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

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.\cite{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.\cite{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.\cite{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.\cite{7}

Though there have been multiple studies on association of ACE gene variation in endurance sports, the results have been contradictory. In one study,\cite{8} it was demonstrated that an excess of I allele in elite middle distance Russian athletes (event duration 1-20 min) while
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.[13] In brief, the method comprised of amplification of the ACE gene using the primers ACE-F: 5’-GCCCTGCAGGTGTCTGCAGCATGT-3’, and ACE-R: 5’-GGATGGCTCTCCCCGCCTTGCTTC-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 $\chi^2$ 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
χ² test, the insertion allele I was found to be higher (56%) in athletes as compared to the controls (48%), with a significant association (χ² = 3.89, P = 0.05). However, the genotype distribution fell short of significance (χ² = 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) in Korean male elite athletes, as well as Kenyan endurance athletes. However, association of the II genotype has been demonstrated in Italian Olympic endurance athletes. In contrast to this study, Amir et al., (2007) 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 subcategorizing 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, 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.

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. 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, where Zoosmann-Diskin (2008) 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, 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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References

1. Gayagay G, Yu B, Hambly B, Boston T, Hahn A, Celermajer D, et al. Elite endurance athletes and the ACE I allele – The role of genes in athletic performance. Hum Genet 1998;103:48-50.
2. Ostrander E, Huson H, Ostrander G. Genetics of athletic performance. Annu Rev Genomics Hum Genet 2009;10:407-29.
3. Zhang X, Wang C, Dai H, Lin Y, Zhang J. Association between angiotensin-converting enzyme gene polymorphisms and exercise performance in patients with COPD. Respir Med 2008;13:683-8.
4. Danser A, Schalekamp M, Bax W, van den Brink A, Saxena P, Riegger G, et al. Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. Circulation 1995;92:1387-8.
5. Enyon N, Alves A, Yamin C, Sagiv M, Duarte J, Oliveira J, et al. Is there an ACE ID – ACTN3 R577X polymorphism interaction that influences sprint performance? Int J Sports Med 2009;30:888-91.
6. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990;86:1343-6.
7. Thompson W, Binder-Macleod S. Association of genetic factors with selected measures of physical performance. Phys Ther 2006;86:585-91.
8. Nazarov I, Woods D, Montgomery H, Shnieder O, Kazakov V, Tomilin N, et al. The angiotensin converting enzyme I/D polymorphism in Russian athletes. Eur J Hum Genet 2001;9:797-801.
9. Amir O, Amir R, Yamin C, Attias E, Enyon N, Sagiv M, et al. The ACE deletion allele is associated with Israeli elite endurance athletes. Exp Physiol 2007;92:881-6.
10. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. Bull World Health Organ 2001;79:373-4.
11. Lindpainter K, Pfeffer M, Kreutz R, Stamper M, Grodstein F, LaMotte F, et al. A prospective evaluation of an angiotensin-converting enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 1995;332:706-11.
12. Oh S. The distribution of I/D polymorphism in the ACE gene among Korean male elite athletes. J Sports Med Phys Fitness 2007;47:250-4.
13. Scott R, Moran C, Wilson R, Onyewara V, Boit M, Goodwin W, et al. No association between Angiotensin Converting Enzyme (ACE) gene variation and endurance athlete status in Kenyans. Comp Biochem Physiol A Mol Integr Physiol 2005;141:169-75.
14. Scanavini D, Bernardi F, Castoldi E, Conconi F, Mazzoni G. Increased frequency of the homozygous II ACE genotype in Italian Olympic endurance athletes. Eur J Hum Genet 2002;10:576-7.
15. Majumder P, Roy B, Bannerjee S, Chakraborty M, Dey B, Mukherjee N, et al. Human-specific insertions/deletions polymorphisms in Indian populations and their possible evolutionary implications. Eur J Hum Genet 1999;7:435-46.
16. Pasha M, Khan A, Kumar R, Ram R, Grover S, Srivastava K, et al. Variations in angiotensin-converting enzyme gene insertion/deletion polymorphism in Indian populations of different ethnic origins. J Biosci 2002;27 Suppl 1:67-70.
17. Zoosmann-Diskin A. The association of the ACE gene and elite athletic performance in Israel may be an artifact. Exp Physiol 2008;93:1220.