USF1 Gene Variants, Cardiovascular Risk, and Mortality in European Americans
Analysis of Two US Cohort Studies

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Objective—A common haplotype of the upstream transcription factor 1 gene (USF1) has been associated with decreased susceptibility to familial combined hyperlipidemia (FCHL) and, paradoxically, with increased risk of cardiovascular disease (CVD) and all-cause mortality.

Methods and Results—We assessed associations between USF1 tagSNPs, CVD risk factors, and aging-related phenotypes using data from 2 large population-based cohorts, Coronary Artery Risk Development in Young Adults (CARDIA) and the Cardiovascular Health Study (CHS), comprising younger and older adults, respectively. In CARDIA, each additional copy of the FCHL low-risk allele was associated with 2.4 mg/dL lower levels of LDL cholesterol (P=0.01) and decreased risk of subclinical atherosclerosis as assessed by coronary artery calcium (odds ratio 0.79; 95%CI 0.63 to 0.98). Whereas there was little association between USF1 genotype and metabolic or CVD traits in older adults from CHS, the USF1 low-risk dyslipidemia allele was associated with higher plasma C-reactive protein and interleukin (IL)-6 levels and with increased risk of mortality, particularly attributable to noncardiovascular causes.

Conclusions—There appears to be a complex and possibly age-dependent relationship between USF1 genotype, atherosclerosis phenotypes, and CVD risk. USF1 may influence mortality through pathways distinct from atherosclerosis. Alternatively, linkage disequilibrium with neighboring polymorphisms in other genes such as F11R may be responsible for the observed USF1 genotype–phenotype associations in older adults. (Arterioscler Thromb Vasc Biol. 2007;27:2736-2742.)

Key Words: USF1 ■ atherosclerosis ■ CVD ■ mortality ■ cholesterol

Upstream transcription factor 1 (USF1) is a member of the ubiquitously expressed beta helix-loop-helix leucine zipper family of transcription factors and regulates a number of genes involved in lipid and glucose metabolism. A haplotype comprised of the common alleles of several intronic SNPs (including rs2073657, rs2073658, and rs3737787) within the gene encoding USF1 (USF1) on chromosome 1q21 has been associated with decreased susceptibility to familial combined hyperlipidemia (FCHL) in pedigrees with early-onset cardiovascular disease (CVD). The FCHL risk haplotype has been associated with differential expression of USF1-regulated genes and lipolytic activity in fat tissue biopsies. Of 40 USF1 target genes examined, apolipoprotein E (APOE) was the most strongly up-regulated in carriers of the USF1 low-risk haplotype.

Komulainen et al recently reported that a SNP tagging the USF1 FCHL risk haplotype was associated with risk of CVD events and all-cause mortality among women in a combined analysis of 2 large Finnish cohorts. Curiously, the direction of association between the allele associated with increased risk of CVD and all-cause mortality in the general population of Finnish women (minor allele of rs2073658) is opposite to that reported for FCHL. Moreover, the reported association was even stronger for total mortality than for CVD risk. In this regard, USF1 regulates not only genes involved in lipid and glucose metabolism, but also genes involved in immune and stress responses, cellular senescence, and carcinogenesis. Therefore USF1 alleles might influence CVD risk or other aging-related phenotypes through additional effects on other gene pathways. Alternatively, the FCHL risk haplotype may be in linkage disequilibrium (LD) with neighboring genes that are causally related to other CVD- or aging-related phenotypes.

To further evaluate the role of the USF1 FCHL risk allele and other common regional variants on atherosclerosis- and aging-related phenotypes in the general population, we ana-
alyzed USF1 genotype associations in 2 large U.S. cohort studies of CVD risk comprised of different age groups. In the Coronary Artery Risk Development in Young Adults (CARDIA) cohort study of apparently healthy young adults, we assessed the relationship between USF1 gene variants and serial quantitative measures of lipids and glucose metabolism among White participants and also with plasma C-reactive protein (CRP) levels and presence of coronary artery calcium (CAC) after 20 years of follow-up. Using baseline and follow-up data from the Cardiovascular Health Study (CHS), we further assessed associations between USF1 gene variants, cholesterol levels, carotid wall thickness, the inflammation biomarkers CRP and intereleukin-6 (IL-6), and risk of incident CVD and all-cause and cause-specific mortality in a longitudinal study of White men and women 65 years and older followed for up to 14 years. Because of reports that USF1 genotype-phenotype associations may differ by sex, body mass index (BMI), and other lipid genes, and the reported influence of USF1 genotype on APOE gene expression, we additionally assessed any evidence of USF1 gene–environment or gene–gene interactions by gender, obesity, and APOE genotype on cholesterol levels. Finally, we evaluated the possibility that LD with neighboring polymorphisms in other genes may be responsible for the observed USF1 genotype-phenotype associations.

