USF1 Contributes to High Serum Lipid Levels in Dutch FCHL Families and U.S. Whites With Coronary Artery Disease

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Objective—Familial combined hyperlipidemia (FCHL) characterized by high serum total cholesterol and/or triglycerides (TGs) is a common dyslipidemia predisposing to coronary artery disease (CAD). Recently, the upstream transcription factor 1 (USF1) was linked and associated with FCHL and TGs in Finnish FCHL families. Here we examined the previously associated rs3737787 SNP in extended Dutch FCHL families (n=532) and in a cohort of US subjects who underwent diagnostic coronary angiography (n=1533).

Methods and Results—In males of the Dutch FCHL families, we observed significant sex-dependent associations between the common allele of rs3737787 and FCHL, TGs, and related metabolic traits (P=0.02 to 0.006). In the U.S. Whites, sex-dependent associations with TGs and related metabolic traits were observed for the common allele of rs3737787 in males (P=0.04 to 0.02) and rare allele in females (P=0.05 to 0.002). This intriguing relationship was further supported by the highly significant genotype x sex interactions observed for TGs in the Dutch and TGs and body mass index (BMI) in U.S. White subjects with CAD (P=0.0005 to 0.00004).

Conclusion—These data show that USF1 influences several cardiovascular risk factors in a sex-dependent manner in Dutch FCHL families and U.S. Whites with CAD. A significant interaction between sex and genotype was shown to affect TGs and BMI. (Arterioscler Thromb Vasc Biol. 2007;27:2222-2227.)

Key Words: upstream transcription factor 1 familial combined hyperlipidemia coronary artery disease association lipids

Familial combined hyperlipidemia (FCHL) is a common familial dyslipidemia with a population prevalence of 1% to 2%. Importantly, FCHL is observed in up to 20% of premature coronary artery disease (CAD) patients. In addition to high serum total cholesterol (TC) and triglycerides (TGs), low plasma levels of high-density lipoprotein cholesterol (HDL-C), small dense low-density lipoprotein particles, and elevated apolipoprotein B (apoB) are also often observed in FCHL.

Association between FCHL and variants of the upstream transcription factor 1 (USF1) located in the FCHL-linked chromosome 1q21 region was originally identified by Pajukanta et al in Finnish FCHL families. Several independent studies have investigated the USF1 variants in samples ascertained for FCHL or risk factors for premature CAD. The common allele of rs3737787 or SNPs in linkage disequilibrium (LD) with this SNP were associated with FCHL or one of its component traits in each of these studies. Komulainen et al investigated USF1 variants in a population-based prospective Finnish cohort and observed an association of USF1 with cardiovascular disease and mortality among females, demonstrating a consequence of USF1 at the population level in Finns. In many of these studies, there is evidence of sex specificity, with the common allele of rs3737787 showing strongest evidence for association with high TGs in males. Interestingly, in females, the minor allele seems to be associated with elevated lipids, as well as cardiovascular disease and mortality. The underlying functional mechanisms explaining this replicated sex specificity are currently unknown.

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USF1 is a ubiquitously expressed member of the basic helix-loop-helix leucine zipper family of transcription factors that bind E-box elements of target gene promoters to regulate downstream genes that have critical lipid and glucose metabolism functions in the liver, pancreas, and adipose tissues. The USF1 gene has been sequenced in 2 independent FCHL cohorts and no missense or nonsense variants have been identified, implying that the causative variant is likely to be within an element affecting the transcription level or splicing of USF1.

Our initial report examined USF1 in Finnish FCHL families, a genetically isolated population. In the current study, we evaluated the effect of rs3737787 on lipids in 2 distinct study samples ascertained for CAD: Dutch FCHL families with a higher burden of undetected coronary atherosclerosis than the population, and a U.S. cohort with documented coronary atherosclerosis. In both study samples, we tested for the association of rs3737787 with lipids. All subjects of the U.S. cohort underwent diagnostic coronary angiography, allowing for assessment of the extent of their coronary atherosclerosis. To the best of our knowledge, this is the first study to evaluate the effect of USF1 on lipids in U.S. Whites with documented significant coronary artery stenosis.

