Original Article

Molecular analysis of genetic variation in angiotensin I-converting enzyme identifies no association with sporting ability: First report from Indian population

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INTRODUCTION: A polymorphism in the angiotensin-converting enzyme (*ACE*) gene was the first performance enhancing polymorphisms (PEPs) to be identified and correlated with athletic abilities. This polymorphism (rs. 5186) is the absence (deletion; D allele), rather than the presence (insertion, I allele) of 287bp Alu repeat element in intron 16. However, the association of *ACE* I/D polymorphism in sports abilities have been contradicted and debated. No study has evaluated the *ACE* gene polymorphism in Indian athletes so far. Hence, the genotype distribution and allelic frequency of *ACE* gene in selected Indian athletic and non-athletic population was studied.
MATERIALS AND METHODS: A total of 147 athletes and 131 controls were genotyped for the ACE gene polymorphism using PCR.
RESULTS: No significant association was observed between the allelic frequencies of *ACE* gene in controls and athletes on a whole, as well as after sub-categorizing the athletes based on the type of sport they played ($P > 0.1$). However, a higher representation of I allele was observed in the athletes.
CONCLUSION: *ACE* genotyping studies need to focus on truly elite athletes of a single sporting discipline, to be able to find an association. The *ACE* I/D polymorphism may not be considered a marker for human performance, but can be further studied in combination with other potent performance enhancing polymorphisms.

Key words: Angiotensin-converting enzyme, athletes, polymorphism

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Introduction

Performance-enhancing polymorphisms (PEPs) are examples of natural genetic variation that affect the outcome of athletic challenges. A polymorphism in the angiotensin-converting enzyme (*ACE*) gene was the first PEP to be identified and correlated with athletic abilities.[1,2] ACE is a part of the renin-angiotensin system. The inactive form of the enzyme; angiotensinogen, is cleaved by renin to produce an inactive angiotensin I, which is subsequently converted into its active form angiotensin II. Angiotensin II is known to influence vasoconstriction, and is responsible for tissue oxygenation and regulation of skeletal muscle efficiency.[3]

A functional polymorphism (rs.5186) of the human *ACE* gene has been identified in which there is absence (deletion; D allele), rather than the presence (insertion, I allele) of 287bp Alu repeat element in intron 16. The absence of this repeat element is associated with higher enzyme activity in both serum and skeletal muscle.[4-6] Reports have associated the D allele with enhanced performance in sprint of power based sports, while the insertion allele I is associated with a predilection to excellence in endurance sports.[7]

Though there have been multiple studies on association of *ACE* gene variation in endurance sports, the results have been contradictory. In one study,[8] it was demonstrated that an excess of I allele in elite middle distance Russian athletes (event duration 1-20 min) while

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another study[9] reported an association of D allele in Israeli elite endurance athletes. However, to the best of our knowledge, no study has evaluated the *ACE* gene polymorphism in Indian athletes.

The aim of this study was to analyze the genotype distribution and allelic frequency of *ACE* gene in selected Indian athletic and non-athletic population, with a specific objective to evaluate if *ACE* gene polymorphism was associated with the sporting ability in National/International level athletes.

Materials and Methods

Ethical approval

The present study was planned in agreement with the World Medical Association *Declaration of Helsinki* (2001).[10] Informed written consent was obtained from each volunteer of study and control group. The research project was reviewed and approved by the SRL-Ethics Committee for ethical clearance.

Subjects

One hundred and forty-seven athletes (106 men and 41 women, age between 14 and 40 years) volunteered to participate in this study. The control group comprised 131 non-athletic healthy individuals randomly selected from the Indian population.

The athletes were categorized by their competitive levels, viz. Regional and National/International. Of the 147 athletes, 69 were top-level athletes of the country, representing India at National and International sporting events, while 78 were Regional (Varsity to State level) athletes, all pursuing their sport for more than 4 years. The top level athletes were subdivided by their sporting excellence, viz. Power Sports (for e.g., running <200 m, swimming 50 m-100 m, artistic gymnastics), mixed pattern sports (for e.g., basketball, tennis, volleyball) and endurance sports (for e.g., running >800 m, swimming >400 m, hockey).

Method

Genomic DNA, which was extracted from the EDTA-whole blood using QIAGEN DNA mini kit. Genotyping of the *ACE* I/D polymorphism was performed using the method previously described.[11] In brief, the method comprised of amplification of the *ACE* gene using the primers ACE-F: 5'-GCCCTGCAGGTGTCTGCAGCATGT-3', and ACE-R: 5'-GGATGGCTCTCCCCGCCTTGTCTC-3'. PCR was performed by initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 94°C for 30s, annealing at 66°C for 1 minute, extension at 72°C for 1 minute, and a final extension step of 10 min at 72°C. The amplified fragment was then electrophoresed in a 2% agarose gel for identifying the genotype.

Data analysis

Allelic frequencies were determined by direct counting. A χ^2 test was used to confirm that the observed genotype frequencies were in Hardy-Weinberg equilibrium. Allelic frequencies amongst National/International level athletes excelling in a specific sporting type (power/endurance/mixed pattern) were compared to the total allelic frequencies in these athletes. *P* values $\leq$ 0.05 were considered to be significant.

