Environmental Mutagens & Carcinogens 22-1 : 22-29 (2002)

Two Genetic Polymorphisms of the Human Lipoprotein Lipase Gene in Korean Patients with Essential Hypertension

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(Received January 17, 2002 / Accepted March 5, 2002)

ABSTRACT **: Essential hypertension is considered to be caused by a complicated combination of genetic and environmental factors. Alterations of lipid metabolism in plasma have been reported to be related to an increased risk of essential hypertension. The purpose of this study was to investigate the relationship between two genetic polymorphisms (*Pvu* II and *Hind* III RFLPs) of the human lipoprotein lipase (LPL) gene and essential hypertension in korean population. In our result, the *Pvu* II RFLP of LPL gene was significantly associated with essential hypertension ($P < 0.05$). Therefore, we suggest that the *Pvu* II RFLP of LPL gene may be useful as a genetic marker for essential hypertension in Korean population.**

Keywords: allele, essential hypertension and lipoprotein lipase

Introduction

Essential hypertension is the result of the interaction of numerous environmental and genetic factors. One factor that is strongly associated with essential hypertension is lipid abnormality such as increased very low and low density lipoprotein and decrease high density lipoprotein (Williams *et al.*, 1989), and several candidate genes became available to study the association of essential hypertension and lipid abnormalities (Frossard *et al.*, 1998; Frossard *et al.*, 1999; Fu *et al.*, 2001; Higashimori *et al.*, 1992; İsbir *et al.*,1997; Munroe *et al.*, 1994).

Lipoprotein lipase (LPL: EC 3.1.1.34) is a glycoprotein synthesized in parenchymal cells of connective tissue, including adipocytes, skeletal and cardiac muscle, and macrophages (Mahoney *et al.*, 1982; Nilsson-Ehle *et al.*, 1980). Through the hydrolysis of triglycerides present in triglyceride-rich lipoprotein, such as chylomicrons and very low-density lipoproteins (VLDL), LPL plays an important role in lipoprotein metabolism. In this reaction, LPL requires apolipoprotein CII (apo CII) as an essential cofactor, thereby releasing free fatty acids that are either used as direct energy or are re-esterified for storage (Harvel *et al.*, 1973; Nilsson-Ehle *et al.*, 1980). Deficiency in LPL activity results in severe chylomicronemia or high level of plasma triglycerides (Babirak *et al.*, 1989).

The LPL gene is composed of 10 exons spread over 30 kb (Oka *et al.*, 1990). To date, numerous LPL gene mutations have been reported and associated with various abnormal lipid phenotypes (Babirak *et al.*, 1989; Wilson *et al.*, 1983; Wilson *et al.*, 1990). As essential hypertension is frequently accompanied by abnormalities in lipid metabolism (Flesch *et al.*, 1994; Fuh *et al.*, 1987; Lopes *et al.*, 1997), the LPL gene is considered a candidate gene that could contribute to the development of this disorder.

Several restriction fragment length polymorphisms (RFLPs) in the LPL gene have been reported (Fisher *et al.*, 1987; Funke *et al.*, 1988; Hegele *et al.*, 1989; Heinzmann *et al.*, 1987; Heinzmann *et al.*, 1991; Li *et al.*, 1988a; Li *et al.*, 1988b), and used as genetic

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markers in clinical association studies. For example, the *Pvu* II RFLP was significantly associated with plasma triglyceride levels (Chamberlain *et al.*, 1989), and the *Hind* III RFLP of the LPL gene with coronary artery disease (Thorn *et al.*, 1990), hypertriglyceridaemia (Chamberlain *et al.*, 1989), total and HDL-cholesterol level (Heinzmann *et al.*, 1991), although the results have not always been concordant in all populations studied.

In view of the importance of LPL in plasma lipid metabolism, we studied the association between two genetic variations (*Pvu* II and *Hind* III RFLPs) of LPL gene and essential hypertension in Korean population. Also, we investigated the relationship between two genetic variations of LPL gene and cardiovascular risk factors.

Materials and Methods

Subjects

We obtained 199 blood samples from the outpatients of Seoul Hygiene Hospital, Seoul, Korea. Of these, 98 essential hypertensive Korean individuals were defined as having a blood pressure above 140/90 mmHg. Subjects with secondary forms of hypertension and those taking antihypertensive drugs were excluded from the study.

