

# Apolipoprotein A5 3'-UTR variants and cardiometabolic traits in Koreans: results from the Korean genome and epidemiology study and the Korea National Health and Nutrition Examination Survey

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**BACKGROUND/OBJECTIVES:** This study aimed to test the association between *APOA5* 3'-UTR variants (rs662799) and cardiometabolic traits in Koreans.

**SUBJECTS/METHODS:** For this study, epidemiological data, *Apolipoprotein A5* (*APOA5*) genotype information, and lymphoblastoid cell line (LCL) biospecimens from a subset of the Ansung-Ansan cohort within the Korean Genome and Epidemiology study (KoGES-ASAS; n = 7,704) as well as epidemiological data along with genomic DNA biospecimens of participants from a subset of the Korea National Health and Nutrition Examination Survey (KNHANES 2011-12; n = 2,235) were obtained. *APOA5* mRNA expression was also measured.

**RESULTS:** *APOA5* rs662799 genotype distributions in both the KoGES-ASAS and KNHANES groups were 50.6% for TT, 41.3% for TC, and 8.1% for CC, which are similar to those in previous reports. In both groups, minor C allele carriers, particularly subjects with CC homozygosity, had lower high-density lipoprotein (HDL) cholesterol and higher triglyceride levels than TT homozygotes. Linear regression analysis showed that the minor C allele significantly contributed to reduction of circulating HDL cholesterol levels [ $\beta = -2.048$ ,  $P < 0.001$ ;  $\beta = -2.199$ ,  $P < 0.001$ ] as well as elevation of circulating triglyceride levels [ $\beta = 0.053$ ,  $P < 0.001$ ;  $\beta = 0.066$ ,  $P < 0.001$ ] in both the KoGES-ASAS and KNHANES groups. In addition, higher expression levels of *APOA5* in LCLs of 64 healthy individuals were negatively associated with body mass index ( $r = -0.277$ ,  $P = 0.027$ ) and circulating triglyceride level ( $r = -0.340$ ,  $P = 0.006$ ) but not significantly correlated with circulating HDL cholesterol level. On the other hand, we observed no significant difference in the mRNA level of *APOA5* according to *APOA5* rs662799 polymorphisms.

**CONCLUSIONS:** The C allele of *APOA5* rs662799 was found to be significantly associated with cardiometabolic traits in a large Korean population from the KoGES-ASAS and KNHANES. The effect of this genotype may be associated with post-transcriptional regulation, which deserves further experimental confirmation.

Nutrition Research and Practice 2018;12(1):61-68; <https://doi.org/10.4162/nrp.2018.12.1.61>; pISSN 1976-1457 eISSN 2005-6168

**Keywords:** Apolipoprotein A-V, metabolic syndrome, triglycerides, Koreans

## INTRODUCTION

Hypertriglyceridemia is a lipid abnormality commonly observed in obese individuals and patients with cardiovascular disease (CVD) or diabetes [1,2]. It is considered to be an independent risk factor for atherosclerosis in patients with type 2 diabetes [3,4]. Apolipoprotein A5 (ApoA5), a protein composed of 366 amino acids and high-density lipoprotein (HDL) primarily secreted from the liver [5,6], is a well-known modulator of circulating triglyceride (TG) levels in both fasting and postprandial states [7]. ApoA5 also inhibits production of very low-density lipoprotein (VLDL) TG [5-7] and stimulates hydrolysis of VLDL TG mediated by lipoprotein lipase (LPL) [6,7], thereby reducing circulating TG levels [5,8]. Consequently, single-nucleotide polymorphisms (SNPs) in *APOA5* have been shown to be involved in TG metabolism in both fasting and postprandial states and are considered as important contributors to the development of obesity, dyslipidemia, CVD, diabetes, and vascular complications in type 2 diabetes [9-13]. Among commonly studied *APOA5* SNPs, -1131T > C (rs662799) and 56C

randial states [7]. ApoA5 also inhibits production of very low-density lipoprotein (VLDL) TG [5-7] and stimulates hydrolysis of VLDL TG mediated by lipoprotein lipase (LPL) [6,7], thereby reducing circulating TG levels [5,8]. Consequently, single-nucleotide polymorphisms (SNPs) in *APOA5* have been shown to be involved in TG metabolism in both fasting and postprandial states and are considered as important contributors to the development of obesity, dyslipidemia, CVD, diabetes, and vascular complications in type 2 diabetes [9-13]. Among commonly studied *APOA5* SNPs, -1131T > C (rs662799) and 56C

This research was supported by the Basic Science Research Program, through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science, and Technology (NRF-2015R1A2A1A15054758).

