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Contribution of *APOA5*−1131C allele to the increased susceptibility of diabetes mellitus in association with higher triglyceride in Korean women

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Abstract

Apolipoprotein A5 (*APOA5*) −1131C allele is associated with higher triglyceride, an independent cardiovascular risk factor and a commonly recognized lipid abnormality in diabetes mellitus (DM). We investigated the association of *APOA5* −1131T>C or S19W with DM. Study subjects were all women and categorized into metabolically healthy controls (n = 2033) and DM subjects (n = 304). Association of *APOA5* −1131T>C with DM was calculated by odds ratio (OR). Anthropometric parameters, fasting glucose, and lipid profiles were measured. C carriers, particularly those with CC homozygote, had higher triglyceride and lower high-density lipoprotein cholesterol in both healthy controls ($P < .001$ and $P < .001$) and DM patients ($P = .002$ and $P = .006$) after the adjustment for age, body mass index, menopause, smoking, and drinking. *APOA5* −1131C allele was associated with an increased risk of DM (OR, 1.61 [95% confidence interval {CI}, 1.23-2.10]; $P < .001$) after adjustment for the above confounders. Further adjustment for fasting triglyceride or/and high-density lipoprotein cholesterol attenuated a little bit, but still significantly increased the risk of DM in C carriers (OR_2, 1.36 [95% CI, 1.02-1.80]; $P = .035$ and OR_3, 1.36 [95% CI, 1.032-1.79]; $P = .029$, respectively). Interestingly, C allele carriers in DM patients showed a positive correlation between fasting glucose and triglyceride after the adjustment ($r = 0.172$, $P = .035$). On the other hand, this significant correlation was not observed in healthy women. Regarding S19W, minor allele was not found in our study population from prescreening test. In conclusion, *APOA5* −1131C allele may contribute to the increased susceptibility of DM in Korean women. In addition, positive correlation between fasting glucose and triglyceride in C carriers of DM patients suggested that C allele in hyperglycemic states may be more susceptible to the risk of cardiovascular disease.

1. Introduction

Apolipoprotein A5 gene (*APOA5*) is involved in triglyceride (TG) metabolism in both fasting and postprandial states in human [1-3]. Among 5 common *APOA5* single nucleotide polymorphisms (SNPs) reported in several populations, −1131T>C and S19W (56C>G) are considered as tag SNPs [4-14]. The frequency of the C allele at −1131T>C is relatively high in East Asian (28%~33%) compared with Western people (9%~16%) [2,4-9]. On the other hand, the minor allele 19W was found more frequently in Western people (6~15%), but was rare in Asian populations (0.1%~3%) [7,10-16]. *APOA5* −1131T>C showed a strong association with the increased risk of cardiovascular disease (CVD) in multiple ethnic populations [2,4-9,16,17]. In our previous studies, *APOA5* −1131C allele was associated with higher TG and contributed to the higher CVD risk in Koreans, independently of common environmental factors [2,18]. S19W is also known to be associated with metabolic

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syndrome and the risk of myocardial infarction [13,16]. However, the results on these relationships are still controversial among studies [16,19-23].

Hypertriglyceridemia is not only a classic risk factor for CVD but also one of the commonly recognized lipid abnormalities in diabetes mellitus (DM). Furthermore, it is an independent risk factor for atherosclerosis in type 2 DM [24,25]. Consequently, the *APOA5* gene has become an interest as a good candidate for the development of vascular complications in type 2 DM or of DM directly. Qi et al [26] reported the strong association of S19W with higher TG levels in type 2 DM women. Dorfmeister et al [27] also showed higher TG levels in type 2 DM patients with −1131C allele in European whites and Indian Asians. Zhai et al [28] reported that the frequency of *APOA5* −1131C allele in DM patients was significantly higher than that of nondiabetic subjects; CC homozygotes had about 3.75 times higher risk of DM compared with TT homozygotes; and DM patients with C allele contributed to the increments in plasma levels of TG, total cholesterol, and low-density lipoprotein (LDL) cholesterol, but had no effect on insulin resistance. Yan et al [29] also showed an association of *APOA5* −1131C allele with the elevated TG in type 2 DM in Chinese population and found a 2-fold increased risk of type 2 DM with coronary heart disease in C allele carries than noncarriers. However, the significantly increased risk was attenuated after adjustment for conventional risk factors. On the other hand, Talmud et al reported no impact of *APOA5* genotype (−1131T>C and S19W) on the risk of development of type 2 DM through the 15-year follow-up study of healthy United Kingdom men (Northwick Park Heart Study II) [30]. However, there are no studies on the relationship of *APOA5* −1131T>C or S19W polymorphism with DM in Korean population. Therefore, our study aimed to investigate the association of *APOA5* −1131T>C or S19W with the risk of DM and their relationship with plasma lipid profile.

