or 31958 as in Cox et al. (2002), has been found to show the largest phenotypic differences between genotypes (Zhu et al., 2000). Although all markers tested in Europeans showed significant differences in ACE levels between each genotype (probably due to linkage disequilibrium between genotypes), the most marked difference in ACE levels in both Afro-Caribbean and European subjects was found between genotypes at A22982G. Absolute linkage disequilibrium between I/D and 22982 has been shown for Caucasian populations (Soubrier et al., 2002) and strong haplotype association has been shown in African populations (Zhu et al., 2000; Cox et al., 2002). The I allele at I/D has been shown to be in linkage disequilibrium with the A allele at A22982G and the D with the G, respectively (Soubrier et al., 2002). Consequently, the A allele has been associated with lower circulating ACE levels than the G allele (Cox et al., 2002; Soubrier et al., 2002), except in one study where the A allele was associated with higher ACE levels (Zhu et al., 2000). The variant at 22982 has been suggested to be a potential functional variant due to the proximity to a splice site (Zhu et al., 2000) which may be influential in the production of alternative splice forms (Sugimura et al., 1998). Although ACE genotype has been shown to have an influence on circulating and tissue ACE levels, it is unclear if this is the mechanism responsible for any association of the ACE genotype and performance. The aim of the present study, therefore, was to describe the association between the ACE genotype at I/D and 22982 and plasma ACE activity and to assess the association between variation in the ACE gene and elite Kenyan endurance athlete status, many of whom were Olympic and World champions and holders of World Records. If the influence of the ACE I/D polymorphism on endurance performance is due to the effects on plasma ACE levels, it is likely that the 22982 polymorphism would also be associated and perhaps more strongly with elite endurance athlete status.

2. Methods

2.1. Subjects

291 elite Kenyan endurance athletes (232 male, 59 female) and 85 control subjects (40 male, 45 female) were included in the present study. 70 of the athletes (59 male, 11 female) had competed internationally representing Kenya (I, N=70) and
comprised many world record holders, Olympic, World and Commonwealth champions. Of the 70 athletes, 42 have won Olympic, World or Commonwealth medals, had a top 3 finish in an international marathon or equivalent road race, or have been ranked in the top 50 runners in the world at their event. Other athletes, classified as National (N), had competed at national level within Kenya (N=221, 173 male, 48 female). All athletes had competed in distances from 3000 m to marathon, where the energy source is predominantly aerobic (Gastin, 2001). Control subjects (C) were students at Kenyatta University and were representative of the Kenyan population in their geographical distribution throughout Kenya (Onywera et al., in press). As some previous studies have identified associations with genotype and performance at the highest performance levels (Nazarov et al., 2001; Yang et al., 2003), international athletes were considered separate from national athletes to ensure that any association was not masked by inadequate classification of athlete status. All controls also provided a supine 10 mL venous blood sample, which was assayed for ACE activity as previously described (Woods et al., 2004). Results were expressed as nmol His-Leu mL⁻¹ min⁻¹.

2.2. DNA extraction and genotype determination

All subjects provided buccal swabs, which were stored in cell lysis solution (0.1 M EDTA, 0.1 M Tris–HCl, 1% SDS). DNA was extracted using a modified version of the Qiagen buccal cell spin protocol (Qiagen Ltd., Crawley, UK). New primers were designed for all genotyping reactions. I/D genotypes were determined by PCR, using a three-primer system; a forward primer which recognised the deletion (D) sequence, a forward primer which recognised the insertion (I) sequence, and a common reverse primer. The forward deletion primer was (5'-CTCTAGACCTGCTGCTATTACAGTC-3'), the forward insertion primer was (5'-CGGGATGGTCTCGATCTC-3') and the common reverse primer was (5'-CCCTCCCATGCCCCA-

3. Results

Of the 22 tests, only international athletes showed a significant deviation from HWE for I/D genotype (P<0.04), showing an excess of heterozygotes. Of the 96 European samples genotyped at both ACE loci, all subjects homozygous at I/D were also homozygous at 22982. Subjects homozygous for the I allele at the I/D locus were homozygous for the A allele at 22982 and likewise for the D allele at I/D with the G allele at 22982. Absolute linkage disequilibrium was assumed between the two loci in Europeans. In the Kenyan samples, linkage disequilibrium was not complete: D=0.12, and D'=0.59.