Male/female (M/F) ratio between two groups was statistically similar (χ^2-test, df = 1, P = 0.35).

Genotyping

Blood samples were obtained after an isolation and determination of lipid profiles were collected in EDTA-containing tubes and were 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 *Pvu* II and *Hind* III PFLPs of LPL gene. Briefly, total 50 μl of the reaction mixture contained 200-400 ng of genomic DNA, 100 ng of each primer, 200 μl of each dNTP, and buffers recommended by the manufacturer. The sequences of the primers for two polymorphisms studied were: (a) For *Pvu* II RFLP; sense primer, 5'-ATCAGGCAATGCGTATGAGGTAA-3' antisense primer, 5'-GAGACACAGATCTCTTAAGAC-3' (Mattu *et al.*, 1994); (b) For *Hind* III RFLP; sense primer 5'-TTTAGGCCTGAAGTTTCCAC-3' antisense primer, 5'-CTCCCTAGAACAGAAGATC-3' (Ahn *et al.*, 1993).

PCR amplification was carried out with automated thermocycler. For *Pvu* II RFLP, the reactions were denatured at 94°C for 1 min, annealed at 61 °C for 45 sec, and extended at 72°C for 45 sec for a total 30 cycles. For *Hind* III RFLP, the reactions were denatured at 95°C for 1 min, annealed at 60°C for 2 min, and extended at 72°C for 2 min for a total 33 cycles. Ten μl of PCR product for two RFLPs was digested overnight with 10 unit of proper restriction enzymes (*Pvu* II or *Hind* III) at 37°C. Digested amplified product were size-fractionated after 2% agarose gel electrophoresis in TBE buffer for 40 min along with molecular markers. Ethidium bromide was incorporated into the gel. The gels were directly photographed on an UV transilluminator and genotyped.

Biochemical assays

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 400E automated analyzer with reagents and calibrators from Boehringer Mannheim. LDL-cholesterol level was calculated by using the formular of Freidwald *et al.*, (1972).

Statistical analyses

Allele frequencies were calculated by gene counting method, and the significant deviation from Hardy-Weinberg equilibrium was analyzed by χ^2-fitness test. The polymorphism information content (PIC) was estimated by the methods of Bostein *et al.* (1980). The odds ratio (OR) and 95% confidence interval (CI) of essential hypertension associated with allelic variation was calculated by logistic regression analysis. The significance of differences in allele or genotype frequencies between normotensives and essential hypertensives was also estimated by the χ^2-independence test. Maximum likelihood estimates (MLE) of haplotype frequencies were obtained by iterative two-steps algorithm called expectation-maximatization (EM). A Monte-Carlo simulation using the Clump (version 1.6) program was performed to test the statistical significance of the association between the haplotype distribution and essential hypertension (Sham and Curtis, 1995). The degree of nonrandom association was determined by calculation of the delta (Δ) (Hill and Robertson, 1968)

and D' (Lewontin, 1964) between the polymorphic sites in the LPL gene. To test the significance of linkage disequilibrium, $n\Delta^2$ value was used as the χ^2 distribution with 1 df (degree of freedom). Comparison of the variables across genotypes was performed using a parametric one-way ANOVA test. Statistical significance was accepted at the P = 0.05 level. All statistical analyses were performed using the MINITAB (version 13) computer program.

Results

LPL Pvu II RFLP

The *Pvu* II polymorphic site of the LPL gene was located in intron 6 of this gene (Fig. 1A), and the genotype patterns of this polymorphism were displayed in Fig. 1B. This polymorphism was detected by digestion with restriction enzyme *Pvu* II after PCR amplification. P1 allele yielded a 431 bp band, and P2 allele gave bands of 222 bp and 209 bp. The genotype and allele frequencies of the *Pvu* II RFLP were shown in Table 1. The genotype frequencies of P1P1, P1P2 and P2P2 were 13, 39 and 48% in normotensives, and 19, 50 and 31% in essential hypertensives, respectively. The heterozygosity and PIC values of *Pvu* II RFLP represented the values of 0.4365 and 0.3412 for normotensives, and 0.4931 and 0.3715 for essential hypertensives, respectively. According to the heterozygosity and PIC values, the *Pvu* II RFLP of the LPL gene showed the relatively high PIC values in the both groups.