2. Subjects and methods

2.1. Study population

All participants were Korean women recruited from a health promotion center during routine checkup at Severance Hospital (January 2007∼October 2008). Exclusion criteria included orthopedic limitations; weight loss/gain over the previous 6 months; or any diagnosis of vascular disease, cancer (clinically or by anamnesis), renal disease, liver disease, thyroid disease, or acute or chronic inflammatory disease. *Metabolically healthy control* was defined as those without disease diagnosed, metabolic syndrome, or fasting impaired glucose. None of them were taking any medications (antihypertensive, antidyslipidemic, antithrombotic, and antidiabetic drugs). Metabolic syndrome definition followed a modification of the National Cholesterol Education Program–Adult Treatment Panel III guideline, Asian-Pacific guideline, and American Diabetes Association guideline. It included at least 3 of the following components: waist circumference greater than 80 cm, TG at least 1.69 mmol/L (150 mg/dL), high-density lipoprotein (HDL) cholesterol less than 1.29 mmol/L (50 mg/dL), blood pressure at least 130/at least 85 mm Hg, or fasting glucose at least 5.56 mmol/L (100 mg/dL); but in fact, fasting glucose greater than 7.0 mmol/L (≥126 mg/mL) was classified as DM according to the American Diabetes Association criteria. In this study, DM includes diagnosis from hospital, taking of oral hypoglycemic agents (non–insulin user) prescribed by a physician, or fasting glucose greater than 7.0 mmol/L at screening. The latter case was rechecked, and the confirmed one was included. Finally, this study included 2033 metabolically healthy controls and 304 DM patients. Written informed consent was obtained from all subjects, and the protocol was approved by the Institute of Review Board of Yonsei University.

2.2. Anthropometric parameters, blood pressure measurements, and blood collection

Body weight and height were measured unclothed and without shoes in the morning. Blood pressure was obtained from the left arm of seated patients with an automatic blood pressure monitor (TM-2654; A&D, Tokyo, Japan) after 20 minutes of rest. After an overnight fasting period, venous blood specimens were collected in EDTA-treated or plain tubes, centrifuged into plasma or serum, and then stored at −70 °C until analysis.

2.3. Genotyping of APOA5 −1131T>C and S19W

Genomic DNA was extracted from 5 mL of whole blood using a commercially available DNA isolation kit (WIZARD Genomic DNA Purification Kit; Promega, Madison, WI) according to the manufacturer's protocol. We first prescreened the 2 *APOA5* SNPs, −1131T>C and S19W (56C>G), in about 20% of study population (n = 480) to see the minor allele frequency. Genotypings of −1131T>C and S19W were performed by SNP-IT assays using single primer extension technology (SNPstream 25K System; Orchid Biosystems, Princeton, NJ). The results of yellow and/or blue color developments were analyzed with enzyme-linked immunosorbent assay reader, and the final genotype calls were made with QCReview program (Orchid Biosciences, Princeton, NJ).

