APOA5 variants and metabolic syndrome in Caucasians[s]

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Abstract *Apolipoprotein A5* (*APOA5*) gene variants were re-
ported to be associated with two components of metabolic
syndrome (MetS): higher TG levels and lower HDL levels.
Moreover, a recent Japanese case-control study found vari-
ant −1131T>C associated with MetS itself. Thus, our study
systematically analyzed the *APOA5* gene for association with
lipid parameters, any other features of MetS, including waist
circumference, glucose-related parameters, blood pressure,
uric acid, and MetS itself in Caucasians. Ten polymorphisms
were analyzed in a large fasting sample of the population-
based Cooperative Health Research in the Region of Augs-
burg (KORA) survey S4 (n = 1,354; southern Germany) and
in a second fasting sample, the Salzburg Atherosclerosis
Prevention Program in Subjects at High Individual Risk
(SAPHIR) study (n = 1,770; Austria). Minor alleles of variants
−1131T>C, −3A>G, c.56C>G, 476G>A, and 1259T>C
were significantly associated with higher TG levels in sin-
gle polymorphism (*P* < 0.001) and haplotype (*P* ≤ 6.6 ×
10^{-6}) analysis. Besides associations with lower HDL levels
in SAPHIR (*P* ≤ 0.001), there were no significant findings
with any other features of MetS. Variant c.56C>G was asso-
ciated with higher risk for MetS [odds ratio (95% confidence
interval) = 1.43 (1.04, 1.99), *P* = 0.03 for KORA and 1.48
(1.10, 1.99), *P* = 0.009 for SAPHIR].**ⓙ** Our study con-
firms the association of the *APOA5* locus with TG and HDL
levels in humans. Furthermore, the data suggest a different
mechanism of APOA5 impact on MetS in Caucasians, as vari-
ant c.56C>G (not analyzed in the Japanese study) and not
−1131T>C, as in the Japanese subjects, was associated with
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Apolipoprotein A5 (*APOA5*) is considered as important
modifying gene for familial combined hyperlipidemia
(FCHL), a disorder characterized by higher plasma tri-
glyceride (TG) levels and lower HDL levels (1, 2). As these
parameters are two components of the metabolic syndrome
(MetS) (3), *APOA5* may also have a role in this complex
disorder. The association of common *APOA5* variants with
higher TG levels was confirmed in several studies (4–19).

However, the underlying mechanisms are still unclear
and cannot be easily explained by an impact on *APOA5*
expression. Although initial overexpression and knockout
mouse models revealed an inverse relationship between the
protein and TG levels (20), studies in humans revealed a
positive correlation between APOA5 and TG levels (21, 22).

Two major haplotypes associated with higher TG levels
are tagged by the rare genotypes of variant −1131T>C
and coding variant c.56C>G, which results in the sub-
stitution of tryptophan for serine at residue 19 within the
predicted signal peptide responsible for APOA5 secre-
tion (20). Thus, c.56C>G is the only common variant with
known influence on APOA5 expression (23).

Recently, variant −1131T>C was identified as the stron-
gest signal for an association with MetS among 158 ana-
lyzed DNA polymorphisms in 133 candidate genes in a
study of 1,788 unrelated elderly (50–75 years) Japanese

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[s] The online version of this article (available at http://www.jlr.org)
contains supplementary data in the form of three tables, one figure.

This article is available online at http://www.jlr.org

individuals admitted in one of five participating hospitals (24). Confirmation and extension of association findings to other populations are mandatory, especially if the original finding was based on a large number of polymorphisms investigated, with its increased probability of false-positives. Furthermore, findings in Japanese populations cannot necessarily be extrapolated to Caucasian populations.

Thus, our study systematically analyzed the genetic information regarding the *APOA5* gene locus for association with lipid parameters and other parameters of MetS in two large fasting samples: 1,354 Caucasian subjects of the population-based Cooperative Health Research in the Region of Augsburg (KORA) survey S4 and 1,770 subjects of the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) study.

