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Association between Genetic Polymorphism of the Human Angiotensin I Converting Enzyme Gene and Athletic Performance

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한국인 운동선수군에서 안지오텐신 전환효소 유전자의 다형성과 심폐 지구력과의 관련성에 관한 연구

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요 약

심폐 지구력은 유전인자에 의해 부분적으로 결정되며, 현재까지 이루어진 연구 결과에 의하면 안지오텐신 전환효소 유전자에 존재하는 다형성과 이 형질 사이에 유의한 관련성이 보고되고 있다. 그러나, 이러한 연구는 주로 서양인을 대상으로 수행되었기 때문에, 유전적 배경이 다른 아시아 집단에 대해서는 아직까지 이렇다 할 연구 성과가 없는 실정이다. 이에, 본 연구에서는 아시아 집단 중에서도 민족적으로 순수한 한국인 집단을 대상으로 안지오텐신 전환효소 유전자에 존재하는 다형성이 한국인 집단에서도 심폐 지구력과 유의한 관련성이 있는 지를 조사하였다. 그러나, 한국인 운동선수군을 대상으로 한 연구에서는 안지오텐신 전환효소 유전자의 다형성이 심폐 지구력을 비롯한 신체 계측치 및 생화학적인 측정치 들과 어떠한 관련성도 나타내지 않았다($P<0.05$). 그러나, 본 연구 대상은 다양한 종목에서 선발된 운동 선수들을 표본으로 하였기 때문에, 단일 종목의 운동 선수군을 대상으로 한 추시가 요구된다.

주요어 : 안지오텐신 전환효소, 심폐 지구력, 유전자형

INTRODUCTION

Cardiovascular function is an important element of athletic performance, and a number of studies have

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suggested that many genes contribute to this function (Rankinen *et al.*, 2001). Until now, it is known that several causative genes contribute to the genetic make-up of athletic performance (Rankinen *et al.*, 2000; Rivera *et al.*, 1997; Rivera *et al.*, 1999). One of the candidate genes is the components of renin-angiotensin system (RAS) (Alvarez *et al.*, 2000; Gayagay *et al.*, 1998). The component genes of RAS include those encoding for renin, angiotensinogen (AGT), angiotensin I converting enzyme (ACE), angiotensin II type1 receptor (AT_1R) and angiotensin II type2 receptor (AT_2R). Angiotensin I is produced from AGT by renin, and it subsequently is converted to angiotensin II by ACE. Angiotensin II increases the blood pressure by causing vasoconstriction, aldosterone secretion and increased sodium and water reabsorption in the kidney. The cellular effects of angiotensin II are mediated by two structurally distinct receptor subtypes, AT_1R and AT_2R (Inagami *et al.*, 1994). Of these receptors, AT_1R largely acts to angiotensin II.

The ACE gene is located on chromosome 17q23 (Soubrier *et al.*, 1988), and insertion/deletion (I/D) polymorphism of this gene is known to reside in intron 16 (Rigat *et al.*, 1992). This polymorphism is composed of the I and D alleles classified by the presence or absence of a 287 bp *alu* repeat sequence, respectively. The D allele of the ACE gene was associated with various cardiovascular diseases such as myocardial infarction (MI) (Cambien *et al.*, 1992; Cambien, 1994; Samani *et al.*, 1996; Tiret *et al.*, 1993), dilated cardiomyopathy (Raynolds *et al.*, 1993), left ventricular hypertrophy (Fatini *et al.*, 2000; Schunkert *et al.*, 1994), coronary artery disease and restenosis after angioplasty (Kaski, 1994), while I allele with athletic performance (Alvarez *et al.*, 2000; Gayagay *et al.*, 1998; Woods *et al.*, 2000). Also, the D allele has been associated with a higher plasma ACE level than that in I allele (Alvarez *et al.*, 2000; Bloem *et al.*, 1996; Cambien, 1994; Cambien *et al.*, 1988; Tiret *et al.*, 1992).

In the present study, we investigated the association between I/D polymorphism of ACE gene and athletic performance in Korean population.

MATERIALS AND METHODS

Study subjects

A total of 170 unrelated individuals were randomly chosen from the students of the department of physical education, the Hanyang University and the outpatients of Seoul Hygiene Hospital, Seoul, Korea. We studied 105 male elite athletes: 15 basketball players, 21 soccer players, 30 baseball players, 12 gymnastics players, 11 volleyball players, 4 >5,000 m middle distant runners, 7 judo players and 5 marathon players. All players were considered to be among the most extreme endurance competitions. In addition, we analyzed 65 male controls. Controls consisted of healthy unrelated volunteers.

