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Association of apolipoprotein A5 concentration with serum insulin and triglyceride levels and coronary artery disease in Korean men

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Abstract

Objective—Whereas the relation between apolipoprotein A5 (*APOA5*) gene polymorphisms and triglycerides (TG) levels is well established, the associations between apoA5 concentrations, TG and coronary artery disease (CAD) remain controversial. Therefore, we investigated these relations in the setting of a case–control study involving Korean males.

Methods—ApoA5, TG, insulin, free fatty acid (FFA) and lipoprotein profiles were determined using a cross-sectional design in 777 healthy controls and 367 CAD patients.

Results—Plasma apoA5 concentration was lower in CAD patients than controls (192.7 ± 5.2 vs. 237.2 ± 3.7 ng/ml, $P < 0.001$). Values in the second and top tertiles of apoA5 were associated with a decreased odds ratio (OR) for CAD when compared with values in the bottom tertile; OR for

apoA5 top tertile was 0.33 (95% CI, 0.23–0.47) in the age- and BMI-adjusted model and 0.35 (95% CI, 0.23–0.56) following additional adjustments for smoking, drinking status, blood pressure, TG and HDL-cholesterol. After adjustment for age and BMI, plasma apoA5 concentration was negatively correlated with serum TG ($r = -0.188$, $P < 0.001$) and insulin ($r = -0.185$, $P < 0.001$) in normotriglyceridemic controls (TG < 150 mg/dL, $n = 509$) whereas apoA5 was positively correlated with serum TG in hypertriglyceridemic controls (TG ≥ 150 mg/dL, $n = 268$) ($r = 0.246$, $P < 0.001$) and total CAD patients ($r = 0.177$, $P < 0.01$). Regardless of TG levels and CAD status, apoA5 concentration was positively correlated with HDL-cholesterol and FFA levels.

Conclusions—Our data supports an inverse association between plasma apoA5 concentrations and CAD risk, probably due to the observed negative correlations of apoA5 with TGs and insulin, although these correlations were affected by TG levels.

Keywords

Apolipoprotein A5; Coronary artery disease; Triglycerides; Insulin

1. Introduction

Apolipoprotein A5 (apoA5) found at very low concentrations in the bloodstream is associated with very low density lipoprotein (VLDL), high density lipoprotein (HDL), and chylomicrons [1–3]. Evidence from genetic studies in humans and experimental models support the important role of ApoA5 in modulating serum triglyceride (TG) concentrations [2,3]. ApoA5 transgenic mice displayed significantly reduced serum TG concentrations [3], and adenovirus vector-mediated transfer of murine apoA5 to mice resulted in a dose-dependent reduction of plasma TG [4], whereas apoA5 gene-knockout mice has significantly increased serum TG concentrations as compared with wild-type mice [3,4]. Although the underlying mechanism for the TG-lowering effect of apoA5 has not been well defined, several lines of evidence indicate that apoA5 stimulates lipoprotein lipase (LPL) activity [5–7].

In multiple human studies, common polymorphisms at the *APOA5* locus have been consistently and significantly associated with variation in the serum TG levels [8–15]. Moreover, more rare *APOA5* deficiencies have been associated with hypertriglyceridemia [7,16], supporting the hypotriglyceridemic activity of apoA5. Thus, *APOA5* genetic variants have been shown to have roles as susceptibility factors in metabolic syndrome [12], cardiovascular disease [8–11], and stroke [13–15], probably through its association with hypertriglyceridemia. However, in terms of its relation with TG concentrations, some groups have reported a modest direct relationship of apoA5 levels with serum TGs while others have reported a slight positive correlation or modest negative correlation [17–20]. Likewise, some researchers have reported the absence of difference in baseline apoA5 levels between healthy controls and future CAD patients in a nested case–control study [21]. In view of the current controversy, our objective was to investigate the relation between apoA5 concentrations and established cardiovascular risk markers in a cross-sectional case–control design involving healthy and CAD Korean males.

2. Subjects and methods

2.1. Subjects

This study included 777 control male subjects and 367 consecutive male patients with CAD aged 30–83 years from an ethnically and geographically homogenous Korean population. Considering the recent reports [20–22] of gender-dependent effect on the relationship between serum apoA5 levels and other-associated markers, the present study was performed with gender-matched (male subjects) case–control study design.