2.4. Serum lipid profiles; apolipoproteins A1, B, and A5; and glucose

Serum TG and total cholesterol were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi, Tokyo, Japan). After using dextran sulfate magnesium to precipitate chylomicron, LDL cholesterol and HDL cholesterol from the supernatant were measured by an enzymatic method. The LDL cholesterol was indirectly estimated in subjects with TG less than 4.52 mmol/L (<400 mg/mL) using the Friedewald formula. In subjects with TG of at least 4.52 mmol/L, LDL cholesterol was

measured by an enzymatic method on a Hitachi 7150 Analyzer directly. Serum apolipoprotein (apo) A1 and apo B were determined by turbidometry at 340 nm using a specific antiserum (Roche, Basel, Switzerland). Plasma apo A5 was measured using an enzyme immunoassay (Human Apolipoprotein A ELISA Kit; Millipore, St Charles, MO). The resultant color reaction was read at 450 nm using a Victor^2 (Perkin Elmer Life Sciences, Turka, Finland). Glucose was measured by using a glucose oxidase method with the Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA).

2.5. Statistical analyses

Statistical analyses were performed with SPSS version 12.0 for Windows (Statistical Package for the Social Science; SPSS, Chicago, IL). Sample size was checked with PS version 2.1 (power and sample size calculation, Nashville, TN). Hardy-Weinberg equilibrium was examined using the Executive SNP Analyzer 1.0 (http://www.istech.info/SilicoSNP/index.html). Genotype distributions and allele frequencies were compared between metabolically healthy controls and DM patients by χ^2 test. The association of genotype with DM was calculated using the odds ratio (OR) [95% confidence intervals {CIs}] with logistic regression model with adjustment for age, body mass index (BMI), smoking, drinking, menopause, fasting TG, etc. Independent *t* test was performed for the comparison of general and biochemical parameters between healthy controls and DM patients. One-way analysis of variance followed by Bonferroni method was used to compare the differences among genotype groups in each subject group. General linear model analysis was also performed when adjusting for compounding factors. Pearson and partial correlation tests were performed to examine the relationship between fasting glucose and TG according to *APOA5* −1131T>C. Each variable was examined for normal distribution patterns, and the significantly skewed variables were log-transformed. For descriptive purposes, mean values are presented using untransformed and unadjusted values. Results are expressed as mean ± SE or percentage, and a 2-tailed value of $P < .05$ was considered statistically significant.

3. Result

3.1. Subject characteristics

Table 1 shows general characteristics of study subjects (N = 2337). Age, BMI, blood pressure, alcohol consumption, and proportions of postmenopause were higher in DM patients (n = 304) than healthy controls (n = 2033). Furthermore, higher levels of TG, total cholesterol, and apo B and lower levels of HDL cholesterol, apo A1, and apo A5 were observed in DM patients. These patterns were still maintained after the adjustment for age, BMI menopause, smoking, and drinking.

Table 1
General characteristics of study population

	Healthy control (n = 2033)	DM (n = 304)	P_0	P_1
Age (y)	54.6 ± 0.3	62.1 ± 0.5	<.001	–
BMI (kg/m^2)	23.4 ± 0.1	25.1 ± 0.2	<.001	–
Postmenopause (%)	64.7	89.4	<.05	–
Current smoking (%)	2.4	2.0	NS	–
Current drinking (%)	31.9	16.2	<.001	–
Systolic blood pressure (mm Hg)	120.8 ± 0.3	132.1 ± 1.1	<.001	<.001
Diastolic blood pressure (mm Hg)	73.8 ± 0.2	76.9 ± 0.6	<.001	<.001
TG (mmol/L)[a]	1.07 ± 0.01	1.71 ± 0.07	<.001	<.001
Total cholesterol (mmol/L)	5.02 ± 0.02	5.04 ± 0.06	NS	.019
HDL cholesterol (mmol/L)	1.56 ± 0.01	1.33 ± 0.02	<.001	<.001
LDL cholesterol (mmol/L)	2.97 ± 0.02	2.95 ± 0.06	NS	NS
Apo A1 (mg/dL)	149.4 ± 0.7	140.8 ± 1.4	<.001	<.001
Apo B (mg/dL)	81.8 ± 0.5	88.5 ± 1.4	<.001	<.001
Apo A5 (mg/dL)[a]	250.4 ± 7.0	187.1 ± 6.6	<.001	<.001
Glucose (mmol/L)[a]	4.64 ± 0.01	7.06 ± 0.03	<.001	<.001

Mean ± SE. NS indicates not significant.

