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Gene-Gene Interaction between *APOA5* and *USF1*: Two Candidate Genes for the Metabolic Syndrome

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Key Words

Metabolic syndrome · Cardiovascular risk · SNP · APOA5 · USF1

Summary

Objective: The metabolic syndrome, a major cluster of risk factors for cardiovascular diseases, shows increasing prevalence worldwide. Several studies have established associations of both apolipoprotein A5 (*APOA5*) gene variants and upstream stimulatory factor 1 (*USF1*) gene variants with blood lipid levels and metabolic syndrome. USF1 is a transcription factor for *APOA5*. **Methods:** We investigated a possible interaction between these two genes on the risk for the metabolic syndrome, using data from the German population-based KORA survey 4 (1,622 men and women aged 55–74 years). Seven *APOA5* single nucleotide polymorphisms (SNPs) were analyzed in combination with six *USF1* SNPs, applying logistic regression in an additive model adjusting for age and sex and the definition for metabolic syndrome from the National Cholesterol Education Program's Adult Treatment Panel III (NCEP (AIII)) including medication. **Results:** The overall prevalence for metabolic syndrome was 41%. Two SNP combinations showed a nominal gene-gene interaction (p values 0.024 and 0.047). The effect of one SNP was modified by the other SNP, with a lower risk for the metabolic syndrome with odds ratios (ORs) between 0.33 (95% CI = 0.13–0.83) and 0.40 (95% CI = 0.15–1.12) when the other SNP was homozygous for the minor allele. Nevertheless, none of the associations remained significant after correction for multiple testing. **Conclusion:** Thus, there is an indication of an interaction between *APOA5* and *USF1* on the risk for metabolic syndrome.

Introduction

The prevalence of the metabolic syndrome has increased in epidemic proportions worldwide [1]. Apolipoprotein A5 (*APOA5*) and upstream stimulatory factor 1 (*USF1*) are genes both related to metabolic syndrome or its characteristic traits [2].

APOA5 is an apolipoprotein discovered independently by two separate research groups [3, 4] in 2001. The corresponding gene is located on chromosome 11 in the *APOA1/C3/A4/A5* gene cluster. The *APOA5* locus is linked to familial combined hyperlipidemia (FCHL) and elevated triglyceride (TG) levels, a known risk factor for cardiovascular diseases (CVD) [5]. FCHL is the most common inherited dyslipidemia with a prevalence of 1–2% in western populations and of about 20% in patients with premature coronary heart disease (CHD) [6]. The key role of APOA5 in the regulation of TG levels has been supported by several epidemiologic and functional studies [7] and confirmed in different ethnic groups, e.g. in Caucasians, Japanese, and Chinese [8].

USF1 is localized on chromosome 1 in another locus linked to FCHL [9]. It encodes for a ubiquitously expressed transcription factor that regulates some 40 genes involved with or actually crucial to glucose and fat metabolism [10]. USF1

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belongs to the family of basic helix-loop-helix leucine zipper transcription factors, dimerizes – most often with USF2 – and binds to palindromic E-box sequence in the promoter area of its target genes. Thus it activates transcription in response to different stimuli. *APOA5* belongs to that group of genes regulated by USF; insulin stimuli reduce the association of USF1 transcription factor to *APOA5* E-box [11]. Associations between *USF1* variants and FCHL [9] and associations of *USF1* SNPs with TG levels and low density lipoprotein (LDL) levels in blood [2] have been found. Associations with CVD and overall mortality in women also have been shown [12].

The changes in blood lipid levels for *APOA5* and *USF1* described above occur similarly in the metabolic syndrome, an important risk factor of CHD morbidity and mortality [2].

Main effects of APOA5 [7] and USF1 were already investigated in KORA S4 [13]. Grallert et al. [7] found significant effects of *APOA5* SNPs on two features of the metabolic syndrome, namely elevated TG levels and lowered HDL levels, but no effects on metabolic syndrome itself. The effects of *USF1* SNPs were investigated by Holzapfel et al. [13] who showed an association with lowered LDL levels.

Having both environmental and genetic risk factors [1], the syndrome's genetic background was our focus. We have examined a potential gene-gene interaction (i.e. deviation from the summation of single effects) between *APOA5* and *USF1* and the metabolic syndrome using data from a large population-based study sample from southern Germany.