MATERIALS AND METHODS

Study populations

The KORA survey S4 is a population-based study of adults performed in southern Germany (25). The KORA survey S4 sample included 1,354 fasting subjects in the 55–74 year age group with an oral glucose tolerance test (OGTT) (26). Newly diagnosed type 2 diabetes was identified in 120 participants by OGTT data. Major population stratification within the KORA studies is unlikely, as there was no major population stratification found between the three large population-based studies from Germany (KORA, Study of Health in Pomerania, and Population Genetic Cohort) (27).

The SAPHIR study is an observational study conducted in Austria from 1999 to 2002 involving 1,770 unrelated fasting subjects: 663 women aged 50–70 years and 1,107 men aged 40–60 years (28, 29). Study participants were recruited by health screening programs at large companies in and around the city of Salzburg. This sample included 57 participants with type 2 diabetes. Individuals from both samples were of Caucasian origin.

Laboratory measurements

Fasting serum was used for all laboratory measurements in both studies. In the KORA S4 study, TG was assessed with the Boehringer glycerol phosphate oxidase-p-aminophenazone assay. Total cholesterol was measured by enzymatic methods (cholesterol oxidase-p-aminophenazone; Roche Diagnostics), HDL cholesterol was measured after precipitation with phosphotungstic acid/Mg^{2+} (Roche Diagnostics), and LDL cholesterol was measured after precipitation with dextran sulfate (Quantolip LDL; Immuno AG). Details on further laboratory measurements of the KORA S4 study are described elsewhere (30).

In the SAPHIR study, TG, total cholesterol, HDL cholesterol, and LDL cholesterol were determined using commercially available assays (Hoffmann-LaRoche GmbH, Vienna, Austria). Details on further laboratory measurements of the SAPHIR study are described elsewhere (31).

Definition of MetS

Diagnosis of MetS in both study samples was based on the definition proposed by the National cholesterol education program (NCEP) Adult Treatment Panel III, including medication (32). Participants were thus diagnosed with MetS if they had three or more of the following five components: *1*) waist circumference of >102 cm for men or >88 cm for women; *2*) fasting serum TG concentration of ≥1.7 mmol/l (150 mg/dl)

or drug treatment for increased TG; *3*) serum HDL cholesterol concentration of <1.0 mmol/l (40 mg/dl) for men or <1.3 mmol/l (50 mg/dl) for women or drug treatment for reduced HDL; *4*) systolic blood pressure of ≥130 mmHg or diastolic blood pressure of ≥85 mmHg or drug treatment for hypertension; and *5*) fasting plasma glucose level of ≥6.1 mmol/l (110 mg/dl) or drug treatment for increased glucose.

Genotyping

Genomic DNA of KORA and SAPHIR participants was extracted from blood leukocytes using the Puregene™ DNA Isolation Kit (Gentra Systems) according to the manufacturer's recommendations.

Genotyping of 10 *APOA5* variants (−9655A>G, −4904C>T, −1131T>C, −1099G>A, −3A>G, c.56C>G, 476G>A, c.457G>A, 1764C>T, and 1259T>C) in KORA S4 and of −1131T>C and 1259T>C in SAPHIR was carried out in the same laboratory by means of matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis of allele-dependent primer extension products as described elsewhere (33). Variant c.56C>G was genotyped in SAPHIR by a 5′ nuclease allelic discrimination (Taqman) assay within the Genotyping Unit of the Gene Discovery Core Facility at the Innsbruck Medical University in Austria.

Statistical analysis

Violation of Hardy-Weinberg equilibrium (HWE) was tested by Pearson's Chi-square test. Quantitative traits that were normally distributed on the original or logarithmic scale were analyzed by linear regression adjusted for age, sex, and body mass index (BMI) with the genotype included *1*) model-free (2 degrees of freedom), *2*) in an additive model, or *3*) in a dominant model.