Determination of anthropometric and biochemical parameters

Blood samples were obtained in EDTA tubes from individuals who had been fasting for 12-16 hour. Systolic and diastolic blood pressures were measured by sphigmomanometer. The mean arterial pressure (MAP) is calculaed by DBP-1/3 (SBP-DBP) (mmHg). The body mass index (BMI) value was calculated by the body weight (kg) devided by the square of the height (m^2). Concentration of plasma total cholesterol (TC) and triglyceride were measured by enzymatic colorimetry methods with commercial kit (Boehringer Mannheim, Germany) and chemistry analyzer. HDL-cholesterol was determined by measuring cholesterol in the supernatant after precipitation of the plasma with $MgCl_2$ and dextran sulfate, with a Gilford Impact 400 E automated analyzer with reagents and calibrators from Boehringer Mannheim. LDL-cholesterol level was calculated by using the formular of Freidwald *et al.* (1972). Plasma LDH and creatine phosphokinase activity were measured by ultraviolet assay.

DNA analysis

Blood samples were collected in EDTA-containing tubes and centrifuged at 1,500 × g for 10 min. Genomic DNA was isolated from buffy coat by the method of Sambrook *et al.* (1989) with slight modification. Polymerase Chain Reaction (PCR) techniques were used for I/D polymorphism of ACE gene (Rigat *et al.*, 1992). Briefly, total 50 μl of the reaction mixture contained 200-400 ng of genomic DNA, 100 ng of each primer, 200 μM of each dNTP, and buffers recommended by the manufacturer. The sequences of the primer for I/D polymorphisms studied were:

sense, 5′-CTGGAGACCACTCCCATCCTTTCT-3′,

nonsense 5′-ATGTGGCCATCACATTCGTCAGAT-3′.

Amplification was carried out with automated thermocycler: one cycle at 94°C for 5 min, 30 cycles at 94°C for 1 min, at 58°C for 1 min and at 72°C for 2 min with a final polymerization at 72°C for 10 min. Amplified PCR products were visualized by 2% agarose gel with ethidium bromide staining. The gels were directly photographed on an UV transilluminator and genotyped. The PCR product of the I/D polymorphism in the ACE gene produces a short (allele D of 190 bp) and a long (allele I of 490 bp) fragments (Fig. 1). Each DD homozygotes were confirmed by a second PCR using insertion-specific primer (Odawara *et al.*, 1997).

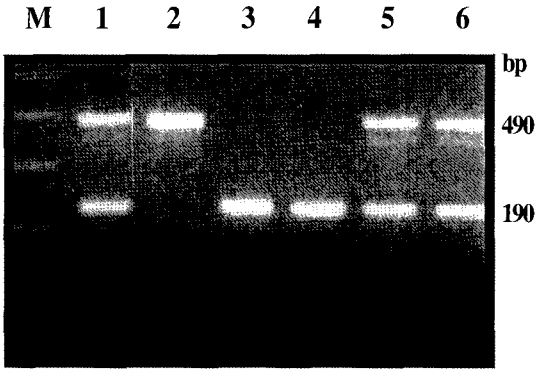

Fig. 1. The I/D polymorphism of the ACE gene. Lane M, size marker; Lanes 1, 5 and 6, ID heterozygotes; lane 2, II homozygote; lane 3 and 4, DD homozygotes.

Statistical analysis

Allele frequencies were estimated by gene counting method. The heterozygosity and polymorphism information content (PIC) values were estimated by the method of Bostein *et al.* (1980). The significance of differences in allele frequencies between populations was also estimated by χ^2-test. The relative ratio of elite athletes associated with allelic variation was expressed in terms of an odds ratio (OR) with 95% confidence interval (CI). One-way ANOVA test was performed to compare the mean levels of biochemical parameters among different genotypes. Statistical significance was accepted at the $P = 0.05$ level. All statistical analysis was performed by the computer program of MINITAB (version 13).