Results

Three genotypes of the *ACE* gene were identified on the basis of the gel pattern, as illustrated in Figure 1. We compared the allelic frequencies of all athletes and controls as described in Table 1. On applying the

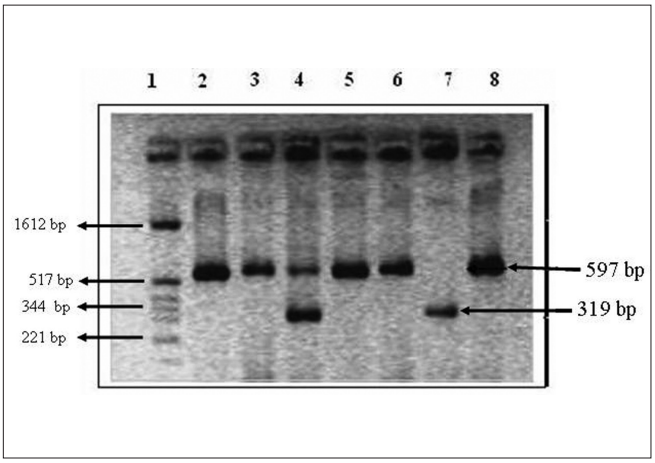

Figure 1: *ACE* genotypes as observed on a 2% agarose gel containing ethidium bromide Illustrating homozygous DD, homozygous II and heterozygous ID genotype of *ACE* gene. Lane 1: DNA ladder (pBR322/ HinfI digest), Lane 2-3, 5-6 and 8: homozygous II genotype, Lane 4: heterozygous ID genotype, Lane 7: homozygous DD genotype

Table 1: Genotype and allelic frequencies of *ACE* in healthy controls and athletes

ACE genotyping	N	Genotype distribution			χ^2	*P*	Allele Frequencies		χ^2	*P*
		II	ID	DD			I allele	D allele		
Athletes	147	53 (36)	30 (41)	64 (23)	4.89*	0.08	166 (56)	128 (44)	3.89#	0.05
Controls	131	41 (31)	44 (34)	46 (35)			126 (48)	136 (52)		

Data in parentheses indicate relative values, *d.f = 2, #d.f = 1

χ^2 test, the insertion allele I was found to be higher (56%) in athletes as compared to the controls (48%), with a significant association (χ^2 = 3.89, *P* = 0.05). However, the genotype distribution fell short of significance (χ^2 =4.89, *P* = 0.08).

Further, we evaluated whether I allele of the *ACE* gene was associated with elite performance in endurance sports. For this, we compared the allelic frequencies in the National/International level power and endurance athletes, where we did not observe any significant association (*P* >0.1), as shown in Table 2.

Thus, no association of ACE genotype could be observed with either power-based or endurance-based athletic performance.

Discussion

The present study revealed no association between ACE genotype and sporting abilities. A similar finding has been observed in study carried out by Oh *et al*., (2007)[12] in Korean male elite athletes, as well as Kenyan endurance athletes.[13] However, association of the II genotype has been demonstrated in Italian Olympic endurance athletes.[14] In contrast to this study, Amir *et al*., (2007)[9] reported the deletion allele 'D' to be associated with the likelihood of being an endurance athlete in their Israeli cohort.

The *ACE* genotype distribution in both control and athlete groups in the present study was in Hardy – Weinberg equilibrium. No significant association was observed between the allelic frequencies of *ACE* gene in controls and athletes on a whole, as well as after sub-categorizing the athletes based on the type of sport they played (*P* > 0.1). However, a higher representation of I allele was observed in the athletes. In an earlier study,[15] a higher frequency of *ACE* insertion allele in various ethnic groups was reported. A higher frequency of I allele is observed in Asiatic and Mongoloid populations, but differs from Americans, Caucasians, and Europeans.[16]

Two parameters have gained importance for observing an association on *ACE* genotyping in athletic performance. First, the association of *ACE* genotype with sporting excellence may be hard to detect amongst a heterogeneous cohort of mixed athletic ability and discipline.[8] We had athletes excelling in different sporting events, ranging from running and swimming to field events like hockey and basketball. We believe that this is the primary reason as to why any significant association could not be observed.

Second, ethnicity or racial closeness of the cohorts of athletes and controls is essential to derive a conclusive association. This has been the source of debate on the work on Israeli endurance athletes;[9] where Zoosmann-Diskin (2008)[17] has commented that association reported by the Israeli group may be an artifact brought about by the uncontrolled mixture of people belonging to different Jewish populations in their sample. An earlier study,[15] which included five different ethnic cohorts, showed ethnic heterogeneity with higher insertion allele frequency.

In conclusion, our data support the earlier findings that subsequent studies need to focus on truly elite athletes (Olympic and/or World Championship winners), that too of a single sporting discipline. Only then can a possible association of *ACE* genotype can be explored and established. The *ACE* I/D polymorphism may not be considered a marker for human performance, but can be further studied in combination with other potent performance enhancing polymorphisms.

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Table 2: Allele frequencies in National/International level athletes based on their sporting excellence

ACE	N	Allele frequencies		χ^2	*P*
		I allele	D allele		
All	69	76 (55)	62 (45)		
Power	7	8 (57)	6 (43)	0.03	>0.1
Mixed	37	41 (55)	33 (44)	0.01	>0.1
Endurance	25	27 (54)	23 (46)	0.04	>0.1

Data in parentheses indicate relative values, #d.f = 1

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