Genetic Association and Interaction Analysis of USF1 and APOA5 on Lipid Levels and Atherosclerosis

Pirkka-Pekka Laurila, Jussi Naukkarinen, Kati Kristiansson, Samuli Ripatti, Tuuli Kauttu, Kaisa Silander, Veikko Salomaa, Markus Perola, Pekka J. Karhunen, Philip J. Barter, Christian Ehnholm, Leena Peltonen

Objective—USF1 is a ubiquitous transcription factor governing the expression of numerous genes of lipid and glucose metabolism. APOA5 is a well-established candidate gene regulating triglyceride (TG) levels and has been identified as a downstream target of upstream stimulatory factor. No detailed studies about the effect of APOA5 on atherosclerotic lesion formation have been conducted, nor has its potential interaction with USF1 been examined.

Methods and Results—We analyzed allelic variants of USF1 and APOA5 in families (n=516) ascertained for atherogenic dyslipidemia and in an autopsy series of middle-aged men (n=300) with precise quantitative measurements of atherosclerotic lesions. The impact of previously associated APOA5 variants on TGs was observed in the dyslipidemic families, and variant rs3135506 was associated with size of fibrotic aortic lesions in the autopsy series. The USF1 variant rs2516839, associated previously with atherosclerotic lesions, showed an effect on TGs in members of the dyslipidemic families with documented coronary artery disease. We provide preliminary evidence of gene-gene interaction between these variants in an autopsy series with a fibrotic lesion area in the abdominal aorta (P=0.0028), with TGs in dyslipidemic coronary artery disease subjects (P=0.03), and with high-density lipoprotein cholesterol (P=0.008) in a large population cohort of coronary artery disease patients (n=1065) in which the interaction for TGs was not replicated.

Conclusion—Our findings in these unique samples reinforce the roles of APOA5 and USF1 variants on cardiovascular phenotypes and suggest that both genes contribute to lipid levels and aortic atherosclerosis individually and possibly through epistatic effects. (Arterioscler Thromb Vasc Biol. 2010;30:346-352.)

Key Words: genes ■ USF1 ■ APOA5 ■ lipids ■ atherosclerosis ■ epistasis

Studying the genetic architecture of complex diseases poses the greatest challenge for genetic analysis. This is largely because of the difficulty in isolating individual gene effects from gene-gene interactions and environmental influences. To go beyond a single gene association, exploration of epistasis and environmentally dependent genetic factors is needed. Epistasis, defined as “deviation from additivity,” is suggested to be a ubiquitous component of the genetic architecture of complex diseases.1 To successfully model disease susceptibility, the observed statistical gene-gene interaction should be linked to biological evidence of the interaction.

In this study we have examined the individual effects of variants in USF1 and APOA5, 2 promising cardiovascular candidate genes, as well as the potential epistasis between their allelic variants on cardiovascular phenotypes. The upstream stimulatory factor (USF1) is a leucine zipper-type transcription factor that governs the expression of multiple genes involved in lipid2 and glucose3 metabolism. USF1 binds so-called E-box elements located in the proximal promoter region of its target genes. The gene was initially associated with familial combined hyperlipidemia,4 and its association has been studied for metabolic syndrome,5,6 cardiovascular disease,7 and the extent of atherosclerotic lesions8,9 as well.

Apolipoprotein A5 (APOA5) was identified in 2001 by comparative sequencing.10 Epidemiological studies in multiple populations support the role of APOA5 in triglyceride (TG) metabolism11-14 and the pathogenesis of metabolic syndrome.15 APOA5 has been identified as a downstream target gene of USF1,16 and being members of the same biological pathway, the study of potential interaction between their DNA sequence variation is warranted.

Here, we report associations of USF1 and APOA5 with lipid levels in Australian ADL families and with aortic atherosclerosis in a Finnish autopsy series. We also provide
preliminary evidence of a potential interaction between these genes. For analysis of genetic contribution to cardiovascular risk factors, we chose an Australian family sample ascertained for atherogenic dyslipidemia (ADL) with extremely well-defined lipid traits, and for end point characterization we selected a unique autopsy series of Finnish middle-aged men featuring detailed measurements of coronary and aortic macrovascular lesions, ideally suited for quantitative studies of atherosclerosis. In addition, as a replication material for the potential epistatic effect, we have used a large Finnish population cohort, FINRISK-97 (FR97). Here, we report associations of USF1 and APOA5 with lipid levels in Australian ADL families and with aortic atherosclerosis in a Finnish autopsy series and we provide preliminary evidence of a potential interaction between these genes.

Materials and Methods

Study Subjects

The Australian ADL families (Table 1) were collected through the large Genetic Epidemiology of Metabolic Syndrome (GEMS) family network study. The Australian GEMS subjects are white and include 139 families (n = 516), mostly consisting of sibling pairs and trios; only 4 families were large enough to be informative for linkage analysis. Families were ascertained via probands with ADL, defined as the 75th percentile and high-density lipoprotein (HDL) cholesterol <25th percentile, both adjusted for sex and age (18 to 82 years). There were 235 affected (46%) and 281 unaffected (54%) individuals. The protocol was approved by the institutional ethics committee, and written consent was obtained from each participant.

