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DNA Polymorphisms and Haplotypes of Apolipoprotein A5's Attribution to the Plasma Triglyceride Levels in Koreans

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Purpose: Recent studies using human and mice reported that apolipoprotein A-V (APOA5) gene plays an important role in controlling triglyceride (TG) concentrations. The purpose of the present study was to investigate the correlation between single nucleotide polymorphisms (SNPs) and haplotypes in the APOA5 gene and TG in subjects and to search for possible associations of the APOA5 gene variants and common haplotypes with hypertriglyceridemia (HTG). **Materials and Methods:** We examined the case-control subjects including 100 HTG patients and 243 unrelated healthy control. The genes were screened for SNPs by direct sequencing in 48 genetically unrelated individuals. Six SNPs (-1390C>T, -1020G>A, -3A>G, V150M, G182C and 1259T>C) were genotyped in case and control populations. **Results:** In this study, our results indicated a strong association between APOA5 SNP -3A>G and G182C and elevated TG levels ($p < 0.001$). Analysis of the SNPs from APOA5 gene has identified major haplotype showing very strong association with HTG, CGGGTT ($p < 0.001$). Likelihood ratio test (LRT) of these six SNPs revealed that haplotypes were strong independent predictors of HTG ($p < 0.001$). Haplotype-trend logistic regression (HTR) analysis revealed a significant association between the CGGGGC (haplotype 2) and CGGGTT (haplotype 4) and HTG (OR = 2.48, 95% CI = 1.06 - 5.76 and OR = 8.54, 95% CI = 2.66 - 27.42, respectively). **Conclusion:** We confirm that the APOA5 variants are associated with triglyceride levels and the haplotype may be strong independent predictors of HTG among Koreans.

Key Words: Apolipoprotein A5, case-control study, hypertriglyceridemia, triglyceride

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INTRODUCTION

Hypertriglyceridemia (HTG) is associated with increased risk of coronary heart disease (CHD) and it is an integral component of the overlapping syndromes of familial combined hyperlipidemia, insulin resistance syndrome, atherogenic lipoprotein profile and hyperapobetalipoproteinemia.[1] Due to the complexity of HTG, there are many possible origins of disorder, including secondary causes such as alcohol, diabetes mellitus, obesity and prescription drugs.[2] However, it is thought that predisposing genetic factors play an important role in TG metabolism and regulation and thus candidate gene are being investigated.[2] TG levels may be altered by a variety of environmental factors, including smoking, obesity, alcohol consumption and exercise. Twin studies have also shown a strong genetic contribution to TG levels.[3] Since TG concentrations do not segregate in a clearly mendelian inheritance fashion in most studies, the majority of studies have sought statistical relation between DNA polymorphisms in candidate genes and TG concentrations in cohorts of unrelated patients.[4]

The main candidate gene for HTG is lipoprotein lipase (LPL), however, extensive studies have failed to provide convincing evidence of common variants that play a major role in HTG.[2] Another candidate is apolipoprotein3 (*APOC3*). *APOC3* is and inhibitor of LPL as well as being an inhibitor of hepatic remnant uptake. Since 1983 association studies have shown HTG to be associated with

APOC3. The association studies have mainly focused on the single-nucleotide polymorphisms (SNPs) that occur within the exons and promoter region of *APOC3*.[2]

Recently, it has been discovered by comparative sequencing that a new apolipoprotein gene, *APOA5*, is located 40 kb downstream of the *APOC3* gene, with SNPs across the locus found to be significantly associated with plasma TG levels.[2] The human *APOA5* gene consists of four exons, three introns and encodes for a 369 amino acid protein.[5] Functional studies have shown that *APOA5* decreases TG, transgenic mice overexpressing the human *APOA5* gene exhibit one-third lower plasma TG levels than controls, while the *APOA5* knockout mice have four-fold elevation in TG levels.[6] When SNPs and their haplotypes across the *APOA5* locus have been studied in humans, some of SNPs were found to be significantly associated with TG in different ethnic populations.[7-11] In humans, a SNP -1131T>C in the promoter region of the *APOA5* gene has been found to be independently associated with TG levels in several populations of various ethnicities.[3] Recently, another polymorphism S19W in the coding region of *APOA5* gene which was designated a third haplotype for this gene, was also reported to be significantly associated with high TG levels in African-Americans, Hispanies and Caucasians.[3] More recently, Kao et al.[11] described a novel variants c.553G>T, which substitutes a cysteine for a glycine residue and is associated with HTG in a Taiwanese Chinese population. Taken together, these reports provide important genetic evidence that *APOA5* is crucial in the human metabolism of TG. However, no studies on the possible association of *APOA5* SNPs and haplotypes with HTG in Koreans have been reported so far.