The primary analysis consisted of four lipid-related traits: TG, LDL, HDL, and total cholesterol levels. Participants taking lipid-lowering drugs (n = 155) were excluded from this quantitative analysis. For the KORA sample, the significance level was corrected for multiple testing of lipid traits and the number of effective loci [$\alpha = 0.05/(4 \times 7) = 0.0017$], which was calculated by spectral decomposition of the correlation matrix of all variants analyzed (34). In the replication step, variants that were significantly associated according to the corrected significance level in KORA S4 were analyzed in the SAPHIR study using the same statistical models. The significance level applied in SAPHIR was corrected analogous to KORA S4.

The secondary analysis investigated further traits of MetS using the same statistical models. Subjects with antidiabetic medication were excluded from the analysis of fasting glucose, 2 h glucose (from OGTT), and fasting insulin. For the analysis of blood pressure, subjects using antihypertensive medication were excluded. Finally, associations of the genotypes with MetS were assessed by logistic regression adjusted for age and sex in all 1,354 (KORA) and 1,770 (SAPHIR) fasting subjects. The significance level for the analysis of MetS was corrected for the number of effective loci (KORA, $\alpha = 0.05/7 = 0.007$; SAPHIR, $\alpha = 0.05/2 = 0.025$). Single variant or haplotype analysis was carried out using SAS (version 9.1; Cary, NC) or the statistical software R (version 2.3.1; haplo.glm procedure), respectively. For details see the supplementary data. Haplotypes with $P < 0.0125$ computed as 0.05/4 were considered statistically significantly associated.

RESULTS

Characteristics of the KORA and SAPHIR samples are shown in **Table 1** stratified by sex and in supplementary

TABLE 1. Characteristics of the study populations

	KORA S4				SAPHIR			
Characteristic	n (Male/Female)	Total	Male	Female	n (Male/Female)	Total	Male	Female
Age (years)	1,354 (697/657)	64.0 ± 5.5	64.1 ± 5.6	63.9 ± 5.3	1,770 (1,107/663)	51.8 ± 6.1	49.2 ± 5.5	56.2 ± 4.3
Body mass index (kg/m^2)	1,348 (693/655)	28.4 ± 4.2	28.2 ± 3.6	28.6 ± 4.7	1,770 (1,107/663)	26.8 ± 4.1	26.9 ± 3.7	26.6 ± 4.7
Primary traits								
TGs (mmol/l)	1,187 (602/585)	1.28 (0.94/1.77)	1.39 (0.98/1.92)	1.20 (0.91/1.62)	1,681 (1,073/608)	1.15 (0.82/1.69)	1.21 (0.85/1.92)[a]	1.06 (0.79/1.45)
HDL cholesterol (mmol/l)	1,195 (606/589)	1.51 ± 0.43	1.36 ± 0.36	1.67 ± 0.43[a]	1,681 (1,073/608)	1.54 ± 0.41	1.43 ± 0.35	1.75 ± 0.42[a]
LDL cholesterol (mmol/l)	1,194 (605/589)	4.00 ± 1.01	3.97 ± 1.00	4.04 ± 1.03	1,681 (1,073/608)	3.78 ± 0.95	3.79 ± 0.93	3.77 ± 0.98
Total cholesterol (mmol/l)	1,196 (607/589)	6.31 ± 1.07	6.18 ± 1.07	6.44 ± 1.06	1,681 (1,073/608)	5.93 ± 1.04	5.85 ± 1.03	6.07 ± 1.05
Secondary traits								
Waist circumference (cm)	1,353 (696/657)	95.6 ± 11.4	100.4 ± 9.5[a]	90.4 ± 11.0	1,728 (1,081/647)	94.6 ± 12.6	98.0 ± 10.5[a]	88.9 ± 13.6
Uric acid (mg/dl)	1,354 (697/657)	5.7 ± 1.4	6.3 ± 1.3[a]	4.9 ± 1.1	1,764 (1,105/659)	5.9 ± 1.4	6.4 ± 1.3[a]	4.9 ± 1.1
Fasting plasma glucose (mg/dl)	1,351 (695/656)	102.2 ± 16.9	105.0 ± 17.9	99.2 ± 15.2	1,736 (1,036/650)	92.5 ± 14.1	93.4 ± 15.2[a]	90.9 ± 11.8
2 h plasma glucose (mg/dl)	1,350 (695/655)	125.9 ± 50.0	127.6 ± 52.8	124.0 ± 46.7	654 (495/159)	102.2 ± 36.8	99.5 ± 37.9	110.7 ± 31.7
Systolic blood pressure (mmHg)	881 (455/426)	133.2 ± 19.4	137.9 ± 18.6[a]	128.0 ± 19.1	1,388 (919/469)	132.8 ± 12.8	134.6 ± 12.4	129.3 ± 12.9
Diastolic blood pressure (mmHg)	881 (455/426)	79.3 ± 9.8	81.2 ± 9.8[a]	77.2 ± 9.4	1,388 (919/469)	81.4 ± 7.7	82.1 ± 7.8	80.1 ± 7.4
Fasting insulin (mU/l)	1,342 (692/650)	10.1 (7.1/14.8)	10.2 (6.9/14.8)	9.9 (7.2/14.4)	1,726 (1,081/645)	6.0 (4.2/8.9)	6.0 (4.0/8.9)	6.0 (4.3/8.8)