The control subjects were recruited from the Health Service Center in the course of a routine check-up visit. The inclusion criteria for the control group were: no history or diagnosis of atherosclerosis, vascular disease, chronic heart failure and arrhythmias, diabetes mellitus, or cancer, and no pathological electrocardiogram patterns. None of the control subjects were taking any medication. Male CAD patients admitted to the Cardiology division, Cardiovascular Center, National Health Insurance Corporation Ilsan Hospital, Goyangsi, Korea were recruited. The inclusion criteria for the CAD patients were: (a) angiographic evidence with 50% occlusion of one or more major coronary arteries or previous myocardial infarction (MI) confirmed according to the World Health Organization (WHO) criteria for symptoms, enzyme elevation, or electrocardiographic changes, (b) absence of nonatherogenic occlusion, such as osteal stenosis and spasm, and (c) no orthopedic limitations and any diagnosis of thyroid or pituitary disease. Exclusion criteria included: (a) abnormal liver or renal function; (b) cancer (diagnosed clinically or by anamnesis); (c) thyroid or pituitary disease; (d) infection determined by medical questionnaire examination and complete blood count; (e) acute or chronic inflammatory disease.

Before participation, the purpose of the study was carefully explained to all participants and their informed consent was obtained. The study protocol was approved by the Institutional Review Board of National Health Insurance Corporation Ilsan Hospital.

2.2. Anthropometric parameters and blood collection

Body weight and height were measured unclothed and without shoes in the morning. Body mass index (BMI) was calculated as body weight in kilograms divided by height in square meters (kg/m^2). Waist circumference was measured with paper tape horizontally at the umbilicus in the standing position after normal expiration. Blood pressure was measured from the left arm of seated patients with an automatic blood pressure monitor (TM-2654, A&D, Tokyo, Japan) after 20 min of rest. The average of three measurements was recorded for each subject.

Venous blood specimens were collected in EDTA-treated or plain tubes after a 12 h 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 were stored at -70°C until analysis.

2.3. Clinical laboratory tests

Fasting serum concentrations of total-cholesterol, TGs, and free fatty acids (FFA) were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd.

Tokyo, Japan). HDL-cholesterol from the supernatant was measured by enzymatic methods. LDL-cholesterol was indirectly estimated in subjects with serum TG concentrations <400 mg/dL using the Friedewald formula. In subjects with serum TG concentrations ≥400 mg/dL, LDL-cholesterol was directly measured by an enzymatic method on a Hitachi 7150 Autoanalyzer. Serum apolipoprotein A-I and B were determined by turbidometry at 340 nm using a specific antiserum (Roche, Switzerland). Fasting glucose was measured by a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, USA) and insulin, by radioimmunoassay using commercial kits from Immuno Nucleo Corporation (Stillwater, MN, USA). Insulin resistance (IR) was calculated with the homeostasis model assessment (HOMA) using the following equation: $\text{HOMA-IR} = \{\text{fasting insulin (}\mu\text{IU/mL)} \times \text{fasting glucose (mmol/L)}\} / 22.5$. Plasma apolipoprotein A5 (apoA5) concentration was measured using an enzyme immunoassay (Human Apolipoprotein AV ELISA Kit, Millipore, MO, USA). The intra- and inter-assay coefficients of variance in apoA5 level were 2.5% and 7.5%, respectively.

2.4. Statistical analysis

Statistical analyses were performed with SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to test the normality of distribution, and the skewed variables (apoA5, triglycerides, insulin, and HOMA-IR) were logarithmically transformed. Results are expressed as mean ± SE and a 2-tailed value of $P < 0.05$ was considered statistically significant.

Differences in clinical and metabolic variables between control subjects and CAD patients were analyzed by Student's *t*-test and a general linear model to adjust for covariates. A χ^2 test was used to test whether there was a difference in cigarette smoking, alcohol consumption, and medication therapy between controls and cases. Plasma apoA5 concentrations were divided into tertiles according to the distribution in the control group. Multiple logistic regression analysis including CAD as a dependent variable and apoA5 level as an independent variable was applied to estimate the independent association of elevated apoA5 with the presence of CAD while simultaneously controlling for confounding factors. Results are presented as odds ratio (ORs) and 95% confidence intervals (CIs) estimated from the logistic regression model initially controlling for age, BMI and subsequently further controlling for blood pressure, cigarette smoking, alcohol consumption, lipid profiles. The lowest apoA5 tertile was used as the reference category. Pearson and partial correlation coefficients were used to examine the relation of apoA5 concentration and lipid variables as well as insulin level. Finally, a multiple stepwise regression analysis was performed to find independent effects with age, BMI, total- and HDL-cholesterol, insulin, and apoA5 as independent variables on serum TG concentration as a dependent variable each in controls and CAD patients.

