

APOA5 variants and metabolic syndrome in Caucasians[§]

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Abstract *Apolipoprotein A5 (APOA5) gene variants were reported 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 variant -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 Augsburg (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 single polymorphism ($P < 0.001$) and haplotype ($P \leq 6.6 \times 10^{-6}$) analysis. Besides associations with lower HDL levels in SAPHIR ($P \leq 0.001$), there were no significant findings with any other features of MetS. Variant c.56C>G was associated 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 confirms 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 variant c.56C>G (not analyzed in the Japanese study) and not -1131T>C, as in the Japanese subjects, was associated with MetS.—Grallert, H., E-M. Sedlmeier, C. Huth, M. Kolz, I. M. Heid, C. Meisinger, C. Herder, K. Strassburger, A. Gehringer, M. Haak, G. Giani, F. Kronenberg, H-E. Wichmann, J. Adamski, B. Paulweber, T. Illig, and W. Rathmann. *APOA5 variants and metabolic syndrome in Caucasians. J. Lipid Res.* 2007. 48: 2614–2621.*

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Apolipoprotein A5 (APOA5) is considered as important modifying gene for familial combined hyperlipidemia (FCHL), a disorder characterized by higher plasma triglyceride (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 substitution of tryptophan for serine at residue 19 within the predicted signal peptide responsible for *APOA5* secretion (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 strongest signal for an association with MetS among 158 analyzed DNA polymorphisms in 133 candidate genes in a study of 1,788 unrelated elderly (50–75 years) Japanese

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^{¶¶} The online version of this article (available at <http://www.jlr.org>) contains supplementary data in the form of three tables, one figure.

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/Mg²⁺ (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

Characteristic	KORA S4				SAPHIR			
	Total	Male	Female	n (Male/Female)	Total	Male	Female	n (Male/Female)
Age (years)	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	1,770 (1,107/663)
Body mass index (kg/m ²)	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	1,770 (1,107/663)
Primary traits								
TGs (mmol/l)	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)	1,681 (1,073/608)
HDL cholesterol (mmol/l)	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	1,681 (1,073/608)
LDL cholesterol (mmol/l)	4.00 ± 1.01	3.97 ± 1.03	4.04 ± 1.03	1,681 (1,073/608)	3.78 ± 0.95	3.79 ± 0.93	3.77 ± 0.98	1,681 (1,073/608)
Total cholesterol (mmol/l)	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	1,681 (1,073/608)
Secondary traits								
Waist circumference (cm)	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	1,728 (1,081/647)
Uric acid (mg/dl)	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	1,764 (1,105/659)
Fasting plasma glucose (mg/dl)	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	1,736 (1,036/650)
2 h plasma glucose (mg/dl)	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	654 (495/159)
Systolic blood pressure (mmHg)	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	1,388 (919/469)
Diastolic blood pressure (mmHg)	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	1,388 (919/469)
Fasting insulin (mU/l)	10.1 (7.1/14.6)	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)	1,726 (1,081/645)

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 1 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. 1. 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/m²) 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				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	0.079	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
	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
c.56C>G	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
	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
c.56C>G	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.

^a1131T>C: AA = AA, Aa = GA, aa = GC; number of minor allele homozygotes in KORA, n = 13; in SAPHIR, n = 6. c.56C>G: AA = GC, Aa = GG, aa = CC; number of minor allele homozygotes in KORA, n = 16; in SAPHIR, n = 3. 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 logaritized variables (TG levels and fasting insulin) are given as geometric mean change estimated by $(\text{EXP}(\log(\text{variable})) - 1) \times 100$.

TABLE 4. Haplotype analysis for TG levels in KORA S4

Haplotype	Total										Male			Female								
	-9655A >G	-4904C >T	-1131T >C	-1099G >A	-3A >G	c.56C >G	476G >A	c.457G >A	1764C >T	1259T >C	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	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	0.98	-7.24, 9.93	0.822	
APOA5_03	2	1	2	1	2	1	2	1	1	2	0.06	14.96	5.4, 25.38	0.002	0.06	15.89	1.68, 32.09	0.028	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	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	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	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 $(EXP(\log(\text{variable}) - 1) \times 100)$. 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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REFERENCES