KORA, Cooperative Health Research in the Region of Augsburg; SAPHIR, Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk; TG, triglyceride. Data are presented as means ± standard deviations or medians (25th/75th percentiles) for traits that were not normally distributed (insulin, TGs).
[a] Sex difference, significantly higher value ($P < 0.05$).

Table I stratified by MetS status and −1131T>C or c.56C>G genotype. Except for fasting insulin and TG levels, all quantitative traits were normally distributed.

Genotyping results of the 10 analyzed APOA5 variants are presented in **Table 2**. The location and linkage disequilibrium (LD) structure of the analyzed variants are presented in supplementary Fig. I. None of the variants violated HWE ($P \geq 0.05$). The genotyping success rates were >94.0% with a discordance of <0.5% in 210 routine duplicates in KORA S4 and >95.7% with no discordance in 64 routine duplicates in SAPHIR. Genotype distribution combined for −1131T>C and c.56C>G is presented in supplementary Table II with and without the exclusion of subjects taking lipid-lowering drugs.

Primary analysis of lipids

TG levels were significantly different between the genotype groups of variants 1259T>C, −3A>G, −1131T>C, and 476G>A ($P \leq 0.001$) and borderline significantly different for c.56C>G. Because −1131T>C is highly correlated with −3A>G ($r^2 = 0.98$), 476G>A ($r^2 = 0.95$), and 1259T>C ($r^2 = 0.84$), **Table 3** presents results for −1131T>C and c.56C>G only. In the additive model, variant −1131T>C showed a statistically significant association with 13.30% ($P = 5.0 \times 10^{-4}$) higher TG levels per copy of the minor allele for −1131T>C. Variant c.56C>G showed a borderline significant difference for TG levels between genotype groups ($P = 0.009$, F-test), with β-estimates in the same range as for variant −1131T>C. However, this association was insignificant after correction for multiple testing [additive model: percentage change of geometric mean (PCGM) = 12.3%; $P = 0.005$]. No significant associations were observed with total, LDL, and HDL cholesterol.

Variants −1131T>C, 1259T>C (data not shown), and c.56C>G were selected for replication in the SAPHIR study (Table 3). A minor allele of variant c.56C>G was significantly associated with higher TG levels in the dominant model (PCGM = 22.08%; $P = 6.7 \times 10^{-7}$). Minor alleles of all three variants were significantly associated with HDL, LDL, or total cholesterol levels (Table 3; 1259T>C data not shown).