Using haplotypes defined by these variants, we have identified the major TG-raising alleles, thus highlighting the relative effect of *APOA5* gene on TG levels. Therefore, this case-control study examines (1) the associations among -1390C>T, -1020G>A, -3A>G, V150M, G182C and 1259T>C (2) the common *APOA5* haplotypes defined by them and (3) the susceptibility of HTG.

MATERIALS AND METHODS

Subjects

One hundred unrelated HTG patients and controls were selected from the Yonsei Cardiovascular genome center, Yonsei Cardiovascular Hospital (Seoul, Korea) and were recruited between 2000 and 2003.

The 48 unrelated subjects selected via health screening at the same hospital and free of any clinical or biochemical signs of HTG, were used as sequencing controls for the study. One hundred patients (70 males and 30 females) with HTG who participated in our genome study were recruited: (a) confirmed by TG levels (≥ 150 mg/dL) (b) were over 20 years of age (c) excluded from genotype-phenotype correlation studies because of the lipid-lowering effects of the drugs. Two hundred forty three control subjects (121 males and 122 females, TG < 150 mg/dL) were randomly selected from volunteering outpatients during their routine health examinations and had no clinical evidence of HTG. The study was approved by the Ethical Committee of the Yonsei University and the subjects have given their informed consents. Clinical and biological features of the cases and controls are shown in Table 1. To examine the associations of the *APOA5* SNPs and haplotypes with TG, two sets of study subjects were used: (1) 48 genetically unrelated individuals for sequencing (all females) and (2) 100 cases (70 males and 30 females) and 243 (121 males and 122 females) controls.

Blood collection

Venous blood specimens were collected in EDTA-treated and plain tubes after a 12h fast. The tubes were immediately covered with aluminum foil and placed on ice until they arrived at the laboratory room (within 1 - 3 h) and stored at -70 ℃ until analysis.

Anthropometric and blood pressure measurements

Body weight and height were measured in the morning while the subjects were unclothed and not wearing shoes. Body mass index (BMI) was calculated as body weight (kg) divided by height (m^2). Blood pressure was read from the left arm

Table 1. Baseline Characteristics of Hypertriglyceridemic Cases and Controls

	Cases (n = 100)	Controls (n = 243)	*p* value*
Age (yrs)	54.8 ± 9.4	44.7 ± 14.5	< 0.001
Gender M/F (% of female)	70/30 (30.0%)	121/122 (50.6%)	0.001
BMI (kg/m^2)	25.4 ± 2.6	22.9 ± 2.9	< 0.001
Total cholesterol (mg/dL)	210.3 ± 41.4	196.5 ± 34.7	0.001
HDL-cholesterol (mg/dL)	67.4 ± 10.8	50.1 ± 11.7	< 0.001
LDL-cholesterol (mg/dL)	118.6 ± 37.4	126.2 ± 31.0	0.079
Triglyceride (mg/dL)	294.5 ± 95.2	91.7 ± 29.2	< 0.001
Apo AI (mg/dL)	128.7 ± 21.4	139.6 ± 25.4	< 0.001
Apo B (mg/dL)	99.2 ± 23.4	82.1 ± 21.1	< 0.001
Glucose (mg/dL)	97.1 ± 20.4	85.6 ± 20.6	< 0.001
Insulin (mg/dL)	10.2 ± 5.0	7.4 ± 4.0	< 0.001
HOMA-IR	2.4 ± 1.3	1.5 ± 0.9	< 0.001
Uric acid (mg/dL)	5.8 ± 1.4	4.8 ± 1.3	< 0.001

BMI, body mass index; HDL-cholesterol, high density lipoprotein cholesterol; LDL-cholesterol, low density lipoprotein cholesterol; Apo AI, apolipoprotein AI; Apo B, apolipoprotein B; HOMA, homeostasis model assessment; M/F, male and female.
Values are given as mean ± S.D.
*p values were calculated by Student t-test for continuous and χ^2 test for categorical variables, respectively.

while subjects remained seated. An average of 3 measurements was recorded for each subject.

