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The *Pst* I Polymorphism of the Human Apolipoprotein AI Gene in Korean Elite Athletes

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Serum lipid and lipoprotein levels are influenced by genetic factors, and exercise increases the concentrations of cardio-protective parameters such as high-density lipoprotein cholesterol (HDL-cholesterol) and apolipoproteinAI (apoAI) in human serum. In the present study, we tested the effect of adaptation to endurance exercise on the association of a genetic polymorphism (*Pst* I RFLP) in the apoAI gene with these biochemical parameters. The genotype and allele frequencies for the *Pst* I RFLP were not significantly different between the elite athletes and sedentary controls (P>0.05). There were also no significant associations between the *Pst* I RFLP of the apoAI gene and the biochemical parameters in elite athletic group. Thus, our results suggest that the *Pst* I RFLP of the apoAI gene was not significantly associated with the serum apoAI and HDL-cholesterol concentrations as well as athletic performance in Koreans.

Serum lipid and lipoprotein levels are influenced by a number of genetic and environmental factors, and environmental factors such as exercise are known to be responsible for about 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, prolonged exercise has been shown to increase serum HDL-cholesterol levels (Williams et al., 1982; Wood et al., 1983).

ApolipoproteinAI (apoAI), the major protein component of HDL, is one of the protective factors for various cardiovascular diseases (Maciejko et al., 1983), and the reverse cholesterol transport process attains this protective function from peripheral tissues to liver (Hill and McQueen, 1997). The gene for apoAI is located on the long arm of chromosome 11 together with the apoCIII and apoAIV genes (Karathanasis, 1986). Several DNA sequence variations in this gene have been reported, and used as genetic markers to investigate the association with various phenotypes (Groenendijk et al., 2001; Kang et al., 2002). Among those studied, *Pst* I RFLP has been reported to be associated with higher serum HDL-cholesterol and apoAI levels in some healthy populations (Kessling et al., 1988), while lower levels in coronary artery disease groups (Ordovas et al., 1986; Paulweber et al., 1988). The data from these studies suggest that the levels of HDL-cholesterol and apoAI may be determined by the interaction between variations associated with the *Pst* I RFLP and second genetic and/or environmental factors.

Until now, there were few report about the relationship between this RFLP of the apoAI gene and serum lipid parameters in elite athletic group. Ziman and Jeenah (1995) failed to uncover an association between *Pst* I RFLP of apoAI gene and serum HDL-cholesterol or apoAI levels in South African marathon group. It could be, however, excluded the possibility that this RFLP of apoAI gene influence serum HDL-cholesterol and apoAI concentrations in other ethnic group.

Using prolonged exercise as an environmental factor, we investigated the association between the serum biochemical parameters and the *Pst* I RFLP of the apoAI gene in specialized 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 genotyped 110 elite male athletes: 15 basketball

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players, 23 soccer players, 30 baseball players, 12 gymnastics players, 15 volleyball players, 4 middle-distant (5,000 m or 10,000 m) runners, 7 judo players and 4 marathon players. In addition, we analyzed 63 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 apoAI concentration was determined by immunoturbidimetric method (COBAS INTEGRA, ROCHE Diagnostics, USA). LDL-cholesterol level was calculated by using the formula of Friedwald et al., (1972). Serum LDH and creatine phosphokinase activity were measured by ultraviolet assay.

DNA analysis

Genomic DNA was prepared from buffy coat of 5 ml blood after lysis of red blood cell (Kang et al., 2000; Bae et al., 2001). Polymerase Chain Reaction (PCR) techniques were used for *Pst* I RFLP of apoAI gene (Ziman and Jeenah, 1995). 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 *Pst* I RFLP studied were:

sense, 5'-GAGCGCTCTCGAGGAGTACAC-3',
anti-sense 5'-GACTGGCTTCCACTGCTGTGC-3'.

Amplification was carried out with DNA thermocycler: one cycle at 93°C for 5 min, 30 cycles at 93°C for 30 sec, at 62°C for 1 min and at 72°C for 2 min with a final polymerization at 72°C for 10 min.

Following amplification, 10 μl of the PCR product were incubated with 10 units of restriction enzyme *Pst* I (Boeringer Mannheim, Germany) at 37°C 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 genotype and allele frequencies between study groups was also estimated by χ^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 SPSSWIN (version 10.0).