Methods

CARDIA Cohort

The CARDIA Study is a prospective cohort study of the development of cardiovascular risk factors in young adults. Beginning in 1985, 5115 participants aged 18 to 30 years were recruited from 4 clinical sites located in Birmingham, Alabama, Chicago, Illinois, Minneapolis, Minnesota, and Oakland, California. For the current study, eligible participants were 1932 self-identified Whites who consented to isolation of genomic DNA at year 10 and for whom an adequate DNA sample and USF1 genotyping data were available. We excluded from all analyses participants with missing genotype data at all SNPs (n=57), with a final sample of 1875 participants. Longitudinal measures of lipids, glucose, and insulin were available from the baseline and year 5, 7, 10, 15, and 20 CARDIA exams. Plasma CRP levels and coronary artery calcium (CAC) were measured at the year 20 examination. In the CARDIA study, 5 SNPs were genotyped, covering all major linkage disequilibrium bins in European-American populations. The 5 SNPs were 1927 (rs2516837), 2005 (rs1556259), 2284 (rs2516838), 4938 (rs2774276), and 7131 (rs3737787), numbering based on GenBank accession number AF542391. The minor allele of SNP 7131 tags the FCHL low-risk haplotype. Additional method details of the CARDIA study are provided described in supplemental Methods (available online at http://atvb.ahajournals.org).

CHS Cohort

The CHS is a prospective population-based cohort study of older adult men and women recruited from 4 US field centers: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania. Four thousand eight hundred eight individuals from the original cohort who self-reported race as White were eligible for the current study. An additional 300 participants for whom DNA was not collected or who did not consent to the use of their DNA were excluded, with a final sample of n=4536. The CHS baseline evaluation included demographic, lifestyle and medical histories, physical examination, fasting blood collection, and measurement of intima- medial thickness (IMT) of the common carotid artery wall. Adjudicated clinical CVD events and mortality occurring through June 30, 2003, were available, which allowed for a maximum of 14 years of follow-up. In CHS, genotyping assays for USF1 SNPs, 1927 (rs2516837), 2005 (rs1556259), 2284 (rs2516838), and 4064 (rs2073655) were performed. Additional method details of the CHS are provided described in supplemental Methods.

Statistical Analysis

Our primary and secondary hypotheses and exploratory analyses related to USF1 variants, and methods for correcting for multiple hypothesis testing, are detailed under supplemental Methods. Hardy-Weinberg equilibrium was assessed by performing Pearson chi-squared Tests. Associations with quantitative traits (BMI, lipid levels, HOMA, CRP, IL-6, and carotid IMT), binary traits (CAC, diabetes), and time-to-event outcomes (incident CVD and mortality) were assessed using multiple linear regression, logistic regression, and Cox regression, respectively. Triglycerides, CRP, IL-6, and HOMA were log-transformed to reduce skewness. To incorporate multiple longitudinal quantitative measures of metabolic traits (BMI, lipid levels, and HOMA) available at years 0, 5, 7, 10, 15, and 20 in the CARDIA study, we assessed associations by using generalized estimating equations (GEE) to adjust for intrasubject corre-
sations (Stata/SE8.2, Stata Corp). All regression or GEE models were minimally adjusted for age, sex, and clinic. Lipid medication users were excluded from models assessing lipid levels. In the time-to-event models, covariates known to be associated with risk of clinical CVD or mortality—diabetes, smoking, hypertension, cholesterol to HDL ratio, BMI, diagnosis of CVD or cancer—were additionally included as adjustment variables.

Individual tag SNP genotypes were as 0, 1, or 2 copies of the minor allele (additive genetic model), with the common homozygous group serving as reference and assuming constant effect size for each additional copy of the minor allele. Covariate-adjusted SNP-specific effect sizes, odds ratios, or hazard ratios were estimated from the regression coefficient (β). Coefficients and SEs were back-transformed for log (Triglycerides) and log (HOMA). SNP effect sizes estimated from longitudinal data in CARDIA using GEE represent the average effect across all follow-up visits. Additional stratified analyses were performed according to gender, carrierness of the APOE2 allele, or obesity (defined as BMI ≥30 kg/m²). Interactions were assessed by introducing a multiplicative term into regression models, and significance was assessed using a Wald test statistic. To test for phenotypic associations with additional SNP markers within the region surrounding the USF1 gene on chromosome 1, we used a Bayesian imputation-based regression method, which combines typed SNP and phenotype data from an association study with information on marker LD from a panel of untyped SNPs across the region from the HapMap genotype and SeattleSNPs resequencing data. The method is described further under supplemental Methods.

Results

At the baseline examination, the mean age of the CARDIA study subjects was 26 years (range 18 to 30 years), and 54% were women. The mean age of the CHS study participants at study entry was 73 years (range 65 to 98 years), and 54% were women (supplemental Table I). As expected, there was a greater prevalence of cardiovascular risk factors and prevalent clinical CVD among the older CHS cohort than among the younger CARDIA cohort.