Measurement of serum lipid profile

Fasting serum total cholesterol and triglyceride concentrations were measured with the use of commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd, Tokyo, Japan). After precipitation of serum chylomicron, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) with dextran sulfate-magnesium, high-density lipoprotein (HDL) cholesterol left in the supernatant fluid was measured by using an enzymatic method. LDL cholesterol was estimated indirectly with the use of the Friedewald formula for subjects with serum TG concentrations.[12] Serum apolipoprotein A-I (apoA1) and B (apoB) were determined by using turbidometry at 340 nm with a specific antiserum (Roche, Basel, Switzerland). Glucose was measured by using a glucose oxidase method with the Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, USA). Insulin was measured by using radioimmunoassay with commercial kits from Immuno Nucleo Corporation (Still-water, MN, USA).[12] Insulin sensitivity was evaluated by the homeostasis model assessment for insulin resistance (HOMA-IR) according to the following equation: IR = [fasting insulin (μIU/mL) × fasting glucose (mmol/L)]/22.5.[13]

Laboratory analysis

DNA sequencing of the APOA5 genes

Sequencing of the *APOA5* gene was performed for a subset of 48 study subjects and gene-specific primer pairs were used to amplify the *APOA5* gene as described in Appendix A.[8] The promoter (- 2000 bp), exons and flanking intron sequences of the *APOA5* gene were screened in randomly selected normal individuals by direct sequencing. The sample sizes were selected on the basis of the following assumptions: the present study would have 99% detection rate to detect SNP over 5% minor allele frequency. DNA fragments of - 400 bp each spanning an exon and the adjacent

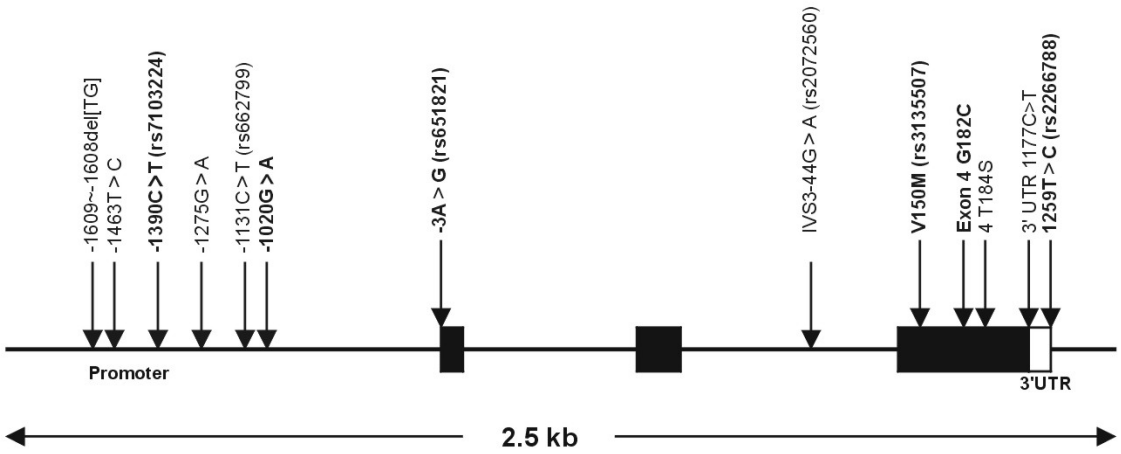

Fig. 1. *APOA5* genomic structure and polymorphism locations. Exons are depicted by boxes in protein-encoding regions with shaded black. The six SNPs used in this analysis are highlighted in bold.

intronic sequences or the proximal promoter sequence were designed based on GenBank sequences (Ref. Genome seq; NM_052968). Polymerase chain reaction (PCR) amplified and sequenced using BigDye Terminator Cycle Sequencing reagents on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA, USA). The Sequencher software (Gene Codes Corporation) was used to align the sequences to facilitate the detection of the polymorphisms. Through direct sequencing of all exons and their boundaries in the *APOA5* gene, 13 polymorphisms (seven in the 5′ flanking region, three in exon 3, one in introns, and two in 3′ UTR, respectively) were identified. Genomic structure and location in the Korean population are shown in Fig. 1.