- Naukkarinen, J., C. Ehnholm, and L. Peltonen. 2006. Genetics of familial combined hyperlipidemia. *Curr. Opin. Lipidol.* **17**: 285–290.
- Shoulders, C. C., E. L. Jones, and R. P. Naoumova. 2004. Genetics of familial combined hyperlipidemia and risk of coronary heart disease. *Hum. Mol. Genet.* **13** (Spec. No. 1): R149–R160.
- Eckel, R. H., S. M. Grundy, and P. Z. Zimmet. 2005. The metabolic syndrome. *Lancet.* **365**: 1415–1428.
- Aouizerat, B. E., M. Kulkarni, D. Heilbron, D. Drown, S. Raskin, C. R. Pullinger, M. J. Malloy, and J. P. Kane. 2003. Genetic analysis of a polymorphism in the human apoA-V gene: effect on plasma lipids. *J. Lipid Res.* **44**: 1167–1173.
- Chaaba, R., N. Attia, S. Hammami, M. Smaoui, S. Mahjoub, M. Hammami, and A. S. Masmoudi. 2005. Association of SNP3 polymorphism in the apolipoprotein A-V gene with plasma triglyceride level in Tunisian type 2 diabetes. *Lipids Health Dis.* **4**: 1.
- Elosua, R., J. M. Ordovas, L. A. Cupples, C. Q. Lai, S. Demissie, C. S. Fox, J. F. Polak, P. A. Wolf, R. B. D'Agostino, Sr., and C. J. O'Donnell. 2006. Variants at the APOA5 locus, association with carotid atherosclerosis, and modification by obesity: the Framingham Study. *J. Lipid Res.* **47**: 990–996.
- Hubacek, J. A., V. Adamkova, R. Ceska, R. Poledne, A. Horinek, and M. Vrablik. 2004. New variants in the apolipoprotein AV gene in individuals with extreme triglyceride levels. *Physiol. Res.* **53**: 225–228.
- Jang, Y., J. Y. Kim, O. Y. Kim, J. E. Lee, H. Cho, J. M. Ordovas, and J. H. Lee. 2004. The $-1131T \rightarrow C$ polymorphism in the apolipoprotein A5 gene is associated with postprandial hypertriglycerolemia; elevated small, dense LDL concentrations; and oxidative stress in nonobese Korean men. *Am. J. Clin. Nutr.* **80**: 832–840.
- Jiang, Y. D., C. J. Yen, W. L. Chou, S. S. Kuo, K. C. Lee, K. C. Chiu, and L. M. Chuang. 2005. Interaction of the G182C polymorphism in the APOA5 gene and fasting plasma glucose on plasma triglycerides in type 2 diabetic subjects. *Diabet. Med.* **22**: 1690–1695.
- Kao, J. T., H. C. Wen, K. L. Chien, H. C. Hsu, and S. W. Lin. 2003. A novel genetic variant in the apolipoprotein A5 gene is associated with hypertriglyceridemia. *Hum. Mol. Genet.* **12**: 2533–2539.
- Lai, C. Q., E. S. Tai, C. E. Tan, J. Cutter, S. K. Chew, Y. P. Zhu, X. Adiconis, and J. M. Ordovas. 2003. The APOA5 locus is a strong determinant of plasma triglyceride concentrations across ethnic groups in Singapore. *J. Lipid Res.* **44**: 2365–2373.
- Lai, C. Q., S. Demissie, L. A. Cupples, Y. Zhu, X. Adiconis, L. D. Parnell, D. Corella, and J. M. Ordovas. 2004. Influence of the APOA5 locus on plasma triglyceride, lipoprotein subclasses, and CVD risk in the Framingham Heart Study. *J. Lipid Res.* **45**: 2096–2105.
- Martin, S., V. Nicaud, S. E. Humphries, and P. J. Talmud. 2003. Contribution of APOA5 gene variants to plasma triglyceride determination and to the response to both fat and glucose tolerance challenges. *Biochim. Biophys. Acta.* **1637**: 217–225.
- Pennacchio, L. A., M. Olivier, J. A. Hubacek, R. M. Krauss, E. M. Rubin, and J. C. Cohen. 2002. Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels. *Hum. Mol. Genet.* **11**: 3031–3038.
- Ribalta, J., L. Figuera, J. Fernandez-Ballart, E. Vilella, C. M. Castro, L. Masana, and J. Joven. 2002. Newly identified apolipoprotein AV gene predisposes to high plasma triglycerides in familial combined hyperlipidemia. *Clin. Chem.* **48**: 1597–1600.
- Talmud, P. J., E. Hawe, S. Martin, M. Olivier, G. J. Miller, E. M. Rubin, L. A. Pennacchio, and S. E. Humphries. 2002. Relative contribution of variation within the APOC3/A4/A5 gene cluster in determining plasma triglycerides. *Hum. Mol. Genet.* **11**: 3039–3046.
- Talmud, P. J., S. Martin, M. R. Taskinen, M. H. Frick, M. S. Nieminen, Y. A. Kesaniemi, A. Pasternack, S. E. Humphries, and M. Svanne. 2004. APOA5 gene variants, lipoprotein particle distribution, and progression of coronary heart disease: results from the LOCAT Study. *J. Lipid Res.* **45**: 750–756.