The alleles of all USF1 polymorphisms showed no significant deviation from Hardy-Weinberg expectations in either cohort. For SNPs 1972, 2005, 2284, and 7131 (or 4064 in CHS), the respective minor allele frequencies in CARDIA and CHS were 0.39 and 0.39, 0.14 and 0.15, 0.34 and 0.33, and 0.26 and 0.28. The extent of LD between SNPs was also similar in the 2 cohorts. The respective pair-wise r² in
USF1 Genotypes and Atherosclerosis-Related Traits in CARDIA

In CARDIA, each additional copy of the minor T allele of USF1 7131 was associated with 2.4 ± 0.95 mg/dL lower total and 2.4 ± 0.95 mg/dL lower LDL cholesterol compared with the major C allele (Table 1). In addition, the minor T allele of SNP 1927 was associated with higher LDL levels. The global multiple SNP-corrected probability value for association between USF1 genotype and total cholesterol and LDL was 0.07 and 0.03, respectively. There was no association between USF1 genotype, HDL, triglycerides, HOMA, CRP, and CAC) the association between the USF1 FCHL risk allele tag SNP 7131 and cholesterol level remained statistically significant (P = 0.03).

Exploratory analyses, the association between total cholesterol and the 7131 T allele was stronger among those with obesity (β = −6.34±2.01 mg/dL; P = 0.002) than without obesity (β = −1.74±1.11 mg/dL; P = 0.12). The p-value of interaction between the FCHL low-risk haplotype tagged by SNP 7131 and obesity on total cholesterol level was 0.07. There was no evidence that any USF1 genotype-metabolic phenotype association differed according to gender.

USF1 Genotypes, Atherosclerosis-Related Traits, and Risk of CVD and Mortality in CHS

In CHS, there was no association between USF1 genotype main effects and baseline lipid levels or risk of diabetes (Table 2). These results did not differ by gender, nor according to history of clinical CVD before baseline (data not shown). Similar to the CARDIA SNP 7131 results, in exploratory analyses, the minor T allele of the FCHL low-risk haplotype tag SNP in CHS (4064) was associated with lower total cholesterol among the subgroup of 785 obese individuals (β = −4.76±2.15 mg/dL; P = 0.03) but there was no association among the 3723 nonobese individuals (β = 0.82±0.96 mg/dL; P = 0.39). The p-value for SNP 4064—
obesity interaction on total cholesterol was 0.02. The association between SNP 4064 minor allele and lower total cholesterol was 0.02. The association between risk of all-cause mortality and the USF1 2284 allele noncarriers (0.33), but also extends strongly into the neighboring gene on chromosome 1 is flanked on either side by regions on untyped SNPs from CARDIA, the Bayesian imputation method showed a significant (P = 0.01). When corrected for testing of all primary phenotypes in CHS (total cholesterol, LDL, HDL, triglycerides, diabetes, incident CVD, and incident mortality) the mortality–USF1 FCHL risk allele tag SNP 4064 association remained statistically significant (P = 0.01). Analysis of Extended USF1 Genomic Region The USF1 gene on chromosome 1 is flanked on either side by 2 genes, F11R and ARHGAP30. Based on analysis of HapMap genotype and SeattleSNPs resequencing data across a 250-kb region encompassing USF1 and the surrounding region on chromosome 1, the FCHL risk haplotype comprises not only several USF1 alleles (4064, 5299, 5892, 7131, 9328), but also extends strongly into the neighboring F11R gene. As shown in the Figure, the extended risk haplotype includes 17 additional SNPs in the F11R gene and 5′ flanking region. Lesser amounts of pair-wise LD (r² ≈ 0.1 to 0.5) were also observed between the USF1 risk variant alleles and SNPs extending further upstream toward the ITLN2 gene (not shown).

By combining information on multi-marker LD across the F11R, USF1, and ARHGAP30 regions on untyped SNPs from the HapMap and SeattleSNPs data with typed USF1 SNP and phenotype data from the CARDIA or CHS cohorts, we used a Bayesian imputation-based regression method to test for genotype associations across all SNPs in the region. In CARDIA, the Bayesian imputation method showed a significant association with LDL across the entire region (P = 0.006). The strongest evidence of association was for the LD cluster containing the FCHL low risk haplotype. At the level of the individual SNP, USF1 5299 (rs2073656) had the single