APOA5 genotyping

Genomic DNA was extracted from peripheral blood cells by Wizard genomic DNA purification kit (Promega, Madison, WI, USA), according to the manufacturer's procedure. We analyzed SNPs having minor allele frequencies of greater than 5%. For the analysis of *APOA5* -1390C>T, -1020G>A, -3A>G, V150M, G182C and 1259T>C polymorphisms, single base extension reaction was performed using SNP-IT (SNP-Identification Technology) assay method[13] and SNP stream 25K system (Orchid Biosciences, Prinston, NJ, USA). Single base extension was expanded by reacting with each dideoxynucleotide marked with fluorescein isothiocyanate (FITC) or biotin prior to renaturation of these primers and each PCR products. We analyzed genotypes, which were colored with yellow and blue, using enzyme-linked immunosorbent assay (ELISA) reader after the reaction was over. Final genotypes were organized with QC Review™ program (Orchid Biosystems). For *APOA5* G182C, the genotyping was performed according to the direct DNA sequencing method.[13] Overall genotyping success rate was over 98 and we randomly selected about 5% sample in total subjects and performed duplication analysis. The genotyping error rate was about under 1% and the results were confirmed by sequencing.

Statistical methods

All data were analyzed with the Statistical Analysis Systems (SAS) software version 9.12 (SAS Institute, Cary, NC, USA). The clinical characteristics of continuous variables were expressed as mean ± S.D. Differences of genotypic and allelic distribution between cases and controls were analyzed by χ^2 test or by Fisher's exact tests. Allele frequencies were estimated by the gene-counting method and an exact test was performed to identify departures from Hardy-Weinberg equilibrium (HWE). Linkage disequilibrium (LD) between pairs of SNPs was assessed using the Lewontin's standardized disequilibrium coefficient (D′) and squared correlation coefficient (r^2).[14] Expectation-maximization (EM) algorithm

was used to estimate the haplotype frequencies. Likelihood ratio test (LRT) was used to test equality of haplotype frequencies between cases and controls.[15] In order to find disease susceptible to specific haplotype, we used haplotype-trend logistic regression (HTR).[16] *P*-values less than 0.05 were considered as indicative of statistical significance.

RESULTS

Characteristics of subjects

Biochemical characteristics of the hypertriglyceridemic and control subjects are summarized in Table 1. Since controls were not selected to match approximately the age and gender distribution of the cases, these two variables did differ significantly between the two populations. Serum concentration of total cholesterol, HDL-C and TG were significantly higher in the group of HTG patients than in the control group. In contrast, there was no significant difference in serum LDL-C level between the two groups. The classical risk factors for the HTG, such as BMI, as well as apo B, glucose and insulin in the HTG patients were significantly higher than for the control subjects.

Linkage disequilibrium in *APOA5*

The measure of LD was calculated for all combinations of SNPs using Lewontin's D′ and R. The linkage disequilibrium among the variants in patients and control subjects is shown in Table 2. Strong LD was found in -1390C>T with other variants and -1020G>A with -3A>G, V150M and G182C variants. The 1259T>C seemed to have least linkage disequilibrium with -1020G>A variants. As shown in Table 2, the -1390C>T and -3A>G variants were in complete LD in these Korean subjects. The LD between -3A>G and G182C was high, but not complete. In contrast, the measurement of R as the confirmation of D′ was not complete.