- Talmud, P. J., J. A. Cooper, H. Hattori, I. P. Miller, G. J. Miller, and S. E. Humphries. 2006. 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.* **49**: 2337–2340.
- Vrablik, M., A. Horinek, R. Ceska, V. Adamkova, R. Poledne, and J. A. Hubacek. 2003. Ser19 \rightarrow Trp polymorphism within the apolipoprotein AV gene in hypertriglyceridaemic people. *J. Med. Genet.* **40**: e105.
- Pennacchio, L. A., M. Olivier, J. A. Hubacek, J. C. Cohen, D. R. Cox, J. C. Fruchart, R. M. Krauss, and E. M. Rubin. 2001. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science.* **294**: 169–173.
- Dallinga-Thie, G. M., A. van Tol, H. Hattori, L. C. van Vark-van der Zee, H. Jansen, and E. J. Sijbrands. 2006. Plasma apolipoprotein A5 and triglycerides in type 2 diabetes. *Diabetologia.* **49**: 1505–1511.
- Vaessen, S. F., F. G. Schaap, J. A. Kuivenhoven, A. K. Groen, B. A. Hutten, S. M. Boekholdt, H. Hattori, M. S. Sandhu, S. A. Bingham, R. Luben, et al. 2006. Apolipoprotein A-V, triglycerides and risk of coronary artery disease: the prospective Epic-Norfolk Population Study. *J. Lipid Res.* **47**: 2064–2070.
- Talmud, P. J., J. Palmen, W. Putt, L. Lins, and S. E. Humphries. 2005. Determination of the functionality of common APOA5 polymorphisms. *J. Biol. Chem.* **280**: 28215–28220.
- Yamada, Y., K. Kato, T. Hibino, K. Yokoi, H. Matsuo, T. Segawa, S. Watanabe, S. Ichihara, H. Yoshida, K. Satoh, et al. 2007. Prediction of genetic risk for metabolic syndrome. *Atherosclerosis.* **191**: 298–304.
- Wichmann, H. E., C. Gieger, and T. Illig. 2005. KORA-gen—resource for population genetics, controls and a broad spectrum of disease phenotypes. *Gesundheitswesen.* **67** (Suppl. 1): 26–30.
- Rathmann, W., B. Haastert, A. Icks, H. Lowel, C. Meisinger, R. Holle, and G. Giani. 2003. High prevalence of undiagnosed diabetes mellitus in southern Germany: target populations for efficient screening. The KORA survey 2000. *Diabetologia.* **46**: 182–189.
- Steffens, M., C. Lamina, T. Illig, T. Bettecken, R. Vogler, P. Entz, E. K. Suk, M. R. Toliat, N. Klopp, A. Caliebe, et al. 2006. SNP-based analysis of genetic substructure in the German population. *Hum. Hered.* **62**: 20–29.
- Esterbauer, H., C. Scheitler, H. Oberkofler, C. Ebenbichler, B. Paulweber, F. Sandhofer, G. Ladurner, E. Hell, A. D. Strosberg, J. R. Patsch, et al. 2001. A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. *Nat. Genet.* **28**: 178–183.
- Heid, I. M., S. A. Wagner, H. Gohlke, B. Iglseider, J. C. Mueller, P. Cip, G. Ladurner, R. Reiter, A. Stadlmayr, V. Mackevics, et al. 2006. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. *Diabetes.* **55**: 375–384.
- Rathmann, W., B. Haastert, C. Herder, H. Hauner, W. Koenig, C. Meisinger, R. Holle, and G. Giani. 2007. Differential association of adiponectin with cardiovascular risk markers in men and women? The KORA survey 2000. *Int. J. Obes. (Lond).* **31**: 770–776.
- Mackevics, V., I. M. Heid, S. A. Wagner, P. Cip, H. Doppelmayr, A. Lejnieks, H. Gohlke, G. Ladurner, T. Illig, B. Iglseider, et al. 2006. The adiponectin gene is associated with adiponectin levels but not with characteristics of the insulin resistance syndrome in healthy Caucasians. *Eur. J. Hum. Genet.* **14**: 349–356.
- Grundy, S. M., J. I. Cleeman, S. R. Daniels, K. A. Donato, R. H. Eckel, B. A. Franklin, D. J. Gordon, R. M. Krauss, P. J. Savage, S. C. Smith, Jr., et al. 2006. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Curr. Opin. Cardiol.* **21**: 1–6.
- Vollmert, C., O. Windl, W. Xiang, A. Rosenberger, I. Zerr, H. E. Wichmann, H. Bickeboller, T. Illig, and H. A. Kretschmar. 2006. Significant association of a M129V independent polymorphism in the 5' UTR of the PRNP gene with sporadic Creutzfeldt-Jakob disease in a large German case-control study. *J. Med. Genet.* **43**: e53.