Material and Methods

Study Population

Data were drawn from the KORA study (Cooperative Health Research in the Region of Augsburg, Germany). KORA was initiated in the Augsburg region in 1996 as a research platform for population-based studies [14, 15]. In detail, we used data of the S4 survey that was performed from 1999 to 2001 and in which information about the participants' cardiovascular risk factors was obtained. KORA S4 included 1,653 participants of the 55- to 74-year age group. Overall, 1,622 subjects (829 men and 793 women) were genotyped successfully and comprised the study population of our analyses. Among those, 1,353 subjects participated in an oral glucose tolerance test (OGTT); in the remaining 269 subjects only fasting glucose levels were measured.

Genotyping

Seven SNPs of the *APOA5* gene and six SNPs of the *USF1* gene, genotyped in KORA S4, were used to investigate the hypothesized gene-gene interaction with the risk of having the metabolic syndrome (table 1). SNPs were selected both on functional (e.g. in or near exons, in promoter regions) and positional (distance <5 kb where possible) basis with simultaneous consideration of validation and frequency. SNPs containing the same information ($r^2 \geq 0.8$) were excluded. Further information like minor allele frequencies, LD plots, and SNP position in the genes had been published in [7] (also see online supplement) for APOA5 and in [13] for USF1 and are also partly available in table 1.

Genotyping was performed with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) as described elsewhere [16]. Assessing the Hardy-Weinberg equilibrium (HWE) with an exact test using a permutation approach developed by Guo and Thompson [17] revealed that no SNP departed from HWE (table 1). Genotyping succeeded with call rates greater than 94.0%, and discordance was less than 0.5% in 210 routine duplicates in KORA S4 [7, 13].

Definition of the Metabolic Syndrome

The metabolic syndrome was diagnosed using the definition provided by the National Cholesterol Education Program's Adult Treatment Panel III (NCEP (AIII)) including medication [adapted from 18] (table 2).

Statistics

The risk for having the metabolic syndrome was estimated by logistic regression and the assumption of an additive genetic model. Risks were displayed as odds ratios (ORs) with 95% confidence intervals (95% CI). Adjustment for age and sex was performed. To assess the gene-gene interaction, i.e. whether the metabolic syndrome risk of one SNP was modified by another SNP, we included both SNPs and additionally an interaction term of both SNPs in each model. Therefore, we combined the seven *APOA5* SNPs with the six *USF1* SNPs and performed the respective logistic regression leading to 42 different models. Each SNP was coded as 0 for homozygous major allele, as 1 for heterozygous, and as 2 for homozygous minor allele. The OR of having a metabolic syndrome per copy of the minor allele for the first SNP (SNP 1) by the value of the other SNP (SNP 2) was drawn from this regression and calculated by exp[estimate(SNP 1) + estimate(SNP 1 × SNP 2) × value(SNP 2)]. The respective 95% CIs were drawn from the logistic regression using the regression coefficients and the covariance matrix.

Furthermore, we performed additional analyses with five quantitative traits of the metabolic syndrome (waist circumference, HDL cholesterol, TG levels, fasting blood glucose, and blood pressure) as endpoints using separate linear regression models. Again, we estimated 42 different models for each of the five quantitative traits with age and sex adjustment including the two SNPs and an interaction term of both SNPs. The increase of the mean quantitative trait per copy of the minor allele for the first SNP (SNP 1) by the value of the other SNP (SNP 2) was drawn from this regression and calculated by estimate(SNP 1) + estimate(SNP 1 × SNP 2) × value(SNP 2) and vice versa for SNP 2. The respective 95% CIs were drawn from the linear regression using the regression coefficient and the covariance matrix.

We assumed interaction effects with p values less than 0.05 as statistically significant. Bonferroni correction for multiple testing was done with a corrected significance level of 0.001 (alpha of 0.05/7 *APOA5*-SNPs × 6 *USF1*-SNPs). All calculations were conducted with the statistical software SAS (SAS V. 9.1 Cary, NC, USA).

Results

The prevalence of the metabolic syndrome according to NCEP (AIII) definition was 41.1% in the overall study population. Most frequently the participants achieved one or two of the five criteria for the metabolic syndrome (24.4 or 23.8%); only 11% of the study population revealed none of the syndrome's characteristics. The most often met criterion for the metabolic syndrome was 'elevated blood pressure or antihypertensive drug treatment' (1,197 persons (74.1% of all participants)) followed by large waist circumference (790 persons (48.8%)), lowered HDL cholesterol (655 participants (40.8%)), elevated TG levels (479 persons (29.6%)), and raised blood glucose levels (425 individuals (26.6%))). According to this observation, antihypertensive drugs were the most frequently taken drugs in the study population (37.2% of the participants compared