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Received: October 23, 2017, Revised: December 16, 2017, Accepted: December 18, 2017

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> G (rs3135506) are considered to be functional-tag SNPs at *APOA5* [12,13]. It has been previously reported that the frequency of the *APOA5* -1131C allele is higher in East Asians (> 25%) than in Westerners (9-16%) [9-13]. The minor C allele of *APOA5* rs662799 is associated with a higher circulating TG level and independently contributes to increased risk of CVD [11,13]. On the other hand, the minor G allele of *APOA5* rs3135506, which is associated with risk of myocardial infarction and metabolic syndrome (MetS), was reported to be more common in Westerners (6-15%) than in Asians (0.1-3%) [12,13].

Conflicting findings on the above-described associations have been reported [14,15], and most studies in Korea have been conducted using small samples, of which the results are not generalizable to the Korean population. Therefore, this study aimed to test the association between *APOA5* 3'-UTR variants of rs662799 as functional-tag SNPs at *APOA5* and cardiometabolic traits using data from subpopulations of the Korean Genome and Epidemiology Study: Ansung-Ansan cohort study (KoGES-ASAS) and the Korea National Health and Nutrition Examination Survey (KNHANES, 2011-2012).

## SUBJECTS AND METHODS

### *Study participants*

This study was conducted using two separate datasets composed of the KoGES-ASAS and KNHANES (2011-2012). The procedure and design of the KoGES-ASAS are described elsewhere [16,17]. With regard to the KoGES-ASAS, 10,030 individuals aged 40-69 years living in the Ansan (urban) and Ansung (rural) districts were recruited for baseline in 2001-2002. The aim was to construct a genomic and epidemiologic database to examine the effects of genetics and the environment on disease prevalence in Koreans. Questionnaire-based interviews were conducted with participants in a community clinic, where they were questioned regarding their socio-demographic status, lifestyle, health, and medical history. They also underwent anthropometric measurements, clinical examinations, as well as biannual follow-up examinations. The current study was based on data collected from a total of 8,841 participants, for whom DNA samples for genotyping were available. Among them, participants with preexisting cancer ( $n = 97$ ), diabetes ( $n = 783$ ), or CVD ( $n = 202$ ) at the time of enrollment in the study were excluded. Participants with TG levels > 600 mg/dL ( $n = 55$ ) were also excluded, leaving 7,704 participants for analysis. This study protocol was approved by the Institutional Review Board of the Korea Centers for Disease Control and Prevention (KCDC; KBP-2016-062) and the Institutional Review Board at Korea University (KU-IRB-16-EX-272-A-1). The KNHANES is a nationwide cross-sectional survey conducted by the KCDC. This survey is composed of three different sections: health examination, health interview, and nutrition survey. Detailed information on the KNHANES is available elsewhere [17]. Among the 16,576 participants (8,518 in 2011 and 8,058 in 2012), those aged < 20 years were excluded. We further excluded those who had been diagnosed with cancer as well as women who were pregnant or breastfeeding. After considering all exclusion criteria, 2,600 participants (492 in 2011 and 2,108 in 2012) whose DNA samples were available for genotyping were included from the study. After

further excluding participants with diabetes ( $n = 278$ ), CVD ( $n = 74$ ), or TG levels > 600 mg/dL ( $n = 13$ ), data on a total of 2,235 participants were finally used for the analysis. The KNHANES was approved by the Institutional Review Board of KCDC (2011-02CON-06-C, 2012-01EXP-01-2C). This study protocol was approved by the Institutional Review Board of KCDC (KBP-2016-062) and the Institutional Review Board at Korea University (KU-IRB-16-EX-137-A-1).

