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SNP Analysis of the Human LDL Receptor and CETP Gene in Korean Subjects with Hypertension

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ABSTRACT **: Essential hypertension is complex disorder influenced by multiple genetic and environmental factors. Alterations of lipid metabolism in plasma have been reported to be related to an increased risk of essential hypertension. The aim of this study was to investigate the relationship between two SNPs of the human LDL receptor and CETP gene and hypertension in Korean population. There were no significant differences in allele and genotype frequencies of two SNPs in normotensives and hypertensives. With respect to *Hinc* II RFLP in the LDL receptor gene, pooled odds ratio value indicated the significant heterogeneity among populations studied by meta-analysis (Breslow-Day test, df = 2, P<0.05). In the case of *Bam* HI RFLP in the CETP gene,. our study is the first report of an association between the SNP of the CETP gene and hypertension, although our result failed to demonstrate the significant association between the *Bam* HI RFLP of the CETP gene and hypertension in Korean population. Further work, using larger sample sizes and various ethnic groups, is required to establish the precise role of these two candidate gene polymorphisms on hypertension.**

Key words: CETP, Hypertension and LDL receptor

Introduction

Blood pressure is also, by itself, a complex variable, regulated by multiple interacting physiologic regulatory systems where multiple genes are likely to be involved in the phenotype (Ward, 1990). Hypertension is frequently associated with plasma lipid abnormalities. This suggest that there may be common underlying genetic determinants between blood pressure regulation and plasma lipid metabolism (Haffner *et al.*, 1992). In this regards, LDL receptor and CETP gene may be one of these determinants.

The LDL receptor is a cell surface glycoprotein that plays a central role in the regulation of cholesterol homeostasis. LDL, the major carrier of plasma cholesterol is removed from the circulation by this protein (Brown and Goldstein, 1984). Then, LDL receptor requires the apo B component of LDL as ligand. Familial hypercholesterolemia is one of the most common single-gene disorders, affecting approximately 1 in 500 people, and is characterized by reduced or absent levels of LDL receptors in the liver (Nicholls *et al.*, 1998). The LDL receptor gene is located on chromosome 19p13.2, and consists of 18 exons (Sudhof *et al.*, 1985). Some studies have been performed in order to demonstrate the relationship between hypertenion and SNP in the LDL receptor gene (Fu *et al.*, 2001; Morris *et al.*, 1994; Zee *et al.*, 1992; Zee *et al.*, 1995). There was a report that the *Hinc* II RFLP of this gene is significantly associated with hypertension in Japanese population (Fu *et al.*, 2001).

The cholesteryl ester transfer protein (CETP) is a glycoprotein (Helser *et al.*, 1987), that plays an important role in the transport of excess cholesterol from peripheral tissues to liver, the only organ capable of catabolizing and excreting cholesterol (Janagin *et al.*, 1987). Free cholesterol from cell membranes is first transferred to HDL particles, where it is esterified by lecithin : cholesterol acyltransferase (LCAT). CETP then catabolizes the transfer

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of the cholesteryl esters to triglyceride-rich lipoproteins such as chylomicrons and very low density lipoproteins, which are subsequently taken up by the liver (Quinet *et al.*, 1993; Stein and Stein, 1986). Plasma CETP deficiency is caused by marked cholesteryl ester accumulation in HDL (Inazu *et al.*, 1993).

The human CETP gene has localized to chromosome 16q13, and a number of SNPs (single nucleotide polymorphisms) have been reported (Agellon *et al.*, 1990; Drayana *et al.*, 1987; Zuliani and Hobbs, 1990). A small number of studies have investigated the association between polymorphisms of the CETP gene and plasma lipid and lipoprotein phenotypes (Kessling *et al.*, 1991; Kondo *et al.*, 1989).

The aims of this study was to investigate the relationship between the SNPs in two candidate genes (LDL receptor and CETP genes) and hypertension in Korean population.

Materials and Methods

Subjects

We obtained 226 blood samples from the outpatients of Seoul Hygiene Hospital, Seoul, Korea. Of these, 96 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.

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 to detect the SNP of each genes. 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 *Hinc* II RFLP of LDL receptor gene; sense primer, 5'-TCTCCTTATCCACTTGTGTGTCTAG-3' antisense primer, 5'-CTTCGATCTCGTACGTAAGCCACAC-3' (Leitersdorf and Hobbs, 1988); (b) For *Bam* HI RFLP of CETP gene; sense primer 5'-TTGTTGAATGAGTG AAAGCC-3' antisense primer, 5'-CACCAAGTTTCCG AGTTTCC-C-3' (Inazu *et al.*, 1990).