The observed genotype distributions were not significantly deviated from those expected for Hardy-Weinberg equilibrium. There were the significant differences in allele and genotype frequencies between normotensives and essential hypertensives by case-control comparison. Table 2 represented the comparison of anthropometrical data and biochemical parameters across the genotypes of the *Pvu* II RFLP in the LPL

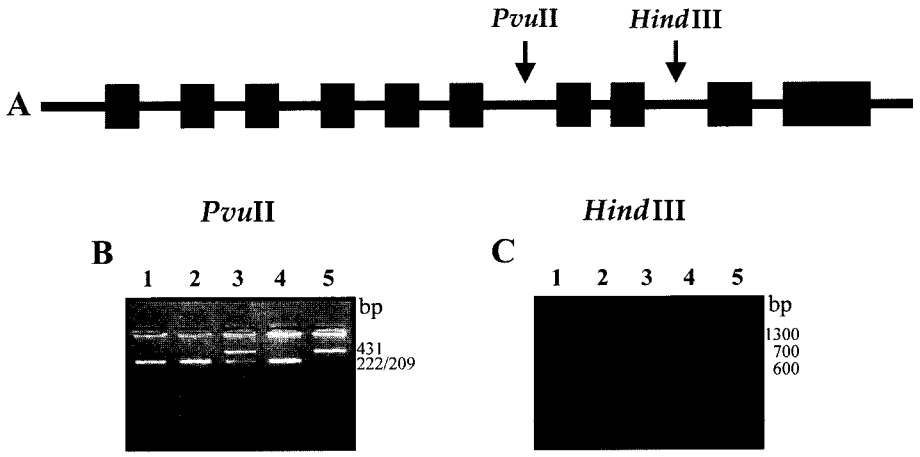

Fig. 1. Schematic diagram of the LPL gene to show the polymorphisms within the gene A) Restriction map of the LPL gene. B) Patterns of *Pvu* II RFLP at the LPL gene. Lane 1, 2 and 4, P2P2 genotypes; lane 3, P1P2 genotype; lane 5, P1P1 genotype. C) Patterns of *Hind*III RFLP at the LPL gene. Lane 1, 3 and 4, H2H2 genotypes; lane 2 and 5, H1H2 genotypes.

Table 1. Genotype and allele frequencies of the *Pvu* II RFLP of the LPL gene in normotensive and essential hypertensive individuals

	Genotype No. (%)			Allele No. (%)		H^1	PIC^2
	P1P1	P1P2	P2P2	P1	P2		
Normotensives	13(13)	39(39)	49(48)	65(32)	137(68)	0.4365	0.3412
Hypertensives	16(19)	43(50)	26(31)	75(44)	95(56)	0.4931	0.3715
X^2		6.2290			5.6065		
P		0.0440			0.0179		
Odds ratio (CI)[3]			1.66 (0.09-2.54)				

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

Table 2. Clinical characteristics of subjects according to genotypes of *Pvu* II RFLP in the LPL gene

Variables	Genotypes		
	P1P1 (No.)[8]	P1P2 (No.)	P2P2 (No.)
Age (year)	58.9 ± 10.7(27)[9]	60.1 ± 11.3(81)	58.9 ± 9.8(75)
BMI (kg/m^2)[1]	23.6 ± 1.9(24)	24.0 ± 2.5(74)	23.3 ± 2.6(75)
Tg (mg/dl)[2]	94.2 ± 44.7(19)	131.5 ± 86.1(70)	133.1 ± 72.8(59)
TC (mg/dl)[3]	157.2 ± 40.2(19)	151.4 ± 35.9(70)	147.2 ± 31.5(59)
LDL-chol (mg/dl)[4]	108.5 ± 36.7(19)	98.5 ± 37.6(70)	92.5 ± 29.8(59)
HDL-chol (mg/dl)[5]	29.8 ± 15.6(19)	26.3 ± 8.5(70)	28.1 ± 9.0(59)
Lp(a) (mg/dl)[6]	15.9 ± 12.0(21)	16.7 ± 12.7(67)	15.7 ± 10.6(68)
Apo AI (mg/dl)[7]	90.3 ± 24.8(10)	93.5 ± 43.0(12)	103.2 ± 32.0(16)
Glucose (mg/dl)	82.0 ± 44.8(11)	75.7 ± 57.1(44)	94.0 ± 89.1(46)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol, [6]Lipoprotein (a), [7]Apolipoprotein AI and [8]Number. [9]Values are mean ± SD (Standard Deviation).

gene. There were no significant associations with any anthropometrical data or biochemical parameters across the genotypes.