[a] Tested by log-transformation. Tested by independent *t* test (P_0: unadjusted) and general linear model analysis with adjustment for age, BMI, and menopausal status (P_1).

3.2. Distribution of *APOA5* −1131T>C or S19W polymorphism

From the prescreening of SNP −1131T>C and S19W, we found that the *APOA5* −1131T>C polymorphism satisfied Hardy-Weinberg equilibrium. The minor C allele frequency at *APOA5* −1131T>C was 0.28, which was consistent with previous observations in a Korean population [2,19]. On the other hand, minor allele of S19W was not observed in our population (C:G = 1:0). Thus, in our study, only *APOA5* −1131T>C SNP was selected as a functional SNP to investigate further analysis. The minor C allele frequency *APOA5* −1131T>C was 0.273 in the whole population. The minor frequency in healthy controls (0.265) was significantly different from that in DM patients (0.327) ($P < .05$). Genotype distribution was in Hardy-Weinberg equilibrium in the entire population as well as in healthy controls and DM patients, separately.

3.3. Lipid profile and glucose according to *APOA5* −1131T>C

Age; BMI; systolic and diastolic blood pressure; and proportions of postmenopause, smoking, and drinking were not significantly different according to *APOA5* −1131T>C in each subject group. Percentages of medications (ie, antihypertensive, lipid-lowering or oral hypoglycemic agents) were not significantly different according to genotype in DM patients (data not shown). C carriers particularly those with CC homozygotes in both healthy controls and DM patients had higher TG levels than noncarriers before (P_0) and after the adjustment for age, BMI, menopause, smoking, and drinking (P_1) (Fig. 1). In

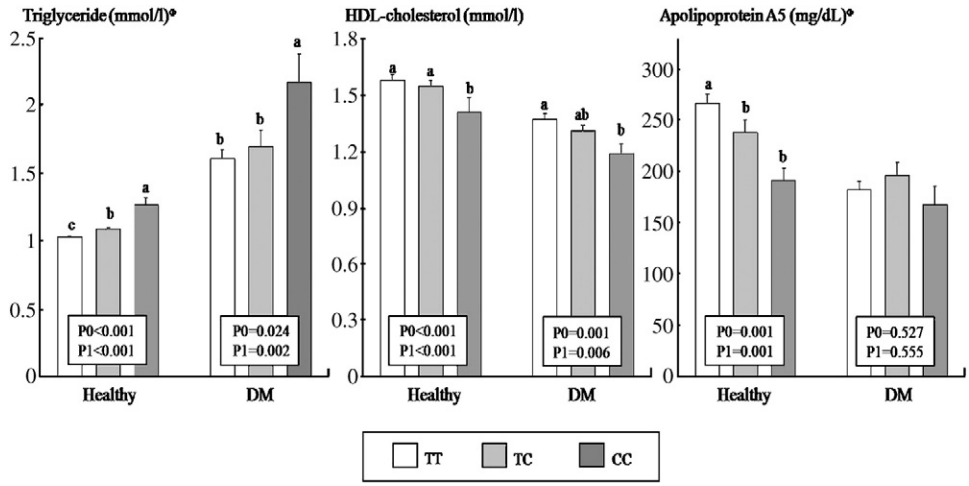

Fig. 1. Triglyceride, HDL cholesterol, and apo A5 according to *APOA5* −1131T>C. Mean ± SE$^{\Phi}$ tested by log-transformation. Tested by 1-way analysis of variance followed by Bonferroni method with or without adjustment in each subject group (P_0: unadjusted; P_1: adjusted for age, BMI, menopause, smoking, and drinking). Sharing the same alphabet in each subject group indicates no significant difference after the adjustment.

addition, CC homozygotes in both subject groups had lower levels of HDL cholesterol than TT or TC genotype. For plasma apo A5 levels, C carriers in healthy controls had significantly lower levels than noncarriers. However, DM patients did not have any genotype-associated differences in apo A5 levels (Fig. 1); differences were also not found after further adjustment for lipid-lowering drug (P = .537) or hypoglycemic drugs (P = .572). In addition, apo A1 levels were lower in CC homozygotes than in TT or TG genotypes among the healthy controls. Fasting levels of total cholesterol, LDL cholesterol, apo B, and glucose were not significantly different according to genotypes in both subject groups (data not shown).

