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The apolipoprotein CIII T2854G variants are associated with postprandial triacylglycerol concentrations in normolipidemic Korean men

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Abstract This study investigated associations between the apolipoprotein (apo) CIII polymorphism and triacylglycerol (TAG) concentrations in fasting and postprandial plasma. Polymerase chain reaction followed by a restriction fragment length genotyping was conducted to assess the allele frequency of the apo CIII T2854G variants in healthy and normolipidemic Korean men ($n=262$). Waist circumference, body mass index (kilograms per meter squared), fasting plasma concentrations of TAG, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), glucose, and insulin were compared across the genotypes. Compared to TT homozygotes and TG heterozygotes, GG homozygotes had 22% higher fasting TAG concentrations, respectively ($p<0.05$). A subgroup of 60 subjects (TT homozygotes = 20, TG heterozygotes = 22, GG homozygotes = 18) were further invited to participate in a high-fat meal test to assess postprandial TAG concentrations. During the high-fat meal test, the GG homozygotes had 21% higher TAG area under the curve (AUC) than the TT homozygotes ($p<0.05$) and 22% higher TAG AUC than the TG heterozygotes ($p<0.05$). In conclusion, this is the first study to show that the apo CIII T2854G variants are associated with elevated postprandial TAG concentrations in the study population of Korean men.

Keywords PCR-RFLP · apo CIII gene polymorphism · Postprandial triacylglycerol

Introduction

Plasma apolipoprotein CIII (apo CIII), which consists of 79 amino acids, is synthesized predominantly in the liver and, to a lesser extent, in the intestine (Breslow 1995). Apo CIII is a major component of very-low-density lipoproteins (VLDL) and chylomicrons and a minor component of high-density lipoproteins (Breslow 1995).

While the functional role of the apo CIII is still unclear, it appears that apo CIII circulates in plasma with triglyceride-rich lipoproteins (TRL) and may interfere with lipolysis. For example, apo CIII inhibits lipoprotein lipase, a key enzyme hydrolyzing the triacylglycerol (TAG) portion of TRL, resulting in a delayed removal of TRL in vitro (Wang et al. 1985) and in vivo (Ginsberg et al. 1986). Apo CIII also interferes with apo E-mediated remnants removal by displacement of apo E from VLDL particles in vivo (Alto-Setälä et al. 1996 and 1992).

The apo AI-CIII-AIV gene cluster, which is located in the long arm of chromosome 11 (Karathanasis 1985), has been known to have more than 20 polymorphic sites (Groenendijk et al. 2001). Among them, the SstI gene polymorphism located in the 3' untranslated region of the apo CIII gene has been consistently implicated in elevated plasma TAG concentrations in several populations such as Caucasians (Paul-Hayase et al. 1992; Stocks et al. 1987; Dammerman et al. 1993) and non-Caucasians (Zeng et al. 1995; Hong et al. 1997; Henderson et al. 1987). In addition, the rare allele of the T2854G polymorphism located in the intergenic region of the apo CIII gene was recently reported to be associated with elevated fasting (Minihane et al. 2002) or postprandial TAG concentrations (Waterworth et al. 1999) in Caucasians. However, little information on the influence of this gene polymorphism on plasma lipids, especially in a postprandial state, is currently available in non-Caucasians such as Koreans. Therefore, the purpose of the current study was to investigate possible

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associations between the apo CIII T2854G variants and fasting lipids and postprandial TAG concentrations in healthy and normolipidemic Korean men.

Materials and methods

Subjects

Apparently healthy and normolipidemic Korean men ($n = 262$ ages 26.6–33.0 years) who had no current major diseases and no family history of coronary heart diseases, were recruited to participate in the study. Participants signed a written consent approved by the Institutional Human Subject Review Board. None was taking medications or hormones affecting lipid and lipoprotein metabolism. No smoking and alcohol consumption were allowed for 24 h prior to the experiment. Weight was assessed with a balance scale and height with a stadiometer; body mass index (BMI) was calculated (kilograms per meter squared). Waist circumference was measured at the umbilicus level. Following an overnight fast, venous blood samples from an antecubital vein were collected into potassium EDTA tubes for genomic DNA extraction and fasting lipid measurements.