APOA5 haplotype analysis

Haplotype analysis performed in the KORA study revealed six haplotypes with frequencies of >5% (**Table 4**). The most frequent haplotype was used as a reference. Haplotype APOA5_3, containing the minor alleles of variants −3A>G, −1131T>C, 476G>A, and 1259T>C, was significantly associated with higher TG levels ($P = 0.002$). Haplotype APOA5_5, tagged by variant c.56C>G, was also significantly associated with higher TG levels (PCGM = 15.2%; $P = 0.003$). This association was stronger in women (PCGM = 22.5%; $P = 0.002$) than in men.

Secondary analysis: MetS and its features

Results of the secondary analysis are presented in supplementary Table III. In both study samples, no significant associations of gene variants with waist circumference,

TABLE 2. Description of investigated polymorphisms within the *APOA5* gene and basic genotyping results

Study	Variant	Alias[a]	Position on Chromosome 11[b]	n	Genotyping Success Rate	Base Change[c]	Amino Acid Change	Minor Allele Frequency	P[d]
		≅			%				
KORA	rs2542061	−9655A>G	116177441	1,581	96.99	A→G		0.50	0.82
	rs633867	−4904C>T	116172690	1,559	95.64	C→T		0.02	0.74
	rs662799	−1131T>C, SNP3	116168917	1,607	98.59	T→C		0.08	0.18
	rs1729411	−1099G>A	116168885	1,589	97.48	G→A		0.13	0.18
	rs651821	−3A>G	116167789	1,596	97.91	A→G		0.07	0.17
	rs3135506	S19W, c56C>G	116167617	1,533	94.05	G→C	W→S	0.06	0.22
	rs2072560	476G>A, SNP2	116167036	1,603	98.34	G→A		0.07	0.25
	rs3135507	c.457G>A, Val 153Met	116166698	1,595	97.85	C→T	V→M	0.03	0.70
	rs619054	1764C>T	116166023	1,608	98.65	G→A		0.26	0.80
	rs2266788	1259T>C, SNP1	116165896	1,597	97.98	T→C		0.08	0.05
SAPHIR									
	rs662799	−1131T>C, SNP3	116168917	1,702	96.16	T→C		0.07	0.57
	rs3135506	S19W, c56C>G	116167617	1,722	97.29	G→C	S→W	0.07	0.06
	rs2266788	1259T>C, SNP1	116165896	1,694	95.71	T→C		0.07	0.42

APOA5, Apolipoprotein A5.
[a] Synonymous names used in the literature.
[b] Positions according to the Ensemble database.
[c] The second base represents the minor allele.
[d] P values of Pearson's test for Hardy-Weinberg equilibrium.

fasting plasma glucose, 2 h plasma glucose, systolic or diastolic blood pressure, fasting insulin, and uric acid levels were found after correction for multiple testing.

A total of 348 KORA S4 subjects (26%) and 502 SAPHIR subjects (28%) fulfilled the NCEP criteria for MetS. In the additive model, the minor allele of variant c.56C>G was associated with an increased risk for MetS [odds ratio (OR) (95% confidence interval {CI}) = 1.43 (1.04, 1.99), $P = 0.03$ for KORA S4, OR (95% CI) = 1.48 (1.10, 1.99), $P = 0.009$ for SAPHIR]. After correction for multiple testing, this association remained significant in SAPHIR only. Minor alleles of variants −4904C>T and c.457G>A, which were not in linkage disequilibrium with any other variant, showed insignificant ORs with MetS in KORA [OR (95% CI) = 1.48 (0.87, 2.54), $P = 0.12$] and SAPHIR [OR (95% CI) = 1.33 (0.85, 2.08), $P = 0.21$]. Variant −1131T>C indicated higher ORs in SAPHIR only [OR (95% CI) = 1.00 (0.75, 1.34), $P = 0.99$ for KORA and OR (95% CI) = 1.24 (0.92, 1.68), $P = 0.16$ for SAPHIR]. No association was observed for any other variant. Combining both study samples, variant −1131T>C was not significantly associated with MetS [OR (95% CI) = 1.03 (0.84, 1.27), $P = 0.77$], but variant c.56C>G was [OR (95% CI) = 1.28 (1.03, 1.58), $P = 0.026$]. Subjects heterozygous in both variants showed insignificant OR (95% CI) of 2.11 (1.00, 4.49), $P = 0.05$.

