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Msp I RFLP of the Human Apolipoprotein AI Gene in Korean Elite Athletes

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ABSTRACT: **Prolonged exercise is known to increase steady-state serum high-density lipoprotein cholesterol (HDL-cholesterol) and apolipoprotein AI(apo AI) concentrations. We investigated the effect of adaptation to endurance exercise on the association of the genetic polymorphism in the apo AI gene with these biochemical parameters. 108 male subjects were randomly selected from a group of elite athletes, and 65 male samples used as sedentary control group from Korean general population. The genetic polymorphism in the apo AI gene locus was detected by polymerase chain reaction(PCR) and DNA digestion with *Msp* I restriction endonuclease. The genotype frequency for the *Msp* I RFLP was significantly different between the elite athletes and sedentary controls($P<0.05$). There were, however, no significant associations between the *Msp* I RFLP of the apo AI gene and the biochemical parameters in elite athletic group. Therefore, our findings indicate that the *Msp* I RFLP of the apo AI gene was not associated with the serum apo AI and HDL-cholesterol concentrations in Korean male elite athletes.**

Keywords : **Apolipoprotein AI, Athletes and Genotype**

Introduction

A number of genetic and environmental factors affect steady-state concentrations of serum lipids, and have been implicated as risk factors for cardiovascular diseases. Environmental factors including exercise are reported to be responsible for as much as 50% of the variance in serum lipid levels, with the remainder of the variance being attributed to genetic differences between individuals (Hamsten *et al.*, 1986; Moll *et al.*, 1989).

Epidemiological studies have shown a negative correlation between serum high-density lipoprotein(HDL) cholesterol level and the development of cardiovascular diseases (Miller *et al.*, 1977) and, exercise has been shown to increase serum HDL-cholesterol levels(Williams *et al.*, 1982; Wood *et al.*, 1983). The level of apolipoprotein A-I(apo AI), the major protein component of HDL, has also been proposed as a negative risk factor for this form of cardiovascular diseases(Maciejko *et al.*, 1983), and the gene encoding apo AI is located on the long arm of chromosome 11 together with the apo CIII and apo AIV genes(Karathanasis, 1986). A common polymorphism described in the apo AI promoter region consists of a G to A substitution at the 75 bp upstream of the transcription start site(Needham *et al.*, 1994). Many studies have found consistent association of this polymorphism with serum HDL levels in several populations (Humphries *et al.*, 1993; Jeenah *et al.*, 1990; Pagani *et al.*, 1990; Paul-Hayase *et al.*, 1992; Sigurdsson *et al.*, 1992; Saha *et al.*, 1994; Talmud *et al.*, 1994; Xu *et al.*, 1993) while not in others(Barre *et al.*, 1994; Needham *et al.*, 1994; Smith *et al.*, 1992). These discrepancies between different studies may be due to differences in ethnic origin, sauce or sample size.

There were few report about the relationship between the genetic variation of the apo AI gene and serum lipid parameters in elite athletic group. Ziman and Jeenah, (1995) failed to uncover an association between *Pst* I RFLP of apo AI gene and serum HDL-cholesterol or apo AI levels in South African marathon group. It could be excluded the possibility that other genetic variations of apo AI gene influence serum HDL-cholesterol and

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apo AI concentrations in elite athletes.

Using prolonged exercise as an environmental factor, we investigated the association between the serum biochemical parameters and the *Msp* I RFLP of the apo AI gene in specialized elite athletic group.

Materials and Methods

Study subjects

A total of 173 unrelated individuals were randomly chosen from the students of the department of physical education, the Hanyang University, Seoul, Korea, and the outpatients of the department of clinical pathology, Seoul Hygiene Hospital, Seoul, Korea.

We studied 108 male elite athletes: 15 basketball players, 22 soccer players, 26 baseball players, 11 gymnastics players, 17 volleyball players, 4 middle-distant (5,000 m or 10,000 m) runners, 9 judo players and 4 marathon players. In addition, we analyzed 65 male sedentary controls.

Determination of anthropometric and biochemical parameters

Blood samples were obtained in EDTA tubes from individuals who had been fasting for 12-16 hr. Systolic and diastolic blood pressures was measured by sphigmomanometer. The mean arterial pressure(MAP) was calculated by DBP 1/3(SBP-DBP)(mmHg). The VO_{2max} index was measured by using motor-driven treadmills (Strømme *et al.*, 1977). The body mass index (BMI) value was calculated by the body weight (kg) divided by the square of the height (m^2). Concentration of total cholesterol (TC), triglyceride and glucose were measured by enzymatic colorimetry methods with commercial kit (Boehringer Mannheim, Germany) and chemistry analyzer. HDL-cholesterol level was determined by measuring cholesterol in the supernatant after precipitation of the serum with $MgCl_2$ and dextran sulfate, with a Gilford Impact 400E automated analyzer with reagents and calibrators from Boehringer Mannheim. Lipoprotein (a) (LP(a)) level was measured by the immunoprecipitation method (SPQ Test System, INCSTAR Corporation, Stillwater, Minnesota, USA) and apo AI concentration was determined by immunoturbidimetric method (COBAS INTEGRA, ROCHE Diagnostics, USA). LDL-cholesterol level was calculated by using the formula of Freidwald *et al.*, (1972). Serum LDH and creatine phosphokinase activity were measured by ultraviolet assay.