3. Results

3.1. Characteristics of controls and patients

General characteristics of 367 CAD patients and 777 healthy controls are shown in Table 1. CAD men were older, heavier, had larger waist to hip ratio and a lower prevalence of

current smoking and alcohol drinking than controls. CAD patients were treated with antihypertensive (95%), antidyslipidemic (65%), hypoglycemic (24%) and antiplatelet (98%) drugs, whereas healthy controls did not take any medication. CAD patients showed higher systolic and lower diastolic blood pressure, lower total-, HDL- and LDL-cholesterol, higher TG, and lower apoA1 and apo-B levels than controls before and after adjustment of age, BMI, smoking and drinking status. Plasma apoA5 concentration was lower in CAD patients (192.7 ± 5.2 ng/ml) than control males (237.2 ± 3.7 ng/ml, $P < 0.001$). CAD patients showed higher levels of glucose, insulin and HOMA-IR than controls.

3.2. Association between plasma apoA5 and CAD

Table 2 shows the association between two upper tertiles of apoA5 concentration in comparison to the bottom tertile and CAD. The OR for the presence of CAD after adjustment for age and BMI was 0.33 (95% CI, 0.23–0.47) when the top tertile of the apoA5 distribution was compared to the bottom tertile (Model 1). After additional adjustment for smoking and drinking status, blood pressure, TG and HDL-cholesterol, a significant association between apoA5 tertiles and risk for CAD remained present (OR, 0.35; 95% CI, 0.23–0.56; Model 5).

3.3. Correlation of apoA5 concentration with serum lipid profiles, FFA and insulin

In controls, apoA5 concentration was correlated negatively with age ($r = -0.234$, $P < 0.001$) and positively with BMI ($r = 0.135$, $P < 0.001$) (Table 3). After adjustment for age and BMI, apoA5 was correlated negatively with insulin and HOMA-IR and positively with total-, HDL-, LDL-cholesterol, apoA1 and FFA. In CAD patients, apoA5 concentration was positively correlated with total-, HDL-, LDL-cholesterol, apoA1, apoB and FFA after adjustment for age and BMI. In contrast to the control group, the case group showed a direct correlation between apoA5 and TG levels after adjustment for age and BMI ($r = 0.177$, $P < 0.01$). Because of the recent publications showing the negative relationship of serum concentrations of apoA5 to TG in healthy normolipidemic individuals [2,20,22], we subdivided control subjects into two groups according to fasting serum TG based on the criteria by the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III [23]: those with hypertriglyceridemia (TG ≥ 150 mg/dL, $n = 268$) and those with normotriglyceridemia (TG < 150 mg/dL, $n = 509$).

After adjustment for age and BMI, in normotriglyceridemic control males, apoA5 concentration was correlated positively with total- ($r = 0.195$, $P < 0.001$), HDL- ($r = 0.286$, $P < 0.001$), LDL-cholesterol ($r = 0.110$, $P < 0.05$), apoA1 ($r = 0.287$, $P < 0.001$) and FFA ($r = 0.293$, $P < 0.001$) concentrations and negatively with serum TG ($r = -0.188$, $P < 0.001$) (Fig. 1), insulin ($r = -0.185$, $P < 0.001$) and HOMA-IR index ($r = -0.146$, $P < 0.01$). In hypertriglyceridemic control males, apoA5 level was positively correlated with total cholesterol ($r = 0.137$, $P < 0.001$), TG ($r = 0.246$, $P < 0.001$) (Fig. 1), apoA1 ($r = 0.206$, $P < 0.01$) and FFA ($r = 0.174$, $P < 0.01$). After adjustment for age and BMI, similar to normotriglyceridemic controls, normotriglyceridemic CAD males ($n = 201$) showed the positive correlation of apoA5 concentration with total- ($r = 0.252$, $P < 0.001$), HDL- ($r = 0.299$, $P < 0.001$), and LDL-cholesterol ($r = 0.182$, $P < 0.05$), apoA1 ($r = 0.260$, $P < 0.001$), and FFA concentrations ($r = 0.317$, $P < 0.001$) and a negative but not significant relation of

apoA5 to TG and insulin. In hypertriglyceridemic CAD males ($n = 166$), apoA5 concentration was positively correlated with total- ($r = 0.345$, $P < 0.001$), HDL- ($r = 0.200$, $P < 0.05$), and LDL-cholesterol ($r = 0.223$, $P < 0.01$), TG ($r = 0.335$, $P < 0.001$) (Fig. 1), apoA1 ($r = 0.281$, $P < 0.001$), apoB ($r = 0.265$, $P < 0.01$) and FFA concentrations ($r = 0.288$, $P < 0.001$) (Table 3).