3.4. Relative risk of DM according to APOA5 −1131T>C genotype

C carriers (TC + CC genotypes) showed a significantly higher risk of DM than noncarriers (TT genotype) before (OR_0, 1.39 [95% CI, 1.09–1.77]; P = .008) and after the adjustment for age, BMI, menopause, smoking, and drinking (OR_1, 1.61 [95% CI, 1.23-2.10]; P < .001). When further adjusted for fasting TG, the increased risk of DM in C carriers was a little bit attenuated but still significant (OR_2, 1.36 [95% CI, 1.02-1.80]; P = .035). In addition, further adjustment for HDL cholesterol still maintained the significance (OR_3, 1.36 [95% CI, 1.032-1.79]; P = .029). However, after adjustments for all the variables including age, BMI, menopause, smoking, drinking, TG, HDL cholesterol, and apo A5, this statistical significance disappeared (P = .264).

3.5. Relationship between fasting glucose and TG in association with APOA5 −1131T>C

Fig. 2 shows the relationship between fasting glucose and TG in association with *APOA5* −1131T>C. Each subject group was subdivided into C carriers (TC + CC genotypes) and noncarriers (TT genotype). In metabolically healthy controls, fasting glucose levels were not significantly associated with TG levels regardless of genotype. Interestingly, in DM patients, C carriers showed a positive correlation between fasting glucose and TG before (r_0 = 0.165, P = .035) and after the adjustment for age, BMI, menopause, smoking, and drinking (r_1 = 0.172, P = .035). However, these patterns were not observed in noncarriers (r_0 = 0.139, P = .102; r_1 = 0.113, P = .219). In addition, significant inverse correlations between apo A5 and TG were observed in both TT (r_1 = −0.275, P = .002) and TC + CC (r_1 = −0.199, P = .030) genotype groups of healthy subjects after the adjustment. However, these relationships were not observed in DM patients (TT: r_1 = −0.143, P = .210; TC + CC: r_1 = 0.071, P = .572). Inverse relationship between TG and HDL cholesterol was found in all subject groups regardless of genotype (TT: r_1 = −0.268, P < .001; TC + CC: r_1 = −0.292, P < .001 in healthy controls and TT: r_1 = −0.368, P < .001; TC + CC: r_1 = −0.484, P < .001 in DM patients). On the other hand, significant genotype-associated correlation between apo A5 and fasting glucose was observed neither in healthy subjects (TT: r_1 = −0.050, P = .571; TC + CC: r_1 = 0.155, P = .092) nor in DM patients (TT: r_1 = 0.070, P = .540; TC + CC: r_1 = 0.028, P = .801).

4. Discussion

This present study shows that *APOA5* −1131C allele may contribute to the increased susceptibility of DM in Korean women. In addition, positive correlation between fasting glucose and TG in C carriers of DM patients suggested that C allele in hyperglycemic states may be more susceptible to the risk of CVD.