Results and Discussion

Genetic differences that may affect athletic fitness or performance began to be investigated at the DNA level in the 1990s (Gayagay et al., 1998). Progress has been rather slow, particularly as a result of the inadequate training of exercise biologists in genomic technologies and their lack of enthusiasm for genetic issues. This may, however, be about to change. Pressure from the major funding agencies has already began to force exercise biologists to incorporate genomics and other molecular biology technologies in their research if they want to remain or become competitive, particularly in light of the advances in the sequencing of the human genome.

Currently available data on the associations between genetic variants and changes in blood lipids or lipoproteins in elite athletic groups are limited to a few candidate genes. Two cross-sectional studies have considered the interactions between apolipoprotein E (apoE) genotypes and physical activity or physical fitness on plasma lipoprotein cholesterol levels (Taimela et al., 1996; ST-Amand et al., 1999). A cross-sectional study reported an interaction between a lipoprotein lipase (LPL) gene polymorphism and regular physical activity on plasma lipoprotein cholesterol (Boer et al., 1999).

In this study, we attempted to address the gene-environmental relationship and its influence on the association between the *Pst* I RFLP of apoAI 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 740 bp product. Digestion with *Pst* I yielded a band of 740 bp, in the presence of P1 allele, while a band of 400 and 340 bp in the presence of P2 allele (Fig. 1).

Table 1 displays the gene frequencies and the values of heterozygosity and PIC for *Pst* I RFLP of the apoAI gene in Korean sedentary controls and pooled elite athletes, respectively. The frequencies of P1P1, P1P2

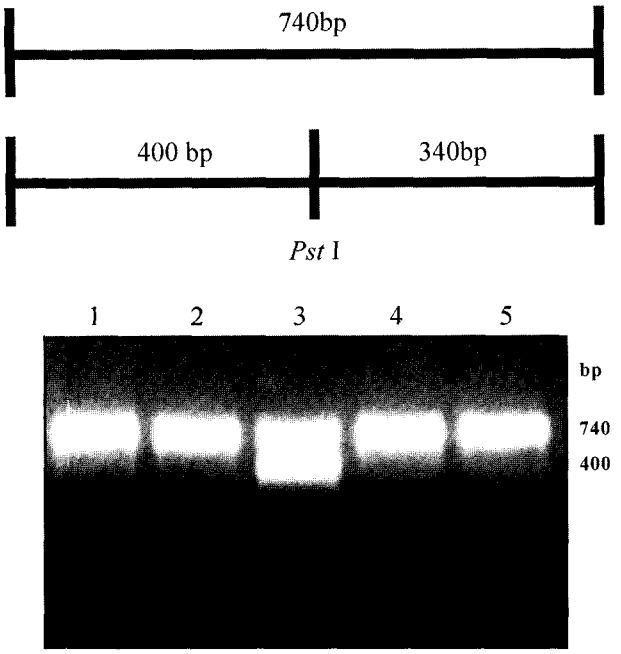

Fig. 1. *Pst* I RFLP patterns of the apoAI gene.
Lane 1 2, 4 and 5, P1P1 genotypes; lane 3, P1P2 genotype.

and P2P2 genotypes were 2, 16 and 82% in sedentary controls, and 3, 14 and 83% in elite athletes, respectively. There were no significant differences in genotype and allele frequencies between two groups ($P>0.05$). Also, these frequencies do not differ from values reported for other population groups at this RFLP (Kessling et al., 1985; Xu et al., 1990; Ziman and Jeenah, 1995).

The heterozygosity and PIC values of *Pst* I RFLP represented the values of 0.1723 and 0.1575 for sedentary controls, and 0.1800 and 0.1638 for elite athletes, respectively. According to the heterozygosity and PIC values, The *Pst* I RFLP indicated the relatively low degree of polymorphism in both groups.

Table 2 represents the distributions of genotype and allele frequencies in the *Pst* I RFLP of the apoAI gene amor g various athletic groups. There were no significant differences in genotype and allele frequencies among sporting disciplines studied ($P>0.05$).