### Table 2. Associations Between USF1 Gene Genotypes and Metabolic Traits and Inflammatory Markers in CHS*

<table>
<thead>
<tr>
<th>Genotype (No. of Subjects)</th>
<th>Total Cholesterol (mg/dL) Beta (SE)</th>
<th>LDL Cholesterol (mg/dL) Beta (SE)</th>
<th>HDL Cholesterol (mg/dL) Beta (SE)</th>
<th>Log Triglycerides (mg/dL) Beta (SE)</th>
<th>Diabetes Beta (SE)</th>
<th>Log CRP (mg/L) Beta (SE)</th>
<th>Log IL-6 (pg/dL) Beta (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USF1 1927</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TC (2158)</td>
<td>−0.04 (0.81)</td>
<td>−0.42 (0.76)</td>
<td>0.27 (0.31)</td>
<td>0.003 (0.009)</td>
<td>1.06 (0.06)</td>
<td>−0.035 (0.022)</td>
<td>−0.025 (0.013)</td>
</tr>
<tr>
<td>TT (707)</td>
<td>P = 0.96</td>
<td>P = 0.57</td>
<td>P = 0.37</td>
<td>P = 0.72</td>
<td>P = 0.36</td>
<td>P = 0.11</td>
<td>P = 0.06</td>
</tr>
<tr>
<td>USF1 2005</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (1109)</td>
<td>0.22 (1.11)</td>
<td>0.34 (1.05)</td>
<td>−0.26 (0.42)</td>
<td>0.012 (0.012)</td>
<td>1.04 (0.09)</td>
<td>0.001 (0.030)</td>
<td>−0.014 (0.018)</td>
</tr>
<tr>
<td>CC (114)</td>
<td>P = 0.85</td>
<td>P = 0.75</td>
<td>P = 0.54</td>
<td>P = 0.35</td>
<td>P = 0.66</td>
<td>P = 0.97</td>
<td>P = 0.44</td>
</tr>
<tr>
<td>USF1 2284</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GC (2024)</td>
<td>0.15 (0.86)</td>
<td>0.06 (0.81)</td>
<td>0.31 (0.32)</td>
<td>−0.020 (0.010)</td>
<td>0.92 (0.06)</td>
<td>−0.010 (0.023)</td>
<td>−0.004 (0.014)</td>
</tr>
<tr>
<td>CC (466)</td>
<td>P = 0.86</td>
<td>P = 0.94</td>
<td>P = 0.34</td>
<td>P = 0.04</td>
<td>P = 0.19</td>
<td>P = 0.66</td>
<td>P = 0.80</td>
</tr>
<tr>
<td>USF1 4064</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CT (1851)</td>
<td>0.06 (0.89)</td>
<td>0.52 (0.84)</td>
<td>−0.62 (0.33)</td>
<td>0.017 (0.010)</td>
<td>1.02 (0.07)</td>
<td>0.052 (0.023)</td>
<td>0.034 (0.015)</td>
</tr>
<tr>
<td>TT (341)</td>
<td>P = 0.95</td>
<td>P = 0.53</td>
<td>P = 0.07</td>
<td>P = 0.09</td>
<td>P = 0.75</td>
<td>P = 0.015</td>
<td>P = 0.02</td>
</tr>
<tr>
<td>Global P</td>
<td>0.98</td>
<td>0.89</td>
<td>0.27</td>
<td>0.22</td>
<td>0.77</td>
<td>0.06</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*All models were adjusted for age, sex, and clinic. Lipid and diabetes regressions additionally adjusted for BMI. Lipid models exclude 247 subjects who were taking lipid-lowering medication. Beta coefficients (or odds ratios) represent change in dependent variable (or risk of disease) relative to common homozygous genotypes. Global P values are shown for the omnibus test of association across all 4 USF1 tagSNPs for a given trait.

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highest posterior probability of association (47%) with LDL. There was 83% probability that at least one SNP in the FCHL risk haplotype cluster affects plasma LDL levels, with much lower probabilities of association for any other SNP in the region. The Bayesian imputation method similarly showed a significant association for total mortality in CHS across the entire F11R-USF1-ARHGAP30 genomic region (P<0.004). The cluster of USF1 and F11R SNPs comprising the FCHL low risk haplotype still had the highest probability of association with mortality (72%); however, a second cluster of