### *General information and anthropometric and biochemical measurements*

Demographic and behavioral data on participants in the KoGES-ASAS and KNHANES (i.e., age, sex, education level, physical activity, cigarette smoking, and alcohol consumption) were obtained from survey questionnaires administered by trained interviewers. Education level was divided into four groups: elementary school, middle school, high school, and university. Current smokers and current drinkers were defined as those who smoked cigarettes or drank alcoholic beverages regularly at the time of the survey. The level of total metabolic equivalents (METs), as a representative of physical activity, was calculated by summing METs during each activity type (2.4 for light, 5.0 for moderate, and 7.5 for intense activities) [18]. For anthropometric and biochemical measurements, procedures and assay methods for the KoGES-ASAS and KNHANES are described elsewhere in detail [17,19]. Height and body weight were measured, from which the body mass index (BMI;  $\text{kg}/\text{m}^2$ ) was calculated as the weight divided by the height squared. Blood pressure (BP) was repeatedly measured by a trained technician using a mercury sphygmomanometer. Two readings were taken on the left and right arms of each subject in the supine position with a 5-min rest between readings. Measurements were recorded to the nearest 2 mmHg, and averages were calculated for systolic and diastolic BPs (SBP and DBP). Hypertension was defined as an SBP of  $\geq 140$  mmHg or DBP of  $\geq 90$  mmHg, previous diagnosis of hypertension as self-reported by the participants, or taking anti-hypertensive medication. In the KoGES-ASAS, blood samples were collected for biochemical analysis after at least 8 h of fasting. Fasting levels of glucose (mg/dL), TG (mg/dL), total cholesterol (mg/dL), and high-density lipoprotein cholesterol (HDL cholesterol, mg/dL) were measured using an automatic analyzer (ADVIA 1650 and 1680; Siemens, Tarrytown, NY, USA). In the KNHANES, blood samples were collected after overnight fasting for the analysis of biochemical markers. Fasting levels of glucose (mg/dL), TG (mg/dL), total cholesterol (mg/dL), and HDL cholesterol (mg/dL) were measured using an automatic analyzer (Hitachi 7600; Hitachi, Tokyo, Japan). In both studies, LDL cholesterol was calculated using the Friedewald equation  $[\text{LDL cholesterol (mg/dL)} = \text{total cholesterol (mg/dL)} - \text{HDL cholesterol (mg/dL)} - (\text{TG (mg/dL)}/5)]$  in subjects with a TG level of < 400 mg/dL [20].

### *Genotyping information*

Detailed information on DNA preparation and genotyping of the KoGES-ASAS is provided elsewhere [19]. Briefly, DNA samples were isolated from the peripheral blood of participants and genotyped using the Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix, Inc., Santa Clara, CA, USA). The accuracy

of genotyping was calculated with Bayesian robust linear modeling using the Mahalanobis distance genotyping algorithm. A total of 352,228 SNPs in 7,704 participants became available after preimputation quality control, namely, 1) exclusion of SNPs with high missing genotype call rates > 5% with a minor allele frequency < 0.01 and not in Hardy-Weinberg equilibrium (HWE,  $P < 1 \times 10^{-6}$ ) and 2) removal of samples with sex mismatch. Genetic principal components were computed in a subset of 304,225 SNPs after excluding additional 48,003 SNPs (not in HWE under a more conservative criterion,  $P < 1 \times 10^{-5}$ ) through the EIGENSTRAT software package. In the present study, a gene variant at *APOA5* (rs662799) was included in the analyses to test its association with cardiometabolic traits. For DNA samples from the KNHANES (Institutional Review Board no. KBP-2016-062, KU-IRB-16-EX-137-A-1), *APOA5* (rs662799) genotyping was screened using the TaqMan fluorogenic 5' nuclease assay (ABI, Foster City, CA, USA). The final volume for the polymerase chain reaction (PCR) was 5  $\mu$ L comprising 10 ng of genomic DNA and 2.5  $\mu$ L of TaqMan Universal PCR Master Mix with 0.13  $\mu$ L of 20  $\times$  assay mix. Thermal cycling conditions were as follows: 50°C for 2 min to activate uracil N-glycosylase and prevent carry-over contamination, 95°C for 10 min to activate DNA polymerase, and 45 cycles of 95°C for 15 s and 60°C for 1 min. PCR was always performed using 384-well plates with a Dual 384-Well GeneAmp PCR System 9700 (ABI), and the endpoint fluorescent readings were obtained on an ABI PRISM 7900 HT Sequence Detection System (ABI). Duplicate samples and negative controls were included to ensure the accuracy of genotyping.

#### *RNA extraction and semi-quantitative reverse transcription-PCR*

To compare mRNA expression levels according to *APOA5* genetic variant (rs662799), lymphoblastoid cell lines (LCLs) of 64 healthy individuals (males,  $n = 27$ ; females,  $n = 37$ ) were obtained from the subgroup of the KoGES-ASAS (Institutional Review Board no. KBP-2016-062, KU-IRB-16-EX-272-A-1). Total RNA was extracted from LCLs using the Ribospin™ Kit (GeneAll, Korea), in accordance with the manufacturer's protocol. cDNA was synthesized from 1  $\mu$ g of RNA using oligo-dT and Superscript™ II reverse transcriptase (Invitrogen, USA). One microgram of cDNA was amplified with quantitative real-time PCR using the SYBR Green PCR Kit (Qiagen, USA). PCR was conducted using the QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA), and the conditions were as follows: 15 min at 95°C, followed by 40 thermal cycles of 94°C for 30 s, 60°C for 20 s, and 72°C for 30 s. Target-specific primers for real-time PCR were designed using Primer Express® software. Sequences of the designed primers were as follows: *APOA5* (sense, 5'-ACG CAC GCA TCC AGC AGA AC-3'; antisense, 5'-TCG GAG AGC ATC TGG GGG TC-3') and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*; sense, 5'-TCC ACC ACC CTG TTG CTG TA-3'; antisense, 5'-ACC ACA GTC CAT GCC ATC AC-3'). Obtained data were analyzed using the comparative cycle threshold ( $C_t$ ) method and were normalized by the *GAPDH* expression value. Melting curves were generated for each PCR reaction to ensure purity of the amplification product. The delta-delta-cycle threshold ( $2^{-\Delta\Delta C_t}$ ) method was used to calculate changes in gene expression as a relative fold difference between experimental and endogenous control samples. Values