Table 1. Characteristics of the SNPs included in the analyses

SNP[a] (dbSNP name)	Function/region of SNP	Genotype	Frequency (%) [frequency among MetS probands (%)]			HWE, p value
			major allele homozygous	heterozygous	minor allele homozygous	
USF1						
rs2774279	synonymous[b] / exon 12 of *ARHGAP30*	C>T	675 (42.19) [381 (40.92)]	733 (45.81) [441 (47.37)]	192 (12.00) [109 (11.71)]	0.75
rs10908821	intron 1 / 3'UTR	C>G	1216 (75.91) [708 (75.97)]	358 (22.35) [207 (22.21)]	28 (1.75) [17 (11)]	0.78
rs2073658	intron 7	G>A	814 (50.75) [461 (49.36)]	666 (41.52) [397 (42.51)]	124 (7.73) [76 (8.14)]	0.45
rs2774276	intron 5	G>C	944 (58.89) [556 (59.53)]	572 (35.68) [325 (34.8)]	87 (5.43) [53 (5.67)]	0.98
rs2516839	5' UTR/ exon 2	A>G	652 (40.78) [393 (42.3)]	737 (46.09) [424 (45.64)]	210 (13.13) [112 (12.06)]	0.94
rs1556259	3' near gene of *USF1* / intron 1	T>C	1217 (75.92) [721 (77.28)]	356 (22.21) [197 (21.11)]	30 (1.87) [15 (1.61)]	0.51
APOA5						
rs2542061	intergenic region	A>G	398 (25.17) [223 (24.32)]	795 (50.28) [469 (317)]	388 (24.54) [225 (24.54)]	0.82
rs633867	3' UTR	C>T	1,493 (95.77) [877 (96.37)]	65 (4.17) [32 (3.52)]	1 (0.06) [1 (0.11)]	0.74
rs1729411	promoter region	G>A	1,188 (74.76) [688 (74.38)]	379 (23.85) [223 (24.11)]	22 (1.38) [14 (1.51)]	0.18
rs662799	promoter	A>G	1,376 (85.63) [817 (87.38)]	218 (13.57) [113 (12.09)]	13 (0.81) [5 (0.53)]	0.18
rs619054	3' UTR in exon 3	G>A	872 (54.23) [499 (53.31)]	627 (38.99) [372 (39.74)]	109 (6.78) [65 (6.94)]	0.80
rs3135507	missense[b] (Met>Val)/ exon 3	C>T	1,500 (94.04) [876 (94.4)]	94 (5.89) [51 (5.5)]	1 (0.06) [1 (0.11)]	0.70
rs3135506	missense[b] (Trp>Ser)/ exon 2 signal peptide	G>C	1,360 (88.71) [809 (89.89)]	165 (10.76) [89 (9.89)]	8 (0.52) [2 (0.22)]	0.22

[a]Bold SNPs are enclosed in the significant combinations in the overall study population.
[b]Amino acid change due to SNP.

Table 2. Definition of the metabolic syndrome from NCEP(AIII) including medication[a]

Waist circumference > 102 cm (men) *or* >88 cm (women)
HDL cholesterol level < 1.04 mmol/l (men) *or* <1.29 mmol/l (women) *or* treatment with lipid lowering drugs
Triglyceride level ≥ 1.7145 mmol/l *or* treatment with lipid lowering drugs
Fasting glucose ≥ 110 mg/dl *or* ensured diabetic (OGTT) *or* treatment with oral antidiabetics *or* insulin
Blood pressure ≥130 / ≥85 mm Hg *or* treatment with antihypertensive drugs

[a]At least any three criteria out of five lead to classification of metabolic syndrome.

with 2.1% receiving insulin treatment, 5.2% receiving oral antidiabetic drug treatment, and 12.2% receiving lipid lowering drug application) (table 3).

The logistic regression analysis revealed overall a lower risk for the metabolic syndrome for one SNP per each copy of the minor allele of the other SNP. We performed this analysis to assess the interaction between two SNPs on the risk of having a metabolic syndrome. Two SNP × SNP interactions changing the risk for the metabolic syndrome were nominal on a significance level of 0.05. These interactions were observed between polymorphisms rs2516839 and rs3135506 (p = 0.0473), and between rs2073658 and rs1729411 (p = 0.0243). The first SNP of a combination denotes the *USF1* gene and the second SNP denotes the *APOA5* gene, what is pursued in the text.