Controls did not differ from the general Kenyan population (Kenyan Central Bureau of Statistics, 2003) in their geographical distribution throughout Kenya (df=2, χ²=5.1, P=0.28). Although athletes differed significantly from controls in ethnic distribution (df=2, χ²=73.2, P<0.001) and geographical distribution throughout Kenya (df=8, χ²=104.9, P<0.001), neither of these factors associated with I/D genotype (Ethnicity: df=2, χ²=0.66, P=0.72; Place of Birth: df=8, χ²=11.4, P=0.18). Nor did 22982 genotype associate with ethnicity or regional
I/D genotype frequencies were similar in both athlete groups and controls (Fig. 1). No significant differences were found in genotype frequencies between subject groups \((df=4, \chi^2=4.1, P=0.39)\). Controls did not differ significantly from national athletes \((df=2, \chi^2=0.6, P=0.74)\) or international athletes \((df=2, \chi^2=2.0, P=0.36)\). Nor did any significant difference arise when athlete groups were combined and compared to controls \((df=2, \chi^2=0.46, P=0.80)\). I allele frequencies were also similar between control and athlete groups \((C: 0.38, N: 0.42, I: 0.38)\), and no significant differences were found between athletes and controls \((df=2, \chi^2=0.97, P=0.62)\).

When groups were separated for gender, male athletes did not differ significantly from male controls in I/D genotype frequencies \((df=2, \chi^2=2.3, P=0.31; I: \chi^2=0.20, P=0.9)\). Low numbers of female athletes \((N=48, I=11)\) did not allow female international athletes to be considered separately, but when all female athletes were compared to controls, no significant difference was found \((df=2, \chi^2=0.05, P=0.98)\).

Whole group ACE activity was \((\text{Mean} \pm \text{SD}) 24.6 \pm 6.9 \text{ nmol His-Leu mL}^{-1} \text{ min}^{-1}\). The mean ages of subjects of each genotype were II and DD: 25 \pm 6 yrs, ID: 31 \pm 9 yrs, II and DD: 28 \pm 9 yrs. AA: 28 \pm 8 yrs, AG: 30 \pm 9 yrs, GG: 29 \pm 9 yrs. The mean ACE activity for each ACE I/D genotype is shown in Fig. 2 and for each 22982 genotype in Fig. 3. I/D genotype was associated with ACE activity (II: 22.20 \pm 6.24, ID: 23.4 \pm 6.83, DD: 27.01 \pm 6.59 nmol His-Leu mL\(^{-1}\) min\(^{-1}\); \(P=0.034\)), and explained almost 13% of the variance in ACE activity levels; II and ID genotypes did not differ significantly in ACE activity, while DD genotype had a significantly higher ACE activity than both II and ID genotypes (Fig. 2). ACE activity was more strongly associated with genotype at 22982 (AA: 20.28 \pm 5.26, AG: 24.81 \pm 6.21, GG: 29.54 \pm 6.6 nmol His-Leu mL\(^{-1}\) min\(^{-1}\); \(P<0.001\)). Genotype at 22982 was calculated to explain over 24% of the variance in ACE activity levels. All genotypes at 22982 differed significantly from each other in ACE activity (Fig. 3). In this East African population, although I/D is associated with ACE activity levels, the association is to a lesser extent than with genotype at 22982.

There was no significant differences in 22982 genotype frequency between groups \((df=4, \chi^2=5.67, P=0.23; \text{Fig. 4})\). Controls did not differ significantly from either athlete group \((df=2, \chi^2=0.11, P=0.95; I: \chi^2=4.45, P=0.11)\). In addition, there was no difference in 22982 genotype frequencies when controls were compared to all athletes combined \((df=2, \chi^2=0.66, P=0.72)\). Frequencies of the A allele at 22982, which associates strongly with lower ACE activities in Africans as the I allele does in Europeans, were similar in athletes and controls \((C: 0.53, N: 0.52, I: 0.41)\), and revealed no significant differences between subject groups \((df=2, \chi^2=5.3, P=0.07)\). As for I/D, separating for gender did not reveal any male specific effect of ACE genotype on elite athlete status when compared to controls.
in either national or international athletes (df=2, N: $\chi^2=0.23, P=0.89$; I: $\chi^2=23.6, P=0.016$). Once again, analysis of combined female athletes against controls did not reveal any association between genotype and athlete status (df=2, $\chi^2=0.10, P=0.95$).