The Helsinki Sudden Death Study (HSDS) consists of 2 prospective series of Finnish white middle-aged men with sudden death, who underwent medicolegal autopsy, of which the latter (n = 300), collected in 1991–1992, was used here. The areas of specific lesion types are expressed as percentages of the total lesion area of the arterial sample. The HSDS was approved by the ethics committee of the University of Helsinki Department of Forensic Medicine. The FR97 population cohort is designed to assess the prevalence and risk factors of cardiovascular diseases in Finland and consists of a random sample of 8141 Finnish whites. The FR97 study was approved by the ethical committee of the National Public Health Institute, and the principles of Helsinki Declaration were followed.

Genotyping and Quality Control

The subjects were genotyped using the Sequenom iPLEX platform (www.sequenom.com) and an in-house developed, array-based genotyping technology. To capture all of the known common allelic diversity of USF1 and APOA5, tag single-nucleotide polymorphisms (SNPs) constituting 5 USF1 and 4 APOA5 haplotypes were selected from the HapMap database and also according to previous studies. Some of the SNPs are redundant in terms of haplotype structure, because 4 APOA5 haplotypes could have been derived from only 3 SNPs and 5 USF1 haplotypes from 4 SNPs, but the SNPs were included here anyway to make our study comparable with previous studies. The PCR and extension primers for the iPLEX assay were designed using the SpectroDESIGNER software version 2.0 (Sequenom Inc), and the genotypes were analyzed by the SpectroTYPER PT 2.0 software. Every SNP genotyped had a genotyping rate >95% and a minor allele frequency >5%. The genotype frequencies of each SNP were in Hardy-Weinberg equilibrium (P > 0.05).

Statistical Analyses

All of the genotypes in the Australian families were checked for correct mendelian transmission using PedCheck version 1.1. No mendelian errors were observed in any of the families. Haplotypes were constructed and their frequencies calculated using the Haploview 4.1 software. Because of the high linkage disequilibrium (LD) structure of the region, haplotypes could be manually assigned unambiguously. Haplotypes <5% frequency were not included in analyses. Haplotype frequencies in the Australian families and HSDS subjects were similar, with the exception that the most common USF1 haplotype in HSDS was the second most common haplotype in Australians and vice versa (Figure). An additive model for haplotype and single-marker association analyses was assumed.

The association analysis for quantitative traits was conducted using the mixed linear model, adjusting for sex, age, and body mass index. In the Australian families and FR97 subjects, TG and HDL cholesterol values were log transformed. Men and women were analyzed separately and jointly, and individuals less than and more than 55 years were also analyzed separately. In the Finnish autopsy series, the variables describing atherosclerosis were square-root transformed, and individuals with no detectable plaque were not included in the analyses, because their inclusion would have led to a heavily skewed distribution. The interaction effect was defined as deviation from an additive model. To control for the family structure in Australian ADL families, numeric family identifications were included in the model as cluster variables and individual level measurements as random variables. Bonferroni correction for multiple testing was used so that the raw P value was multiplied by the number of genetic markers tested. Although each marker was tested for 2 phenotypes, TG and HDL cholesterol, we did not perform an additional multiplication of 2 for the corrected P values, because the correlation between TG and HDL cholesterol was strong (in Australian ADL families; Pearson r = -0.65; P = 5 × 10^-646). The statistical analyses were carried out with SPSS software (version 15.0).

Results

Association of USF1 and APOA5 Markers With TGs and HDL Cholesterol in Australian ADL Families

We genotyped a total of 6 USF1 tag SNPs and 4 APOA5 tag SNPs in 139 Australian families (n = 516) ascertained for ADL and were able to construct 5 USF1 and 4 APOA5 haplotypes (Figure). Because both USF1 and APOA5 have been previously strongly associated with plasma lipid levels, we tested quantitative association of their variants with