are expressed as fold changes relative to the control and as mean  $\pm$  standard deviation (SD).

#### *Statistical analysis*

Statistical analyses were performed using Stata SE 12.0 (Stata Corp., Carolina, USA). First, the distribution of variable values was investigated. Fasting blood glucose and TG levels were log-transformed to mimic a Gaussian distribution. Descriptive statistics of all variable values are presented as mean  $\pm$  standard error (SE) for continuous variables and as a number and percentage for categorical variables. Effect allele frequency was calculated using the following formula: (counts of heterozygotes + 2  $\times$  count of homozygotes of effect allele)/(2  $\times$  total count). HWE equilibrium was tested using Stata. Mean differences in variables among the three different rs662799 genotype groups were compared using one-way analysis of variance after considering potential covariates for continuous variables and using chi-squared test for categorical variables. Pearson's correlation analysis was conducted to evaluate relationships between the mRNA abundance of *APOA5* in LCLs of participants and selected variables. To estimate the effect of genotype on cardiometabolic risk factors, a linear regression model was used in an additive scale model, with adjustments for potential covariates. Results are presented as estimated regression coefficients  $\beta$  with 95% confidence interval (CI) for continuous variables. Corresponding *P*-values are also provided.

## RESULTS

#### *Genotype distribution and comparisons of characteristics of study participants according to the APOA5 rs662799 polymorphism*

Table 1 presents the general information and cardiovascular risk parameters of the two study populations. Mean values of age as well as proportions of sex, smoking status, and hypertension (the KoGES-ASAS: 31.3% vs. the KNHNAES: 29.2%) were similar between the two study population groups. Genotype distribution of the *APOA5*-1131T > C (rs662799) in the population was in Hardy-Weinberg equilibrium. The minor C allele frequency was 0.288 in each population group as well as in total population, which is consistent with previous observations in the Korean population [10-12]. Genotype distributions of *APOA5* rs662799 in the total population were 50.6% for TT, 41.3% for TC, and 8.1% for CC (50.7% for TT, 41.4% for TC, and 7.9% for CC in the KoGES-ASAS and 50.4% for TT, 40.9% for TC, and 8.7% for CC in the KNHNAES, respectively). Table 2 presents the lifestyle and socio-economic information of the study participants according to the *APOA5* rs662799 polymorphism. There were no significant differences in age, proportions of sex, education level, MET, smoking status and drinking status in either the KoGES-ASAS or KNHANES group.

#### *Associations between the APOA5 rs662799 polymorphism and cardiovascular risk factors*

Table 3 presents cardiovascular risk parameters of the study participants according to the *APOA5* rs662799 polymorphism. In both the KoGES-ASAS and KNHANES groups, minor C allele carriers, particularly CC homozygotes, had lower HDL cholesterol levels and higher TG levels than TT homozygotes. On the other

**Table 1.** General information and cardiovascular risk parameters of study participants

	KoGES-ASAS (n = 7,704)	KNHANES (n = 2,235)
Lifestyle and socio-economic factor		
Age (yrs)	51.7 ± 0.1	48.6 ± 0.3
Male (% , n)	46.8 (3,598)	46.0 (1,027)
Education level (%) <sup>1)</sup>	32.3 / 23.1 / 31.1 / 13.3	20.0 / 11.3 / 37.0 / 31.7
Metabolic equivalent (hr)	19.3 ± 0.2	13.3 ± 0.3
Smoking (never/ex-/current) (%)	59.6 / 14.7 / 25.7	57.1 / 19.9 / 23.0
Drinking (never/ex-/current) (%)	46.3 / 4.8 / 48.9	10.4 / 13.2 / 76.5
Cardiovascular risk factor		
Systolic blood pressure (mmHg)	120.8 ± 0.2	118.7 ± 0.3
Diastolic blood pressure (mmHg)	80.0 ± 0.1	76.6 ± 0.2
Body mass index (kg/m <sup>2</sup> )	24.49 ± 0.04	23.86 ± 0.07
Fasting blood glucose (mg/dL)	83.8 ± 0.1	93.8 ± 0.2
Total cholesterol (mg/dL)	190.6 ± 0.4	190.4 ± 0.7
HDL cholesterol (mg/dL)	45.0 ± 0.1	49.9 ± 0.2
LDL cholesterol (mg/dL)	115.4 ± 0.4	115.3 ± 0.7
Triglyceride (mg/dL)	153.3 ± 0.9	127.6 ± 1.7
APOA5 rs662799 polymorphism		
Minor C allele frequency	0.286	0.286
TT/TC/CC (%)	50.7 / 41.4 / 7.9	50.4 / 40.9 / 8.7