Case-control association studies with individual SNPs

The distribution of genotypes and alleles of the two groups are shown in Table 3. The genotype distributions for all variants were in HWE. However, a slight departure from HWE was estimated for -1020G>C ($p = 0.041$). Although -1390C>T and -1020G>A genotype frequencies did not differ between the hypertriglyceridemic and control groups, differences in -3A>C and G182C genotype frequencies were statistically significant (Table 3, $p < 0.001$). Likewise, the allele frequencies of -3A>G and G182C obtained by gene counting showed significant differences between these two groups ($p < 0.001$, data not shown). We also found significant associations between TG levels and the three polymorphisms (-3A>G, G182C and 1259T>C), but not -1390T>C, -1020G>A and V150M polymorphic sites in subjects (Table 3, $p < 0.001$). The minor allele of each of these three polymorphisms (-3A>G, G182C and

Table 2. Linkage Disequilibrium of *APOA5* SNPs

	SNPs	|D′|					
		-1390C>T	-1020G>A	-3A>G	V150M	G182C	1259T>C
	-1390C>T	-	1.00	1.00	1.00	1.00	1.00
	-1020G>A	0.07	-	1.00	1.00	1.00	0.84
r	-3A>G	0.22	0.15	-	1.00	0.97	0.99
	V150M	0.17	0.11	0.34	-	1.00	1.00
	G182C	0.10	0.07	0.46	0.16	-	1.00
	1259T>C	0.18	0.10	0.78	0.27	0.17	-

The linkage disequilibrium is measured by estimates of Lewontin's |*D′*| (upper diagonal) and *r* (lower diagonal).

Table 3. Distribution of Genotypes and Alleles for *APOA5* -1390C>T, -1020G>A, -3A>G, V150M, G182C, 1259T>C in Cases and Controls

SNP	Genotype	Frequency (%)		*p* value*
		Cases	Controls	
-1390C>T	CC	82.1	78.9	0.710
	CT	17.8	20.6	
	TT	0.0	0.4	
-1020G>A	GG	93.9	89.4	0.374
	AG	4.8	9.7	
	AA	1.2	0.8	
-3A>G	AA	30.9	50.2	< 0.001
	AG	52.3	44.3	
	GG	16.6	5.4	
V150M	GG	69.7	59.2	0.195
	AG	27.0	36.9	
	AA	3.1	3.7	
G182C	GG	69.7	86.1	< 0.001
	GT	28.1	13.8	
	TT	2.0	0.0	
1259T>C	TT	54.2	61.7	0.038
	CT	36.1	35.2	
	CC	9.6	2.9	

*p value was obtained from χ^2-test.

1259T>C) was associated with higher TG levels ($p < 0.001$, data not shown).

Association between *APOA5* haplotype and HTG

There were possible haplotypes derived from all polymorphic sites in both hypertriglyceridemic patients and control. Common haplotypes and their relative frequencies are shown in Table 4. The most common haplotype 1 (CGAGGT) and haplotype 3 (CGAAGT) distribute more frequently in controls. In contrast, haplotype 2 (CGGGGC) and haplotype 4 (CGGGTT) distribute more commonly in the cases than the controls. Haplotype 4 is distinguished from the common haplotype 1 by two nucleotide substitutions (-3A >G and G182C) and was shown to be associated with increased plasma TG concentrations. The frequency of haplotype 4 was significantly higher in HTG than in control subjects ($p < 0.001$). By likelihood ratio chi-square test, Table 4 shows the significance testing results. For testing for haplotypes and disease association, the chi-square value for haplotypes was 19.5-still highly significant ($p < 0.001$). Thus the results clearly demonstrate strong evidence for linkage disequilibrium between the disease and the polymorphic sites. Table 5 shows the results of HTR. As a result of the stepwise selection, the haplotype 2 and haplotype 4 were a statistically significant effect on HTG (OR = 2.48, 95% CI = 1.06 - 5.76, OR = 8.54, 95% CI = 2.66 - 27.42).

DISCUSSION

Clinical studies in different populations have shown that some variations in the *APOA5* gene

Table 4. The Distribution of Haplotypes in *APOA5* (-1390C>T, -1020G>A, -3A>G, V150M, G182C and 1259T>C) and Results of Likelihood Ratio Tests

Haplotype*	EM estimators†			*p* value
	Case	Control	Combined	
CGAGGT (1)	27.0	33.6	31.8	0.170
CGGGGC (2)	26.9	20.3	22.2	0.070
CGAAGT (3)	16.6	22.2	20.7	0.110
CGGGTT (4)	15.1	6.7	9.1	< 0.001
TGAGGT (5)	8.8	10.8	10.3	0.500
CAAGGT (6)	3.6	5.7	5.1	0.300
2 log L (*df*)	-259.05 (*df* = 27)	-641.42 (*df* = 28)	-910.30 (*df* = 28)	
Likelihood ratio statistic			19.65	
p value from exact test			< 0.001	