34. Nyholt, D. R. 2004. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am. J. Hum. Genet.* **74**: 765–769.
35. van der Vliet, H. N., M. G. Sammels, A. C. Leegwater, J. H. Levels, P. H. Reitsma, W. Boers, and R. A. Chamuleau. 2001. Apolipoprotein A-V: a novel apolipoprotein associated with an early phase of liver regeneration. *J. Biol. Chem.* **276**: 44512–44520.
36. Jakel, H., M. Nowak, A. Helleboid-Chapman, J. Fruchart-Najib, and J. C. Fruchart. 2006. Is apolipoprotein A5 a novel regulator of triglyceride-rich lipoproteins? *Ann. Med.* **38**: 2–10.
37. Kahn, R., J. Buse, E. Ferrannini, and M. Stern. 2005. The metabolic syndrome: time for a critical appraisal. Joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetologia.* **48**: 1684–1699.
38. Ayyobi, A. F., and J. D. Brunzell. 2003. Lipoprotein distribution in the metabolic syndrome, type 2 diabetes mellitus, and familial combined hyperlipidemia. *Am. J. Cardiol.* **92**: 27J–33J.
39. Lewis, G. F. 2002. Lipid metabolism. *Curr. Opin. Lipidol.* **13**: 97–99.
40. Eichenbaum-Voline, S., M. Olivier, E. L. Jones, R. P. Naumova, B. Jones, B. Gau, H. N. Patel, M. Seed, D. J. Betteridge, D. J. Galton, et al. 2004. Linkage and association between distinct variants of the APOA1/C3/A4/A5 gene cluster and familial combined hyperlipidemia. *Arterioscler. Thromb. Vasc. Biol.* **24**: 167–174.
41. Mar, R., P. Pajukanta, H. Allayee, M. Groenendijk, G. Dallinga-Thie, R. M. Krauss, J. S. Sinsheimer, R. M. Cantor, T. W. de Bruin, and A. J. Lusis. 2004. Association of the APOLIPOPROTEIN A1/C3/A4/A5 gene cluster with triglyceride levels and LDL particle size in familial combined hyperlipidemia. *Circ. Res.* **94**: 993–999.
42. van der Vleuten, G. M., A. Isaacs, W. W. Zeng, E. ter Avest, P. J. Talmud, G. M. Dallinga-Thie, C. M. van Duijn, A. F. Stalenhoef, and J. de Graaf. 2007. Haplotype analyses of the APOA5 gene in patients with familial combined hyperlipidemia. *Biochim. Biophys. Acta.* **1772**: 81–88.
43. Holle, R., M. Happich, H. Lowel, and H. E. Wichmann. 2005. KORA—a research platform for population based health research. *Gesundheitswesen.* **67 (Suppl. 1)**: 19–25.
44. Grallert, H., C. Huth, M. Kolz, C. Meisinger, C. Herder, K. Strassburger, G. Giani, H. E. Wichmann, J. Adamski, T. Illig, et al. 2006. IL-6 promoter polymorphisms and quantitative traits related to the metabolic syndrome in KORA S4. *Exp. Gerontol.* **41**: 737–745.