All of those combinations were associated inversely with the risk for the metabolic syndrome, with increasing numbers

Table 3. Descriptive data of the study population

	Number of participants[a]			Mean ± standard deviation		
	overall	women	men	overall	women	men
Age, years	1,622	793	829	64 ± 5 (range 55–74)		
Waist circumference, cm	1,622	793	825	96.1 ± 11.8	91.0 ± 11.6	101.0 ± 9.8
HDL, mmol/l	1,620	792	828	1.49 ± 0.42	1.63 ± 0.44	1.35 ± 0.35
TG, mmol/l	1,620	785	820	1.49 ± 1.07	1.53 ± 1.00	1.76 ± 1.13
Fasting glucose, mg/dl	1,618	791	827	108 ± 33	105 ± 33	111 ± 32
Blood pressure, mm Hg	1,616	791	825	136.5 ± 20.5 / 80.5 ± 10.6	132.3 ± 20.0 / 78.5 ± 10.1	140.5 ± 20.1 / 82.4 ± 10.6
				Number of participants (% of all participants)		
				overall	women	men
Drug treatment[b]						
Insulin	1,617	791	826	33 (2.02%)	19 (2.40%)	14 (1.69%)
Oral antidiabetics	1,617	791	826	84 (5.19%)	33 (4.17%)	51 (6.17%)
Lipid lowering drugs	1,617	791	826	197 (12.18%)	89 (11.25%)	108 (13.08%)
Antihypertensives	1,617	791	826	602 (37.23%)	302 (38.18%)	300 (36.32%)
Metabolic syndrome (NCEP (AIII))	1,604	786	818	659 (41.08%)	300 (38.17%)	359 (43.89%)

[a]Number of persons used from KORA S4 study
[b]Number of persons with defined drug treatment.

of the minor alleles of *APOA5* and *USF1*. For the case with the lowest p value for interaction, rs2073658 × rs1729411, the OR of having a metabolic syndrome per copy of the minor allele for rs2073658 by rs1729411 was 0.81 (95% CI = 0.67–0.97) in homozygous state for the major allele (GG), 0.54 (95% CI = 0.34–0.86) in heterozygous state (GA); and 0.36 (95% CI = 0.16–0.81) in homozygous state for the minor allele (AA) (table 4). Conversely, for rs1729411, the OR was 0.74 (95% CI = 0.55–0.99) with rs2073658 in homozygous state for the major allele (GG); 0.49 (95% CI = 0.27–0.89) in heterozygous state (GA); and 0.33 (CI = 0.13–0.83) in homozygous state for the minor allele (AA). All other ORs are presented in table 4. These results did not remain nominal after Bonferroni correction.

Moreover, we performed a sex-stratified analysis and obtained different trends for associations of SNPs combinations compared to the overall analysis – in contrast to the overall analysis all of them associated with higher risk of metabolic syndrome presented with the minor alleles of *APOA5* and *USF1*. Due to the stratification, the number of cases decreased accordingly (partially even no case with homozygous minor allele for both SNPs), and thus ORs were very high (data not shown). Consequently, these results were not processed further, because of their uncertainty.

Furthermore, the quantitative traits of the metabolic syndrome were applied for analysis. One of the two interactions, namely rs2073658 × rs1729411, was also nominally associated with waist circumference (table 5). Significances were in a comparable dimension as the main analyses and similarly failed Bonferroni correction for multiple testing.

We also applied the other model assumptions (dominant, recessive) which revealed very similar results (data not shown).

Discussion

Our results from KORA S4 indicate a possible gene-gene interaction between *APOA5* and *USF1* variants and the metabolic syndrome in two combinations. The results showed a trend for association, if we refrained from Bonferroni correction. Persons who were homozygous for minor alleles in both SNPs of one of the found interactions seemed to have a lower risk for the metabolic syndrome than those homozygous for the major alleles or heterozygous. This conclusion implies that individuals with the homozygous genotype for the minor alleles of both SNPs of a found interaction combination have the lowest risk for metabolic syndrome in this context (ORs ranged between 0.33 and 0.40; table 4).

To our knowledge, these associations have not been investigated before.

According to Moore and Williams [19] gene-gene interactions play a relevant role in the genetic architecture of common complex diseases and can be seen from statistical and from biological view. Stating a relationship between those views occurs as a great challenge thereby. As we focused on a statistical interaction on population level we merely consider some aspects subsequently about explanations of the statistical effect and about biological plausibility.