The exact test statistic for testing the haplotypes at these markers for association with disease status is calculated as 19.65 = 2 (-641.42 - 259.05 + 910.30) with degrees of freedom 27 = 28 + 27 - 28, which has a $p < 0.001$.
*Denote haplotype corresponding to pair of CGAGGT, CGGGGC, CGAAGT, CGGGTT, TGAGGT, CAAGGT, CGAGTT.
†More than 1% of frequency.

Table 5. Odds Ratios of Hypertriglyceridemia and 95% Confidence Intervals (CI) in Haplotype Trend Logistic Regression Results

# of SNPs	Selected haplotype	OR	95% CI
6	CGGGGC (Haplotype 2)	2.48	1.06 - 5.76
	CGGGTT (Haplotype 4)	8.54	2.66 - 27.42

OR, odds ratio; CI, confidence interval.

Appendix A. The Specific Primer Pairs Used to Amplify the *APOA5* Gene

Primer name	Sequence (5′ - 3′)	Annealing temperature (℃)
APOA5 -1390C>T -Forward	TTTTATGTGGCAGCAGATGA	55
APOA5 -1390C>T -Reverse	ATTTCCACTACCATCATTGCA	
APOA5 -1390C>T -SNP Primer	CAACAGGTCTTTCTACCACACTCCT	
APOA5 -1020G>A -Forward	ATCTCATCTTCACCCTGCC	55
APOA5 -1020G>A -Reverse	TTCACTACAGGTTCCGCAG	
APOA5 -1020G>A -SNP Primer	TTGTTCCAGGATTGTTTCATAAAGA	
APOA5 -3A>G -Forward	TTCTTCCCCTAACCAGGC	55
APOA5 -3A>G -Reverse	AAAGAAGAGCCAGAGCCC	
APOA5 -3A>G -SNP Primer	AGCACGGCAGCCATGCTTGCCATTA	
APOA5 V150M -Forward	TGATGGAGCAGGTGGCCC	55
APOA5 V150M -Reverse	AAGCCCAAGCCTCGTCC	
APOA5 V150M -SNP Primer	TGGGCCTTGGTGTCTTCCCCCACCA	
APOA5 G182C -Forward	CAGGAAACAGCTATGACCACTGGCCAGGGCTAGGAC	55
APOA5 G182C -Reverse	TGTAAAACGACGGCCAGTGCCTTCAGCGTGAGCTTC	
APOA5 1259T>C -Forward	CTGTGCAGGACAGGGAGG	55
APOA5 1259T>C - everse	TGAATCTAATGCATCCAGATTG	
APOA5 1259T>C -SNP Primer	ATTGGGGAGTCGCAGGAGGCTGGAT	

are strongly associated with plasma TG levels. In the Taiwan Han Chinese population, the result of case-control study demonstrated that *APOA5* -1131C and 553T alleles are positively associated with increased TG and 553T allele and -1131C/553T haplotype are associated with increased risk of HTG.[6] Recently, Liu et al.[7] reported that both the -1131T>C and S19W variants in *APOA5* are significantly associated with HTG in Chinese populations and contribute to the variation in human plasma TG levels. Pennacchio et al.[17] reported that a minor haplotype of *APOA5* (1259C, IVS3+476A and -1131C) was associated with a 20-30% elevation in plasma TG levels in Caucasian men and women. They also identified another *APOA5* haplotype (1259T, IVS3+476G, 56G and -1131T) which was independently associated with high plasma TG levels in African-American, Hispanic and Caucasians. While polymorphism in *APOA5* -1131T>C had a significant independent effect on the TG level in Japanese, this association was not significant in a population-based Spanish control group.[11] This result indicates that influence of polymorphism in the *APOA5* on TG level is different in different ethnicities.