Genotyping

Genomic DNA was extracted from plasma buffy coats using QiaAmp DNA Blood Mini Kit (Qiagen, CA, USA). The extracted DNA was stored at -80°C until analysis. The T2854G allele variant of the apo CIII gene was determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as previously described (Waterworth et al. 1999), with modifications.

In brief, polymerase chain reaction was performed in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA) with a 20- μl reaction volume containing 30–50 ng of genomic DNA, 20 pmol of each primer, and 1 \times Taq PCR master mix (Qiagen, Valencia, CA, USA). The Taq PCR master mix provides a final concentration of 2.5 U of Taq DNA polymerase, PCR buffer (1.5 mM MgCl_2), and 200 μM each dNTPs. PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s, and extension at 72°C for 30 s, and final extension at 72°C for 7 min. The primer sequences were as follows: forward 5'-CAA CAG GAG CTG TCC TTC AGT TCT GCC -3' and reverse 5'-GGT CAG TCC AGA GGT CAG AGT CAG GAG GAG-3'.

The PCR products were digested with the restriction enzyme Alw26I (MBI Fermentas Inc, Hanover, MD, USA), separated by electrophoresis using low melting temperature 2.5% agarose gel (ICN Biomedicals Inc, Aurora, OH, USA) and visualized by ethidium bromide staining (Sigma, St. Louis, MO, USA). The rare allele was detected by the presence of a cutting site. Genotyping was successfully completed for all the 262 subjects.

High-fat meal test

A subgroup of 60 subjects (TT homozygotes = 20, TG heterozygotes = 22, and GG homozygotes = 18) was further invited to participate in postprandial TAG response to a high-fat meal. No exercise was permitted 48 h before the test, and no alcohol or smoking was allowed for 24 h before the test. Following an overnight fast, subjects arrived at the Clinical Laboratory Unit. An indwelling cannula was inserted into the antecubital vein of the forearm and a fasting sample was taken (0 h). After consumption of the standard fat meal consisting of 22 g dairy milk protein, 56 g carbohydrates, 66 g dairy milk fat (of which 42 g was saturated), and 417 mg cholesterol, venous blood samples were collected at 2, 3, 4, and 6 h to assess postprandial TAG concentrations.

Postprandial TAG during the high-fat meal test was expressed as area under the curve (AUC) calculated by the trapezoidal rule.

Fasting lipid, glucose, and insulin measurements

All blood samples were collected into potassium EDTA tubes. Samples were separated by centrifugation at 4,600 g for 10 min and the plasma was removed and stored at -80°C until analysis. Total cholesterol and TAG concentrations were measured by enzymatic colorimetry methods with commercial kits (Boehringer Mannheim, Mannheim, Germany). High-density lipoprotein cholesterol (HDL-C) concentration was determined by measuring cholesterol in the supernatant after precipitation of apo B-containing lipoproteins with magnesium chloride and dextran sulfate (Sigma, St. Louis, USA). Low-density lipoprotein cholesterol (LDL-C) concentration was calculated using the Friedewald formula (1972). Fasting glucose was determined by an enzymatic method (Beckman Diagnostics, Fullerton, CA, USA), and fasting insulin concentration was determined using the IMx Insulin Assay (Abbott Labs, Abbott Park, IL, USA).

Statistical analyses

The statistical program used for the analyses was SPSS-PC version 10.1. The distributions of the dependent variables were checked for normality prior to the analyses by plotting standardized residuals for each variable from regression models using age as an independent variable. Because TAG was not normally distributed, a \log_{10} transformation was performed for statistical analyses.