PCR amplification was carried out with automated thermocycler. For *Hinc* II RFLP of LDL receptor gene, the reactions were denatured at 94°C for 1 min, annealed at 56 °C for 1 min, and extended at 72°C for 1 min for a total 35 cycles. For *Bam* HI RFLP of CETP gene, the reactions were denatured at 94°C for 30 sec, annealed at 57°C for 1 min, and extended at 72°C for 1 min for a total 30 cycles. Ten μl of PCR product for two RFLPs was digested overnight with 10 unit of proper restriction enzymes (*Hinc* II or *Bam* HI) at 37°C. Digested amplified product were size-fractionated after 10% polyacrylamide (for *Hinc* II RFLP) or 2% agarose gel electrophoresis (for *Bam* HI RFLP).

Biochemical analysis

Plasma total cholesterol (TC), triglyceride (TG), lipoprotein (a) (Lp(a)) and apolipoprotein AI (apo AI) levels were measured enzymatically using an Hitachi 736 automatic analyzer. Plasma HDL-cholesterol level was measured after heparin-manganese precipitation of the apo B containing lipoproteins. LDL-cholesterol level was calculated by using the formular of Freidwald *et al.*, (1972).

Data analysis

Allele frequencies were estimated by genotyping counting. The Hardy-Weinberg equilibrium was tested 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 hypertension associated with allelic variation was calculated by logistic regression analysis. The significance of differences in allele or genotype frequencies between normotensives and hypertensives was also estimated by the χ^2-independence test. A Monte-Carlo simulation using the Clump (version 1.6) program was performed to test the statistical significance of the association between the combined genotype distribution and hypertension (Sham and Curtis, 1995). The significance of differences between the average of the variables in different genotypes was performed by a parametric one-way ANOVA test or Students t-test. All calculated P values were accepted at the $P = 0.05$ level.

Meta-analysis

To perform the meta-analysis, the pooled odds ratio (OR) and 95% confidence interval (CI) were calculated by Mantel-Haenszel technique (Mantel and Haenszel,

1959). Hetergeneity was tested by Breslow-Day method (Breslow and Day, 1980). All statistical analyses of the meta-analysis were performed by the SAS Please consider including the source of the software (version 6.12) computer program.

Results

LDL receptor / Hinc II RFLP

The *Hinc* II polymorphic site of the LDL receptor gene was located in exon 12 of this gene, and the genotype patterns of this polymorphism were displayed in Fig. 1. This polymorphism was detected by digestion with restriction enzyme, *Hinc* II after PCR amplification. H1 allele yielded a 133 bp band, and H2 allele revealed bands of 98 bp and 35 bp. The genotype and allele frequencies of the *Hinc* II RFLP in the LDL receptor gene were shown in Table 1. The genotype frequencies of H1H1, H1H2 and H2H2 were 1, 25 and 74% in normotensives, and 2, 26 and 72% in hypertensives, respectively. The heterozygosity and PIC values of *Hind* III RFLP represented the values of 0.2130 and 0.1903 for normotensives, and 0.1913 and 0.1730 for hypertensives,

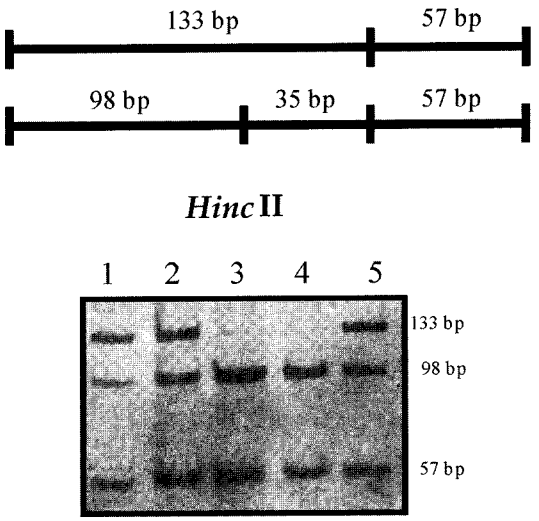

Fig. 1. *Hinc* II RFLP of LDL receptor gene. Lane 1, 2 and 5, H1H2 genotypes; Lane 3 and 4, H2H2 genotypes.