| Table 1. Clinical Characteristics of Australian ADL Families (n = 516) |
|-----------------|-----------------|-----------------|
| Clinical Characteristic | Men (n = 281) | Women (n = 235) | Men + Women (n = 516) |
| Age, y | 46.8 ± 11.8 | 46.2 ± 15.6 | 46.5 ± 13.7 |
| Body mass index, kg/m² | 28.10 ± 3.85 | 27.30 ± 5.31 | 27.70 ± 4.62 |
| Triglycerides, mmol/L | 2.83 ± 0.96 | 1.99 ± 0.91 | 2.45 ± 1.00 |
| Cholesterol, mmol/L | 5.69 ± 1.09 | 5.54 ± 1.02 | 5.62 ± 1.06 |
| HDL cholesterol, mmol/L | 1.00 ± 0.25 | 1.26 ± 0.38 | 1.12 ± 0.34 |
| Low-density lipoprotein cholesterol, mmol/L | 3.50 ± 1.03 | 3.43 ± 0.99 | 3.47 ± 1.01 |
| Waist:hip ratio | 0.96 ± 0.06 | 0.89 ± 0.11 | 0.93 ± 0.10 |
| Glucose, mmol/L | 5.31 ± 0.90 | 5.08 ± 1.27 | 5.20 ± 1.10 |
| Insulin, μIU/L | 12.87 ± 14.30 | 11.60 ± 11.70 | 12.30 ± 13.10 |
| Systolic blood pressure, mm Hg | 139 ± 18.1 | 133 ± 20.9 | 136 ± 19.8 |
| Diastolic blood pressure, mm Hg | 84.0 ± 12.1 | 82.0 ± 12.5 | 83.0 ± 12.4 |
| Atherogenic dyslipidemia, n (%) | 153 (54) | 82 (35) | 235 (46) |
| CAD, n (%) | 43 (15.0) | 18 (7.7) | 61 (12.0) |

Data are presented as mean ± SD unless otherwise specified.
plasma TGs and HDL cholesterol, the component traits of ADL. Three APOA5 variants (rs3135506, rs662799, and rs10750097), associated previously with TG, showed significant association with TGs and HDL cholesterol in the Australian families (Table 2). The most common APOA5 haplotype, G-A-C-A (f=73%), was identified to be protective with its carriers featuring low TG (P=0.0003) and high HDL cholesterol (P=0.0001) levels (Table S1, please see the online Data Supplement at http://atvb.ahajournals.org). We also identified APOA5 risk haplotypes, C-A-C-G and G-G-C-G, associated with a lipid profile opposite to that of the carriers of the protective haplotype. To examine whether the effect of rs10750097, although it is the SNP of highest statistical significance, could only be attributable to its LD with rs3135506 and rs662799, the former SNP possessing previously reported functional evidence and the latter having earlier been shown to influence TG independently, we constructed a linear model including all 3 of the rs10750097 “risk allele” (G) containing haplotypes (G-A-T-G, G-G-C-G, and C-A-C-G) in the model. In this model, the effects of G-G-C-G and C-A-C-G haplotypes on TG were significant (P=0.006 and P=0.001, respectively), unlike the effect of G-A-T-G (P=0.452). The TG raising alleles of rs3135506 (C) and rs662799 (G) are always associated with the risk allele (G) of rs10750097, as is the non-significant T allele of rs17120035. This observation implies that rs10750097 being the most significant SNP could be accounted for by the rs10750097 risk allele (G) reflecting the combined effects of rs3135506 and rs662799 risk alleles (C and G, respectively) while the functionality of rs10750097 could be ruled out by the non-significant effect of G-A-T-G haplotype, also possessing the G-allele of rs10750097. In line with previous reports, our data thus suggest that the significance of rs10750097 is only a reflection of its tight LD structure with rs3135506 and rs662799, the variants with truly independent lipid associations.

The USF1 variant rs2516839, located in the 5′ untranslated region, was weakly associated with TG levels (P=0.047), especially in men (P=0.017), but no other USF1 SNP or haplotype showed any association with these lipid traits in the entire sample (Table 2 and Table S1). Because Lee et al showed that the effect of USF1 on lipid levels is enhanced in subjects with coronary artery disease (CAD), we performed association tests of USF1 variants on TG and HDL cholesterol in the Australian subjects with documented CAD (n=61). We observed association for 2 of the 6 USF1 SNPs; the carrierrship of major alleles of rs2516839 and rs1556259 was associated with higher TGs (P=0.0078 and P=0.0022, respectively) and lower HDL cholesterol levels (P=0.01 and P=0.02, respectively; Table 3). Association analysis for USF1 haplotypes in CAD patients revealed a parallel trend; a risk haplotype, C-C-C-T-A-A, composing the major alleles of both rs2516839 and rs1556259, was associated with elevated TGs (P=0.0007 and P=0.0022, respectively) and reduced HDL cholesterol (P=0.0002) and a protective haplotype, C-C-C-C-G-G, harboring minor alleles of the aforementioned SNPs, was linked to lower TGs (P=0.0002) and higher HDL cholesterol (P=0.001; Table S2). These findings imply that USF1 risk alleles might further exacerbate the already deteriorated cardiovascular status of the CAD patients.

In the Australian ADL families, association analyses were also conducted for men and women separately, because sex-specific effects of USF1 have been reported previously. No significant genotype-related sex effects on lipid levels were observed in the whole sample, but in the subset of CAD patients, significant association signals were only obtained for men (rs2516839, P=0.002 and rs1556259, P=0.0008), which is most likely attributable to the larger portion of men (n=46) from the CAD subset (n=65; data not shown). No age-specific effects of USF1 genotypes on lipid levels were observed.