Because of the complexity of the regulation of serum TG levels, stepwise multiple linear regression analysis was performed to find independent variables defining TG levels. A multiple regression analysis showed that total- (standardized $\beta = 0.365$, $P < 0.001$), HDL-cholesterol (standardized $\beta = -0.245$, $P < 0.001$), insulin (standardized $\beta = 0.194$, $P < 0.001$), and apoA5 (standardized $\beta = -0.135$, $P = 0.001$) were independent variables determining TG levels in normotriglyceridemic controls (adjusted $r^2 = 0.244$, $P < 0.001$), HDL-cholesterol (standardized $\beta = -0.337$, $P < 0.001$), apoA5 (standardized $\beta = 0.236$, $P < 0.001$), total-cholesterol (standardized $\beta = 0.224$, $P < 0.001$), and insulin (standardized $\beta = 0.143$, $P = 0.011$) in hyper-triglyceridemic controls (adjusted $r^2 = 0.210$, $P < 0.001$) and total- (standardized $\beta = 0.513$, $P < 0.001$), HDL-cholesterol (standardized $\beta = -0.421$, $P < 0.001$), insulin (standardized $\beta = 0.133$, $P = 0.001$), age (standardized $\beta = -0.102$, $P = 0.012$) and apoA5 (standardized $\beta = 0.101$, $P = 0.020$) in CAD patients (adjusted $r^2 = 0.425$, $P < 0.001$). Standardized β values indicate the regression coefficients on log-transformed TG by 1-unit change in lipid profiles as well as age and insulin level.

4. Discussion

In this case-control study, apoA5 concentrations were lower in patients with CAD compared with healthy controls and elevated apoA5 concentrations were associated with reduced risk of CAD, which persisted after adjustment for other risk factors, including age, BMI, smoking and drinking status, blood pressure, TG and HDL-cholesterol. The reduced apoA5 concentration observed in the case group might be partly ascribed to the hyperinsulinemia and insulin resistance occurring in these patients. There is some evidence supporting that insulin resistance down-regulates apoA5 expression and that average apoA5 concentration is lower among type 2 diabetic patients [20,24,25]. Moreover, plasma apoA5 levels have been found to be decreased following insulin infusion in men [21]. In the current study, apoA5 concentrations were negatively related to insulin concentration and HOMA-IR in healthy controls, after adjustment for age and BMI. However, the present findings are in contrast with a previous study in a White population [21], which reported an association of reduced apoA5 concentration with decreased LPL level, but not with increased risk of future CAD. This discrepancy could be attributed to differences in study design (*i.e.* nested case-control study vs. cross-sectional case-control study). Other difference between the present study and that of Vassen et al. [21] relates to the measurement of apoA5 concentration. Whereas the previous study determined apoA5 level in nonfasting serum that was obtained at a nonuniform time of the day [21], the present study analyzed apoA5 concentration in 12-h fasting serum that was obtained at 8:00 to 9:00 AM to prevent diurnal variation and differences in the time span since the last meal. This approach was taken to avoid the potential confounding effect of the postprandial state known to increase both plasma apoA5 and TG levels [26]. Interestingly, Vaseen et al. found the association between the APOA5

–1131T > C polymorphism, which has a functional role in the expression of the *APOA5* gene [23], and increased risk of CAD.

ApoA5 is known to play an important role in the regulation of circulating TG levels through its direct interaction with LPL [5–7,27]. ApoA5 appears to facilitate the interaction between circulating lipoprotein particles and proteoglycans at the vascular wall with the subsequent activation of proteoglycan-bound LPL. This process results in increased LPL-mediated plasma TG hydrolysis. In addition, apoA5 was found to stabilize dimeric LPL or, less likely, modulate the shape of TG-rich lipoproteins, making them a better substrate for LPL. The negative correlation between apoA5 and TG levels was also observed in normolipidemic healthy controls of the current study. Our result agrees with three previous studies by O'Brien et al. [2], Ishihara et al. [20] and Zhao et al. [22] in healthy subjects and support that TG concentration is affected by serum apoA5 in humans. However, multiple factors might contribute to the increased TG levels in normolipidemic healthy controls since multiple regression analysis showed that total- and HDL-cholesterol, insulin, and apoA5 were independent variables determining TG levels. Furthermore, in contrast to normolipemic controls, there was a positive correlation between apoA5 and TG levels in hyperlipidemic controls with serum TGs of more than 150 mg/dl. Differences in the correlation between human serum apoA5 and TG according to fasting TG level may support recent publications suggesting that the correlation in humans may not be nearly straightforward as in mice. These findings suggested that fasting TG levels might be an important impact factor to explain the relationship with apoA5.