Table 1. Genotype and allele frequencies of *Hinc* II RFLP of the LDL receptor gene in normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)		H[1]	PIC[2]
	H1H1	H1H2	H2H2	H1	H2		
Normotensives	1(1)	32(25)	92(74)	33(13)	215(87)	0.2130	0.1903
Hypertensives	2(2)	25(26)	69(72)	29(15)	163(85)	0.1913	0.1730
X^2		0.7098		0.2889			
P		0.7012		0.5909			
Odds ratio(CI)[3]			1.16(0.68-1.99)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.
There were no statistically significant differences in genotype and allele frequencies between normotensives and essential hypertensive subjects.

Table 2. Clinical characteristics of subjects according to genotypes of *Hinc* II RFLP at the LDL receptor gene

Variables	Genotypes		
	H1H1(No.)[8]	H1H2(No.)	H2H2(No.)
Age (year)	58.8 ± 9.5 (3)[9]	61.1 ± 10.4 (56)	40.0 ± 0.0 (157)
BMI (kg/m^2)[1]	23.8 ± 2.7 (2)	23.8 ± 2.4 (50)	22.5 ± 0.0 (147)
Tg (mg/dl)[2]	133.6 ± 83.1 (2)	114.5 ± 70.6 (40)	104.0 ± 0.0 (117)
TC (mg/dl)[3]	151.8 ± 34.9 (2)	150.6 ± 19.1 (40)	158.0 ± 0.0 (117)
LDL-chol (mg/dl)[4]	98.1 ± 36.6 (2)	101.6 ± 26.4 (40)	97.2 ± 0.0 (117)
HDL-chol (mg/dl)[5]	26.8 ± 9.4 (2)	26.1 ± 6.0 (40)	40.0 ± 0.0 (117)
[a]Lp(a) (mg/dl)[6]	**16.6 ± 12.2 (1)**	**14.0 ± 14.8 (39)**	**11.9 ± 0.0 (114)**
Apo AI (mg/dl)[7]	90.7 ± 31.2 (1)	130.0 ± 9.8 (16)	0.0 ± 0.0 (43)

[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).
[a]Statistically significant difference (one-way ANOVA test, P = 0.026). However, when we combined H1H1 and H1H2 genotype for statistical analysis becauce of the small number of H1H1 individual (n = 1), this significance disappeared (Students t-test, P > 0.05).

respectively. According to the heterozygosity and PIC valuesthe *Hinc* II RFLP of the LDL receptor gene showed the relatively low PIC values in the both groups. The observed genotype distributions were not significantly deviated from those expected for Hardy-Weinberg equilibrium. There were the no significant differences in allele and genotype frequencies between normotensives and hypertensives by case-control comparison. Table 2 represented the comparison of anthropometrical data and biochemical parameters across the genotypes of the *Hinc* II RFLP in the LDL receptor gene. There was the significant associations with plasma Lp(a) level across the genotypes (one-way ANOVA test, $P = 0.026$). Thereafter, we combined the H1H1 and H1H2 subjects for statistical analysis because of the small number of H1H1 individual ($n = 1$). Then, this significance disappeared (Student's t-test, $P > 0.05$).

CETP/Bam HI RFLP

The *Bam* HI polymorphic site of the CETP gene was located in intron 9 of this gene, and the genotype patterns of this polymorphism were displayed in Fig. 2. This polymorphism was detected by digestion with restriction enzyme, *Bam* HI after PCR amplification. B1 allele yielded a 460 bp band, and B2 allele gave bands of 390 bp and 70 bp. The genotype and allele frequencies

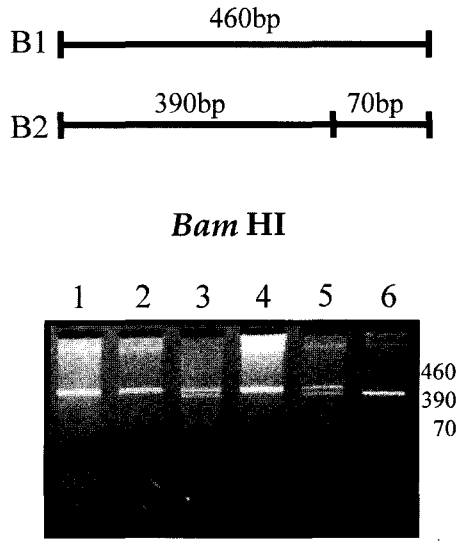

Fig. 2. *Bam*HI RFLP patterns of CETP gene. Lane 1, 2 and 4 B1B1 genotypes; Lane 3 and 5, B1B2 genotypes; Lane 6, B2B2 genotype.