From biological view there is no physical interaction between USF1 and APOA5 proteins (but protein-DNA interplay), but a statistical interaction may also occur without physical interaction of biomolecules, if these have an impact on the same phenotype as APOA5 and USF1 do have on blood lipid levels.

None of the investigated *APOA5* SNPs are located within the E-box, where USF dimers physically collaborate with the DNA [11]. Therefore the statistical interaction effects did not suggest biological plausibility simply by variations at the locus of physical interaction by a sequence alteration.

Two of the SNPs included in the interaction calculation cause a missense exchange. Namely, these are rs3135506 (Trp>Ser) and rs3135507 (Met>Val). The missense exchange rs3813609 (Val>Leu) is represented by another *USF1*-SNP (rs2516839, which participates in SNP combinations with interaction effect). None of the others change the amino acid sequence. SNP rs3135506 alters the cleavage site of signal sequence and, thus, lowers translocation and secretion of the APOA5 protein [20]. The interaction combination including rs3135506 showed the lowest significance of all found nominal combinations (p = 0.0473) (table 4). Altogether, amino acid changes might contribute to a biological background of the statistical effects seen here.

There is seemingly none of the *USF1* SNPs located in any of the protein domains responsible for dimerization or domains needed for transactivation. Dimerization then might not be affected by the variations, but an influence on the complex transcription machinery is conceivable.

Via presence of insulin, USF1/USF2 dimers would be phosphorylated and subsequently non-functional for *APOA5* transcription initiation [11]. Maybe the SNP variants influence this regulation mechanism positively or negatively and, thus, contribute to the interaction effect.

The major fraction of USF1 functions as heterodimers with USF2 [2]. Thus, an influence of *USF2* variations on the suggested interaction between *APOA5* and *USF1* should be considered. Actually, there are SNPs in the *USF2* gene, and one of them, rs45614038, even causes a missense mutation (*www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId = 7392 and chooseRs = all in Jan 2009*).

These advisements make us consider that either there are yet unknown regulatory regions, where these SNPs are located, or the underlying cause is by far more complex than variations in any regulatory regions of these genes. So we also consider contributions in generating the statistical interaction effect by the very complex regulatory machinery of and between *APOA5* and *USF1* and by other affected genes or proteins that take part in the metabolic pathways regulated by APOA5 after *USF1* stimulation. For example, Prieur et al. [21] described an activation of *APOA5* synergistically accomplished by thyroid hormone receptor and *USF1*. Further examples of regulators of *APOA5* are RORα, PPARα, and LXR [8]. Recently, Nowak et al. [22] could demonstrate the

Table 4. Results from gene-gene interaction analysis for risk of the metabolic syndrome[a]

Number	SNP	rs-number	Estimate	Standard error	p value	OR for SNP 1 by SNP 2			OR for SNP 2 by SNP1		
						SNP 2 = 0 (95% CI)	SNP 2 = 1 (95% CI)	SNP 2 = 2 (95% CI)	SNP 1 = 0 (95% CI)	SNP 1 = 1 (95% CI)	SNP 1 = 2 (95% CI)
1564	SNP 1	rs2073658	–0.21	0.09	0.026						
	SNP 2	rs1729411	–0.30	0.15	0.045	0.81 (0.67–0.97)	0.54 (0.34–0.86)	0.36 (0.16–0.81)	0.74 (0.55–0.99)	0.49 (0.27–0.89)	0.33 (0.13–0.83)
	SNP 1 × SNP 2	rs2073658 × rs1729411	–0.41	0.18	0.024						
1497	SNP 1	rs2516839	0.06	0.08	0.472						
	SNP 2	rs3135506	–0.04	0.23	0.870	1.06 (0.90–1.25)	0.66 (0.38–1.14)	0.40 (0.15–1.12)	0.96 (0.62–1.50)	0.60 (0.25–1.40)	0.37 (0.10–1.36)
	SNP 1 × SNP 2	rs2516839 × rs3135506	–0.48	0.24	0.047						

[a]Logistic regression model included the covariates sex (dichotomous), age, SNP 1, SNP 2 and SNP 1 × SNP 2 (all continuous). SNP coding: 0 = homozygous for major allele, 1 = heterozygous, 2 = homozygous for minor allele. After correction for multiple testing, none of the interactions remained significant.