APOA5 and Aortic Atherosclerosis in HDSDS

The HDSDS is composed of 300 men (aged 33 to 70 years) who underwent medicolegal autopsy. Because many of

Figure. Haplotype structure of the genes. A, USF1 haplotypes and their frequencies in HDSDS autopsy series and GEMS Australian ADL families, respectively. The LD values between SNPs are expressed as r², in the order of HSDS/GEMS, respectively (modified from Kristiansson et al). B, APOA5 haplotypes and their frequencies in HDSDS autopsy series and GEMS Australian ADL families, respectively. The LD values between SNPs are expressed as r², in the order of HSDS/GEMS, respectively.
the individuals (27%) died of cardiovascular causes, their postmortem macrovascular status (abdominal aorta, coronary arteries, and cerebral arteries) was subjected to careful pathologic characterization. We showed previously in HSDS subjects that the risk allele of rs3135506 for the specific lesion types constituting the total lesion area in the abdominal aorta, namely, fatty streaks, calcified lesions, fibrous lesions, and complicated lesions, as defined in Reference 18. We observed the strongest association of rs3135506 with fibrous lesions (P=0.003; Table 4). Association analyses for APOA5 haplotypes were conducted for the aforementioned phenotypes as well, but because each SNP specifically tags exactly 1 APOA5 haplotype, the results of the haplotype associations were virtually identical to those of single-marker analyses and were, thus, redundant (Tables S4 and S5).

**Gene-Gene Interaction Analysis Between USF1 and APOA5**

Because USF1 is a transcription factor that regulates the expression of dozens of downstream target genes,2 with APOA5 being one of them, it is tempting to hypothesize that an interaction in a DNA sequence variation of the genes might underlie the reported molecular interaction.16 We first modeled the situation by surveying USF1 and APOA5 alleles for independent effects on atherosclerosis in the HSDS sample. As stated above, the APOA5 SNP rs3135506 was

| Table 2. Association of APOA5 and USF1 Variants With TGs and HDL Cholesterol in Australian ADL Families (n=516) |
| Variable (n=516) | MAF, % | 1/2* | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | P† |
| APOA5 | | | | | | | | | | | | | | |
| rs3135506 | 12 | G/C | 2.33±0.07 | 2.82±0.21 | 3.84±0.53 | 0.0047‡ | 1.13±0.02 | 1.07±0.02 | 0.88±0.09 | 0.07 |
| rs662799 | 7 | A/G | 2.34±0.08 | 2.83±0.20 | 5.30±2.90 | 0.006 | 1.13±0.02 | 1.07±0.04 | 0.95±0.05 | 0.37 |
| rs17120035 | 8 | C/T | 2.44±0.08 | 2.50±0.17 | NA | 0.67 | 1.13±0.02 | 1.04±0.03 | NA | 0.01 |
| rs10750097 | 27 | A/G | 2.21±0.08 | 2.63±0.14 | 3.15±0.27 | 0.0009‡ | 1.16±0.02 | 1.08±0.02 | 1.00±0.04 | 0.001‡ |
| USF1 | | | | | | | | | | | | | | |
| rs10908821 | 14 | C/G | 2.46±0.09 | 2.47±0.14 | 1.91±0.29 | 0.79 | 1.13±0.02 | 1.07±0.02 | 1.17±0.13 | 0.26 |
| rs2073658 | 24 | C/T | 2.36±0.09 | 2.55±0.12 | 2.92±0.55 | 0.59 | 1.12±0.02 | 1.11±0.03 | 1.08±0.07 | 0.68 |
| rs2774276 | 26 | C/G | 2.46±0.09 | 2.53±0.13 | 1.80±0.16 | 0.50 | 1.12±0.02 | 1.11±0.02 | 1.18±0.07 | 0.77 |
| rs2516839 | 41 | T/C | 2.45±0.14 | 2.63±0.11 | 1.95±0.11 | 0.047 | 1.14±0.03 | 1.10±0.02 | 1.14±0.04 | 0.81 |
| rs1556259 | 15 | A/G | 2.50±0.09 | 2.31±0.11 | 2.43±0.31 | 0.22 | 1.12±0.02 | 1.12±0.03 | 1.08±0.06 | 0.43 |
| rs2774279 | 34 | G/A | 2.41±0.11 | 2.49±0.12 | 2.42±0.17 | 0.35 | 1.10±0.02 | 1.14±0.02 | 1.11±0.04 | 0.34 |

| Data are presented as mean±SE. NA indicates genotype not observed; MAF, minor allele frequency. |
| *Data show major and minor alleles of the variant, respectively. Major allele is represented by “1” and minor allele by “2.” |
| †The critical P value for 5% significance is 0.005 using Bonferroni correction for 10 tests. |
| ‡P value is smaller than the critical P value using Bonferroni correction. |