Table 3. Genotype and allele frequencies of *Bam* HI RFLP of the CETP gene in normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)		H^1	PIC^2
	B1B1	B1B2	B2B2	B1	B2		
Normotensives	53(56)	33(35)	9(9)	139(73)	51(27)	0.3927	0.3156
Hypertensives	55(60)	31(34)	5(6)	141(77)	41(23)	0.3491	0.2881
X^2		1.1570		0.9296			
P		0.5610		0.3350			
Odds ratio(CI)[3]		1.26(0.79-2.03)					

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.
There were no statistically significant differences in genotype and allele frequencies between normotensives and essential hypertensive subjects.

Table 4. Clinical characteristics of subjects according to genotypes of *Bam* HI RFLP at the CETP gene

Variables	Genotypes		
	B1B1(No.)[8]	B1B2(No.)	B2B2(No.)
Age (year)	60.7 ± 11.4(105)[9]	58.5 ± 11.0 (63)	59.4 ± 13.1 (14)
BMI (kg/m^2)[1]	23.7 ± 2.6 (96)	23.8 ± 2.1 (57)	23.1 ± 2.3 (14)
Tg (mg/dl)[2]	123.7 ± 62.2 (79)	130.0 ± 68.0 (49)	141.9 ± 90.7 (12)
TC (mg/dl)[3]	158.2 ± 35.88(79)	147.8 ± 38.7 (49)	141.2 ± 44.2 (12)
LDL-chol (mg/dl)[4]	105.2 ± 34.4 (79)	94.2 ± 34.4 (49)	86.5 ± 49.7 (12)
HDL-chol (mg/dl)[5]	27.6 ± 8.7 (79)	27.6 ± 10.7 (49)	26.3 ± 9.3 (12)
Lp(a) (mg/dl)[6]	17.6 ± 14.1 (81)	15.6 ± 13.9 (53)	19.3 ± 11.9 (12)
Apo AI (mg/dl)[7]	93.9 ± 33.5 (29)	100.3 ± 34.1 (15)	118.5 ± 8.5 (2)

[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).

of the *Bam* HI RFLP in the CETP gene were shown in Table 3. The genotype frequencies of B1B1, B1B2 and B2B2 were56, 35 and 9% in normotensives, and 60, 34 and 6% in hypertensives, respectively. The heterozygosity and PIC values of *Bam* HI RFLP represented the values of 0.3927 and 0.3156 for normotensives, and 0.3491 and 0.2881 for hypertensives, respectively. According to the heterozygosity and PIC values, *Bam* HI RFLP showed a reasonably high degree of polymorphism in the both groups. The observed genotype distribution of this RFLP was not significantly deviated from Hardy-Weinberg equilibrium. There were no significant differences in allele or genotype frequencies between normotensives and hypertensive by case-control comparison. Table 4 represented the comparison of anthropometrical data and biochemical parameters across the genotypes of the *Bam* HI RFLP in the CETP gene. There were no significant associations with any anthropometrical data or biochemical parameters across the genotypes.

Combined genotype analysis

The combined effect of two SNPs in the LDL receptor and CETP gene on the risk of hypertension was measured by combined genotype analysis (Table 5). There were no significant differences in combined genotype distributions between normotensive and hypertensives (Monte-Carlo simulation, $T_2 = 5.0260$, df = 3, P>0.05).

Discussion

In this study, we failed to demonstrate significant association between the *Hinc* II RFLP of the LDL receptor gene and hypertension or other cardiovascular risk factors in Korean population. Therefore, it is unlikely that this SNP may influence the etiology of hypertension or other cardiovascular diseases in our subjects. To further assess the association of the *Hinc* II RFLP in the LDL receptor gene with hypertension, we performed the meta-analysis from 3 studies (Fig. 3). In total, the studies contain 535 normotensives and 508 hypertensive subjects. The overall allele frequencies of H1 and H2 were 21 and 79% in normotensives, and 20 and 80% in hypertensives, respectively. The Mantel-Haenszel odds ratio and 95% CI across all studies is 0.72 (0.58-0.90), suggesting the 39% increase of H2 allele. The reason for this may be due to the high frequency of H2 allele in Japanese hypertensives with relatively large sample size (Fu *et*

Table 5. Combined genotype analysis of LDL receptor (*Hinc* II) and CETP (*Bam* HI) gene

Combined genotypes		Normotensives (No.)	Hypertensives (No.)
Hinc II	*Bam* HI		
H1H1	B1B1	0.000000 (0)	0.000000 (0)
H1H1	B1B2	0.000000 (0)	0.023256 (2)
H1H1	B2B2	0.012500 (1)	0.000000 (0)
H1H2	B1B1	0.100000 (8)	0.186047 (16)
H1H2	B1B2	0.137500 (11)	0.058140 (5)
H1H2	B1B2	0.062500 (5)	0.011628 (1)
H2H2	B2B2	0.412500 (33)	0.430233 (37)
H2H2	B2B2	0.250000 (20)	0.244186 (21)
H2H2	B2B2	0.025000 (2)	0.046512 (4)
Total		1.000000 (80)	1.000000 (86)

There was no significance in combined genotype frequencies between normotensives and hypertensive groups (Monte-Carlo simulation, $T_2 = 5.0260$, df = 3, P = 0.1701).