Risk assessment of Pvu II RFLP

For P1P1 genotype, our result had odds ratio, relative risk, sensitivity, specificity, positive predictive value, negative predictive value and total predictive value of 1.57, 1.26, 0.19, 0.87, 0.55, 0.56 and 0.56, respectively, while for P1 allele, 1.66, 1.31, 0.44, 0.68, 0.54, 0.59 and 0.57, respectively.

LPL Hind III RFLP

The *Hind* III polymorphic site of the LPL gene was located in intron 8 of this gene (Fig. 1A), and the genotype patterns of this polymorphism were displayed in Fig. 1C. This polymorphism was detected by digestion with restriction enzyme *Hind* III after PCR amplification. H1 allele yielded a 1,300 bp band, and H2 allele gave bands of 700 bp and 600 bp. The genotype and allele

Table 3. Genotype and allele frequencies of the *Hind* III RFLP of the LPL gene in normotensive and essential hypertensive individuals

	Genotype No. (%)			Allele No. (%)		H[1]	PIC[2]
	H1H1	H1H2	H2H2	H1	H2		
Normotensives	6(6)	32(32)	62(62)	44(22)	156(78)	0.3432	0.2843
Hypertensives	1(1)	38(39)	59(60)	40(20)	156(80)	0.3249	0.2721
χ^2		4.1400		0.1501			
P		0.1260		0.6984			
Odds ratio (CI)[3]			1.10(0.681.78)				

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

Table 4. Clinical characteristics of subjects according to genotypes of *Hind* III RFLP in the LPL gene

Variables	Genotypes		
	H1H1 (No.)[8]	H1H2 (No.)	H2H2 (No.)
Age (year)	51.9 ± 8.8(7)[9]	60.0 ± 10.2(68)	59.7 ± 11.6(119)
BMI (kg/m^2)[1]	24.8 ± 1.8(7)	23.8 ± 2.5(61)	23.5 ± 2.5(110)
Tg (mg/dl)[2]	99.3 ± 26.2(6)	121.2 ± 89.8(57)	132.3 ± 66.8 (88)
TC (mg/dl)[3]	141.5 ± 27.4(6)	149.5 ± 39.1(57)	152.6 ± 34.0 (88)
LDL-chol (mg/dl)[4]	98.0 ± 26.2(6)	98.1 ± 39.2(57)	98.6 ± 33.0 (88)
HDL-chol (mg/dl)[5]	23.7 ± 6.6(6)	26.8 ± 11.2(57)	27.5 ± 8.9 (88)
Lp(a) (mg/dl)[6]	16.6 ± 9.0(7)	16.3 ± 13.4(52)	15.6 ± 10.7 (97)
Apo AI (mg/dl)[7]	59.6 ± 0.0(1)	104.4 ± 29.4(13)	99.6 ± 34.6 (33)
Glucose (mg/dl)	69.0 ± 51.0(4)	75.8 ± 61.8(34)	91.5 ± 80.9 (60)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol, [6]Lipoprotein (a), [7]Apolipoprotein AI and [8]Number. [9]Values are mean ± SD (Standard Deviation).

frequencies of the *Hind* III RFLP were shown in Table 3. The genotype frequencies of H1H1, H1H2 and H2H2 were 6, 32 and 62% in normotensives, and 1, 39 and 60% in essential hypertensives, respectively. The heterozygosity and PIC values of *Hind* III RFLP represented the values of 0.3432 and 0.2843 for normotensives, and 0.3249 and 0.2721 for essential hypertensives, respectively. According to the heterozygosity and PIC values, *Hind* III RFLP showed a relatively low degree of polymorphism in the both groups compared with the *Pvu* II RFLP.

The observed genotype distribution of this RFLP was significantly deviated from Hardy-Weinberg equilibrium in essential hypertensives (χ^2-fitness test, $P<0.05$). There were no significant differences in allele or genotype frequencies between normotensives and essential hypertensive by case-control comparison. Table 4 represented the comparison of anthropometrical data and biochemical parameters across the genotypes of the *Hind* III RFLP in the LPL gene. There were no significant associations with any anthropometrical data or biochemical parameters across the genotypes.