Methods

Study Subjects

Dutch FCHL Families
All subjects provided written informed consent and the study design was approved by the Human Investigation Review Committee of the University Hospital, Utrecht. The 27 Dutch FCHL families (532 individuals genotyped) were recruited at the Lipid Clinic of the Utrecht Academic University Hospital, the Netherlands. The inclusion and exclusion criteria of the probands were described previously. The Finnish age-sex specific 90th population percentiles of TC, TGs, and apolipoproteins were used to classify the Dutch families because Dutch specific population percentiles were unavailable. Subjects with TC or TG levels greater than the Finnish age-sex specific 90th percentiles were classified as FCHL affected. The National Cholesterol Education Program Adult Treatment Panel III criteria based on abdominal obesity, TGs, HDL-C, blood pressure, and fasting glucose assessments were used to classify the metabolic syndrome (MS) status.

GeneBank Cohort
All subjects gave written informed consent, and the Institutional Review Board of Cleveland Clinic Foundation approved the study protocol. A total of 1533 sequential subjects from the GeneBank cohort, a prospective clinical study designed to research cardiovascular disease, were genotyped. Details of the GeneBank study design are described in detail elsewhere. Briefly, the GeneBank subjects represent consenting sequential subjects age 18 years or older undergoing diagnostic cardiac catheterization at the Cleveland Clinic. Accordingly, CAD was considered present (n=1114 "CAD") if significant angiographic evidence of coronary artery stenosis (≥50%) in 1 or more major coronary vessels was noted, and considered absent (n=418 "nonCAD") if no evidence of significant angiographic coronary artery stenosis was found. Ethnicity was determined by self report and included maternal and paternal ethnicity. The number of non-White subjects (n=165) was insufficient for ethnicity stratified analysis. Therefore, they were excluded from the association analysis to avoid false-positive results attributable to population stratification, resulting in 1368 Whites analyzed.

Myocardial infarction (MI) status was classified as previously described (453 affected subjects). The revascularization status was determined by history of coronary artery bypass graft (CABG; 474 subjects) or percutaneous coronary intervention (PCI; 445 subjects).

Genotyping
The rs3737787 SNP was genotyped using the Pyrosequencing technique on the PSQ HS96A platform. The SNP was in Hardy-Weinberg equilibrium in the total study sample and in every subcategory (males, females, CAD, and nonCAD subjects) of the GeneBank cohort, and in the Dutch FCHL founders.

Biochemical Analysis
In the Dutch FCHL families, lipids and other metabolic phenotypes were measured as described previously. Lipid-lowering drug treatments were withheld for 3 weeks before phenotypic data measurements. In the GeneBank study sample, fasting lipid and basic metabolic panels were collected using standard methods as described elsewhere.

Statistical Analysis

Dutch FCHL Families
In the Dutch families, the PedCheck program was used to detect Mendelian errors. We tested SNP rs3737787 for association with qualitative traits using the haplotype-based haplotype relative risk (HHRR) test in the ANALYZE package, a helper program used to run the HHRR test. The Association Given Linkage option (option 22) of Mendel 7.0, assuming dominance and the empirical variance option-e of the family-based association test (FBAT) were used to test for association given linkage for TGs and FCHL. Mendel determines the direct effect of the risk allele, whereas FBAT is a transmission distortion based test. Using these 2 programs, which are based on different modeling assumptions and use different data attributes, allows for thorough assessment of association. The quantitative association analyses of age- and sex-adjusted residual values of BMI were performed using FBAT. Genotype by sex interaction was tested by the penetrance estimation (option 14) of Mendel using a logistic regression model assuming dominant disease transmission, binary TG outcomes, and genotypes as allele counts, with or without the inclusion of the genotype interaction with sex. Probability values were generated by the likelihood ratio statistic with 1 degree of freedom.