KoGES-ASAS, Korean Ansong-Ansan cohort within the Korean Genome and Epidemiology study; KNHANES, Korea National Health and Nutrition Examination Survey; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

The values were described as mean ± SE for a continuous variable or frequency (% , n) for a categorical variable.

<sup>1)</sup> Educational level: (elementary school / middle school / high school / university) (%).

**Table 2.** Lifestyle and socio-economic information of study participants according to the APOA5 rs662799 polymorphism

	KoGES-ASAS				KNHANES			
	T/T (n = 3,903)	T/C (n = 3,190)	C/C (n = 611)	P-value <sup>1)</sup>	T/T (n = 1,127)	T/C (n = 913)	C/C (n = 195)	P-value <sup>1)</sup>
Age (yrs)	51.7 ± 0.1	51.9 ± 0.2	51.4 ± 0.4	0.386	48.6 ± 0.5	48.1 ± 0.5	50.5 ± 1.1	0.132
Male (% , n)	46.3 (1,807)	47.3 (1,508)	46.3 (283)	0.701	47.0 (530)	45.4 (414)	42.6 (83)	0.458
Education level (%) <sup>2)</sup>	32.2 / 23.8 / 31.0 / 13.0	32.6 / 22.6 / 31.0 / 13.8	31.6 / 21.2 / 34.7 / 12.5	0.444	19.1 / 11.5 / 35.9 / 33.6	21.1 / 10.5 / 38.3 / 30.1	19.8 / 14.4 / 37.4 / 28.3	0.373
Metabolic equivalent (hr)	19.4 ± 0.3	19.3 ± 0.3	18.9 ± 0.7	0.753	13.1 ± 0.4	13.7 ± 0.5	12.3 ± 0.9	0.329
Smoking status (%) <sup>2)</sup>	60.0 / 14.7 / 25.3	58.9 / 14.9 / 26.2	60.9 / 14.0 / 25.1	0.854	55.5 / 20.2 / 24.3	58.7 / 18.6 / 22.7	59.4 / 24.1 / 16.6	0.102
Drinking status (%) <sup>2)</sup>	47.6 / 4.7 / 47.8	45.1 / 5.0 / 49.8	44.8 / 4.4 / 50.7	0.269	10.2 / 13.8 / 76.0	11.0 / 12.3 / 76.8	8.6 / 13.4 / 78.1	0.750

KoGES-ASAS, Korean Ansong-Ansan cohort within the Korean Genome and Epidemiology study; KNHANES, Korea National Health and Nutrition Examination Survey. The values were described as mean ± SE for a continuous variable and as frequency (% , n) for a categorical variable.

<sup>1)</sup> Statistical differences were determined using chi square test for categorical variables and one-way analysis of variance (ANOVA) for continuous variables with Bonferroni's multiple correction ( $P < 0.05$ ).

<sup>2)</sup> Educational level (%): elementary school / middle school / high school / university; smoking status (%): never/ex/current smokers; drinking status (%): never/ex/current drinkers.