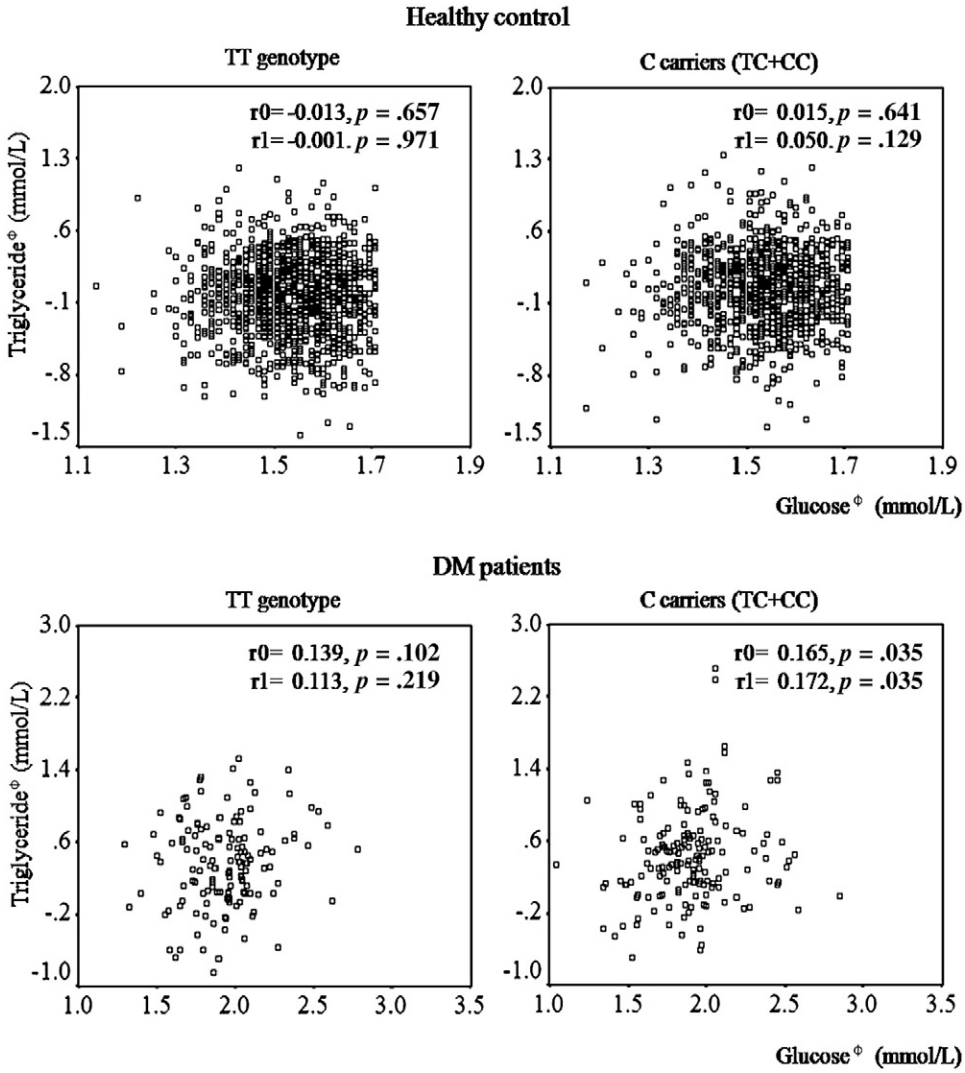

Fig. 2. Relationship between fasting glucose and TG levels according to *APOA5* −1131T>C (r_0: unadjusted; r_1: adjusted for age, BMI, menopause, smoking, and drinking).

Our results are similar with the reports of Zhai et al [28] and Yan et al [29] for Chinese people reporting the higher risk of DM in C carriers (2~3.75 times). However, differently from ours, the significant association shown in the study of Yan et al was attenuated after the adjustment for the conventional risk factors such as sex, HDL cholesterol, TG, smoking, etc. In addition, the 15-year follow-up study of United Kingdom healthy men (Northwick Park Heart Study II) showed no impact of *APOA5* genotype (1131T>C or S19W) on the risk of development of type 2 DM [30]. This discrepancy may be due to the differences in subject characteristics, disease severity, ethnicity, etc. Our subjects were all Korean women, and our diabetic patients did not have any CVD. On the other hand, other studies included both men and women [29] or only men [30]; and in some, diabetic patients had coronary heart disease [29]. *APOA5* −1131T>C is associated with the increased risk of CVD in multiple ethnic populations [2,4,18,31]. However, the results of these relationships are still controversial among studies. Hubacek et al [16] demonstrated that −1131T>C and S19>W may influence the risk of myocardial infarction, but they did not affect plasma remnant particles concentrations [19]. According to Lai et al [20], −1131C allele was associated with a higher hazard ratio for CVD (×1.85) in women; but this association was not observed in men. Hsu et al [21] also showed that −1131C homozygotes carriers were found frequently in coronary artery disease (CAD) patients than age-/sex-matched controls, but that the association between −1131T>C and the risk for CAD disappeared in multiple logistic regression analysis. Lee et al [22] reported no association of apo A5 gene polymorphisms or haplotype with CAD as determined by angiography. Possible explanations for this discrepancy may be related to the ethnicity or the selection of CAD patients or controls. In addition, the differences in allele frequency among populations might be also considered. It is well known that minor C allele