DNA analysis

Genomic DNA was isolated from buffy coat by the method of Kunkel *et al.*, (1977). Polymerase Chain Reaction(PCR) techniques were used for *Msp* I RFLP of apo AI gene (Needham *et al.*, 1994; Pagani, *et al.*, 1990). Briefly, total 50 ml of the reaction mixture contained 200-400 ng of genomic DNA, 100 ng of each primer, 200 mM of each dNTP, and buffers recommended by the manufacturer. The sequences of the primer for *Msp* I RFLP studied were:

sense, 5'-CACCCGGGAGACCTGCAAGC-3',
nonsense 5-TCTAAGCAGCCAGCTCTTGCA-3.

Amplification was carried out with DNA thermocycler: one cycle at 96°C for 5 min, 30 cycles at 94°C for 1 min, at 55°C for 1 min and at 72°C for 2 min with a final polymerization at 72°C for 10 min.

Following amplification, 10 ul of the PCR product were incubated with 10 units of restriction enzyme *Msp* I (Boeringer Mannheim, Germany) at 37 for 18 hours. Digested PCR products were genotyped by the electrophoresis using 2% agarose gel with 0.5X TBE buffer.

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 study groups was also estimated by c^2-test. 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 analyses were performed by the computer program of dBSTAT for windows (version 3.0).

Results and Discussion

The candidate gene approach in the study of cardiovascular risk factors has been plagued with conflicting results. These are highlighted in association studies between genetic variations of apo AI gene and changes in serum lipid and apolipoprotein levels (Ziman and Jeenah, 1995). Association studies are also, complicated by gene-gene and gene-environment interactions. Pedersen and Berg, (1989, 1990) have shown, in two separate studies, that the interaction between

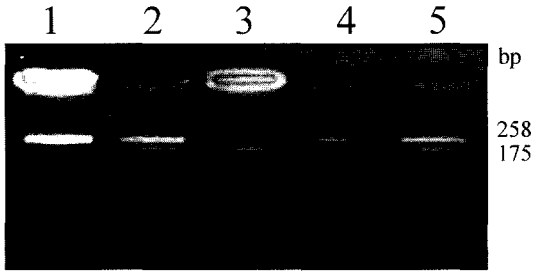

Fig. 1. *Msp* I RFLP patterns of the apo AI gene. Lane 1, AA genotype; lane 2, 4 and 5, GA genotypes; lane 3, GG genotype.

variation at the LDL receptor and apo E loci enhances the association with changes in serum lipid levels. In this study, we attempted to address the gene-environmental relationship and its influence on the association between the *Msp* I RFLP of apo AI gene and serum lipid changes, by investigating this association in a group of highly trained elite athletes of genetically homogeneous Korean origin.

PCR amplification of genomic DNA produced a 258 bp product. Digestion with *Msp* I yielded three bands of 175, 79 and 4 bp, in the presence of G allele, while two bands of 254 and 4 bp in the presence of A allele. The 4 bp fragment was not visualized because it ran off the gel during electrophoresis (Figure 1).

In the present study, we attempted to clarify the distribution of *Msp* I RFLP of the apo AI gene in Korean male elite athletes. Table 1 displays the gene frequencies and the values of heterozygosity and PIC for *Msp* I RFLP of the apo AI gene in Korean sedentary controls and pooled elite athletes, respectively. The frequencies of GG, GA and AA genotypes were 63, 29 and 8% in sedentary controls, and 40, 56 and 4% in elite athletes, respectively. There was the significant difference in genotype frequency between two groups ($P<0.05$). The genotype distribution of GA heterozygotes was 56% in elite athletes (n=60) compared with only 29% in sedentary controls (n=19). The excess of GA heterozygotes in elite athletes resulted in the significant deviation from Hardy-Weinberg equilibrium ($P<0.05$). Chance effect might be operating in *Msp* I RFLP of the apo AI gene in elite athletes. Accordingly, we can only set a limited potential value on the difference in the genotype distribution between elite athletes and sedentary controls. Further investigations with large sample size are required to confirm this association.

The derived allele frequency from genotype distribution was not significantly different between sedentary controls and elite athletes. The heterozygosity and PIC values of *Msp* I RFLP represented the values of 0.3466 and 0.2866 for sedentary controls, and 0.4381 and 0.3421 for elite athletes, respectively. According to the heterozygosity and PIC values, The *Msp* I RFLP showed the reasonably high degree of polymorphism in both groups.