Table 5. Results from gene-gene interaction analysis with the quantitative traits of metabolic syndrome[a]

Number	SNP 1 * SNP 2	Estimate	Standard error	p value	Mean increase for SNP 1 by SNP 2			Mean increase for SNP 2by SNP1		
					SNP 2 = 0	SNP 2 = 1	SNP 2 = 2	SNP 1 = 0	SNP 1 = 1	SNP 1 = 2
Fasting blood glucose level										
1547	rs2774276 × rs633867	–12.17	6.16	0.05	1.93 (–0.86 to 4.72)	–10.25 (–23.08 to 2.58)	–22.42 (–40.34 to –4.50)	–13.08 (–23.29 to –2.87)	–25.25 (–42.14 to –8.36)	–37.43 (–59.02 to –15.84)
1574	rs2774276 × rs3135507	–11.13	5.60	0.05	1.38 (–1.39 to 4.15)	–9.75 (–21.55 to 2.05)	–20.89 (–37.34 to –4.44)	–9.74 (–18.64 to –0.84)	–20.88 (–36.08 to –5.68)	–32.01 (–51.59 to –12.43)
1577	rs1556259 × rs3135507	16.37	7.68	0.03	–2.92 (–6.35 to 0.51)	13.45 (–2.42 to 29.32)	29.82 (7.64 to 52.00)	–0.05 (–7.60 to 7.50)	16.32 (–1.34 to 33.98)	32.69 (8.88 to 56.50)
HDL level										
1600	rs3737787 × rs662799	0.08	0.04	0.04	0.01 (–0.02 to 0.04)	0.10 (–0.26 to 0.46)	0.18 (–0.33 to 0.69)	0.12 (0.05 to 0.19)	0.20 (–0.31 to 0.71)	0.29 (–0.43 to 1.01)
1597	*rs2073658* × rs662799	0.09	0.04	0.04	0.01 (–0.02 to 0.04)	0.10 (–0.26 to 0.46)	0.19 (–0.32 to 0.70)	0.12 (0.05 to 0.19)	0.21 (–0.30 to 0.72)	0.30 (–0.42 to 1.02)
1586	*rs2073658* × rs651821	0.08	0.04	0.05	0.01 (–0.02 to 0.04)	0.10 (–0.26 to 0.46)	0.18 (–0.33 to 0.69)	0.12 (0.05 to 0.19)	0.21 (–0.31 to 0.73)	0.29 (–0.44 to 1.02)
1585	rs1556259 × rs651821	–0.14	0.06	0.03	0.01 (–0.03 to 0.05)	–0.13 (–0.56 to 0.30)	–0.26 (–0.86 to 0.34)	0.04 (–0.02 to 0.10)	–0.10 (–0.59 to 0.39)	–0.23 (–0.93 to 0.47)
Waist circumference										
1580	rs3737787 × *rs1729411*	1.97	0.92	0.03	0.65 (–0.28 to 1.58)	2.62 (–0.14 to 5.38)	4.58 (0.79 to 8.37)	0.58 (–0.89 to 2.05)	2.54 (–0.72 to 5.80)	4.51 (0.14 to 8.88)
1578	**rs2073658 × rs1729411**	2.00	0.93	0.03	0.74 (–0.19 to 1.67)	2.73 (–0.03 to 5.49)	4.73 (0.93 to 8.53)	0.64 (–0.83 to 2.11)	2.63 (–0.64 to 5.90)	4.63 (0.25 to 9.01)
1517	rs3813609 × *rs3135506*	2.49	1.19	0.04	0.52 (–0.30 to 1.34)	3.01 (–0.03 to 6.05)	5.50 (1.28 to 9.72)	1.81 (–0.43 to 4.05)	4.30 (0.05 to 8.55)	6.79 (1.22 to 12.36)
1509	**rs2516839 × rs3135506**	2.49	1.21	0.04	0.50 (–0.33 to 1.33)	2.98 (–0.09 to 6.05)	5.47 (1.20 to 9.74)	1.85 (–0.38 to 4.08)	4.34 (0.08 to 8.60)	6.83 (1.23 to 12.43)
1511	rs2774279 × *rs3135506*	–2.45	1.10	0.03	–0.69 (–1.52 to 0.14)	–3.14 (–6.03 to –0.25)	–5.59 (–9.59 to –1.59)	–1.87 (–4.27 to 0.53)	–4.32 (–8.59 to –0.05)	–6.78 (–12.32 to –1.24)

[a]Linear regression model included the covariates sex (dichotomous), age, SNP 1, SNP 2 and SNP 1 × SNP 2 (all continuous), SNP coding: 0 = homozygous for major allele, 1 = heterozygous, 2 = homozygous for minor allele. SNPs italics were also significant in the analysis with the metabolic syndrome, combinations in bold were also found in the analysis with the metabolic syndrome. After correction for multiple testing, none of the interactions remained significant.

glucose dependency of *APOA5* activation by USF dimers via a dephosphorylation mechanism.