| Table 3. Association USF1 Variants With TGs and HDL Cholesterol in CAD Patients of Australian ADL Families (n=61) |
| USF1 (n=61) | MAF, % | 1/2* | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | P† |
| USF1 | | | | | | | | | | | | | | |
| rs10908821 | 13 | C/G | 2.82±0.18 | 2.75±0.30 | NA | 0.81 | 0.93±0.03 | 0.93±0.05 | NA | 0.87 |
| rs2073658 | 28 | C/T | 2.73±0.25 | 2.95±0.21 | 2.43±0.17 | 0.93 | 0.98±0.04 | 0.87±0.04 | 0.95±0.05 | 0.27 |
| rs2774276 | 22 | C/G | 2.86±0.17 | 2.78±0.29 | 1.50 | 0.56 | 0.89±0.03 | 0.98±0.04 | 1.00 | 0.58 |
| rs2516839 | 40 | T/C | 2.92±0.27 | 2.97±0.23 | 1.64±0.25 | 0.007§ | 0.88±0.04 | 0.91±0.03 | 1.19±0.07 | 0.01 |
| rs1556259 | 15 | A/G | 3.03±0.18 | 2.20±0.23 | 2.40 | 0.0022‡ | 0.88±0.03 | 1.06±0.06 | 1.00 | 0.02 |
| rs2774279 | 35 | G/A | 2.31±0.16 | 3.27±0.26 | 2.85±0.30 | 0.012 | 0.99±0.04 | 0.89±0.04 | 0.88±0.03 | 0.15 |

| Data are presented as mean±SE. NA indicates genotype not observed; MAF, minor allele frequency. |
| *Data show major and minor alleles of the variant, respectively. Major allele is represented by “1” and minor allele by “2.” |
| †The critical P value for 5% significance is 0.008 using Bonferroni correction for 5 tests. |
| ‡P value is smaller than the critical P value using Bonferroni correction. |
associated with the fibrotic lesion area in the plaque in the present study and was, therefore, tested for interaction. The USF1 variant rs2516839 was chosen because of its previous association with atherosclerosis in this study sample.8 Although no interaction between rs3135506 and rs2516839 was observed for the total plaque area in the abdominal aorta, a significant interaction effect (P=0.0028) emerged as the SNPs were tested for fibrotic lesion area, the best associating phenotype for APOA5 SNP rs3135506. This putative interaction appears to be synergistic in nature, because the APOA5 risk allele seems to reinforce the USF1 risk allele effect in fibrotic lesion formation (Table 5). To see whether the potential epistatic effect between these variants could be replicated for lipids, we tested it in Australian ADL families with CAD (n=61) for TG and HDL cholesterol levels, because the effect of USF1 was most pronounced in CAD patients. A suggestive interaction between the same variants was evidenced (for TG: P=0.033; for HDL cholesterol: P value was nonsignificant), again demonstrating that the carriers of APOA5 rs3135506 and USF1 rs2516839 risk alleles had the most adverse cardiovascular phenotype and the highest TG levels, in agreement with the results in HSDS subjects (Table 5).

Prompted by this finding, we also tested gene-gene interactions between all of the other USF1 and APOA5 SNPs for TG and HDL cholesterol in Australian families and fibrotic lesions of the abdominal aorta in HSDS subjects. After testing, the interaction between rs2516839 and rs3135506 remained the most significant (data not shown).

To examine whether this suggestive interaction effect between APOA5 rs3135506 and USF1 rs2516839 could be replicated for lipids in a larger sample, we genotyped these SNPs in a Finnish FR97 population cohort (n=7939). Although no interaction between the variants was detected for TG levels in FR97 CAD patients (P=0.34; n=1065), we observed an interactive effect of these SNPs on HDL cholesterol levels (P=0.008; Table 5), a trait correlating well with TG (Pearson r=−0.514; P=6×10−80 for FR97). The carriers of risk alleles of rs3135506 and rs2516839 had the lowest HDL cholesterol levels, representing the most atherogenic phenotype. As such, this outcome is in line with the results of the Finnish autopsy study and the Australian ADL family study, although caution must be exercised when interpreting these results because of the lack of replication of the interaction on the exactly same phenotypes.

### Discussion

In this study we have replicated the association of previously identified APOA5 variants with TG and HDL cholesterol levels in Australian ADL families, as well as observed a novel APOA5 association with carefully quantified fibrotic lesion areas in the abdominal aorta of men in a Finnish autopsy series. We have shown that USF1 variants associate with the aforementioned lipid parameters in Australians with diagnosed CAD, in agreement with Lee et al.7 We have also provided initial evidence of the genetic interaction between the best associating USF1 and APOA5 SNPs, rs2516839 and rs3135506, respectively; in the HSDS autopsy series for the percentage of fibrous lesions in the abdominal aorta, in Australian CAD patients for TGs, and in a cohort of Finnish CAD patients for HDL cholesterol, although this interaction was not replicated for TG.