Table 2 represents the distributions of allele and genotype in the *Msp* I RFLP of the apo AI gene among various athletic groups. There were no significant differences in genotype and allele frequencies among sporting disciplines studied.

Table 3 presents the comparision of anthropometric data and intermediate phenotypes across *Msp* I RFLP of the apo AI gene in elite athletes. There were no significant differences in anthropometric data or intermediate phenotypes across the genotypes. These observations indicate that prolonged exercise is not an environmental factor affecting variation in serum lipid or apo AI levels associated with the *Msp* I in Koreans.

Further studies are required to elucidate the genetic background responsible for the elevation of serum HDL-cholesterol and apo AI concentrations in elite athletes.

Table 1. Genotype and allele frequencies of the *Msp* I RFLP in the apo AI gene between controls and elite athletes

	Genotype No. (%)			Allele No. (%)		H^1	PIC^2
	GG	GA	AA	G	A		
Controls	41(63)	19(29)	5(8)	101(78)	29(22)	0.3466	0.2866
Athletes	43(40)	60(56)	5(4)	146(68)	70(32)	0.4381	0.3421
Chi-square		11.3387		3.5735			
Probability		0.0035		0.0587			
Odds ratio(CI)[3]			1.67(1.01-2.76)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.

Table 2. Distribution of *Msp* I RFLP of apo AI gene in normal controls and elite athletic groups

Subjects	Apo AI/*Msp* I				
	Genotypes			Alleles	
	GG	GA	AA	G	A
Controls (n=65)	41(63)	19(29)	5(8)	101(78)	29(22)
Athletes (n=108)	43(40)	60(56)	5(4)	146(68)	70(32)
Basketball (n=15)	7(47)	7(47)	1(6)	21(70)	9(30)
Soccer (n=22)	9(41)	11(50)	2(9)	29 (66)	15(34)
Baseball (n=26)	12(46)	13(50)	1(4)	37(71)	15(29)
Gymnastics (n=11)	7(64)	4(36)	0(0)	18(82)	4(18)
Volleyball (n=17)	3(18)	13(76)	1(6)	19(56)	15(44)
Runner (n=4)[1]	1(25)	3(75)	0(0)	1(62)	3(38)
Judo (n=9)	4(44)	5(56)	0(0)	13(72)	5(28)
Marathon (n=4)	0(0)	4(100)	0(0)	4(50)	4(50)
Total (n=173)	84(48)	79(46)	10(6)	247(71)	99(29)

[1]>5,000m distance runner.

Table 3. The comparison of the anthropometric data and intermediate phenotypes according to Apo AI/*Msp* I RFLP in elite athletes

Variables	Genotypes		
	GG(No.)[13]	GA(No.)	AA(No.)
Age (year)	20.2 ± 1.0(34)	20.4 ± 1.2(48)	20.2 ± 1.3(5)
BMI (kg/m^2)[1]	23.4 ± 2.0(34)	22.7 ± 1.9(48)	22.3 ± 1.1(5)
VO_{2max} (ml/kg/min)	55.5 ± 1.6(34)	55.9 ± 1.5(48)	56.3 ± 0.8(5)
SBP (mmHg)[2]	118.4 ± 6.4(34)	119.4 ± 9.1(51)	120.8 ± 6.3(5)
DBP (mmHg)[3]	74.1 ± 6.2(34)	71.3 ± 7.0(51)	76.8 ± 4.1(5)
MAP (mmHg)[4]	88.9 ± 5.4(34)	87.3 ± 6.7(51)	91.2 ± 4.8(5)
Tg (mg/dl)[5]	110.0 ± 83.8(43)	98.6 ± 67.3(60)	104.0 ± 60.3(5)
TC (mg/dl)[6]	178.5 ± 51.7(43)	173.6 ± 30.9(60)	175.8 ± 24.0(5)
LDL-chol (mg/dl)[7]	99.4 ± 54.8(43)	93.4 ± 27.8 (60)	82.4 ± 51.8(5)
HDL-chol (mg/dl)[8]	57.0 ± 12.0(43)	57.4 ± 12.9(60)	56.4 ± 8.7(5)
Apo AI (mg/dl)[9]	96.9 ± 25.7(43)	111.4 ± 37.9(60)	89.3 ± 21.3(5)
Lp (a) (mg/dl)[10]	8.7 ± 8.9(21)	8.5 ± 9.4(27)	7.7 ± 0.9(3)
CPK (IU/l)[11]	559.3 ± 843.8(42)	575.5 ± 942.8(60)	374.2 ± 173.2(5)
LDH (IU/l)[12]	455.3 ± 86.5(43)	463.2 ± 116.2(60)	440.6 ± 74.7(5)
Glucose (mg/dl)	54.3 ± 16.3(43)	57.1 ± 14.2(60)	46.2 ± 10.1(5)

[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]Apolipoprotein AI, [10]Lipoprotein (a), [11]Creatine phosphokinase and [12]Lactate dehydrogenase and [13]Number. Value are mean ± SD (standard deviation).

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