Results were expressed as mean \pm SD. Allelic frequencies were estimated by the genotype frequencies. Univariate analysis of variance followed by Bonferroni test was used to compare measured fasting lipids among the genotypes. The postprandial response was compared among the genotypes by univariate analysis of variance followed by Bonferroni test (1) for each time point TAG and (2) for AUC TAG. Statistical significance was tested at $p = 0.05$.

Results

Figure 1 illustrates the examples of the apo CIII T2854G variants. Table 1 represents the allele frequencies of the T2854G variants. The frequency of the rare allele G was 0.37, which was comparable with those of Caucasians (Minihane et al. 2002; Waterworth et al. 1999).

Table 2 represents physical and laboratory profiles of the participants according to the genotypes. There were no significant differences in average age, BMI, and waist circumference among the genotypes. With respect to fasting plasma, however, the finding that the GG homozygotes had 22% higher TAG concentrations than TT homozygotes ($p < 0.05$) and the TG heterozygotes ($p < 0.05$) was similar to those of previous studies. In addition, the further analyses classified by BMI (> 25 or < 25) showed that compared to either the TT homozygotes or the TG heterozygotes, the GG homozygotes had still significantly elevated fasting TAG concentrations independent of the dichotomy (data not shown).

Table 3 represents the physical and laboratory profiles of the subgroup who participated in the high-fat meal test. With respect to the postprandial TAG concentrations, it was of particularly interest to find that the GG

Fig. 1 Examples of the apo CIII T2854G variants. Lanes 1, 18, 33: 25-bp size markers. Lanes 7, 14, 21, 23, 32: TT homozygotes. Lanes 2, 4, 5, 8–13, 15, 17, 20, 22, 24, 25, 27–29, 31: TG heterozygotes. Lanes 3, 6, 16, 19, 26, 30: GG homozygotes

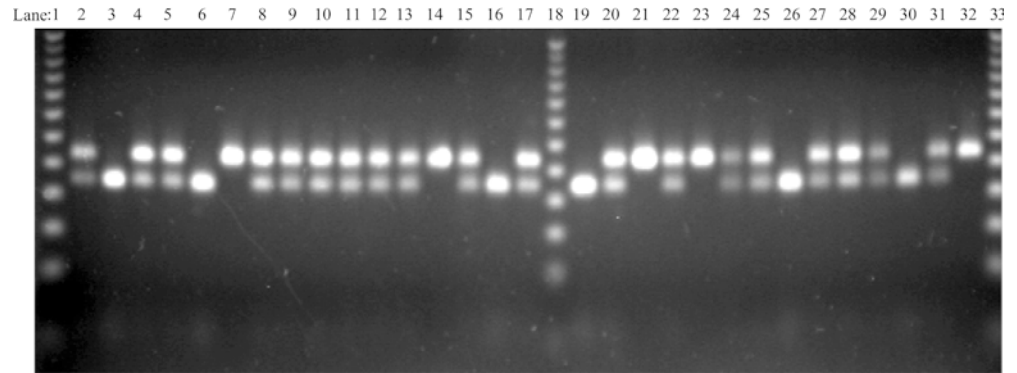


Table 1 The genotype and allele frequencies of the apolipoprotein (apo) CIII T2854G variants

Genotype/ allele	Current study	Minihae et al. 2002	Waterworth et al. 1999
TT	99 (0.38)	55 (0.41)	284 (0.40)
TG	133 (0.51)	62 (0.46)	348 (0.49)
GG	30 (0.11)	18 (0.13)	85 (0.11)
T	166 (0.63)	86 (0.64)	458 (0.64)
G	96 (0.37)	49 (0.36)	259 (0.36)
Total	262	135	717

homozygotes had 21% higher TAG AUC than the TT homozygotes ($p < 0.05$) and 22% higher TAG AUC than the TG heterozygotes ($p < 0.05$) during the test. The plot of the test according to the genotypes is shown in Fig. 2. In addition, there was a tendency for the GG homozygotes to have higher TG and waist circumference than the TT homozygotes or the TG heterozygotes, but neither TG nor waist circumference was significantly different across the genotypes, as shown in Table 3.

