Frequency of the Angiotensin - Converting Enzyme (ACE) Gene Polymorphism in the General Population and the Elite Endurance Students in Korea

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Recently it was reported that an Insertion / Deletion polymorphism in the gene coding for Angiotensin - Converting Enzyme (ACE) is associated with human capacity for physical performance. This study was performed to genotyping of the *ACE* gene to determine the correlation between elite endurance performance and *ACE* I/D gene polymorphism. DNA sample was obtained from peripheral blood, hair roots and mouth epithelial cell in 739 general population and 200 elite athletic performance students. The ACE gene was amplified by polymerase chain reaction (PCR) using allele specific oligonucleotide primers. 155, 525 bp and 237 bp PCR products indicating the presence of insertion(I) and deletion(D) alleles, respectively, were clearly resolved after electrophoresis on a 2% agarose gel with ethidium bromide. Of the 200 elite athletic performance population subjects, 68 (34%) showed *ACE* genotype I I, 100 (50%) genotype ID and 32 (16%) genotype DD. Of the 739 general population subjects, 259 (35.1%) showed *ACE* genotype I I, 363 (49.1%) genotype ID and 117 (15.8%) genotype DD. Therefore *ACE* I/D gene polymorphism was not associated with human capacity for physical performance.($p > 0.05$)

Key words : angiotensin-converting enzyme, insertion / deletion polymorphism, physical performance

INTRODUCTION

Specific genetic markers that might contribute to human capacity for physical performance have not been reported, but candidate genes might be found the gene encoding Angiotensin - Converting enzyme (ACE). ACE is a peptidyldipeptide hydrolase belonging to the class of the zink metallopeptidase (Hubert *et al.*, 1990). Main function of this enzyme are converting angiotensin I (inactive form) into the vasoactive and aldosterone-stimulating peptide angiotensin II (active form) and to inactivate bradykinin (Erdos and Skidgel, 1987). ACE is found as a membrane-bound enzyme anchored to the cell membrane through a signal transmembrane domain located near its carboxyterminal extremity (Costeroucse *et al.*, 1992).

ACE gene spans 21 Kilobase(kb) is located on the chromosome 17q23 and it consists of 26 exons and 25 introns (Niimi *et al.*, 1998). There are three genotypes I I, ID, DD according to the insertion / deletion polymorphism of the *ACE* gene that consist of presence or absence of a 287 bp insertion on intron 16 on chromosome 17q23 of the human gene (Rigat *et al.*, 1992). An insertion allele of the gene is associated with improved elite endurance performance (Gayagay *et al.*, 1998 ; Montgomery and Marshall, 1998.) but deletion allele is known to be a risk factor of coronary heart disease (Evans *et al.*, 1998).

The *ACE* I/D polymorphism was associated with difference in serum ACE concentration as well as ACE activity (Triet *et al.*, 1992 ; Bloem *et al.*, 1996). The marker I allele appeared as it is always associated with the major - gene alleles characterized by lower ACE concentration and ACE activity (Triet *et al.*, 1992).

This study analyzed the polymorphism associated with *ACE* gene and compared elite endurance students with general population.

METHODS

Subject selection

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The *ACE* I / D gene polymorphism was analyzed in 939 (men ; 301, women ; 638) Korean individuals. Two hundred students of the Korean National University and High School of Physical Education were selected because they have a elite endurance performance (men ; 159, women ; 41). For control, we recruited 739 healthy general population (men ; 142, women ; 597). We received peripheral blood, hair roots, mouth epithelial cell specimens labeled with name, sex and age.

Determination of the ACE genotype

The *ACE* genotype of the all subjects was determined by polymerase chain reaction (PCR) amplification of the respective fragment for the D and I alleles from intron 16 of the *ACE* gene and size fraction by electrophoresis. Genomic DNA was extracted from peripheral blood, hair roots and mouth epithelial cell with standard techniques and PCR was performed with allele specific primer in a final volume of 20 ㎕, containing 10X PCR buffer, 1mM dNTP mixture, 1Unit of Taq polymerase (TaKaRa), Primermix (Genotech) and 100 ng/㎕ Genomic DNA. The DNA was amplified for 30 cycles with denaturation 94℃ for 45 sec, annealing at 60℃ for 45 sec, and extension at 72℃ for 45 sec, followed by final extension at 72℃ for 5 min (DNA thermal cycler TaKaRa, Japan). PCR products were electrophoresed in 2% agarose gels with ethidium bromide. The amplification products of the I and D alleles were identified by ultraviolet transillumination as distinct bands (I allele : 525, 155 bp, D alllele : 237 bp) (Fig. 1).