The relevance of investigating the connection between *USF1* and *APOA5* was already underlined by a review of Naukkarinen et al. [23] in 2006 that summarizes genetic findings on the risk for FCHL. They found both genes with very interesting clues not only on the risk for FCHL but also on the metabolic syndrome based on the disorders´ overlapping molecular pathogenesis.

Gene-gene interaction has already been identified for other genes. For example, Pessi et al. [24] found an interaction effect between *FCGR2A* and *CRP* on the intima media thickness in young Finnish men with a lack of independent effects of *FCGR2A* polymorphisms on IMT, and an investigation in French Canadians [25] showed significant interaction effect of gene variants in the VLDL catabolism.

Overall, we suppose that we have not found the causal or functional SNPs for the interaction effect, but possibly markers for it.

Our study is strengthened by the selection of the participants from a large homogeneous population-based sample with high-quality phenotyping (e.g. anthropometric measures, OGTT). However, our findings could be only chance discoveries. It should be considered that there were very few participants with both *USF1* and *APOA5* SNPs in homozygous state for the minor allele in all found combinations with nominal interactions (no individual with GG in rs2516839 and concomitant CC in rs3135506; one individual with AA in rs2073658 and concomitant AA in rs1729411, and only a few persons in some heterozygous combinations out of about 1,600 individuals) (data not shown). Therefore, our results should be reproduced in larger study samples, especially to figure out the sex-stratified effects.

Moreover, our study population shows a high prevalence of the metabolic syndrome compared to numbers reported for different populations. This likely might be due to the older age and the high mean of the BMI in the group. Day et al. [26] also reported a prevalence of 40% with the NCEP (AIII) definition in a US group of probands aged 60 to 69 years; thus the age effect seems to explain the prevalence we found.

In conclusion, we found a suggestive gene-gene interaction between *APOA5* and *USF1* by statistical analysis of data from the KORA study. However, the underlying mechanisms are speculative.

Finally, we would appreciate that other research groups resume our work and either replicate our findings in a proper and well powered sample or focus on biological causes of the found statistical effect.

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Disclosure

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References

1 Candib LM: Obesity and diabetes in vulnerable populations: reflection on proximal and distal causes. Ann Fam Med 2007;5:547–556.
2 Shoulders CC, Naoumova RP: USF1 implicated in the aetiology of familial combined hyperlipidaemia and the metabolic syndrome. Trends Mol Med 2004; 10:362–365.
3 van der Vliet HN, Sammels MG, Leegwater AC, Levels JH, Reitsma PH, Boers W, Chamuleau RA: Apolipoprotein A-V: a novel apolipoprotein associated with an early phase of liver regeneration. J Biol Chem 2001;276:44512–44520.
4 Pennacchio LA, Olivier M, Hubacek JA, Cohen JC, Cox DR, Fruchart JC, Krauss RM, Rubin EM: An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. Science 2001;294:169–173.
5 Ribalta J, Figuera L, Fernandez-Ballart J, Vilella E, Castro CM, Masana L, Joven J: Newly identified apolipoprotein AV gene predisposes to high plasma triglycerides in familial combined hyperlipidemia. Clin Chem 2002;48:1597–1600.
6 Eichenbaum-Voline S, Olivier M, Jones EL, Naoumova RP, Jones B, Gau B, Patel HN, Seed M, Betteridge DJ, Galton DJ, Rubin EM, Scott J, Shoulders CC, Pennacchio LA: Linkage and association between distinct variants of the APOA1/C3/A4/A5 gene cluster and familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol 2004;24:167–174.
7 Grallert H, Sedlmeier EM, Huth C, Kolz M, Heid IM, Meisinger C, Herder C, Strassburger K, Gehringer A, Haak M, Giani G, Kronenberg F, Wichmann HE, Adamski J, Paulweber B, Illig T, Rathmann W: APOA5 variants and metabolic syndrome in Caucasians. J Lipid Res 2007;48:2614–2621.
8 Jakel H, Nowak M, Helleboid-Chapman A, Fruchart-Najib J, Fruchart JC: Is apolipoprotein A5 a novel regulator of triglyceride-rich lipoproteins? Ann Med 2006;38:2–10.
9 Pajukanta P, Lilja HE, Sinsheimer JS, Cantor RM, Lusis AJ, Gentile M, Duan XJ, Soro-Paavonen A, Naukkarinen J, Saarela J, Laakso M, Ehnholm C, Taskinen MR, Peltonen L: Familial combined hyperlipidemia is associated with upstream transcription factor 1 (USF1). Nat Genet 2004;36:371–376.