Discussion

While the rare alleles of XmnI and SstI polymorphic sites within the apo AI-CIII-AIV complex were previ-

ously associated with increased TAG concentrations in Korean hypertriglyceridemic subjects (Hong et al. 1997), this is the first study to report that the GG homozygotes of the apo CIII T2854G gene polymorphism may be implicated in elevated postprandial TAG concentrations during a high-fat meal test.

The finding—that compared to the TT homozygotes and the TG heterozygotes, the GG homozygotes had significantly higher TAG AUC during the high-fat meal test—suggests that the G allele may have an impact on TAG metabolism via an impaired lipolysis and thereby TAG concentrations in circulating fasting plasma. Therefore, it is speculated that the polymorphic site itself, due to its intergenic location, may be functionally important in postprandial lipolysis (Minihane et al. 2002; Waterworth et al. 1999). Second, it is possible that the polymorphic impact modulates plasma TAG concentrations by linkage disequilibrium with other functional sequences in the apo AI-CIII-AIV gene cluster (Waterworth et al. 1999). Third, there was a tendency for the GG homozygotes to have a relatively larger waist circumference than the TT homozygotes or the TG heterozygotes, suggesting a possible interaction between the rare allele and central obesity in TAG metabolism and thereby its concentrations in circulating plasma. However, this possible interaction between this polymorphism and abdominal obesity is yet to be established in a large sample.

Table 2 Comparisons of anthropometric and laboratory measures across the genotypes, adjusted for age and body mass index (BMI) ($n = 262$). TAG triacylglycerol, Waist waist circumference, AUC area under the curve, CI confidence interval. HDLC high-density lipoprotein cholesterol, LDLC low-density lipoprotein cholesterol

Variables	Genotype			95% CI	<i>p</i> value
	TT	TG	GG		
Age, year	29.7 ± 1.5	30.2 ± 1.4	30.0 ± 1.2	29.8–30.2	0.071
Waist, cm	82.8 ± 15.1	79.9 ± 16.1	83.5 ± 12.7	79.2–83.0	0.143
BMI, kg/m ²	25.5 ± 7.2	24.0 ± 7.3	26.3 ± 6.9	23.7–25.5	0.279
*TAG, mM	1.18 ± 0.40	1.19 ± 0.46	1.52 ± 0.64	1.16–1.28	0.001
TC, mM	4.00 ± 0.80	3.92 ± 0.71	4.15 ± 0.91	3.87–4.06	0.452
HDLC, mM	1.18 ± 0.26	1.18 ± 0.26	1.07 ± 0.25	1.14–1.20	0.127
LDLC, mM	2.52 ± 0.73	2.56 ± 0.97	2.69 ± 0.80	2.45–2.66	0.559
Glucose, mM	5.06 ± 0.37	5.13 ± 0.40	5.08 ± 0.36	5.01–5.16	0.664
Insulin, pM	107.48 ± 61.70	103.31 ± 49.59	128.06 ± 43.42	102.49–123.59	0.232