The positive correlation between apoA5 and TG levels in hypertriglyceridemic controls is in agreement with previous findings of Schaap et al. [17] in patients with markedly elevated TGs. The present observation of direct correlation between apoA5 and fasting FFA concentrations in both normotriglyceridemic and hypertriglyceridemic controls may indicate that the presumed apoA5-stimulated triglyceride hydrolase activity of LPL does not normalize circulating triglyceride levels in hypertriglyceridemic individuals. Furthermore, apoA5 concentration positively related to TG in CAD patients even with higher TG and lower apoA5 concentrations compared to controls. This result is in line with the finding of Ishihara et al. [20] who reported lower apoA5 concentration in patients with type 2 diabetes than in normal healthy controls and a positive correlation of apoA5 with TG in diabetic patients. In addition, there are several evidences that apoC3, a LPL inhibitor, rather than apoA5 is the main driver of serum TG levels in patients with severe hypertiglyceridemia or type 2 diabetes [17,18,28,29].

APOA5 gene appears to be predominantly expressed in the liver, where its gene product is secreted into blood circulation residing on HDL and VLDL particles [1]. Although apoA5 mainly seems to affect VLDL metabolism [6], the circulating apoA5 is mainly present on HDL as a low abundant plasma apolipoprotein (approximately 0.1%, w/w of apoA1) [1,30]. In this study, apoA5 concentration was positively correlated to HDL-cholesterol and apoA1 levels, similar to previous findings of Zhao et al. [22] and Ishihara et al. [20]. The limitation of this study, as always in case-control studies in which exposure and outcome are collected at one point in time, relates to the difficulty to assess the time sequence of the described associations. Moreover, because gender is an important risk factor for CAD, in the present

study we specifically focused on a representative group of middle-aged Korean men, so that results cannot be generalized to women or to other ethnic, age or geographical groups. Despite this limitation, our results clearly show the association of elevated concentration of apoA5 with reduced CAD risk. Furthermore, serum apoA5 concentration was negatively correlated with TG and insulin, but positively correlated with HDL-cholesterol and FFA after adjustment for age and BMI in normotriglyceridemic controls with serum TGs of less than 150 mg/dL. Moreover, the negative correlations of apoA5 with TG and insulin were affected by fasting serum TG levels partly due to the insufficient LPL-stimulatory activity of apoA5 in hypertriglyceridemia. The results from this study highlight the need for additional human studies to clarify the functional role of apoA5.

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References

1. van der Vliet HN, Sammels MG, Leegwater AC, et al. Apolipoprotein A-V: a novel apolipoprotein associated with an early phase of liver regeneration. *J Biol Chem.* 2001; 276:44512–20. [PubMed: 11577099]
2. O'Brien PJ, Alborn WE, Sloan JH, et al. The novel apolipoprotein A5 is present in human serum, is associated with VLDL, HDL, and chylomicrons, and circulates at very low concentrations compared with other apolipoproteins. *Clin Chem.* 2005; 51:351–9. [PubMed: 15528295]
3. Pennacchio LA, Olivier M, Hubacek JA, et al. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science.* 2001; 294:169–73. [PubMed: 11588264]
4. van der Vliet HN, Schaap FG, Levels JH, et al. Adenoviral overexpression of apolipoprotein A-V reduces serum levels of triglycerides and cholesterol in mice. *Biochem Biophys Res Commun.* 2002; 295:1156–9. [PubMed: 12135615]
5. Merkel M, Loeffler B, Kluger M, et al. Apolipoprotein AV accelerates plasma hydrolysis of triglyceride-rich lipoproteins by interaction with proteoglycan-bound lipoprotein lipase. *J Biol Chem.* 2005; 280:21553–60. [PubMed: 15774484]
6. Schaap FG, Rensen PC, Voshol PJ, et al. ApoAV reduces plasma triglycerides by inhibiting very low density lipoprotein-triglyceride (VLDL-TG) production and stimulating lipoprotein lipase-mediated VLDL-TG hydrolysis. *J Biol Chem.* 2004; 279:27941–7. [PubMed: 15090553]
7. Grosskopf I, Baroukh N, Lee SJ, et al. Apolipoprotein A-V deficiency results in marked hypertriglyceridemia attributable to decreased lipolysis of triglyceride-rich lipoproteins and removal of their remnants. *Arterioscler Thromb Vasc Biol.* 2005; 25:2573–9. [PubMed: 16166565]
8. Szalai C, Keszei M, Duba J, et al. Polymorphism in the promoter region of the apolipoprotein A5 gene is associated with an increased susceptibility for coronary artery disease. *Atherosclerosis.* 2004; 173:109–14. [PubMed: 15177130]
9. Hsu LA, Ko YL, Chang CJ, et al. Genetic variations of apolipoprotein A5 gene is associated with the risk of coronary artery disease among Chinese in Taiwan. *Atherosclerosis.* 2006; 185:143–9. [PubMed: 16054149]
10. Talmud PJ, Martin S, Taskinen MR, et al. APOA5 gene variants, lipoprotein particle distribution, and progression of coronary heart disease: results from the LOCAT study. *J Lipid Res.* 2004; 45:750–6. [PubMed: 14729863]