DISCUSSION

The present study investigated 10 polymorphisms covering the *APOA5* locus for association with lipid parameters, NCEP-defined MetS, and its features in KORA S4. Minor alleles of −1131T>C and variant c.56C>G were associated with higher TG levels, as reported previously (5, 20, 35, 36). Both associations were replicated in the SAPHIR study. Furthermore, associations with lower HDL and higher LDL and total cholesterol levels were found for −1131T>C and c.56C>G in the SAPHIR population but not in KORA S4. Variant c.56C>G was associated with higher risk of MetS in the SAPHIR study, with similar ORs in the KORA study or in combined analysis. Although association with MetS was convincingly statistically significant in SAPHIR, this association did not remain significant in KORA after applying correction for multiple testing.

Especially findings with MetS deserve some attention, as a result of the recent description of an association of −1131T>C with MetS (OR, 1.45–2.07) in a Japanese study investigating a large number of polymorphisms in 133 candidate genes (24). Our results could not confirm this association.

Although the association of *APOA5* variants with TG levels could be replicated in different ethnicities, the complexity of polygenic susceptibilities of MetS might be an explanation for the lack of association of −1131T>C with MetS in Caucasians. The comparability of the Japanese case-control study with our population-based study samples could be biased by different study designs. Whereas the age range was similar in both studies, the MetS definition in the Japanese study included a body mass index threshold (≥ 25 kg/m^2) instead of waist circumference thresholds. We decided to use waist circumference criteria because mean body mass index was rather high in our populations. Another difference was the selection of control subjects, which were free from any MetS component in the Japanese study. Furthermore, the definition of MetS is controversial, especially concerning different ethnicities (37). Genetic differences were reflected in −1131T>C minor allele frequencies of 35.3% in Japanese versus 7.4% in Caucasians.

We were able to find an association of another functionally relevant polymorphism (c.56C>G) with MetS. This could be attributable to the fact that MetS is a strongly lipid-driven or lipid-influenced syndrome involving three of five components. Two of these components are clear lipid com-