### Table 3. Associations Between USF1 Genotypes, Incident CVD, Mortality, and Cause-Specific Mortality in CHS

<table>
<thead>
<tr>
<th>Genotype (No. of Subjects)</th>
<th>Incident CVD HR (95% CI)</th>
<th>All-Cause Mortality HR (95% CI)</th>
<th>CVD Mortality HR (95% CI)</th>
<th>Non-CVD Mortality HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USF1 1927</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (1671)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>TC (2158)</td>
<td>0.98 (0.91-1.06)</td>
<td>0.95 (0.90-1.01)</td>
<td>0.94 (0.85-1.03)</td>
<td>0.96 (0.89-1.04)</td>
</tr>
<tr>
<td>TT (707)</td>
<td>P=0.68</td>
<td>P=0.13</td>
<td>P=0.17</td>
<td>P=0.36</td>
</tr>
<tr>
<td>USF1 2005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (3314)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>TC (1109)</td>
<td>0.99 (0.89-1.10)</td>
<td>1.01 (0.91-1.07)</td>
<td>0.92 (0.81-1.05)</td>
<td>1.03 (0.93-1.14)</td>
</tr>
<tr>
<td>CC (114)</td>
<td>P=0.89</td>
<td>P=0.71</td>
<td>P=0.22</td>
<td>P=0.56</td>
</tr>
<tr>
<td>USF1 2284</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG (2042)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>GC (2024)</td>
<td>1.02 (0.94-1.11)</td>
<td>0.98 (0.92-1.04)</td>
<td>1.06 (0.96-1.17)</td>
<td>0.93 (0.86-1.01)</td>
</tr>
<tr>
<td>CC (466)</td>
<td>P=0.63</td>
<td>P=0.56</td>
<td>P=0.25</td>
<td>P=0.10</td>
</tr>
<tr>
<td>USF1 4064</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (2342)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>CT (1851)</td>
<td>1.00 (0.91-1.09)</td>
<td>1.09 (1.02-1.16)</td>
<td>1.02 (0.91-1.13)</td>
<td>1.13 (1.04-1.23)</td>
</tr>
<tr>
<td>TT (341)</td>
<td>P=0.96</td>
<td>P=0.01</td>
<td>P=0.77</td>
<td>P=0.004</td>
</tr>
<tr>
<td>Global P</td>
<td>0.91</td>
<td>0.13</td>
<td>0.44</td>
<td>0.004</td>
</tr>
</tbody>
</table>

HR=hazard ratio.

*HR and 95%CI derived from Cox proportional hazard models, with common homozygous genotype as the reference group. All models adjusted for baseline age, sex, clinic, BMI, smoking status, diabetes, hypertension, total cholesterol/HDL cholesterol ratio, systolic blood pressure, history of coronary heart disease, stroke, cancer. Global P values are shown for the omnibus test of association across all 4 USF1 tagSNPs for a given trait.

Figure. Linkage disequilibrium (LD) between SNPs typed in HapMap Europeans in a ~70-kb region containing USF1 and F11R. Numbers refer to nucleotide position on chromosome 1. USF1 and F11R are indicated as horizontal lines at the top of the figure. The asterisk at position 159 276 147 indicates USF1 7131 (rs373787), the FCHL tagSNP typed in CHS. The grid in the lower part of the figure shows the extent of LD between USF1 7131 and a block of SNPs located in the F11R gene.
SNPs in LD with USF1 4064 but extending further toward F11R and ITLN2 (including rs836, rs11265540, and rs4596920) had a nearly 50% posterior probability that at least one SNP from the cluster is associated with mortality in CHS. These data suggest that although 1 (or more) of the USF1 FCHL-related risk variants is most likely responsible for the LDL cholesterol association observed in CARDIA younger adults, there appears to be a more complex pattern of genetic association with mortality in CHS older adults; several distinct variants in the region may be related to risk of mortality, but there is considerable uncertainty about exactly which SNP(s) are responsible for the association.

**Discussion**

Our results from 2 large US cohort studies of CVD risk suggest age-dependent associations between common variation around the USF1 gene and several atherosclerosis- and aging-related phenotypes. The minor allele of a SNP that tags a common FCHL low-risk USF1 haplotype was associated with lower total and LDL cholesterol levels and lower risk of subclinical atherosclerotic disease in younger white adults from the CARDIA study. In older healthy white adults from CHS, we observed little association with cholesterol levels or subclinical or clinical CVD; however, the same low-risk FCHL USF1 allele was associated with increased risk of mortality during CHS follow-up and showed some evidence of association with a proinflammatory phenotype.

Several studies have shown that USF1 variants confer susceptibility to dyslipidemia in families with early-onset CVD or FCHL.6 Our findings from CARDIA suggest that the low-risk FCHL USF1 haplotype contributes modestly to interindividual differences in plasma total cholesterol and LDL levels measured longitudinally during early adulthood, and also to the occurrence of early-onset coronary atherosclerosis (CAC) in otherwise healthy young white adults. Interestingly, there was little attenuation of the CAC association on adjustment for cholesterol level, suggesting that USF1 might influence CVD risk through effects on other atherogenic pathways besides lipids.

The absence of an observed main effect of USF1 tagSNP genotype on cholesterol level or CVD risk in older adults from CHS suggests that the influence the USF1 risk haplotype on these atherosclerosis-related phenotypes may be stronger during early adulthood. It is also possible that USF1 gene–gene or gene–environment interaction may explain some of the heterogeneity of results between studies. Consistent with this hypothesis, the USF1 genotype–lipid phenotype association was stronger among obese than nonobese individuals in both CARDIA and CHS and among APOE2 allele carriers versus noncarriers in CHS. Putt et al similarly observed an interaction between USF1 gene variants and BMI or other lipid-related genes on LDL levels in healthy young European male offspring of CVD cases.8 In the FINRISK cohort, USF1 tagSNP genotype at SNP 1927 (rs2516837) was associated with higher cholesterol levels among incident CVD cases but not among those without CVD.4 Taken together, these results suggest the importance of effect modification by age (or menopausal status), adiposity, other genes related to lipid metabolism, and perhaps other CVD risk factors on the association between USF1 polymorphism and cholesterol levels in the European-American population.