Table 2. Distribution of the *Pst* I RFLP of apoAI gene in normal controls and elite athletic groups

Subjects	Apo AI/*Pst* I				
	Genotypes			Alleles	
	P1P1	P1P2	P2P2	P1	P2
Controls(n=63)	1(1.6)	10(15.9)	52(82.5)	12(9.5)	114(90.5)
Athletes(n=110)	3(2.7)	16(14.5)	91(82.7)	22(10.0)	198(90.0)
Basketball(n=15)	1(6.7)	2(13.3)	12(80.0)	4(13.3)	26(86.7)
Soccer(n=23)	0(0.0)	4(17.4)	19(82.6)	4(8.7)	42(91.3)
Baseball(n=30)	1(3.3)	4(13.3)	25(83.3)	6(10.0)	54(90.0)
Gymnastics(n=12)	0(0.0)	2(16.7)	10(83.3)	2(8.3)	22(91.7)
Volleyball(n=15)	0(0.0)	2(13.3)	13(86.7)	2(6.7)	28(93.3)
Runner(n=4)[1]	1(25.0)	0(0.0)	3(75.0)	2(25.0)	6(75.0)
Judo(n=7)	0(0.0)	1(14.3)	6(85.7)	1(7.1)	13(92.9)
Marathon(n=4)	0(0.0)	1(25.0)	3(75.0)	1(12.5)	7(87.5)
Total(n=173)	4(2.3)	26(15.0)	143(82.7)	34(9.8)	312(90.2)

[1]>5,000 m middle distance runner.

Table 3 presents the comparision of anthropometric data and intermediate phenotypes across *Pst* I RFLP of the apoAI gene in elite athletes. There were no significant differences in anthropometric data or intermediate phenotypes across the genotypes. These observations may imply that prolonged exercise is not an environmental factor affecting variation in serum lipid or apoAI levels associated with the *Pst* I in Korean elite athletes as well as South African marathon players (Ziman and Jeenah, 1995), and furthermore, exercise would also not be the major factor responsible for the contrasting data obtained in various association studies between the *Pst* I RFLP and serum lipid or lipoprotein levels (Ordovas et al., 1986; Kessling et al., 1988).

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 serum lipid or lipoprotein levels and the *Pst* I RFLP of the apoAI gene in specialized athletes of Asian origin.

Table 1. Genotype and allele frequencies of the *Pst* I RFLP in the apoAI gene between controls and elite athletes

	Genotype No. (%)			Allele No. (%)			
	P1P1	P1P2	P2P2	P1	P2	H^1	PIC^2
Controls	1(1.6)	10(15.9)	52(82.5)	12(9.5)	114(90.5)	0.1723	0.1575
Athletes	3(2.7)	16(14.5)	91(82.7)	22(10.0)	198(90.0)	0.1800	0.1638
Chi-square		0.2723		0.0205			
Probability		0.8727		0.8861			
Odds ratio(CI)[3]			1.06(0.50-2.21)				

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

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

Variables	Genotypes		
	P1P1(No.)[13]	P1P2(No.)	P2P2(No.)
Age(year)	19.0±0.0(2)	20.8±1.0(12)	20.3±1.2(74)
BMI(kg/m^2)[1]	23.7±0.6(2)	23.1±2.3(12)	22.0±2.0(75)
VO_{2max}(ml/kg/min)	55.7±0.5(2)	55.4±1.9(12)	55.8±1.6(75)
SBP(mmHg)[2]	115.0±9.9(2)	119.5±8.1(13)	119.2±8.0(77)
DBP(mmHg)[3]	70.0±11.3(2)	73.1±7.6(13)	72.6±6.8(77)
MAP(mmHg)[4]	85.0±4.2(2)	88.5±7.1(13)	88.1±6.1(77)
Tg(mg/dl)[5]	74.0±21.2(3)	82.0±26.9(16)	109.0±79.7(91)
TC(mg/dl)[6]	171.3±4.0(3)	193.1±77.3(16)	171.4±28.3(91)
LDL-chol(mg/dl)[7]	89.2±5.3(3)	119.1±79.4(16)	89.9±29.6(91)
HDL-cho (mg/dl)[8]	66.0±6.2(3)	57.5±7.4(16)	57.4±13.0(91)
ApoAI(mg/dl)[9]	144.3±61.2(3)	98.3±21.6(16)	103.1±32.7(91)
Lp(a)(mg/dl)[10]	5.2±0.0(1)	8.8±12.9(9)	8.3±7.6(46)
CPK(IU/l)[11]	559.3±843.8(3)	575.5±942.8(16)	374.2±173.2(90)
LDH(IU/l)[12]	462.0±33.0(3)	403.7±63.7(16)	468.1±101.8(91)
Glucose(mg/dl)	72.7±17.0(3)	52.8±11.9(16)	54.9±15.1(91)

[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. Values are mean±SD (standard deviation)

Further studies using other candidate genes are required to elucidate the genetic background responsible for the elevation of serum HDL-cholesterol and apoAI concentrations in elite athletes.

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