GeneBank Cohort
Statistical analyses were performed using the SPSS 15.0 software. Because the samples represent sequential consenting subjects enrolled in the study and were not selected on the basis of any given trait, multivariate linear regression specified by the model \( y_i = \alpha + \beta_1 X_{1i} + \beta_2 X_{2i} + \epsilon_i \) was used to assess the effects of rs3737787 on continuous traits (TGs, BMI, glucose, and 0 to 4 number of vessels with ≥50% stenosis). Log transformations were performed for traits with nonnormal distributions. Presence or absence of CAD was used as a covariate in the combined analyses of CAD and nonCAD groups. To evaluate the effect of rs3737787 on CAD, MI, and revascularization (CABG, PCI), logistic regression was performed using the covariates of age, sex, blood pressure, dyslipidemia, and BMI when significant. We were not able to adjust for smoking given the lack of data for more than 450 individuals. Trait values outside of 3.5 standard deviations from the adjusted mean were considered outliers and therefore excluded. A dominant model of inheritance coded as C/C=0 and T/T, C/T=1 was used, as the original linkage signal was obtained with a dominant model. Genotype by sex interactions were tested using 2 factor ANOVA by inclusion of the genotypes, sex, and their interaction term in the model \( y_i = \alpha + \beta_{sex} X_{1i} + \beta_{sexSex} X_{2i} + \beta_{sexMale} X_{3i} + \beta_{sexMale} X_{4i} + \epsilon_i \).

Results

Association Analysis of rs3737787 in Dutch FCHL Families
The phenotypic characteristics of the 27 Dutch FCHL families are described in Table 1. The rs3737787 SNP, with a
Results were obtained by the HHRR test.
A negative Z score indicates the common C risk allele.
allele in females. The MAF of rs3737787 in all members was 0.27, similar to the Dutch (MAF/H11005 0.27). In the Finnish FCHL families the MAF was 0.34. No association was observed between rs3737787 and TC levels.

We did not consider the effect of BMI as a covariate, but rather we tested it as a separate trait, because the same genetic factors may contribute to BMI and TG levels. We observed the same pattern of sex- and allele-specific associations for BMI as for TGs in the CAD group (P/male/H11005 0.02, P/female/H11005 0.007; Figure, B). In females with CAD, we also observed higher fasting glucose values in carriers of the rare allele (P/H11005 0.009). However, no association was obtained between rs3737787 and fasting glucose values in males. Taken together, our results demonstrate the consequence of rs3737787 variant on several metabolic risk factors in CAD.

We also tested whether allele frequencies or genotype groups (using a dominant model) of rs3737787 differed between the CAD and nonCAD subjects. No differences were observed, which may partially reflect the small sample size and younger age of the nonCAD subjects (Table 1). We also performed logistic regression analyses of CAD, MI, and revascularization status using the significant covariates. The number of vessels with ≥50% stenosis was also tested as a continuous trait. No significant results were obtained in these analyses.

### Sex-Dimorphic Association of rs3737787 in FCHL and CAD

Our data both in the Dutch FCHL families and the GeneBank cohort provide consistent evidence for a significant sex-specific association of the common allele with TGs in males (Tables 2 and 3). Next, we stratified the mean TG levels by rs3737787 genotype and sex for the Dutch FCHL families and the GeneBank cohort (Table 4). Interestingly, in FCHL and CAD affected males, TG levels are higher in the common CC genotype carriers than in the rare T allele carriers. In females of the GeneBank cohort with CAD, the rare T allele carriers have higher TGs, although this effect is not observed in the females with FCHL. No differences in mean TGs were observed between genotype groups in FCHL and CAD unaffected individuals. To further investigate this intriguing relationship between rs3737787 genotype and sex, we examined the effect of genotype x sex interaction on the traits. In the Dutch FCHL families, we used the Mendel program (see Methods), and observed a significant rs3737787 genotype x sex interaction that influences the qualitative TG affection (P/H11005 0.0003; Table 4). The binary TG affection was tested because the Dutch FCHL families were ascertained through a proband and a first degree relative with high TC or TGs, and the variance of TG levels in these families is reduced and thus limited for effective quantitative analysis. In the GeneBank cohort, univariate analysis of variance showed that quantitative values of both TGs and BMI are significantly affected by the rs3737787 genotype x sex interaction (P=0.0005, 0.00004, respectively; Table 4 and Figure). The effect of rs3737787 genotype on TGs in the Dutch FCHL families and the GeneBank cohort is thus sex-dependent. This significant interaction between the genotype and sex (Figure) also describes the results of the association analyses implicating different risk alleles between males and females.