Although the association we observed between the USF1 low-risk dyslipidemia allele and increased mortality in older White adults from CHS may at first seem paradoxical, these results confirm similar USF1 genotype-mortality association findings from the FINRISK study.7 Our analysis of cause-specific mortality in CHS suggests that the association with low-risk dyslipidemia allele may be stronger for non-CVD deaths than for CVD deaths, which were mainly attributable to cancer. In addition, we were unable to confirm the association between USF1 genotype and incident CVD events reported by Komulainen et al.4 One possible reason is that CHS participants tended to be somewhat older than FINRISK participants (mean age 73 years versus 58 years).

The above observations raise the possibility that in older adults USF1 genotype may influence aging or aging-related diseases through pathways distinct from lipid metabolism or atherosclerosis. Such pleiotropy may not be surprising given that USF1 is a ubiquitous transcription factor that regulates the expression of many other genes, including those involved in aging and carcinogenesis (such as telomerase, p53, BRCA2) and the immune response. The USF1 risk haplotype has also been associated with upregulation of immune response genes in fat biopsy specimens taken from FCHL patients.1,2,15 In this regard, we noted an association between the USF1 risk allele and increased levels of 2 proinflammatory markers, CRP and IL-6, both of which are strong predictors of total mortality in CHS (Jenny 2007). In contrast, there was no association with CRP levels in the younger CARDIA cohort.

Because of the close proximity of USF1 to other genes and the extensive linkage disequilibrium within this genomic region, particularly between the FCHL low-risk alleles and multiple SNPs in F11R (see Figure), another possible explanation for the differences in phenotypic associations observed between study populations is that F11R-related or other functional variants may have phenotypic affects that are differentially expressed according to age. F11R encodes junctional adhesion molecule A or F11 receptor, which has multiple roles in inflammation, acting as a ligand for the integrin LFA1 (involved in leukocyte transmigration across endothelium), a platelet receptor, a reovirus receptor, and a regulator of epithelium tight junction assembly.9 Although our association results provide some preliminary evidence for involvement of F11R (or other regional variants) in aging-related phenotypes in CHS, further dissection of the relative contribution of these genes will require more detailed molecular genetic and functional analysis of this genomic region.

Strengths of the current study include (1) the comparison of 2 different age groups using large ethnically-homogeneous cohorts with extensive longitudinal data on a number of CVD risk factors and aging-related traits, including a large number of clinical events in CHS, and (2) the use of tagSNPs with excellent coverage of common patterns of genetic variation across the USF1 region. However, several potential limitations should be noted. Because USF1 genotyping in CARDIA and CHS was performed in different laboratories using different genotyping platforms, 2 different tagSNPs (7131
and 4064) were typed as proxies for the FCHL risk haplotype. Current functional evidence suggests that SNP rs2073658 within intron 7 may be the etiologic variant within the USF1 risk haplotype.\(^1,2\) Genotype data available from European-American populations indicate that the tagSNPs assayed in our 2 cohorts are in complete LD both with each other and with rs2073658. Thus, failure to type this SNP directly is neither likely to have adversely affected our power to detect associations, nor to account for any observed genotype-phenotype association differences between the 2 cohort studies. Moreover, there was no evidence that the extent of LD differed between CARDIA and CHS populations. As with any indirect association study using common tagSNPs, we cannot exclude the possibility of associations with rare variants within USF1. Correlation of a typed SNP with a causal untyped variant, or interactions between the typed variant and other genetic loci, could additionally explain associations of opposite alleles with the same phenotype in different studies.\(^10\) By using a method that imputes untyped genotype data in a candidate gene association study sample using information on multi-marker LD from HapMap data,\(^7\) we obtained some evidence that the true susceptibility effect on mortality may be attributable, at least in part, to neighboring polymorphisms in other genes. Finally, even though we adjusted our results for multiple testing, some of our associations might be attributable to chance. On the other hand, evidence for association of the same FCHL risk allele with similar phenotypes in other studies supports the notion that this common USF1 haplotype has true, albeit modest, effects on atherogenic and aging-related traits in otherwise healthy adults from the general US population.

In summary, a common USF1 haplotype is associated with lower cholesterol levels and decreased risk of early-onset coronary atherosclerosis in young adults. In older adults, the same variant is associated with increased proinflammatory marker levels and increased mortality. We hypothesize that these findings might reflect age-dependent and pleiotropic effects of USF1 genotype on several distinct gene pathways, or alternatively, other gene variants in the region, which ultimately influence the risk of aging-related disease through both atherosclerotic and non-atherosclerotic mechanisms. Further investigation of the biologic role of USF1 in human aging and longevity, the relationship between USF1 and F11R gene variants and other aging-related phenotypes, and interactions between USF1 polymorphisms and additional lifestyle factors or other USF1-regulated genes (related to lipid metabolism, inflammation, or tumorigenesis), are all relevant areas for future study.