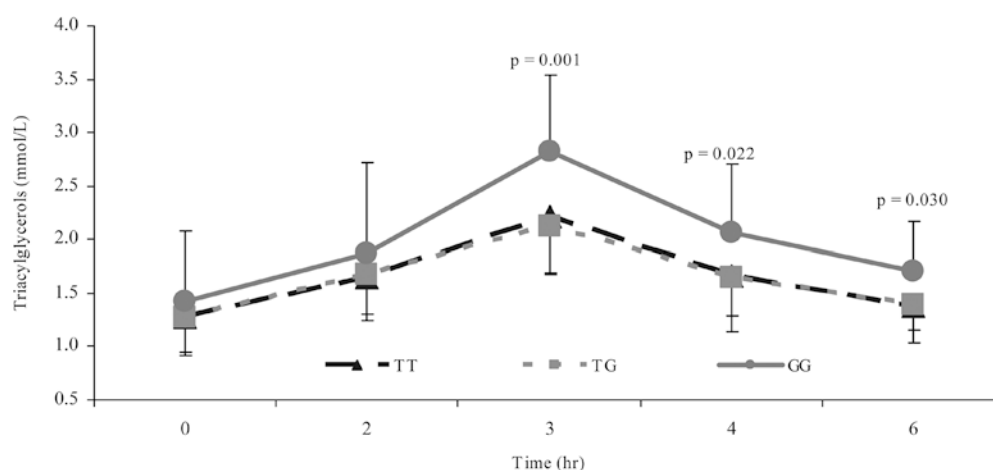
*TAG was log₁₀-transformed for statistical analysis, but actual means and SD are presented. For TAG, GG is significantly different from TT ($p < 0.05$) and TG ($p < 0.05$)

Table 3 Comparison of physical and biochemical measures across the subgroup participating in the high-fat meal test ($n=60$). *Waist* waist circumference, *BMI* body mass index, *TAG* triacylglycerol, *AUC* area under the curve, *CI* confidence interval, *HDLC* high-density lipoprotein cholesterol, *LDLC* low-density lipoprotein cholesterol

Variables	Genotype			95% CI	<i>p</i> value
	TT ($n=20$)	TG ($n=22$)	GG ($n=18$)		
Age, year	30.7 ± 0.9	31.3 ± 1.2	30.8 ± 3.3	30.4–31.4	0.556
Waist, cm	83.7 ± 11.4	84.0 ± 14.6	89.1 ± 6.7	82.4–88.5	0.281
BMI, kg/m ²	23.8 ± 4.4	24.1 ± 5.4	25.7 ± 4.0	23.7–25.7	0.418
TAG, mM	1.43 ± 0.09	1.44 ± 0.11	1.51 ± 0.13	1.43–1.49	0.064
*AUC, mM/h	3.84 ± 0.91	3.81 ± 0.77	4.65 ± 1.48	3.79–4.36	0.028
TC, mM	4.24 ± 0.76	4.45 ± 0.81	4.40 ± 0.64	4.17–4.56	0.659
HDLC, mM	1.21 ± 0.30	1.17 ± 0.20	1.09 ± 0.28	1.09–1.23	0.376
LDLC, mM	2.38 ± 0.61	2.60 ± 0.74	2.51 ± 0.71	2.33–2.68	0.590
Glucose, mM	5.06 ± 0.40	5.09 ± 0.33	5.09 ± 0.37	4.99–5.17	0.934
Insulin, pM	116.38 ± 76.07	112.79 ± 59.91	132.89 ± 42.98	104.81–135.23	0.538

*For AUC, GG is significantly different from TT ($p < 0.05$) and TG ($p < 0.05$)

Fig. 2 Postprandial triacylglycerol concentrations across the genotypes



Although the rare allele of the apo CIII T2854G gene polymorphism was associated with elevated TAG concentrations in the fasting and postprandial state in this study population, caution is necessary because the physiologic significance of the mutation has yet to be examined. First, while the rare allele (S2) of the apo AII-CIII-AIV complex was positively associated with apo CIII and TAG concentrations in healthy individuals (Shoulders et al. 1991), whether or not a similar correlation between the T2854G polymorphism and plasma apo CIII concentrations exists still needs to be investigated in future studies.

In summary, postprandial hyperlipemia has been complicated in the metabolic syndrome (Gott Jr 1998; Jeppesen et al. 1995) consisting of visceral obesity, hyperlipemia, insulin resistance, glucose intolerance, and hypertension, which collectively increases the risk of cardiovascular disease (CVD). In the currently study, we report that the GG homozygotes of the apo CIII T2854G gene polymorphism had elevated TAG concentrations not only in the fasting but also in the postprandial state in the study population, implying a potential contribution of this polymorphism to the variation in susceptibility to the CVD.

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