Our study affirms the contribution of genetic variants in USF1 and APOA5 to variation on lipid levels. The USF1 variant showing the most convincing evidence of association in our study sample is rs2516839, with its major allele associating with a higher percentage of fibrotic lesion area in men from the HSDS autopsy series8 and elevated levels of TGs in CAD patients from Australian ADL families. This allele (T) has been associated previously with higher TG and cholesterol levels in families from Utah ascertained for type 2 diabetes mellitus,28 whereas the protective genotype (CC) of the present study has shown suggestive association with a lower risk of metabolic syndrome in Chinese subjects.5 Rs2516839 is located in the 5’ untranslated region of USF1, raising the possibility that its 2 alleles might differentially affect the stability of USF1 mRNA or modulate its transport outside the nucleus, thus altering the availability of the

### Table 4. Association of APOA5 Variant rs3135506 With Lesion Types in the Abdominal Aorta in HSDS Subjects (n=300)

<table>
<thead>
<tr>
<th>Variable</th>
<th>GG</th>
<th>GC</th>
<th>N†</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calciﬁed lesion area</td>
<td>4.12±0.38</td>
<td>3.99±1.14</td>
<td>239</td>
<td>0.85</td>
</tr>
<tr>
<td>Fatty streaks</td>
<td>13.0±0.61</td>
<td>16.0±1.93</td>
<td>286</td>
<td>0.10</td>
</tr>
<tr>
<td>Fibrotic lesions</td>
<td>6.07±0.31</td>
<td>9.48±1.27</td>
<td>261</td>
<td>0.003†</td>
</tr>
<tr>
<td>Complicated lesions</td>
<td>7.42±0.71</td>
<td>7.26±1.68</td>
<td>193</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Data are presented as mean±SE.

*The critical P value for 5 % significance is 0.01 using Bonferroni correction for 4 tests.

†Individuals with lesion area >0 of the particular lesion type were included in the analyses.

### Table 5. Joint Effects of USF1 rs2516839 and APOA5 rs3135506 on Fibrotic Lesion Areas of Abdominal Aorta in Finnish HSDS Autopsy Series, TG Levels in Australian CAD Patients, and HDL Cholesterol Levels in Finnish CAD Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>USF1 rs2516839</th>
<th>APOA5 rs3135506</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSDS: lesion area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 1</td>
<td>5.82±0.56</td>
<td>95</td>
</tr>
<tr>
<td>1 2</td>
<td>5.62±0.43</td>
<td>114</td>
</tr>
<tr>
<td>2 2</td>
<td>4.41±0.58</td>
<td>44</td>
</tr>
<tr>
<td>GEMS CAD: TG, mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 1</td>
<td>2.89±0.31</td>
<td>16</td>
</tr>
<tr>
<td>1 2</td>
<td>3.02±0.24</td>
<td>20</td>
</tr>
<tr>
<td>2 2</td>
<td>1.46±0.21</td>
<td>7</td>
</tr>
<tr>
<td>FR97 CAD: HDL cholesterol, mmol/L</td>
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<td></td>
</tr>
<tr>
<td>1 1</td>
<td>1.23±0.02</td>
<td>371</td>
</tr>
<tr>
<td>1 2</td>
<td>1.25±0.02</td>
<td>418</td>
</tr>
<tr>
<td>2 2</td>
<td>1.18±0.03</td>
<td>135</td>
</tr>
</tbody>
</table>

Data are presented as mean±SE unless otherwise specified.
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functional USF1 protein. The risk allele might, consequently, contribute to an adverse pattern of USF1 target gene expression. The hypothesis that rs2516839 might actually be the functional variant cannot be ruled out, because this variant is located in a region with an eye-catching peak of phylogenetic conservation (http://genome.ucsc.edu). It must be noted that no association of USF1 polymorphisms was detected in the Australian families when association tests were performed on the whole sample (n=516); the contribution of USF1 variants on lipids was observable only in patients with diagnosed CAD (n=61). The lack of association between USF1 and lipid levels in the entire sample may be attributable to the small sample size, but it may also point to USF1 variants not having a major impact on lipid levels in the general population. The effect of USF1 is more pronounced in subjects already diagnosed with CAD, implying that other risk factors, whether genetic or environmental, are crucial to the early stage pathogenesis of CAD, and only later does USF1 significantly contribute to lipid levels. The lack of association for rs2073658 in the Australian families is noteworthy. This variant was associated with familial combined hyperlipidemia and TG in men in the original study4 and others,5,29 and it was also shown to influence the expression of USF1 target genes.2 This may point to an existence of 2 independent functional variants, as the role of rs2516839 on cardiovascular phenotypes has been established.5,28

The associations of APOA5 variants rs3135506, rs662799 (in LD with rs2072560), and rs10750097 with TGs, observed here for the first time in the Australian population, have been well established, and APOA5 has emerged as one of the most solid candidate genes affecting plasma TGs in the general population. APOA5 polymorphisms have been associated previously with various disease end points, such as common carotid artery intima-media thickness,30 large-vessel associated ischemic stroke,31 and increased cerebral infarction risk.32 Elevated plasma TG levels have been solidly established as a risk factor for atherosclerosis,33,34 yet there is only one previous study linking the APOA5 polymorphism to the progression of atherosclerosis.35 To the best of our knowledge, this is the first study to associate allelic variant of APOA5 with atherosclerotic plaque size and their composition, specifically the percentage of fibrotic lesion area in the abdominal aorta.