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Disclosures
None.

References
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SUPPLEMENTAL METHODS

CARDIA cohort

The CARDIA Study is a prospective cohort study of the development of cardiovascular risk factors in young adults. Beginning in 1985, 5,115 participants aged 18–30 years were recruited from four clinical sites located in Birmingham, Alabama, Chicago, Illinois, Minneapolis, Minnesota, and Oakland, California. CARDIA was recruited to be balanced on age, sex, ethnicity and educational attainment. Participants were re-examined at six follow-up examinations with overall retention rates among surviving participants of 91% at year 2, 86% at year 5, 81% at year 7, 79% at year 10, and 74% at year 15, and 72% at year 20. The number of lipid medication users in CARDIA at years 0, 5, 7, 10, 15, and 20 were 8, 7, 13, 43, and 132, respectively. These individuals were excluded from analyses assessing USF1 genotype associations with lipid phenotypes. All procedures were conducted under institutionally approved protocols for use of human subjects, and all subjects provided written informed consent.

Fasting plasma lipid levels were measured at the University of Washington Northwest Lipid Research Clinic Laboratory (Seattle, Washington) using standard enzymatic procedures. HDL cholesterol concentration was determined after dextran sulfate–magnesium precipitation. LDL cholesterol was calculated by using the Friedewald equation. Fasting insulin concentration at baseline was determined using a modification of the immunoassay techniques of Herbert et al. (LINCO Research, Inc., St. Louis, Missouri). Fasting glucose level was measured using the hexokinase method. Insulin resistance was calculated by the homeostasis model assessment (HOMA) \((\text{fasting plasma insulin} \times \text{fasting plasma glucose})/22.5\). Plasma CRP levels were measured on 1,599 eligible white participants at the year 20 exam by immunonephelometry.
Coronary artery calcium (CAC) was measured in 1,480 eligible white participants at the year 20 exam by electron beam computerized tomography scanning using methods that have been previously described. Whereas quantitative coronary artery calcium scores were obtained, scores were highly skewed within the cohort, with most participants having scores of zero; therefore, we defined CAC dichotomously as present/absent.

In the CARDIA study, 5 SNPs were genotyped, covering all major linkage disequilibrium bins in European-American populations. The 5 SNPs were 1927 (rs2516837), 2005 (rs1556259), 2284 (rs2516838), 4938 (rs2774276), and 7131 (rs3737787), numbering based on GenBank accession number AF542391. The minor allele of SNP 7131 tags the FCHL low-risk haplotype. Genotyping was performed using the SNPlex method under standard conditions and as previously described in detail.

**CHS cohort**

The CHS is a prospective population-based cohort study of older adult men and women recruited from four U.S. field centers: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania. Those eligible to participate included all persons 65 years of age or older living in the household of each individual sampled. The original cohort (n=5,201) was recruited from 1989 to 1990 and additional minority cohort (n=687) was recruited from 1992 to 1993. In CHS, there were 243 lipid medication users at the baseline exam. Lipid medication users were excluded from models assessing lipid levels. All
procedures were conducted under institutionally approved protocols for use of human subjects, and all subjects provided written informed consent.

Plasma total cholesterol, HDL cholesterol, and triglycerides were measured under fasting conditions by enzymatic methods at a central laboratory. Diabetes was defined as history of diabetes, use of hypoglycemic agent or insulin, or fasting glucose 126 mg/dL. Intima-medial thickness (IMT) of the common carotid artery wall was determined at the baseline examination by high-resolution B-mode ultrasonography as previously described. Baseline CRP and IL-6 were measured on stored EDTA plasma using high-sensitivity ELISAs (C.V. ~6%). In CHS, genotyping assays for USF1 SNPs, 1927 (rs2516837), 2005 (rs1556259), 2284 (rs2516838), and 4064 (rs2073655) were performed on genomic DNA samples by TaqMan 5’ nuclease allelic discrimination assay on an ABI 7900 under standard conditions. SNP 4064 is a tagSNP for the FCHL low-risk haplotype, and is in perfect linkage disequilibrium with tagSNP 7131, which was typed in CARDIA. A TaqMan assay could not be developed for tagSNP 4938.