4. Discussion

The results of the present study do not support an association between elite endurance athlete status and variation in the ACE gene amongst Kenyans. No significant differences in genotype or allele frequency at either of the loci tested were found between elite endurance athletes and controls. ACE genotype was independent of ethnicity and regional distribution of subjects. Although some studies report differences in the allele frequency of performance genes such as ACE and Alpha-actinin-3 when the very best athletes were compared to controls (Nazarov et al., 2001; Yang et al., 2003), no significant differences in genotype or allele frequency were found in the present study even when only the international athletes (i.e. including many Olympic and World champion distance runners) were compared to controls.

Despite some disparity amongst past reports, as reviewed by Jones et al (Jones et al., 2002), ACE I/D genotype has been associated with exercise performance: the I allele has been associated with endurance phenotypes and the D allele with power. Therefore, it may have been expected that the I allele would be more frequent amongst elite endurance athletes than controls. However, many of the studies supporting this hypothesis have been conducted in Caucasian subjects where the I/D genotype is strongly associated with ACE activity (Rigat et al., 1990), with no associations in ‘black’ African subjects, where the link between ACE I/D genotype and ACE activity is not as strong (Zhu et al., 2000). African populations show higher levels of haplotype diversity (Reich et al., 2001) and are therefore more useful in assessing associations with SNPs (single nucleotide polymorphisms) by allowing contrasts to be seen where a pair of SNPs may be in tight linkage disequilibrium in European populations. It has been shown that the I/D polymorphism is not in linkage disequilibrium with many other potentially functional polymorphisms in the ACE gene, especially in African subjects (Rieder et al., 1999). Even in European populations there is a major genetic subdivision which separates the Deletion clade into two distinct haplotypes: H1 and H7 (Rieder et al., 1999). Such findings may help further explain some of the controversy in the literature regarding the association between ACE genotype and physical performance. A polymorphism at nt (or nucleotide) 22982 has been shown to be the variant with the largest deviation in ACE levels between opposing homozygotes in Afro-Carribean subjects in a study of seven polymorphisms spanning 13 Kb (Zhu et al., 2000). This polymorphism is within 6 bp of an exon splice junction and may cause alternative splice forms (Zhu et al., 2000), which may have a functional role in the inter-genotype variation in circulating ACE levels. This is in agreement with the results of the present study where genotype at 22982 is more strongly associated with variation in ACE levels than genotype at I/D. It can be seen in Fig. 3 that the G allele is associated with higher circulating ACE activity than the A allele. Data from the European subjects has shown that the I allele is in complete linkage disequilibrium with the A allele at 22982. However, this is not the case in the Kenyan subjects of the present study where linkage disequilibrium between the two loci is 0.58. This may explain why there is no association between I/D genotype and elite Kenyan athlete status. However, when genotype at 22982 is considered, once again there is no association with elite Kenyan endurance athlete status despite the genotype at this locus explaining over 24% of the variance in ACE activity levels in this population.

Previous findings that many of the most successful East African athletes reside and train at altitude (Scott et al., 2003) may have supported a role for the ACE gene in the determination of their success in distance running. The ACE I allele has been associated, although not conclusively (Dehnert et al., 2002) with the ability to tolerate high altitude conditions (Woods and Montgomery, 2001) as well as with endurance phenotypes (Jones et al., 2002). It has been considered therefore that there may have been a role for the ACE gene in the success of Kenyan athletes, many of whom live and train at altitudes of over 2500 m (Onywera et al., in press). It is conceivable that there has been selection for the I allele amongst such a population for the altitude tolerance phenotype it may confer. The association of the I allele with endurance performance phenotypes may have adapted this population toward endurance performance. However, the present results do not support this hypothesis.

Although some studies have shown that ACE genotype has an influence on physical performance and cardiovascular mediators of physical performance such as ventricular
endurance athlete status. Currently, there is controversy over the influence of ACE genotype on endurance performance, and this study does not support a role for ACE gene variation on the inter-individual or inter-population differences in endurance performance. The absence of an association between either I/D or A22982G genotype and elite Kenyan athlete status also suggests that the ACE gene does not contribute significantly to the phenomenal success of Kenyan endurance runners in international distance running competition. To date, this is the largest and most homogenous genetic association study involving elite athletes and finds no association between ACE gene variation and elite endurance athlete status in Kenyan distance runners.

Acknowledgements

The authors acknowledge the invaluable assistance of Athletics Kenya. Mrs. Heather Collin is acknowledged for her excellent technical assistance. The co-operation of all subjects is greatly appreciated. HM is funded by the Portex Endowment at the Institute of Child Health, London.

References