**Table 3.** Cardiovascular risk parameters of study participants according to the APOA5 rs662799 polymorphism

	KoGES-ASAS				KNHANES			
	T/T (N = 3,903)	T/C (N = 3,190)	C/C (N = 611)	P-value <sup>1)</sup>	T/T (N = 1,127)	T/C (N = 913)	C/C (N = 195)	P-value <sup>1)</sup>
Systolic BP (mmHg)	120.7 ± 0.3	121.0 ± 0.3	120.4 ± 0.7	0.778	118.8 ± 0.5	118.2 ± 0.5	119.9 ± 1.1	0.391
Diastolic BP (mmHg)	79.9 ± 0.2	80.0 ± 0.2	79.7 ± 0.5	0.770	76.5 ± 0.3	76.5 ± 0.3	77.2 ± 0.7	0.673
Body mass index (kg/m <sup>2</sup> )	24.5 ± 0.1	24.5 ± 0.1	24.6 ± 0.1	0.808	24.0 ± 0.1	23.8 ± 0.1	23.4 ± 0.2	0.050
Fasting glucose (mg/dL)	83.6 ± 0.2	84.0 ± 0.2	84.4 ± 0.4	0.055	94.1 ± 0.3	93.4 ± 0.3	94.3 ± 0.7	0.233
Total cholesterol (mg/dL)	190.4 ± 0.6	190.5 ± 0.6	192.4 ± 1.5	0.399	189.4 ± 1.0	191.3 ± 1.2	192.2 ± 2.4	0.344
HDL cholesterol (mg/dL)	46.1 ± 0.2 <sup>a</sup>	44.3 ± 0.2 <sup>b</sup>	41.7 ± 0.4 <sup>c</sup>	< 0.001	51.1 ± 0.4 <sup>a</sup>	49.1 ± 0.4 <sup>b</sup>	47.0 ± 0.8 <sup>b</sup>	< 0.001
LDL cholesterol (mg/dL)	116.0 ± 0.5	114.8 ± 0.6	114.5 ± 1.4	0.234	115.2 ± 0.9	115.7 ± 1.1	114.4 ± 2.2	0.870
Triglyceride (mg/dL) <sup>2)</sup>	142.0 ± 1.1 <sup>a</sup>	160.0 ± 1.5 <sup>b</sup>	190.9 ± 4.0 <sup>c</sup>	< 0.001	117.3 ± 2.2 <sup>a</sup>	134.4 ± 2.6 <sup>b</sup>	155.1 ± 6.2 <sup>c</sup>	< 0.001

KoGES-ASAS, Korean Ansong-Ansan cohort within the Korean Genome and Epidemiology study; KNHANES, Korea National Health and Nutrition Examination Survey; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

The values were described as mean ± SE.

<sup>1)</sup> Statistical differences were determined using one-way analysis of variance (ANOVA) for continuous variables with Bonferroni's multiple correction ( $P < 0.05$ ).

<sup>2)</sup> Tested after log-transformation.

**Table 4.** Contribution of minor C allele in the *APOA5* rs662799 polymorphism to cardiovascular risk (circulating HDL-cholesterol and triglyceride)

	KoGES-ASAS		KNHANES	
	Estimates	P-value <sup>1)</sup>	Estimates	P-value <sup>2)</sup>
HDL cholesterol (mg/dL)	-2.048 (-2.398, -1.699)	< 0.001	-2.199 (-2.929, -1.469)	< 0.001
Triglycerides (mg/dL) <sup>3)</sup>	0.053 (0.046, 0.060)	< 0.001	0.066 (0.051, 0.081)	< 0.001

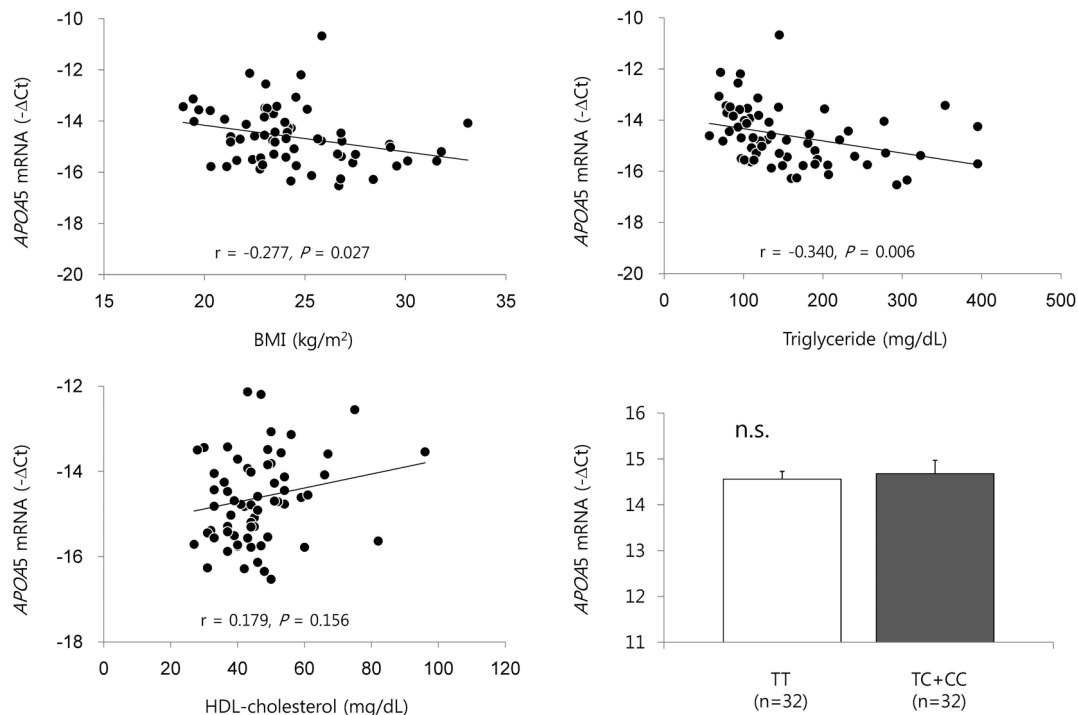
KoGES-ASAS, Korean Ansung-Ansan cohort within the Korean Genome and Epidemiology study; KNHANES, Korea National Health and Nutrition Examination Survey; BP, blood pressure; HDL, high-density lipoprotein.