Details of event ascertainment during CHS follow-up have been published. Incident CVD (fatal or nonfatal MI or fatal CHD, and fatal or nonfatal ischemic stroke events) and all-cause mortality were analyzed as primary endpoints. Clinical events, including fatal events due to all causes, were adjudicated by physician review panel according to medical records, death certificates, supplemented by Medicare utilization data. The National Death Index was used to provide complete mortality follow-up. Adjudicated events occurring through June 30, 2003, were available, which allowed for a maximum of 14 years of follow-up.
Primary and secondary hypotheses and exploratory analyses related to \textit{USF1} variants

Based on the previous literature, our primary objective was to test whether the \textit{USF1} FCHL risk allele (tagged by SNP 7131 in CARDIA or SNP 4064 in CHS) was associated with (a) cholesterol, triglyceride and insulin resistance traits in the general population (CARDIA and CHS) and (b) incident CVD events and total mortality in older adults during follow-up (CHS). Secondarily, we hypothesized that the same variants might be associated with inflammation phenotypes (CRP, IL-6) and subclinical atherosclerosis (CAC, carotid IMT). Finally, we performed several additional exploratory analyses: (a) the role of gender, obesity, and \textit{APOE} genotype as modifiers of the association between \textit{USF1} genotype and cholesterol levels; (b) whether \textit{USF1} genotype association in CHS differed according to cause-specific mortality (CVD vs. non-CVD) or type of clinical CVD event (MI vs. stroke); (c) whether other \textit{USF1} tagSNPs besides the FCHL risk allele were associated with CVD and aging-related phenotypes.

\textbf{Multiple hypothesis test correction}

To correct our primary and secondary analyses in CARDIA and CHS for testing of multiple, correlated quantitative and binary traits, we used a procedure that computes p-values while retaining the original correlation structure under the null hypothesis by simulation from a multivariate normal distribution.\textsuperscript{9} By simulating the data 10,000 times under the null and comparing the results to the original p-values, the probability of observing a \textit{p}-value as small as the original minimum was estimated, given the correlation between tests. We also adjusted for testing multiple SNPs by performing a test based on the global \textit{p}-value obtained from linear regression of phenotype on all \textit{USF1} SNP genotypes simultaneously.\textsuperscript{10} For our exploratory or
sensitivity analyses, we did not perform formal multiple testing correction due to the exploratory nature of these hypotheses.

**Phenotypic association with other SNPs in the extended genomic region surrounding USF1**

To test for phenotypic associations with additional SNP markers within the region surrounding the *USF1* gene on chromosome 1, we used a Bayesian imputation-based regression method,\textsuperscript{11} which combines typed SNP and phenotype data from an association study (in this case, *USF1* genotypes from CARDIA or CHS) with information on multi-marker LD from a panel of untyped SNPs across the region from the HapMap and SeattleSNPs re-sequencing data. Using LD and haplotype inference, this method imputes genotypes of the CARDIA or CHS study subjects at untyped SNPs in an extended region surrounding *USF1*, allowing for uncertainty in the imputed genotypes. Permutation-based p-values for individual SNPs (typed and untyped) as well as the global test of significance for all SNPs in the region were estimated using 10,000 permutations. The strength of evidence for phenotypic association at each typed and untyped SNP was estimated by calculating a Bayes Factor (BF). By multiplying the BF by the odds of association over a range of priors and using model averaging, the results were summarized as the posterior probability of association for each typed or untyped SNP in the candidate region.
References:


Supplemental Table 1. Baseline characteristics of white CARDIA and CHS participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CARDIA</th>
<th>CHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>1,932</td>
<td>4,536</td>
</tr>
<tr>
<td>Mean age, years (range)</td>
<td>25.6 (18 – 30)</td>
<td>72.8 (65 – 98)</td>
</tr>
<tr>
<td>Female sex</td>
<td>1023 (53)</td>
<td>2580 (56.7)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>483 (25)</td>
<td>497 (10.9)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.7 ± 4.1</td>
<td>26.4 ± 4.5</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>176 ± 32</td>
<td>212 ± 40</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>108 ± 30</td>
<td>130 ± 36</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>52 ± 13</td>
<td>54 ± 16</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>78 ± 56</td>
<td>144 ± 79</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>109 ± 11</td>
<td>135 ± 21</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>68 ± 9</td>
<td>70 ± 11</td>
</tr>
<tr>
<td>Diagnosis of hypertension</td>
<td>54 (3)</td>
<td>2530 (56)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>9 (0.5)</td>
<td>657 (14.5)</td>
</tr>
<tr>
<td>HOMA*</td>
<td>50.8 ± 44.8</td>
<td>94 ± 232</td>
</tr>
<tr>
<td>Prevalent CVD†</td>
<td>12 (0.6)</td>
<td>995 (22)</td>
</tr>
</tbody>
</table>

Data are presented as number (%) or mean ± standard deviation, unless otherwise indicated.

*Insulin resistance was calculated by the homeostasis model assessment (HOMA) = (fasting plasma insulin × fasting plasma glucose)/22.5.

†Self-reported myocardial infarction, stroke, angina, transient ischemic attack, claudication, or revascularization procedure.