In this study, we have characterized the association between a genetic variant in *APOA5* and HTG. The sequencing analysis identified many SNPs which show a marked change in allele frequency between the case HTG population and the control normolipidemic population, indicating that variation of the *APOA5* genes is associated with the occurrence of HTG. Present studies confirm that the -3A>G, G182C and 1259T>C in *APOA5* is associated with elevated TG levels not only in normal control subjects but also in patients with HTG. The minor allele frequency in -3A>G and 1259T>C was 16.6 and 9.6%, respectively. These frequencies were nearly 2-fold higher than those in Caucasians. This indicates that different ethnicity might entail different polymorphism. The -3A>G, which is in strong LD with -1131T>C and is located in the Kozak sequence preceding the predicted translation start codon, potentially affects the rate of *APOA5* translation and could be a candidate.[3] However, no functional study has supported the claim that either of these two polymorphisms is functional variant. Although various polymorphisms of the *APOA5* gene are known to be associated with HTG, the role of the G182C polymorphism had not been evaluated previously. In Taiwanese cohorts, Jiang YD et al.[18] examined the G182C polymorphism of the *APOA5* gene affects plasma triglycerides in both non-diabetic and diabetic groups, independent of age, gender, fasting plasma glucose, BMI and total cholesterol. Although the minor allele of G182C polymorphism is a powerful predictor for HTG, the exact cause for such an association is unknown.

By using a gene-wide haplotype approach, which is increasingly recognized as the future model for genetic association study, we could provide strong evidence that implicates the *APOA5* gene's involvement in susceptibility to HTG in our study. Haplotype analysis shows that there is a marked difference between case and control haplotypes at the *APOA5* locus. All the tests performed over the haplotypes were based on the LRT analysis. Using this LRT analysis, we observed a highly significant association between the common haplotypes and HTG ($p < 0.001$). However, the more powerful score test for regression can be used to perform a test for additive effects of the haplotypes. In particular, the HTR test showed that as a result of the stepwise selection, the haplotype 2 and 4 had a statistically significantly effect on disease status. The search for genetic regions associated with complex diseases, such as HTG is an important challenge that may lead to better diagnosis and treatment.

In this study, the ages and genders between the case-control groups are different, therefore it could influence that an effects of gene on the appearance of HTG is biased. Considering the Mendelian randomization mechanism, on the other hand, we can not assert that the distributions of gene variations for ages and genders in this study are different. Consequently, in the association with gene and HTG, we conclude that it is rarely possible to be affected by the gender and the age. More interestingly, Zhao et al.[19] found the correlation between human serum *APOA5* and TG was affected by gender. In their study, serum *APOA5* concentration was higher in female than in male though without statistic significance, and it was related negatively to TG

concentration in female but not in male. Overall, they found that human serum *APOA5* concentration was very low and was negatively correlated with TG, BMI and age, but positively correlated with HDL-C. Moreover, these correlations were affected by gender.

One possible limitation of the present study is a relatively small samples of cases and control subjects and this lead to insufficient statistical power to detect the presumably modest effects in phenotypes that may be associated with *APOA5* variation. Further studies are needed to establish the mechanisms of action associated with the presence of this allelic variation. Moreover, given the number of other loci and environmental factors known to influence TG concentrations, it will be highly relevant to examine how the effect of this *APOA5* polymorphism and haploytype are modified by the presence of other genetic and environmental factors. Pennacchio et al.[4] remarked the specific role of *APOA5* in plasma TG transport has not been defined and therefore the mechanisms underlying the association between the SNP and haplotypes and plasma TG concentrations are not known.

In order to imply that two SNPs are in LD one has to look at both D′ and r^2. If D′ is one and r^2 is different from one the SNPs are said to be in complete LD. If r^2 and thus also D′ is one the SNPs are said to be in perfect LD. The fact that D′ is high for all the SNPs is a good thing, hence the numbers of haplotypes decrease and the power will increase. R^2 may be used in order to exclude some SNPs from the analysis. However, this is not the case in the present study.

In conclusion, this study revealed that *APOA5* -3A>G, G182C and 1259T>C variants and haplotypes (CGGGTT and CGGGGC) are associated with TG. We also found that there was a significant association between these haplotypes and increasing susceptibility of HTG in Koreans. This finding suggests that the *APOA5* polymorphisms and haplotypes could be used as predictors for HTG for Koreans in the future. In addition, these results suggest that the *APOA5* gene variability is an important determinant of TG levels.

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