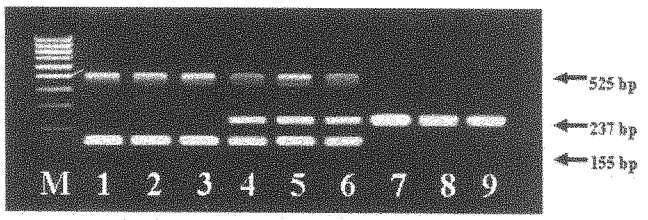

Fig. 1. Ethidium bromide stained gel showing the three *ACE* genotype 155, 525 bp correspond to the Insertion allele (I), 237 bp to Deletion allele (D).
M : DNA size marker (100 bp) Lane 1 - 3 : ACE I I type
Lane 4 - 6 : ACE ID type Lane 7 - 9 : ACE DD type

Statistical analysis

Statistical Significance was assessed by χ^2 analysis using the SAS for the ACE genotype. A value of $p<0.05$ was considered.

RESULT

We examined the distribution of the *ACE* genotypes among elite endurance students and compared it to match general population. The relative frequency of *ACE* genotype did not show significant difference between elite endurance students and general population (Table 1).

DISCUSSION

Serum ACE concentration is very stable within the individual, but there is large inter - individual difference, and approximately 50% of the variability of serum ACE between individuals is the result of an I / D polymorphism (O`Dell *et al.*, 1995). Individuals homozygous for D allele have serum ACE concentration about 50% higher than individuals homozygous for the I allele. And ACE I I genotype have serum ACE activity which is approximately two - thirds that of DD genotype (Lechin *et al.*, 1995).

Possession of the *ACE* gene polymorphic marker D has been shown to be a risk factor for coronary artery disease (Ruiz et al., 1994). Homozygous for D allele is associated with myocardial infarcton, dilated cardiomyopathy, restenosis after coronary angioplasty, ventricular hypertrophy, sudden cardiac death (Morris, 1996) and progressive deterioration of renal function (Marre *et al.*, 1997). Whereas, the association of the I allele with improved endurance might have derived from variable

Table 1. Frequency of the *ACE* genotypes and Insertion (I) / Deletion (D) allleles in the populations by χ^2 analysis, elite endurance students did not have a significant I I genotype compared with general population (p = 0.783). n=number of subjects studied

Group	ACE genotype			ACE allele	
	II	ID	DD	I	D
elite endurance students(n=200)	68 (34.0%)	100(50.0%)	32(16.0%)	236(59.0%)	164(41.0%)
general population (n=739)	269(35.1%)	63(49.1%)	117(15.8%)	881(59.6%)	597(40.4%)

increases in substrate delivery due to increase in cardiac output and muscle capillary density ; from changes in the nature of substrate used, due to a different shift stored fatty acids as fuel, or in the efficiency of substrate utilization relating to altered muscle fiber type, from altered mitochondrial density, or from raised muscle myglobin content. Elevated catecholamine, cortisol and growth hormone concentration may also increase the availability of oxidative fuel (Montgomery and Marshall, 1998).

A strong relationship between *ACE* genotype and physical performance has been observed by other studies. We believed that *ACE* I I genotype was correlation with the athletic performance in elite endurance students. And we examined genotyping of *ACE* gene of elite endurance students and the general population. But previous report (Montgomery and Marshall, 1998 ; Gayagay *et al.*, 1998) suggested that an allelic variant of the gene encoding ACE was significantly over - represented in elite British mountaineers and Australian national rowers. But our result showed that no significant difference was observed among *ACE* I I genotype and human capacity for physical performance (x^2 test=0.076 , DF = 1 P = 0.783). Therefore, the angiotensin converting enzyme gene I/D polymorphism does not seem to be a useful marker for human capacity for physical performance in Korea. The making of an elite athlete is complex and includes a range of environmental and behavioral factor and genetic predisposition to athletism which also might be important.

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