RESULTS

Genotype distribution

In the present study, we attempted to clarify the distribution of I/D polymorphisms in the ACE gene in Korean population. Table 1 displays the gene fre-

Table 1. Genotype and allele frequencies of the insertion/deletion polymorphism in the ACE gene between controls and elite athletes

	Genotype no. (%)			Allele no. (%)			
	II	ID	DD	I	D	H[1]	PIC[2]
Controls	13(20)	43(66)	9(14)	69(53)	61(47)	0.4981	0.3741
Athletes	25(24)	69(66)	11(10)	119(57)	91(43)	0.4911	0.3705
Chi-square		0.6490		0.4190			
Probability		0.7230		0.5180			
Odds ratio (CI)[3]			1.16(0.74-1.79)				

[1] Heterozygosity was calculated as $H = 1 - \Sigma pi^2$ (p; allele frequency).
[2] Polymorphism Information Content was calculated as $PIC = 1 - \Sigma pi^2 - \Sigma\Sigma^2 pi^2 pj^2$ (p; allele frequency).
[3] 95% Confidence Interval.
Frequency is given as a percentage in parenthesis.

quencies and the values of heterozygosity and PIC for I/D polymorphisms of the ACE gene in Korean normal controls and pooled elite athletes, respectively. In the case of I/D polymorphism, the genotype and allele frequencies were not significantly different between two groups, respectively. The frequencies of II, ID and DD genotypes were 20, 66 and 14% in normal controls, and 24, 66 and 10% in elite athletes, respectively. The heterozygosity and PIC values of I/D polymorphism represented the values of 0.4981 and 0.3741 for normol controls, and 0.4911 and 0.3705 for elite athletes, respectively. According to the heterozygosity and PIC values, I/D polymorphism showed the reasonably high degree of polymorphism in both groups.

Table 2. Distribution of ACE insertion/deletion genotypes in normal controls and elite athletic groups

Subjects	ACE				
	Genotypes			Alleles	
	II	ID	DD	I	D
Controls (n=65)	13 (26)	43 (59)	9 (15)	69 (53)	61 (47)
Athletes (n=105)	25 (24)	69 (66)	11 (10)	119 (57)	91 (43)
Basketball (n=15)	6 (40)	9 (60)	0 (0)	21 (70)	9 (30)
Soccer (n=21)	4 (19)	14 (67)	3 (14)	22 (52)	20 (48)
Baseball (n=30)	7 (23)	19 (63)	4 (14)	33 (55)	27 (45)
Gymnastics (n=12)	3 (25)	6 (50)	3 (25)	12 (50)	12 (50)
Volleyball (n=11)	1 (9)	10 (91)	0 (0)	12 (55)	10 (45)
Runner (n=4)1	1 (25)	3 (75)	0 (0)	1 (62)	3 (38)
Judo (n=7)	0 (0)	6 (86)	1 (14)	6 (43)	8 (57)
Marathon (n=5)	3 (60)	2 (40)	0 (0)	3 (80)	2 (20)
Total (n=170)	38 (22)	112 (66)	20 (12)	188 (55)	152 (45)

[1] > 5,000 m distance runner.

Table 2 represents the distributions of genotype and allele frequencies in the ACE gene among various athletic groups. Among sporting disciplines studied, we found the highest excess of I allele in marathon runners although statistically not significant.

Association with biochemical parameters

Table 3 presents the comparision of anthropometric data and intermediate phenotypes across I/D polymorphism in elite athletes. There were no significant differences in anthropometric data or intermediate phenotypes across the genotypes, respectively. Therefore, I/D polymorphism of ACE gene was not significantly associated with any anthropometric data or biochemical parameters in our subjects.

Table 3. The comparison of the anthropometric data and intermediate phenotypes according to ACE insertion/deletion genotypes in elite athletes

Variables	Genotypes		
	II (No.)[11]	ID (No.)	DD (No.)
Age (year)	20.5±1.0 (23)	20.1±1.1 (53)	20.5±1.3 (8)
BMI (kg/m^2)[1]	22.4±2.1 (23)	23.0±1.9 (54)	23.4±2.2 (8)
SBP (mmHg)[2]	118.7±7.1 (23)	118.9±8.1 (55)	120.8±9.8 (8)
DBP (mmHg)[3]	69.8±7.4 (23)	72.5±7.2 (55)	73.8±6.8 (8)
MAP (mmHg)[4]	86.1±5.8 (23)	87.9±6.7 (55)	89.5±6.8 (8)
Tg (mg/dl)[5]	111.4±95.7 (25)	104.2±71.3 (69)	91.1±31.4 (11)
TC (mg/dl)[6]	188.2±64.8 (25)	169.8±28.2 (69)	170.7±22.4 (11)
LDL-chol (mg/dl)[7]	106.1±67.0 (25)	90.2±30.1 (69)	92.4±24.5 (11)
HDL-chol (mg/dl)[8]	59.7±11.2 (25)	57.6±13.3 (69)	58.4±10.8 (11)
CPK (IU/l)[9]	531.9±986.1 (25)	607.0±925.5 (68)	421.3±381.0 (11)
LDH (IU/l)[10]	479.5±97.4 (25)	458.8±110.3 (69)	404.6±58.9 (11)