### Discussion
High serum lipid levels predispose to CAD. In this study, we examined the relationship between the USF1 variant,
Table 4. The Effect of Genotype and Sex on TGs in Dutch FCHL Families and the GeneBank Cohort

<table>
<thead>
<tr>
<th>Study Sample</th>
<th>CC</th>
<th>TX</th>
<th>P Value*</th>
<th>Sex Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Dutch FCHL Affected</td>
<td>3.7±2.0</td>
<td>2.3±1.1</td>
<td>3.3±2.3</td>
<td>2.2±0.9</td>
</tr>
<tr>
<td></td>
<td>(34)</td>
<td>(64)</td>
<td>(31)</td>
<td>(42)</td>
</tr>
<tr>
<td>Dutch FCHL Unaffected</td>
<td>1.6±0.6</td>
<td>1.2±0.4</td>
<td>1.6±0.6</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td></td>
<td>(99)</td>
<td>(91)</td>
<td>(84)</td>
<td>(59)</td>
</tr>
<tr>
<td>GeneBank CAD</td>
<td>2.0±1.3</td>
<td>2.0±1.2</td>
<td>1.8±1.1</td>
<td>2.5±1.8</td>
</tr>
<tr>
<td></td>
<td>(397)</td>
<td>(143)</td>
<td>(364)</td>
<td>(101)</td>
</tr>
<tr>
<td>GeneBank nonCAD</td>
<td>1.9±1.2</td>
<td>1.6±0.9</td>
<td>1.9±2.1</td>
<td>1.5±0.9</td>
</tr>
<tr>
<td></td>
<td>(91)</td>
<td>(84)</td>
<td>(82)</td>
<td>(81)</td>
</tr>
</tbody>
</table>

Data represent the mean TGs, mmol/L±SD (n). CC indicates homozygotes of the common allele; TX, carriers of the rare allele; ns, P value > 0.1.

*The P values represent the results of the HHRR test for qualitative TGs in the Dutch FCHL families and multivariate regression analysis for TGs in the GeneBank cohort.

†The genotype x sex interaction results were obtained using the penetrance estimation (option 14) of Mendel16 in the Dutch families and by 2 factor ANOVA in the GeneBank cohort, as described in Methods.

rs3737787, and lipid traits in Dutch FCHL families and in U.S. Whites who underwent diagnostic coronary angiography. This study shows that this common variant of USF1 influences serum lipid levels in a similar sex-dependent manner both in FCHL and in subjects with significant angiographic evidence of CAD. In the Dutch FCHL families, rs3737787 showed a significant association with FCHL and its component traits in males. In the GeneBank cohort, we show for the first time that USF1 influences lipid and metabolic traits in U.S. White subjects with angiographically-verified CAD in a sex-dependent manner. Moreover, we show that a genotype by sex interaction significantly influences TGs in the Dutch FCHL families, and TGs and BMI in the GeneBank cohort. Importantly, this is the first time that a sex-genotype interaction is statistically demonstrated in FCHL.

USF1 was originally identified as an FCHL candidate gene in a sex-dependent manner in a family-based study. Additionally, the association with USF1 has been replicated for the FCHL, TGs, and low density lipoprotein cholesterol (LDL-C) traits, in several independent studies. The association results for type 2 diabetes mellitus (T2DM), which shares linkage to the 1q21–23 region using the T2DM status as an end point have been mostly negative, although, these studies did not address the sex-specific effect of USF1 (reviewed in reference). In the current study, we replicated the association between USF1 and FCHL and its component traits in Dutch FCHL families. Moreover, to the best of our knowledge, this is the first study showing that USF1 has a sex-specific effect on lipid and metabolic traits in unrelated U.S. White subjects with CAD. In female U.S. Whites with CAD, the rare allele was associated with risk, whereas in males of both study samples, the common allele of rs3737787 conferred risk. This result was also described by the significant genotype x sex interaction influencing TG levels in both study samples. The molecular mechanisms underlying this sex-specific allelic difference are currently unknown. However, hormonal factors may contribute to allele- and sex-specific differences in the expression of USF1 or the regulation of its target genes. The results of the association analyses, as well as the significant sex-genotype interaction detected in the present study, demonstrate the importance of underlying sex specific differences in CAD, which have been well established in previous epidemiological studies.