11. Lai CQ, Corella D, Demissie S, et al. Dietary intake of n-6 fatty acids modulates effect of apolipoprotein A5 gene on plasma fasting triglycerides, remnant lipoprotein concentrations, and lipoprotein particle size: the Framingham Heart Study. *Circulation*. 2006; 113:2062–70. [PubMed: 16636175]
12. Yamada Y, Kato K, Hibino T, et al. Prediction of genetic risk for metabolic syndrome. *Atherosclerosis*. 2007; 191(2):298–304. [PubMed: 16806226]
13. Maász A, Kisfali P, Szolnoki Z, Hadarits F, Melegh B. Apolipoprotein A5 gene C56G variant confers risk for the development of large-vessel associated ischemic stroke. *J Neurol*. 2008; 255(5):649–54. [PubMed: 18274806]
14. Havasi V, Szolnoki Z, Talián G, et al. Apolipoprotein A5 gene promoter region T-1131C polymorphism associates with elevated circulating triglyceride levels and confers susceptibility for development of ischemic stroke. *J Mol Neurosci*. 2006; 29(2):177–83. [PubMed: 16954607]
15. Maasz A, Kisfali P, Jaromi L, et al. Apolipoprotein A5 gene IVS3 + G476A allelic variant confers susceptibility for development of ischemic stroke. *Circ J*. 2008; 72(7):1065–70. [PubMed: 18577813]
16. Priore Oliva C, Pisciotta L, Li Volti G, et al. Inherited apolipoprotein AV deficiency in severe hypertriglyceridemia. *Arterioscler Thromb Vasc Biol*. 2005; 25:411–7. [PubMed: 15591215]
17. Schaap FG, Nierman MC, Berbée JF, et al. Evidence for a complex relationship between apoA-V and apoC-III in patients with severe hypertriglyceridemia. *J Lipid Res*. 2006; 47:2333–9. [PubMed: 16861622]
18. Dallinga-Thie GM, van Tol A, Hattori H, et al. Plasma apolipoprotein A5 and triglycerides in type 2 diabetes. *Diabetologia*. 2006; 49:1505–11. [PubMed: 16752169]
19. Talmud PJ, Cooper JA, Hattori H, et al. The apolipoprotein A-V genotype and plasma apolipoprotein A-V and triglyceride levels: prospective risk of type 2 diabetes. Results from the Northwick Park Heart Study II. *Diabetologia*. 2006; 49:2337–40. [PubMed: 16917759]
20. Ishihara M, Kujiraoka T, Iwasaki T, et al. A sandwich enzyme-linked immunosorbent assay for human plasma apolipoprotein A-V concentration. *J Lipid Res*. 2005; 46:2015–22. [PubMed: 15961791]
21. Vassen SF, Schaap FG, Kuivenhoven JA, et al. Apolipoprotein A-V, triglycerides and risk of coronary artery disease: the prospective Epic-Norfolk Population Study. *J Lipid Res*. 2006; 47:2064–70. [PubMed: 16769999]
22. Zhao SP, Hu S, Li J, et al. Association of human serum apolipoprotein A5 with lipid profiles affected by gender. *Clin Chim Acta*. 2007; 376:68–71. [PubMed: 16962087]
23. The Expert Panel on Detection Evaluation Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*. 2001; 285:2486–97. [PubMed: 11368702]
24. Nowak M, Helleboid-Chapman A, Jakel H, et al. Insulin-mediated down-regulation of apolipoprotein A5 gene expression through the phosphatidylinositol 3-kinase pathway: role of upstream stimulatory factor. *Mol Cell Biol*. 2005; 25:1537–48. [PubMed: 15684402]
25. Hirano T, Hayashi T, Adachi M, et al. Marked decrease of apolipoprotein AV in both diabetic and nondiabetic patients with end-stage renal disease. *Metabolism*. 2007; 56:462–3. [PubMed: 17379001]
26. Pruneta-Deloche V, Ponsin G, Groisne L, et al. Postprandial increase of plasma apoAV concentrations in Type 2 diabetic patients. *Atherosclerosis*. 2005; 181:403–5. [PubMed: 16039297]
27. Pennacchio LA, Rubin EM. Apolipoprotein A5, a newly identified gene that affects plasma triglyceride levels in humans and mice. *Arterioscler Thromb Vasc Biol*. 2003; 23:529–34. [PubMed: 12615678]
28. Henneman P, Schaap FG, Havekes LM, et al. Plasma apoAV levels are markedly elevated in severe hyper-triglyceridemia and positively correlated with the APOA5 S19W polymorphism. *Atherosclerosis*. 2007; 193:129–34. [PubMed: 16777114]