[1]Body Mass Index, [2]Systolic blood pressure, [3]Diastolic blood pressure, [4]Mean arterial pressure, [5]Triglyceride, [6]Total cholesterol, [7]LDL-cholesterol, [8]HDL-cholesterol, [9]Creatine phosphokinase, [10]Lactate dehydrogenase and [11]Number. Value are mean±SD (standard deviation).

DISCUSSION

Many genetic and environmental factors contribute to athletic performance (Rankinen *et al.*, 2000). Specific candidate genes have been tested for association with athletic performance (Alvarez *et al.*, 2000; Dionne *et al.*, 1993; Gayagay *et al.*, 1998; Murakami *et al.*, 2001; Rivera *et al.*, 1998; Wolfarth *et al.*, 2000). Nevertheless, the genetic variations responsible for athletic performance remain largely unknown (Gagnon *et al.*, 1997), and the success to date in identifying causative genes has been very limited. It appears that DNA polymorphisms in the candidate genes may play a significant role as useful genetic markers in the association study.

Gayagay *et al.* (1998) firstly reported an excess of I allele in the ACE gene in Australian rowers, and the study by Alvarez *et al.* (2000) confirmed the previous result in Spanish elite athletes. Other studies, however, reported no association between this allele and athletic performance (Rankinen *et al.*, 2000; Taylor *et al.*, 1999). The discrepant results reported for the same gene may be due to different criteria used in selection of study subjects, different sample size or racial differences in study samples. In the relationship between I allele of ACE gene and endurance performance, inconsistent results have been documented in mainly Caucasian population (Alvarez *et al.*, 2000; Gayagay *et al.*, 1998; Myerson *et al.*, 1999; Rankinen *et al.*, 2000; Taylor *et al.*, 1999), while to our knowledge, the report in Asian population is scanty. Because the I allele frequency of the ACE gene is various among different ethnic groups studied (Barley *et al.*, 1994; Johanning *et al.*, 1995), it could not excluded the possibility that the ethnic difference may exist. Especially, it is the trend that the frequency of I allele in Asian population (0.53-0.59) is higher (Hong *et al.*, 1997) than that of Caucasian population (0.41-0.50) (Cambien, 1994; Johanning *et al.*, 1995). Thus, these association studies emphasize the importance of using a homogeneous ethnic group in the selection of the study samples.

The present study revealed an excess of I allele in the ACE gene among marathon runners in ethnically homogeneous Korean population, although there were no significant differences in I allele frequency between normal controls and pooled elite athletic group. Myerson *et al.* (1999) reported that the frequency of I allele is higher among longer distance runners than controls, and rises with distance run. This trend of rising I allele frequency, from short to longer distance, may imply the possibility that I allele of ACE gene influence endurance performance through improvements in cardiovascular function such as regulation of plasma ACE concentration and blood pressure. Therefore, it is likely that I allele in the ACE gene could exercise the favorable effect on endurance performance in Koreans as well as Caucasians (Alvarez *et al.*, 2000; Gayagay *et al.*, 1998; Myerson *et al.*, 1999).

Recently, Woods *et al.* (2001) performed the association study using swimmers as an athletic group in British population. They showed the significant excess of D allele in this group, suggesting the possible role of D allele in power sports. The excess of D allele in power sports and the significant association of I allele with endurance performance requires the attention in sample design. It is necessary to use a homogeneous sample with the same sporting discipline to clarify the relationship between I/D polymorphism in the ACE gene and endurance performance. Unfortunately, our cohort was composed of the athletes from diverse sporting disciplines as the study subjects, and consequently caused by small sample size in each sporting discipline.

Nevertheless, this study is the first report of an association between the endurance performance and I/D polymorphism of the ACE gene in Asian population. Therefore, our study will contribute to clarification of genetic basis of endurance performance, and provide a basis for further investigation, although it is yet premature to draw any conclusion about the genetic susceptibility of endurance performance in Korean elite athletes.

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