The strongest associating APOA5 variant in the present study is rs3135506, a nonsynonymous SNP located in the second exon of the gene. Carrying the minor allele of rs3135506 leads to impaired signal peptide activity of APOA5 via amino acid replacement,25 thus reducing the amount of functioning protein. Our preliminary evidence of epistasis between USF1 and APOA5 might, thus, be a result of a differential level of USF1 protein and its effect on APOA5 secretion. This preliminary interaction was observed in 3 independent cohorts and for 3 different yet highly correlated phenotypes, namely, fibrous lesions of the abdominal aorta, TG, and HDL cholesterol. It is noteworthy that the epistatic of APOA5 rs3135506 and USF1 rs2516839 on TG in Australian CAD patients was not replicated in Finnish CAD patients (P=0.34; n=1065), but instead found for HDL cholesterol, a phenotype correlating with TG (Pearson r=−0.514; P=6×10−80). Even if the correlation between TG and HDL cholesterol was highly significant, the proportion of variability in TG accounted for by HDL cholesterol is only 26% (r^2=0.514^2=0.26), thus making the lack of replication on TG more comprehensible. The fact that exactly the same phenotype was not replicated in each cohort is most likely indicative of the difference in their ascertainment, as well as the complex nature of the phenotypes under study. Although the evidence in the present study for this interaction is not fully compelling, genes with established biological interplay are worthy starting points for studies looking for epistatic interactions.

To conclude, our current findings support the role of both APOA5 and USF1 in lipid metabolism and establish a novel association of APOA5 with atherosclerotic plaque composition. Our data also offer preliminary evidence of potential epistasis between these genes, hopefully providing encouragement for future interaction studies on transcription factors and their target genes.

Acknowledgments

We thank Minttu Jussila and Minna Suvela for their expert technical assistance.

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Disclosures

L.P. is a member of the board of directors of Orion Corporation.

References


Genetic Association and Interaction Analysis of USF1 and APOA5 on Lipid Levels and Atherosclerosis

Pirkka-Pekka Laurila, Jussi Naukkarinen, Kati Kristiansson, Samuli Ripatti, Tuuli Kauuttu, Kaisa Silander, Veikko Salomaa, Markus Perola, Pekka J. Karhunen, Philip J. Barter, Christian Ehnholm and Leena Peltonen

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### Table I. Association of APOA5 and USF1 haplotypes (Figure 1) with triglycerides and HDL-cholesterol in Australian ADL families (n=516).

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Triglycerides (mmol/L)</th>
<th>HDL-cholesterol (mmol/L)</th>
<th>P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>f</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>APOA5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-A-C-A</td>
<td>73 %</td>
<td>3.29±0.31</td>
<td>2.63±0.14</td>
<td>2.44±0.07</td>
</tr>
<tr>
<td>C-A-C-G</td>
<td>12 %</td>
<td>2.32±0.07</td>
<td>2.79±0.21</td>
<td>3.84±0.53</td>
</tr>
<tr>
<td>G-A-T-G</td>
<td>8 %</td>
<td>2.43±0.08</td>
<td>2.52±0.18</td>
<td>NA</td>
</tr>
<tr>
<td>G-G-C-G</td>
<td>7 %</td>
<td>2.41±0.08</td>
<td>2.67±0.23</td>
<td>5.30±2.90</td>
</tr>
<tr>
<td>USF1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-C-T-A-A</td>
<td>34 %</td>
<td>2.42±0.11</td>
<td>2.52±0.12</td>
<td>2.43±0.18</td>
</tr>
<tr>
<td>C-T-C-T-A-G</td>
<td>24 %</td>
<td>2.36±0.09</td>
<td>2.56±0.12</td>
<td>2.91±0.55</td>
</tr>
<tr>
<td>C-C-C-G-G</td>
<td>15 %</td>
<td>2.52±0.09</td>
<td>2.31±0.12</td>
<td>2.48±0.33</td>
</tr>
<tr>
<td>G-C-G-C-A-G</td>
<td>15 %</td>
<td>2.48±0.09</td>
<td>2.47±0.14</td>
<td>1.91±0.29</td>
</tr>
<tr>
<td>C-C-C-A-G</td>
<td>11 %</td>
<td>2.48±0.08</td>
<td>2.51±0.21</td>
<td>1.53±0.28</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.

*The critical p-value for 5 % significance is 0.006 using Bonferroni correction for nine tests.