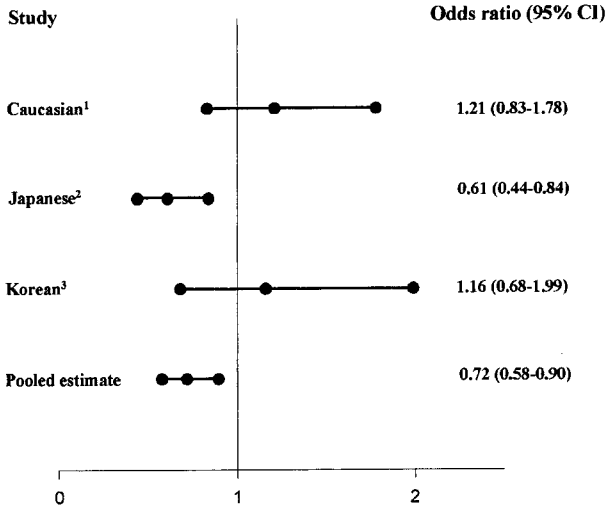

Fig. 3. Meta-analysis of *Hinc* PFLP in the in the LDL receptor gene [1]Zee *et al.*, 1995; [2]Fu *et al.*, 2001; [3]*Present study.*

Table 6. Comparison of allele frequencies of Hinc II RFLP in the LDL receptor gene among various ethnic groups

Populations	Sample Number	Allele frequencies		P[1]	References
		H1	H2		
(Normotensives)					
Caucasian	101	0.42	0.58	<0.05	Zee *et al.*, 1995
Japanese	310	0.17	0.83	NS[2]	Fu *et al.*, 2001
Korean	124	0.13	0.87		Present study
(Hypertensives)					
Caucasian	112	0.47	0.53	<0.05	Zee *et al.*, 1995
Japanese	300	0.11	0.89	NS	Fu *et al.*, 2001
Korean	96	0.15	0.85		Present study

[1]Prabability, [2]Not significant.

Table 7. Comparison of allele frequencies of *Bam* HI RFLP at the CETP gene from various ethnic groups

Populations	Sample number	Allele frequencies		P[1]	Reference
		B1	B2		
Japanese	30	0.93	0.07	<0.05	Inazu *et al.*, 1990
Korean	206	0.77	0.23	NS[2]	Song *et al.*, 1997
Korean	95	0.73	0.27		Present study

[1]Probability, [2]Not significant.

al., 2001). However, there is significant evidence against homogeneity of the Mantel-Haenszel odd ratio value (Breslow-Day test, df = 2, P = 0.001). The difference in genetic background may significantly not contribute to this genetic heterogeneity. H1 allele frequency of LDL receptor gene in Korean population is similar to that in Japanese population, but significantly lower than that in Caucasian population (Table 6). Nevertheless, the odds ratio values on hypertension of *Hinc* II RFLP is significantly different between Koreans and Japaneses (Breslow-Day test, df = 1, P = 0.044), rather the odds ratio value of Koreans is similar to that of Caucasians (Breslow-Day test, df = 1, P>0.05). Accordingly, we could not ignore the effect of sampling bias. When the difference in frequency of H1 allele in two group is about 1%, then a study of about 17,000 cases and a similar number of control subjects is required to have 80% power to detect a difference at a probability of 0.05 between controls and cases. So far, none of the studies have been anywhere near this size. Thus, our result waits the precise confirmation from large-scale prospective cohort study.

In the case of *Bam* HI RFLP in the CETP gene, there was also no significant association with any cardiovascular risk factors as well as hypertension in Korean population. Therefore, it is unlikely that this SNP contributes the risk of hypertension or other cardiovascular disease in Korean population. The *Bam* HI RFLP of CETP gene was only reported in Asian population (Inazu *et al.*, 1990) (Table 7), and to our knowledge, no data have been available regarding the role of the CETP gene in hypertension. Thus, studies in other racial or ethnic groups will be of great interest.

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