Tested by a linear regression model for cardiovascular risk factors

<sup>1)</sup> Sex, age, area, education level, metabolic equivalent, smoking status, and drinking status were adjusted for analysis using Ansan-Ansung data

<sup>2)</sup> Sex, age, year, education level, metabolic equivalent, smoking status, and drinking status were adjusted for analysis using KNHANES data

<sup>3)</sup> Tested after log-transformation



**Fig. 1.** Relationships between *APOA5* mRNA expression and body mass index, circulating triglyceride, and high-density lipoprotein cholesterol levels and/or mRNA abundance of *APOA5* according to the *APOA5* rs662799 genotype. Results are expressed as  $r$  (correlation coefficient) or mean  $\pm$  SE tested by Pearson's correlation analysis or independent t-test (non-parametric test); n.s., indicates no statistically significant differences in the values among the groups.

hand, there were no significant differences in BP, BMI, fasting glucose, and total and LDL cholesterol according to the *APOA5* rs662799 polymorphism. To estimate the effect of genotype on circulating levels of TG and HDL cholesterol, a linear regression model was constructed with adjustments for potential covariates. Results show that the minor C allele significantly contributed to reduction of circulating HDL cholesterol levels [ $\beta = -2.048$  (CIs: -2.398, -1.699),  $P < 0.001$ ;  $\beta = -2.199$  (CIs: -2.929, -1.469),  $P < 0.001$ ] as well as elevation of circulating TG levels [ $\beta = 0.053$  (CIs: 0.046, 0.060),  $P < 0.001$ ;  $\beta = 0.066$  (CIs: 0.051, 0.081),  $P < 0.001$ ] in both the KoGES-ASAS and KNHANES groups, respectively (Table 4).

#### Relationship between *APOA5* mRNA expression and BMI, circulating TG, or the *APOA5* rs662799 genotype

To investigate the relationship between *APOA5* gene expression level and blood parameters, we measured the mRNA abundance of *APOA5* in LCLs of participants ( $n = 64$ , 27 males

and 37 females;  $54.1 \pm 1.1$  years) using quantitative real-time reverse transcription polymerase chain reaction (RT-PCR). As shown in Fig. 1, a higher *APOA5* expression level (-ΔCt) in LCLs was negatively associated with BMI ( $r = -0.277$ ,  $P = 0.027$ ) and circulating TG level ( $r = -0.340$ ,  $P = 0.006$ ). However, *APOA5* mRNA expression level (-ΔCt) was not significantly correlated with circulating HDL cholesterol level ( $r = 0.179$ ,  $P = 0.156$ ). On the other hand, no significant difference was observed in *ApoA5* mRNA level (-ΔCt) between *APOA5* rs662799 TT homozygotes ( $n = 32$ ) and C allele carriers (*APOA5* rs662799 TC + CC,  $n = 32$ ).

## DISCUSSION

While the association between the *APOA5* rs662799 (T/C) polymorphism and blood lipid profile remains controversial, we confirmed previous studies on Koreans [9,10] that reported that the minor C allele in *APOA5* rs662799 is significantly associated with elevation of TG and reduction of HDL cholesterol levels

in a large Korean population, from subsets of the KoGES-ASAS and KNHANES. Our results are in line with a recent meta-analysis that showed that the *APOA5* rs662799 C allele is associated with elevated circulating TG levels, regardless of ethnicity [21]. Other recent meta-analyses reported that the *APOA5* rs662799 C allele and CC genotype confer increased risks for the development of coronary artery disease (CAD) [22] and ischemic stroke [23], indicating a possible mediating role for circulating TG in the association between the risk variant at *APOA5* and the atherosclerotic process.