†P-value smaller than the critical p-value using Bonferroni correction.

‡Number of copies of the given haplotype are denoted by 0, 1, and 2, respectively.
Table II. Association of USF1 haplotypes (Figure 1) with triglycerides and HDL-cholesterol in CAD patients of Australian ADL families (n=61).

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>f (%)</th>
<th>Triglycerides (mmol/L)</th>
<th>HDL-cholesterol (mmol/L)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>USF1 C-C-T-A-A</td>
<td>34 %</td>
<td>2.31±0.16</td>
<td>3.27±0.26</td>
<td>2.86±0.35</td>
</tr>
<tr>
<td>C-T-C-T-A-G</td>
<td>28 %</td>
<td>2.73±0.25</td>
<td>2.95±0.21</td>
<td>2.43±0.17</td>
</tr>
<tr>
<td>C-C-C-G-G</td>
<td>15 %</td>
<td>3.04±0.19</td>
<td>2.20±0.23</td>
<td>2.40</td>
</tr>
<tr>
<td>G-C-G-A-G</td>
<td>13 %</td>
<td>2.83±0.17</td>
<td>2.75±0.34</td>
<td>NA</td>
</tr>
<tr>
<td>C-C-G-A-G</td>
<td>9 %</td>
<td>2.83±0.16</td>
<td>2.82±0.55</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.
†The critical p-value for 5 % significance is 0.006 using Bonferroni correction for nine tests.
‡P-value smaller than the critical p-value using Bonferroni correction.
‡Number of copies of the given haplotype are denoted by 0, 1, and 2, respectively.
Table III. Association of APOA5 variants with total lesion area in coronary arteries and abdominal aorta in HSDS subjects (n=300).

<table>
<thead>
<tr>
<th></th>
<th>Total lesion area in coronary arteries, n=282 §</th>
<th>Total lesion area in abdominal aorta, n=286 §</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAF* 1/2†</td>
<td>P-value‡</td>
</tr>
<tr>
<td></td>
<td>11 1 12 2 2</td>
<td>1 1 1 2 2</td>
</tr>
<tr>
<td>APOA5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3135506</td>
<td>6 % G/C</td>
<td>16.7±0.75 18.2±2.03 NA 0.65</td>
</tr>
<tr>
<td>rs662799</td>
<td>6 % A/G</td>
<td>16.1±0.75 19.4±2.02 NA 0.08</td>
</tr>
<tr>
<td>rs17120035</td>
<td>9 % C/T</td>
<td>16.6±0.79 17.0±1.62 NA 0.89</td>
</tr>
<tr>
<td>rs10750097</td>
<td>21 % A/G</td>
<td>16.3±0.97 17.0±1.06 18.9±3.73 0.74</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.
NA; genotype not observed.
*MAF; minor allele frequency
†Major and minor alleles of the variant, respectively. Major allele is represented by ‘1’ and minor allele by ‘2’.
‡The critical p-value for 5 % significance is 0.01 using Bonferroni correction for four tests.
§Individuals with lesion area greater than zero were included in the analyses.
Table IV. Association of APOA5 haplotypes (Figure 1) with total lesion area in coronary arteries and abdominal aorta in HSDS subjects (n=300).

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>f</th>
<th>Total lesion area in coronary arteries, n=282†</th>
<th>Total lesion area in abdominal aorta, n=286†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>G-A-C-A</td>
<td>75 %</td>
<td>17.1±3.23</td>
<td>16.3±1.07</td>
</tr>
<tr>
<td>C-A-C-G</td>
<td>6 %</td>
<td>15.7±0.75</td>
<td>18.2±2.03</td>
</tr>
<tr>
<td>G-A-T-G</td>
<td>9 %</td>
<td>16.3±0.81</td>
<td>14.8±1.38</td>
</tr>
<tr>
<td>G-G-C-G</td>
<td>6 %</td>
<td>15.7±0.75</td>
<td>18.6±2.01</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.

*The critical p-value for 5 % significance is 0.01 using Bonferroni correction for four tests.

† Individuals with lesion area greater than zero were included in the analyses.
Table V. Association of APOA5 haplotype C-A-C-G (Figure 1) with lesion types in the abdominal aorta in HSDS subjects (n=300).

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>APOA5 haplotype C-A-C-G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carrier</td>
</tr>
<tr>
<td>Calcified lesion area</td>
<td>3.99±1.14</td>
</tr>
<tr>
<td>Fatty streaks</td>
<td>16.0±1.93</td>
</tr>
<tr>
<td>Fibrotic lesions</td>
<td>9.48±1.27</td>
</tr>
<tr>
<td>Complicated lesions</td>
<td>7.26±1.68</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.

*The critical p-value for 5 % significance is 0.01 using Bonferroni correction for four tests.
†P-value smaller than the critical p-value using Bonferroni correction.
‡Individuals with lesion area greater than zero of the particular lesion type were included in the analyses.