TABLE 3. Primary analysis in KORA S4 and SAPHIR

Study and Variant	Trait	n	P[b]	aa Versus AA[a]			Aa Versus AA[a]			Additive			Dominant		
				β	95% CI	P	β	95% CI	P	β	95% CI	P	β	95% CI	P
KORA S4															
−1131T>C	TGs (%)[c]	1,163	**8.3×10^{-4}**	54.23	16.02, 105.04	0.003	10.29	1.9, 19.36	0.015	13.30	5.62, 21.54	**5.0×10^{-4}**	12.66	4.31, 21.67	0.002
	HDL cholesterol (mmol/l)	1,171	0.486	−0.02	−0.26, 0.21	0.847	−0.04	−0.11, 0.03	0.233	−0.03	−0.09, 0.02	0.259	−0.04	−0.1, 0.02	0.232
	LDL cholesterol (mmol/l)	1,170	0.051	0.60	−0.07, 1.26	0.081	0.16	−0.02, 0.33	0.081	0.18	0.03, 0.34	0.021	0.18	0.01, 0.35	0.038
	Total cholesterol (mmol/l)	1,172	0.008	0.85	0.19, 1.51	0.012	0.18	0, 0.36	0.054	0.23	0.07, 0.4	0.005	0.22	0.04, 0.4	0.015
c.56C>G	TGs (%)[c]	1,108	0.009	−0.71	−33.69, 48.69	0.973	14.72	5.15, 25.15	0.002	12.31	3.61, 21.75	0.005	14.07	4.72, 24.25	0.003
	HDL cholesterol (mmol/l)	1,116	0.424	0.03	−0.3, 0.37	0.844	−0.05	−0.12, 0.02	0.197	−0.04	−0.1, 0.03	0.268	−0.04	−0.11, 0.03	0.221
	LDL cholesterol (mmol/l)	1,115	0.230	0.05	−0.84, 0.94	0.916	0.17	−0.02, 0.36	0.087	0.15	−0.03, 0.32	0.107	0.16	−0.03, 0.35	0.090
	Total cholesterol (mmol/l)	1,116	0.048	0.09	−0.85, 1.02	0.856	0.25	0.05, 0.45	0.014	0.22	0.03, 0.41	0.020	0.25	0.05, 0.44	0.015
SAPHIR															
−1131T>C	TGs (%)[c]	1,596	**1.0×10^{-6}**	4.17	−33.61, 63.46	0.859	22.68	13.7, 32.37	**1.5×10^{-7}**	20.51	12.09, 29.57	**5.1×10^{-7}**	22.19	13.34, 31.73	**2.0×10^{-7}**
	HDL cholesterol (mmol/l)	1,596	**0.001**	−0.31	−0.62, −0.01	0.046	−0.08	−0.13, −0.03	**0.002**	−0.09	−0.14, −0.04	**3.8×10^{-4}**	−0.09	−0.14, −0.04	**7.6×10^{-4}**
	LDL cholesterol (mmol/l)	1,596	0.040	0.01	−0.82, 0.84	0.984	0.18	0.04, 0.32	**0.011**	0.16	0.03, 0.3	0.016	0.18	0.04, 0.32	**0.012**
	Total cholesterol (mmol/l)	1,596	**0.011**	−0.25	−1.16, 0.65	0.581	0.23	0.08, 0.38	**0.003**	0.20	0.05, 0.34	**0.009**	0.22	0.07, 0.37	**0.005**
c.56C>G	TGs (%)[c]	1,630	**3.0×10^{-6}**	3.94	−42.05, 86.41	0.897	21.25	12.53, 30.64	**4.6×10^{-7}**	20.05	11.64, 29.08	**8.9×10^{-7}**	22.08	12.88, 32.03	**6.7×10^{-7}**
	HDL cholesterol (mmol/l)	1,630	0.057	0.06	−0.33, 0.46	0.756	−0.06	−0.11, −0.01	0.018	−0.06	−0.11, −0.01	0.026	−0.06	−0.11, −0.01	0.021
	LDL cholesterol (mmol/l)	1,630	0.042	1.11	0.04, 2.18	0.043	0.11	−0.03, 0.24	0.124	0.13	0, 0.27	0.050	0.12	−0.01, 0.26	0.080
	Total cholesterol (mmol/l)	1,630	0.010	1.26	0.1, 2.42	0.034	0.17	0.02, 0.32	0.027	0.19	0.05, 0.34	**0.009**	0.18	0.03, 0.33	0.016

CI, confidence interval. Estimated associations in linear regression models between the *APOA5* polymorphisms and lipid parameters are shown.

[a] 1131T>C: AA = AA, Aa = GA, aa = GG; number of minor allele homozygotes in KORA, n = 13, in SAPHIR, n = 16. c.56C>G: AA = GG, Aa = GC, aa = CC; number of minor allele homozygotes in KORA, n = 16, in SAPHIR, n = 16. Significant *P* values are shown in boldface. Corrected significance levels are as follows: for KORA, $P < 0.0017$; for SAPHIR, $P < 0.0125$.

[b] *P* value from *F*-test.

[c] β values of logarithmized variables (TG levels and fasting insulin) are given as geometric mean change estimated by $(EXP(\log(variable)) - 1) \times 100$.