Haplotype analysis

The haplotype distribution and linkage disequilibrium statistic values reflecting the extent or statistical significance of nonrandom associations between the two polymorphic sites were shown in Table 5. There were no significant differences in haplotype distributions between normotensive and essential hypertensives. However, significant linkage disequilibrium was detected in only normotensive group.

Table 5. Haplotype frequencies and linkage disequilibrium statistics (D', Δ) between pairs of two RFLPs in the LPL gene

Haplotypes		Normotensives	Hypertensives
Pvu II	*Hind* III		
P1	H1	0.174714	0.181550
P1	H2	0.154956	0.262895
P2	H1	0.045066	0.028327
P2	H2	0.625264	0.527229
Total chromosomes		182	162
D		0.525213	0.083689
D'		0.693984	0.145214
χ^2		50.204394	1.134618
P		1.3854×10^{-12}	0.286792

There were no significant differences in haplotype frequencies between normotensive and essential hypertensive individuals (Monte-Carlo simulation, $T_1 = 7.096$, $df = 3$, $P = 0.0690$, simulation number = 10,000). The significant linkage disequilibrium was only detected in normotensive group.

Discussion

Essential hypertension is known to be caused by polygenes, and its phenotypic expression is modulated by various environmental factors (Bae *et al.*, 2001). Recent advances in molecular biology have allowed investigation of the role of candidate genes for essential hypertension. The association study revealed that the genes encoding for the components of renin-angiotensin system such as angiotensinogen and angiotensin I converting enzyme (ACE) are genetic risk factors for essential hypertension, but these associations were not denied in Korean population (Cho *et al.*, 1996; Hong *et*

Table 6. Comparison of allele frequencies of *Pvu*II RFLP in the LPL gene from various ethnic groups

Populations	Sample number	Allele frequencies		Reference
		P1	P2	
Caucasians				
American	49	0.41	0.59	Fisher et al., 1987
American	34	0.38	0.62	Li *et al.*, 1988a
English	86	0.46	0.54	Chamberlain *et al.*, 1989
American	15	0.43	0.56	Johnson *et al.*, 1990
English	93	0.44	0.55	Thorn *et al.*, 1990
American	539	0.46	0.54	Ahn *et al.*, 1993
Russian	180	0.52	0.48	Stepanov *et al.*, 1993
English	97	0.49	0.51	Munroe *et al.*, 1994
Austalian	75	0.49	0.51	Mitchell *et al.*, 1994
Dutch	152	0.49	0.51	Reymer *et al.*, 1995
Mongolians				
Japanese	41	0.28	0.72	Chamberlain *et al.*, 1989
Japanese	50	0.22	0.78	Gotoda *et al.*, 1989
Japanese	70	0.27	0.73	Gotoda *et al.*, 1992
Korean	101	0.32	0.68	Present study

Table 7. Comparison of allele frequencies of *Hind* III RFLP in the LPL gene from various ethnic groups

Populations	Sample number	Allele frequencies		Reference
		H1	H2	
Caucasians				
American	131	0.33	0.67	Heinzmann *et al.*, 1987
English	86	0.41	0.59	Chamberlain *et al.*, 1989
English	108	0.42	0.58	Thorn *et al.*, 1990
English	539	0.24	0.76	Ahn *et al.*, 1993
Dutch	145	0.28	0.72	Reymer *et al.*, 1995
Austalian	72	0.33	0.67	Mitchell *et al.*, 1994
English	98	0.27	0.73	Munroe *et al.*, 1994
Mongolians				
Japanese	41	0.34	0.72	Chamberlain *et al.*, 1989
Japanese	70	0.24	0.76	Gotoda *et al.*, 1992
Korean	100	0.22	0.78	Present study

al., 2000).

Alterations of lipid metabolism in plasma have been reported to be related to an increased risk of essential hypertension (Bønaa and Thelle, 1991; Flesch *et al.*, 1994; Fuh *et al.*, 1987; Hjermann *et al.*, 1978; Lee *et al.*, 1986), and LPL has a critical role in the control of plasma lipid metabolism. Therefore, the genetic variations in the LPL gene may be related to essential hypertension as well as to abnormal lipid metabolism.