29. Alborn WE, Prince MJ, Konrad RJ. Relationship of apolipoprotein A5 and apolipoprotein C3 levels to serum triglycerides in patients with type 2 diabetes. *Clin Chim Acta*. 2007; 378:154–8. [PubMed: 17188674]
30. van Dijk KW, Rensen PC, Voshol PJ, et al. The role and mode of action of apolipoproteins CIII and AV: synergistic actors in triglyceride metabolism? *Curr Opin Lipidol*. 2004; 15:239–46. [PubMed: 15166778]

Fig. 1.

Correlation between apoA5 and triglycerides in healthy controls and CAD patients. r_0 : unadjusted, r_1 : adjusted for age and BMI.

Table 1

General characteristics of controls and CAD patients.

	Controls (<i>n</i> = 777)	CAD patients (<i>n</i> = 367)	<i>P</i> ^a
Age (years)	51.4 ± 0.3	56.7 ± 0.5	<0.001
Body mass index (kg/m ²)	24.5 ± 0.1	25.1 ± 0.2	0.001
Waist to hip ratio	0.90 ± 0.0	0.93 ± 0.0	<0.001
Current smoker, <i>n</i> (%)	253 (32.6)	89 (24.5)	<0.001
Current drinker, <i>n</i> (%)	602 (77.9)	202 (55.3)	<0.001
Antihypertensive therapy, <i>n</i> (%)	–	348 (94.8)	–
Antidyslipidemic therapy, <i>n</i> (%)	–	235 (64.5)	–
Antiplatelet therapy, <i>n</i> (%)	–	359 (97.8)	–
Hypoglycemic therapy (%)	–	88 (24.0)	–
Systolic BP (mmHg)	125.1 ± 0.6	130.7 ± 0.9	<0.001
Diastolic BP (mmHg)	79.2 ± 0.4	76.4 ± 0.6	<0.001
Total cholesterol (mg/dL)	191.8 ± 1.2	163.8 ± 2.3	<0.001
HDL-cholesterol (mg/dL)	51.3 ± 0.5	45.0 ± 0.6	<0.001
LDL-cholesterol (mg/dL)	113.0 ± 1.1	86.8 ± 1.9	<0.001
Triglyceride (mg/dL) ^b	139.8 ± 2.9	168.6 ± 6.5	<0.001
ApolipoproteinA1 (mg/dL)	143.1 ± 1.1	129.6 ± 1.3	<0.001
ApolipoproteinB (mg/dL)	85.1 ± 1.0	69.4 ± 1.2	<0.001
ApolipoproteinA5 (ng/mL) ^b	237.2 ± 3.7	192.7 ± 5.2	<0.001
Free fatty acid (uEq/L)	489.5 ± 8.5	509.3 ± 12.5	0.187
Glucose (mg/dL)	92.2 ± 0.5	114.6 ± 2.2	<0.001
Insulin ((μIU/ml)) ^b	8.1 ± 0.1	10.4 ± 0.4	<0.001
HOMA-IR ^{b, c}	1.9 ± 0.0	3.0 ± 0.2	<0.001

Data are reported as mean ± S.E. or *n* value (%).^aTested by Student's *t*-test or χ^2 test (two-sided).^bTested by logarithmic transformation.^cHOMA IR = {fasting insulin (μU/mL) × fasting glucose (mg/dL)}/22.5.