TABLE 4. Haplotype analysis for TG levels in KORA S4

Haplotype	−9655A>G	−4904C>T	−1131T>C	−1099G>A	−3A>G	c.56C>G	476C>A	c.457G>A	1764C>T	1259T>C	Total				Male				Female			
											Frequency	Estimate (%)	95% CI (%)	P	Frequency	Estimate (%)	95% CI (%)	P	Frequency	Estimate (%)	95% CI (%)	P
APOA5_01	2	1	1	1	1	1	1	1	2	1	0.26	1.91	−3.68, 7.83	0.511	0.26	−2.69	−10.79, 6.14	0.538	0.26	6.11	−1.17, 13.93	0.103
APOA5_02	2	1	1	1	1	1	1	1	1	1	0.15	−2.66	−8.93, 4.05	0.429	0.15	−7.37	−16.33, 2.54	0.141	0.14	0.98	−7.24, 9.93	0.822
APOA5_03	2	2	2	2	2	2	1	1	1	2	0.06	14.96	5.4, 25.38	**0.002**	0.06	15.89	1.68, 32.09	0.028	0.06	15.00	2.78, 28.68	0.015
APOA5_04	1	1	1	2	1	1	1	1	1	1	0.13	6.60	−0.6, 14.32	0.074	0.13	9.94	−1.27, 22.43	0.085	0.13	1.98	−6.65, 11.41	0.665
APOA5_05	1	1	1	1	1	**2**	1	1	1	1	0.06	15.24	4.91, 26.59	**0.003**	0.06	10.49	−3.47, 26.46	0.148	0.05	22.54	7.85, 39.24	**0.002**
Rare	a	a	a	a	a	a	a	a	a	a	0.05	9.62	−2.08, 22.73	0.111	0.05	6.74	−10.23, 26.92	0.461	0.04	12.34	−1.92, 28.67	0.094
Reference	1	1	1	1	1	1	1	1	1	1	0.30				0.28				0.31			

TG levels are given as geometric mean change, estimated by (EXP(log(variable) − 1) × 100. Highly correlated variants are highlighted in gray. Significance level for haplotype analysis corrected for multiple testing was P = 0.0125. Haplotype tagging variants and statistically significant values are shown in boldface. Haplotype coding: 1 = common allele, 2 = minor allele. Haplotypes with frequency <=0.02 were included in the rare group.
a Any allele.

ponents correlated not only with each other but also with other components of MetS. Therefore, it is possible that an association found between a genetic variant and TG and HDL levels extends to an association with MetS only marginally triggered by the other components. This interpretation is also supported by an association of c.56C>G and FCHL, which might share etiological overlap with MetS (38, 39).

In most (15, 40, 41) but not all (42) studies investigating Caucasian FCHL subjects, variant −1131T>C was associated with FCHL. More consistent results were reported for c.56C>G, showing increased transmission of the G allele in FCHL subjects (40, 41). However, the major impact on serum TG levels by homozygote c.56C>G found in several studies (14, 16, 19, 40) could only be confirmed in SAPHIR.

In combination with c.56C>G, −1131T>C might have an effect on MetS risk in Caucasians too, as estimated MetS risk was increased in subjects heterozygous for minor alleles of both variants.

Haplotype analysis supports the findings of the single variant analysis. Estimated haplotypes are in agreement with the haplotypes reported by Pennacchio et al. (14), and the additional variants included do not appear to provide further information.

The KORA survey S4, being representative of the general population of Augsburg, Germany, benefits from the high-quality phenotyping of the internationally approved Monitoring Trends and Determinants on Cardiovascular Diseases/KORA surveys (43). Our analyzed sample included fasting values of parameters relevant to MetS. The SAPHIR study, being representative of the working population of Salzburg, Austria, provides fasting measurements as well. Thus, both populations are exceptionally applicable to the detection of associations with MetS (total power of 97.5% to detect OR of 1.5) or related traits (29, 44). The ability to include replication in a larger sample ensures the observed associations. Significance level was strictly corrected for multiple testing to avoid false-positive results, which are a major problem in genetic epidemiological association studies.

CONCLUSION

Our investigation of the association between *APOA5* variants and features of MetS in one of the largest Caucasian study samples to date provides strong support for an association with TG and HDL levels. The association of variant c.56C>G with MetS might be driven mainly by an association with TG and HDL levels.

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