10 Putt W, Palmen J, Nicaud V, Tregouet DA, Tahri-Daizadeh N, Flavell DM, Humphries SE, Talmud PJ: Variation in USF1 shows haplotype effects, gene : gene and gene : environment associations with glucose and lipid parameters in the European Atherosclerosis Research Study II. Hum Mol Genet 2004;13:1587–1597.
11 Nowak M, Helleboid-Chapman A, Jakel H, Martin G, Duran-Sandoval D, Staels B, Rubin EM, Pennacchio LA, Taskinen MR, Fruchart-Najib J, Fruchart JC: Insulin-mediated down-regulation of apolipoprotein A5 gene expression through the phosphatidylinositol 3-kinase pathway: role of upstream stimulatory factor. Mol Cell Biol 2005;25:1537–1548.
12 Komulainen K, Alanne M, Auro K, Kilpikari R, Pajukanta P, Saarela J, Ellonen P, Salminen K, Kulathinal S, Kuulasmaa K, Silander K, Salomaa V, Perola M, Peltonen L: Risk alleles of *USF1* gene predict cardiovascular disease of women in two prospective studies. PLoS Genet 2006;2:e69.
13 Holzapfel C, Baumert J, Grallert H, Müller A, Thorand B, Khuseyinova N, Herder C, Meisinger C, Hauner H, Wichmann H, Koenig W, Illig T, Klopp N: Genetic variants in the *USF1* gene are associated with LDL cholesterol levels and incident T2DM in women: results from the MONICA/KORA Augsburg case-cohort study, 1984–2002. Eur J Endocrinol 2008;159:407–416.
14 Holle R, Happich M, Lowel H, Wichmann HE: KORA – a research platform for population based health research. Gesundheitswesen 2005;67(suppl 1): S19–S25.
15 Wichmann HE, Gieger C, Illig T: KORA-gen – resource for population genetics, controls and a broad spectrum of disease phenotypes. Gesundheitswesen 2005;67(suppl 1):S26–S30.
16 Vollmert C, Windl O, Xiang W, Rosenberger A, Zerr I, Wichmann HE, Bickeboller H, Illig T, Kretzschmar HA: 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 2006;43:e53.
17 Guo SW, Thompson EA: Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 1992;48:361–372.
18 Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C: Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004; 109:433–438.
19 Moore JH, Williams SM: Traversing the conceptual divide between biological and statistical epistasis: systems biology and a more modern synthesis. Bioessays 2005;27:637–646.
20 Talmud PJ, Palmen J, Putt W, Lins L, Humphries SE: Determination of the functionality of common APOA5 polymorphisms. J Biol Chem 2005;280: 28215–28220.
21 Prieur X, Huby T, Coste H, Schaap FG, Chapman MJ, Rodriguez JC: Thyroid hormone regulates the hypotriglyceridemic gene *APOA5*. J Biol Chem 2005; 280:27533–27543.
22 Nowak M, Helleboid-Chapman A, Jakel H, Moitrot E, Rommens C, Pennacchio LA, Fruchart-Najib J, Fruchart JC: Glucose regulates the expression of the apolipoprotein A5 gene. J Mol Biol 2008; 380:789–798.
23 Naukkarinen J, Ehnholm C, Peltonen L: Genetics of familial combined hyperlipidemia. Curr Opin Lipidol 2006;17:285–290.
24 Pessi T, Eklund C, Huhtala H, Raitakari OT, Juonala M, Kahonen M, Viikari JS, Lehtimaki T, Hurme M: *CRP* and *FCGR2A* genes have an epistatic effect on carotid artery intima-media thickness: the Cardiovascular Risk in Young Finns Study. Int J Immunogenet 2008;36:39–45.
25 Brisson D, St Pierre J, Santure M, Hudson TJ, Despres JP, Vohl MC, Gaudet D: Genetic epistasis in the VLDL catabolic pathway is associated with deleterious variations on triglyceridemia in obese subjects. Int J Obes (Lond) 2007;31:1325–1333.
26 Day C: Metabolic syndrome, or what you will: definitions and epidemiology. Diab Vasc Dis Res 2007; 4:32–38.