In this study we investigated the rs3737787 variant because this SNP along with rs2073658, in tight LD, have been the most associated SNPs in the previous studies. Furthermore, Komulainen et al examined 6 haplotype-tagging SNPs and observed that rs2073658 was the statistically most significant signal for CAD in females of the Finnish population based cohort.

Recently, van der Vleuten et al evaluated the role of rs3737787 and rs2073658 SNPs in an independent Dutch FCHL cohort. Consistent with our results, they also observed association between rs3737787, rs2073658 variants, and TC, apoB, and FCHL. However, association with TGs, or an apparent sex effect was not observed in these families. Although some differences in the diagnostic, inclusion, and exclusion criteria were used in ascertainment of the 2 Dutch FCHL study samples, the relatively low numbers of subjects in these families, which are difficult to diagnose for the complex FCHL phenotype, may have influenced the differences in the outcome of the 2 studies. As FCHL is a complex disease with multiple genes, environmental factors, and their interactions contributing to the phenotype, differences in diagnosis, ascertainment and analysis of different FCHL cohorts may also contribute to differences in the results. Furthermore, as discussed by van der Vleuten et al, identification of USF1 as a modifier gene in their study sample may be of importance as this Dutch study sample did not reveal any significant or suggestive linkage signals at the genome-wide level.

Numerous genetic and environmental factors are involved in the etiology of CAD. Therefore, the combined effect of the rs3737787 risk allele and additional genetic and environmental risk factors is likely to be more pronounced, and thus more easily detectable, in study samples that are enriched for CAD risk factors. This may explain the detection of the TG and BMI associations in CAD subjects, but not in the nonCAD subjects of the GeneBank cohort, as well as the robust association observed with TGs in Finnish, Mexican and Dutch FCHL families that were ascertained for elevated TGs. Our results are in line with a previous study that demonstrated an interaction between SNPs in LD with rs3737787 and BMI in determining LDL-C and fasting
glucose levels in cases with a family history of premature CAD, but not in the controls.\(^4\)

The lack of association with the CAD, MI, and revascularization phenotypes may reflect the fact that the study design is not optimal for a traditional case-control analysis because of the relatively small number of nonCAD subjects, ie, subjects without significant stenosis by angiography. This may result in insufficient power to detect genotypic effects, particularly in a sex-specific manner. Furthermore, in case-control studies of late-onset diseases such as CAD, older control subjects who are less likely to harbor predisposing genetic risk factors are preferable. However, in the current study, the nonCAD group was approximately 10 years younger than the CAD group, this being a significant difference in age. Hence, the significance of \(rs3737787\) variant in CAD needs to be addressed in future studies using stratified analysis of larger case–control samples for CAD.

USF1 is an especially relevant candidate for FCHL and more generally for CAD because its target genes encode apolipoproteins and important enzymes involved in lipid metabolism, such as hepatic lipase and hormone sensitive lipase.\(^3\) Therefore, an alteration in the expression level or function of USF1 may influence a variety of metabolic features in addition to serum lipid levels, as evidenced by the observed association with the MS in the Dutch families and component traits of the MS in the GeneBank cohort. Furthermore, in study samples ascertained for various CAD risk factors and in a population-based prospective cohort, significant associations have been reported between \(rs3737787\) (or SNPs in LD) and metabolic traits that predispose to CAD, including peak glucose level during oral glucose tolerance test, TGs, TC, LDL-C, apoB and adiposity indices.\(^2\)–\(^7\) Together, these studies provide further support for the hypothesis that \(rs3737787\) is involved in the complex CAD phenotype.

In conclusion, we have identified significant, sex-specific associations between USF1 and risk factors that predispose to CAD in Dutch FCHL families and U.S. Whites with angiographically verified CAD.

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Disclosures
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References
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