Table 2

Odds ratios (OR) of CAD associated with apoA5 concentration.

ApoA5 (ng/mL)	Tertile 1 (<181.0)	Tertile 2 (181.0/< 265.0)	Tertile 3 (265.0)	<i>P</i>
Model 1	1.0	0.58 (0.43–0.78)	0.33 (0.23–0.47)	<0.001
Model 2	1.0	0.59 (0.44–0.81)	0.35 (0.24–0.50)	<0.001
Model 3	1.0	0.56 (0.40–0.79)	0.33 (0.22–0.48)	<0.001
Model 4	1.0	0.56 (0.40–0.78)	0.31 (0.21–0.46)	<0.001
Model 5	1.0	0.59 (0.42–0.83)	0.35 (0.23–0.56)	<0.001

Data are ORs (95% CI) for association of CAD with plasma apoA5 levels, tested by multivariable logistic regression analysis after various adjustments. Tertiles are based on data from control subjects. *P* indicates *P* value for linearity between ApoA5 tertiles and CAD risk. ApoA5 levels and triglyceride levels were log-transformed before analysis. Model 1 adjusted for age and BMI. Model 2 adjusted for age, BMI, smoking and drinking status. Model 3 adjusted for age, BMI, smoking, drinking status, systolic BP and diastolic BP. Model 4 adjusted for age, BMI, smoking, drinking status, systolic BP, diastolic BP and TG. Model 5 adjusted for age, BMI, smoking, drinking status, systolic BP, diastolic BP, TG, and HDL-C.

Table 3

Correlation coefficients of log-apoA5 levels with lipid profiles and insulin level.

	Log-apoA5				Log-apoA5				Cases with TG <150mg/dL				Cases with TG ≥150mg/dL			
	All controls		Controls with TG <150mg/dL		Controls with TG ≥150mg/dL		All cases		Cases with TG <150mg/dL		Cases with TG ≥150mg/dL		Cases with TG <150mg/dL		Cases with TG ≥150mg/dL	
	r	r ^a	r	r ^a	r	r ^a	r	r ^a	r	r ^a	r	r ^a	r	r ^a	r	r ^a
Age	-0.234***		-0.244***		-0.201**		-0.088		-0.072		-0.066		-0.072		-0.066	
BMI	0.135***		0.107*		0.174**		0.065		-0.005		0.106		-0.005		0.106	
Total-C	0.174***	0.169***	0.183***	0.195***	0.137*	0.132*	0.321***	0.312***	0.249***	0.252***	0.360***	0.345***	0.249***	0.252***	0.360***	0.345***
HDL-C	0.181***	0.219***	0.255***	0.286***	0.123*	0.124*	0.219***	0.235***	0.290***	0.299***	0.205**	0.200*	0.290***	0.299***	0.205**	0.200*
LDL-C	0.079*	0.079*	0.107*	0.110*	0.021	0.017	0.206***	0.203***	0.177*	0.182*	0.231**	0.223**	0.177*	0.182*	0.231**	0.223**
TG ^b	0.003	-0.042	-0.169***	-0.188***	0.254***	0.246***	0.188***	0.177**	-0.114	-0.103	0.349***	0.335***	-0.114	-0.103	0.349***	0.335***
ApoA1	0.223***	0.261***	0.248***	0.287***	0.175*	0.206**	0.283***	0.281***	0.261***	0.260***	0.288***	0.281***	0.261***	0.260***	0.288***	0.281***
ApoB	0.069	0.038	0.019	-0.004	0.089	0.077	0.221***	0.211***	0.123	0.127	0.276	0.265**	0.123	0.127	0.276	0.265**
Free fatty acid	0.229***	0.259***	0.254***	0.293***	0.168**	0.174**	0.304***	0.317***	0.300***	0.317***	0.284***	0.288***	0.300***	0.317***	0.284***	0.288***
Insulin ^b	-0.048	-0.109**	-0.125**	-0.185***	0.074	0.030	0.020	-0.001	-0.086	-0.093	0.091	0.070	-0.086	-0.093	0.091	0.070
HOMA-IR ^b	-0.016	-0.074*	-0.090*	-0.146**	0.103	0.064	0.112*	0.098	0.020	0.021	0.165*	0.152	0.020	0.021	0.165*	0.152

^a Controlled for age and BMI.^b Tested by logarithmic transformation.* $P < 0.05$.** $P < 0.01$.*** $P < 0.001$.