frequency at *APOA5* −1131 SNP is higher in East Asian (33% in Chinese and Japanese and 28%~30% in Koreans) compared with Western people (9% in whites and 16% in Hispanics) [2,4-9]. On the other hand, the minor allele 19W was found in Western people (6%~8% in whites and African Americans and 15% in Hispanics); but it is rare in Asian populations (0.1% in Chinese, 1.7% in Malays, and 3.1% in Asian Indians) [7,14,15]. In our study, minor allele frequency of −1131T>C was 0.273, which was in accordance with our previous report, whereas 19W allele was not found in our subjects from the prescreening test.

Hypertriglyceridemia, a classic risk factor for CVD, is also commonly observed in DM and is an independent risk factor for cardiovascular complication in type 2 DM [24,25]. In our study, TG levels were higher in DM patients than healthy controls. When subdivided into genotypes, TG levels were higher in C carriers, particularly those with CC homozygotes, than noncarriers in both subject groups; and the significances were maintained after the adjustment for confounders (ie, age, BMI, smoking, drinking, and menopause). −1131T>C SNP is localized within the promoter region of *APOA5*, and it has the potential to affect gene expression of *APOA5* [14]. Apolipoprotein A5 is known to play an important role in the regulation of circulating TG by directly interacting with lipoprotein lipase and inducing lipoprotein lipase–mediated hydrolysis of plasma TG [32,33]. It is reported that *APOA5* expression is enhanced through peroxisome proliferator-activated receptor α (*PPAR*-α) as a crucial regulator, and the peroxisome proliferator response element is required to confer the function of *PPAR*-α activator [34,35]. According to functional analyses of the *APOA5* promoter region in human hepatocytes [34,35], the peroxisome proliferator response element is located approximately 328 base pairs from −1131T>C. Therefore, −1131T>C may represent the variant showing a strong association with TG levels.

In our study, apo A5 levels were lower in DM patients than healthy controls; and negative correlation between apo A5 and TG was observed in healthy controls. Several studies reported that apo A5 levels are lower in CAD or DM patients than healthy people [35-38]. According to Nowak et al [39], apo A5 expression was down-regulated by insulin resistance; and the plasma levels were reduced after insulin infusion. In addition, when human *APOA5* gene was expressed in transgenic mice, plasma concentration of TG was decreased by 70%, whereas *APOA5* gene knockout mice had 4-fold elevation of plasma TG levels [1]. However, some other studies showed conflicting results on this association. [40-42]. Dallinga-Thie et al [40], Kahri et al [41], and Alborn et al [42] reported that apo A5 levels were higher in type 2 DM compared with control groups and that plasma apo A5 levels were positively associated with plasma TG in type 2 DM patients. This discrepancy among studies may be related to the differences in subject characteristics (ie, severity of disease or symptoms), species, relation of other functional sites, etc. Prieur et al [43] showed the differential regulation of the human vs the mouse *APOA5* gene by *PPAR*-α on the apo A5 expression and the metabolism of TG-rich lipoproteins. Kahri et al [41] demonstrated that the increase of apo A5 during postprandial lipemia paralleled the response of TG-rich lipoproteins in type 2 DM and suggested that apo A5 production in the liver is increased as a regulatory mechanism to cover the increase need of postprandial lipolysis. It may be consistent with the reports showing the elevated apo A5 concentration in patients with severe hypertriglyceridemia [34,35].