ApoA5 consists of four exons and encodes a 366-amino-acid protein, and *APOA5* rs662799 is located in the promoter region of *APOA5* proximal to the *APOA1/C3/A4* gene cluster on chromosome 11 [5,6,12,13]. Regarding the function of ApoA5 in TG metabolism, it was previously demonstrated that *APOA5* overexpression in mice resulted in elevation of plasma ApoA5 levels and marked reduction of circulating TG levels [6]. There is also evidence that serum TG levels were remarkably increased 4-fold in mice with *APOA5* knockout [5]. In addition, several plausible proposals have been made regarding the biological mechanisms by which genetic variants at *APOA5* affect TG metabolism in humans. For example, the minor C allele is thought to impair ribosomal translation efficiency, thereby reducing the level of ApoA5 translated from this mRNA [24,25]. In line with this, previous studies have reported that the *APOA5* rs662799 C allele is associated with decreased circulating ApoA5 levels or activity, which may result in an impaired interaction with LPL activity and increased circulating TG levels in certain metabolic contexts [26,27]. Alternatively, a peroxisome proliferator response element in the promoter region of *APOA5* was identified as a target gene for peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ) [28]. This indicates a possible role for the PPAR $\alpha$  pathway in the association between ApoA5 and circulating TG. In addition, the association of the *APOA5* rs662799 polymorphism with CAD was suggested to be attributable to linkage disequilibrium with *APOC3* variants or to other closely linked genetic variations [29].

In this study, we observed that the minor C allele significantly contributed to reduction of circulating HDL cholesterol as well as elevation of circulating TG levels in a large Korean population. While mRNA levels of *APOA5* expressed from LCLs of healthy individuals were negatively associated with BMI as well as circulating TG, mRNA abundance was not significantly different according to the *APOA5* rs662799 polymorphism. This result suggests that the genotype effect was more or less weak and this SNP might modulate *APOA5* expression at the post-transcriptional level. Indeed, it was very recently proposed that the miR-binding site created by the rare c.\*158C allele in *APOA5* 3' UTR causes liver post-transcriptional down-regulation of *APOA5* by miR-485-5p, at least partially accounting for the subsequent elevation of plasma TG levels in humans [30], which warrants further experimental confirmation. While evidence of the role of ApoA5 in LPL-mediated TG metabolism has been generally accepted, it has been additionally speculated that the composition of gut microbiota is related to circulating ApoA5 levels or *APOA5* polymorphisms as well as related metabolic properties in Koreans [31,32]. Lim et al. suggested that *APOA5* rs651821 polymorphisms may contribute to compositional

changes in MetS-related gut microbiota [31]. It was observed that the minor C allele in *APOA5* rs651821, which increases circulating TG levels and MetS incidence, was significantly associated with reduced abundance of *Bifidobacterium* and its parent taxon Actinobacteria, independent of the individual's MetS status [31]. ApoA5 is known to be produced mainly in the liver but is also expressed at a low level in the intestine, and its function is to modulate chylomicron production [33,34]. Therefore, carriers of the minor C allele of *APOA5* rs651821 have different lipid properties in the gut compared with non-carriers, which may lead to alteration of gut microbiota composition as mentioned above [31], suggesting a role for microbiome pattern in the regulation of ApoA5. More interestingly, Oliva *et al.* recently suggested that *APOA5* genetic and epigenetic variabilities may jointly regulate circulating TG levels [35]; minor allele carriers of *APOA5* SNPs (rs662799, rs3135506, and 724C > G) had significantly higher circulating TG levels (by an average of 57.5%) than non-carriers [35]. At the same time, *APOA5* promoter and exon 3 were hypermethylated, whereas exon 2 was hypomethylated. In particular, exon 3 methylation was positively correlated with circulating TG levels and a lipoprotein profile linked to atherogenic dyslipidemia [35]. Taking these findings together, circulating TG levels were the highest in minor allele carriers of at least one *APOA5* SNP, possibly together with a high methylation percentage in exon 3 ( $\geq 82\%$ ) among the population, which was not experimentally proven in the present study. Since blood TG levels are strongly affected by not only genetic susceptibility but also environmental factors such as diet and lifestyle, this result may enhance our understanding of the effect of *APOA5* on TG levels.

Despite a lack of experimental data supporting the observed association between *APOA5* gene polymorphism and cardiometabolic traits, this study was able to confirm that the minor C allele of *APOA5* rs662799 is significantly associated with cardiometabolic traits and, more specifically, negatively associated with circulating HDL cholesterol and positively associated with TG level in a large Korean population from the KoGES-ASAS and KNHANES. In addition, this genotype may exert its effect via post-transcriptional regulation, which further requires experimental confirmation. Our results should add to our current understanding that *APOA5* might be a useful target for clinical therapeutic intervention.

## ACKNOWLEDGMENT

Bioresources for the study were provided by the National Biobank of Korea and the Centers for Disease Control and Prevention, Republic of Korea (KBP-2016-062).

## CONFLICT OF INTEREST

The authors declare no potential conflicts of interests.

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