Munroe *et al.* (1994) firstly reported the relationship between the genetic variations of the LPL gene and essential hypertension in Caucasian population, but failed to detect the significant association. Other study however, reported the significant linkage between a genetic locus at or near the LPL gene locus and the variation of systolic blood pressure in Chinese families (Wu *et al.*, 1996). The discrepant results reported for the same gene may be due to different criteria used in selection of study subjects, difference in study methods or racial differences in study sample.

In this study, we has found the significant association between the *Pvu* II RFLP of the LPL gene and essential hypertension in Korean population. The P1 allele was more frequent in essential hypertensives than in normotensives. However, this RFLP did not result in any amino acid substitution because this polymorphic site is located in intron 6 of this gene, and therefore, this significant association detected in our study may be due to linkage disequilibrium between P1 allele and a significant causative allele. Also, considerable caution is needed in interpreting statistical significance with the *Pvu* II RFLP observed in the present study. The odds ratio for P1 allele with P2 allele is estimated to be 1.66, with a wide 95% CI (95% CI 0.09-2.54). In order to verify the 66% increase in essential hypertension risk with the P1 allele, about 119 individuals would be required in each group of cases and controls, with 80% statistical power at a 5% type I error probability in Korean subjects. Unfurtunately, sample size in our study was not reached this standard particularly in essential hypertensive group. Accordingly, we can only set a limited potential value on this, and further investigations are required into whether these findings are applicable to the prediction of essential hypertension in Korean population. With respect to anthropometrical data and biochemical parameter, any significant association was not observed in our study group. Thus, the *Pvu* II RFLP of the LPL gene may not contribute to the variation of any cardiovascular risk factors in Korean population.

In the case of *Hind* III RFLP, there was no significant association with any cardiovascular risk factors as well as essential hypertension in Korean population. The observed genotype distribution of this RFLP was not in Hardy-Weinberg equlibrium. Because *Hind* III RFLP of the LPL gene is located in intron 8 and have no effect on the protein structure or function of this gene, this deviation from Hardy-Weinberg equilibrium may not be due to natural selection. Therefore, founder effect might be operating in the *Hind* III RFLP of the LPL gene in Korean population.

By pair-wise haplotype analysis, the significant linkage disequilibrium between two polymorphic sites was detected in normotensives. This finding suggests that the haplotype occurred by two polymorphisms decreases the information content for linkage analysis, while it did not require the large sample size to perform the association study. Therefore, association study may be better than linkage analysis to discover the disease susceptibility gene in the case of two polymorphisms in the LPL gene. Unlike to normotensives, this significant linkage disequilibrium was not detected in essential hypertensives. This may be explained by the significant association between the *Pvu* II RFLP of the LPL and essential hypertension in our study. In other word, the discrepancy in the association with essential hypertension between two polymorphic sites might bring about the modification of linkage disequilibrium between the study groups.

The allele distribution of *Pvu* II RFLP in the LPL gene was different between Caucasians and Monglians, but considerably similar within same racial group (Table 6). The reason for this phenomenon may be explained by different genetic background of two racial groups. It seems to be important for carefully designed studies to minimize the racial heterogeneity of the case and control populations. In contrast, the allele distribution of *Hind* III RFLP in the LPL gene was relatively uniform between Caucasians and Mongolians (Table 7). The H2 allele frequency was higher than the H1 allele frequency in all populations studied. It is likely that this RFLP arose before the divergence of man into different racial groups. Because of the possible absence of selective forces at this locus, neither allele may have progressed to fixation.

In conclusion, the *Pvu* II RFLP of the LPL gene appears to increase the risk for essential hypertension in

Korean population. Therefore, this RFLP may be an useful genetic marker for the pathogenesis of essential hypertension in Korean population, although further studies using large sample size will be required to clarify the precise role of the LPL gene for essential hypertension. Also, association studies in other Asian populations including Japanese population, will be of great interest, because the distribution of the *Pvu* II RFLP in Japanese population showed the similar pattern with that in Koreans

Acknowledgments

The human blood samples were kindly supplied by Dr. Cho Seung Hee, Clinical Pathology, Seoul Hygiene Hospital, Seoul, Korea.

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