In addition, our study showed that apo A5 levels were lower in C carriers, particularly those with CC genotype, than noncarriers of healthy controls; but these were not observed in DM patients. As stated above, lower apo A5 levels were also associated with C allele at *APOA5* −1131 [2,4,18,31,34,35]; but we lost the association in DM patients. It is assumed that hyperinsulinemia or insulin resistance, which commonly occurs in DM patients, might significantly down-regulate apo A5 expression so much as to mask the effect of *APOA5* −1131T>C on plasma apo A5 levels. However, we could not conclude it because we have not measured the insulin levels in our subjects. Another possible explanation may be related to the report of Talmud et al [44]. According to their report, there is no significant difference in the relative luciferase expression in Huh7 cells transiently transfected with a −1131T construct compared with the −1131C. Similar results were also observed in SNP 1891T>C and −3A>G, and all these SNPs are included in *APOA5*2* (−1131T>C, −3A>G, 751G>T, and 1891T>C). In addition, −1131T>C had strong linkage disequilibrium with the *APOC3* −482C>T variant, which disrupts the insulin responsiveness of *APOC3* leading to raised TG levels [7,45,46]. Thus, it is needed to consider *APOC3* SNPs on the plasma levels of TG and apo A5 beyond the *APOA5* −1131T>C.

Furthermore, fasting glucose levels were not significantly different according to genotypes in both subject groups. It is out of accordance with the study of Nowak et al [47] showing the up-regulation of *APOA5* gene by D-glucose and by upstream stimulatory factor through phosphatase activation. Regarding the discrepancy between the results, we assumed that no association in our results may need more explanation compared with their works performed in transfected cell line system. On the other hand, the similar result with ours was observed in the report of Zhai et al [28] that showed no significant genotype association with profiles of insulin resistance in fasting level in both healthy controls and type 2 DM patients. Our previous study for healthy controls and CAD patients also showed no significant genotype association with fasting levels of glucose and insulin resistance calculated as homeostasis model assessment [18]. However, Chiu et al [48] reported interesting result that *APOA5* V150M was not associated with fasting glucose levels, but was significantly associated with higher first- and second-phase insulin responses during hyperglycemic clamping. This result was from a different SNP not

measured in our study, but it suggested that measuring the postprandial levels of glucose or insulin during hyperglycemic clamping or the oral glucose tolerance test rather than fasting state will be more relevant to examine the direct association of *APOA5* −1131T>C with glucose or insulin response. On the other hand, interestingly, a positive correlation between fasting glucose and TG levels was observed in C carriers of DM patients, but not in noncarriers before and after the adjustment. These results may suggest that C carriers in hyperglycemia are more susceptible to hypertriglyceridemia, which may increase the risk of CVD.

The present investigation has several limitations. Study subjects were Korean women; thus, the results may not be applicable to men or other ethnic samples whose clinical and biochemical characteristics may differ from those in our population. Second, this case-control study is not designed for assessing the time sequential associations because the exposure and outcomes are collected at one point in time. Third, the information of insulinemia or insulin resistance is needed for more accurate explanation for the genotype-phenotype. Fourth, postprandial glucose test or hyperglycemic clamping test may be needed to investigate the direct contribution of *APOA5* −1131C allele to the regulation of glucose and insulin status. In addition, the analysis of other SNPs in *APOA5* gene (ie, V150M, G185C) or other related genes (ie, *APOA1/C3/A4*) needs to be considered for deciphering the association with the risk of DM [1,23].

Despite these limitations, our findings may support that *APOA5* −1131C allele may contribute to the increased susceptibility of DM in Korean women. In addition, a positive correlation between fasting glucose and TG levels observed in C carriers of DM patients may suggest that C carriers in hyperglycemia are more susceptible to hypertriglyceridemia, which may increase the risk of CVD.

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