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Common coding variants of the *HNF1A* gene are associated with multiple cardiovascular risk phenotypes in community-based samples of younger and older European-American adults: the Coronary Artery Risk Development in Young Adults study and the Cardiovascular Health Study

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Abstract

Background—The transcription factor hepatocyte nuclear factor 1 (*HNF-1*) α regulates the activity of a number of genes involved in innate immunity, blood coagulation, lipid and glucose transport and metabolism, and cellular detoxification. Common polymorphisms of the *HNF-1* α gene (*HNF1A*) were recently associated with plasma C-reactive protein (CRP) and gamma-glutamyl transferase (GGT) concentration in middle-aged to older European-Americans (EA).

Methods and Results—We assessed whether common variants of *HNF1A* are associated with CRP, GGT, and other atherosclerotic and metabolic risk factors, in the large, population-based CARDIA study of healthy young European-American (EA; n=2,154) and African-American (AA; n=2,083) adults. The minor alleles of Ile27Leu (*rs1169288*) and Ser486Asn (*rs2464196*) were associated with 0.10 to 0.15 standard deviation units lower CRP and GGT levels in EA. The same *HNF1A* coding variants were associated with higher LDL cholesterol, apolipoprotein B, creatinine, and fibrinogen in EA. We replicated the associations between *HNF1A* coding variants and CRP, fibrinogen, LDL cholesterol, and renal function in a second population-based sample of EA adults 65 years and older from the Cardiovascular Health Study. The *HNF1A* Ser486Asn and/or Ile27Leu variants were also associated with increased risk of subclinical coronary atherosclerosis in CARDIA and with incident coronary heart disease in CHS. The Ile27Leu and Ser486Asn variants were 3-fold

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less common than in EA. There was little evidence of association between *HNF1A* genotype and atherosclerosis-related phenotypes in AA.

Conclusions—Common polymorphisms of *HNF1A* appear to influence multiple phenotypes related to cardiovascular risk in the general population of younger and older EA adults.

Keywords

atherosclerosis; genetics; C-reactive protein; HNF-1; gamma glutamyl transferase

INTRODUCTION

The transcription factor hepatocyte nuclear factor (HNF)-1 α is expressed in the liver, kidney, and endocrine pancreas and regulates a number of genes involved in innate immunity, blood coagulation, lipid and glucose transport and metabolism, and cellular detoxification [1-8]. Sequence variants of the gene encoding HNF-1 α *HNF1A* (also known as *TCF1*) have been associated with several distinct cardiovascular disease (CVD) risk factors and metabolic phenotypes. Rare, heterozygous mutations of *HNF1A* are responsible for the autosomal dominant disorder mature-onset diabetes of the young type 3 (MODY3) [9]. In several recent genome-wide analyses, common variants of the *HNF1A* region on chromosome 12 were associated with circulating levels of fibrinogen [10], C-reactive protein (CRP) [11,12], and gamma-glutamyl transferase (GGT) [13]. Whether *HNF1A* variants are associated with these or other atherosclerotic and metabolic phenotypes in independent community-based samples that include non-Caucasian populations is unknown.

Inflammation and thrombosis biomarkers such as CRP and fibrinogen are correlated with one another and tend to cluster with other atherosclerotic and metabolic risk factors such as cholesterol, insulin resistance, as well as with markers of oxidative stress such as GGT [14, 15]. Genetic co-regulation by transcription factors such as HNF-1 α might explain some of the correlation between atherosclerotic phenotypes. In addition, variants of genes that pleiotropically influence several etiologic pathways may be good candidates for association with complex, multi-factorial vascular phenotypes such as coronary atherosclerosis. This is an important goal clinically since identification of genetic markers that predict coronary disease may ultimately allow targeting of susceptible individuals for aggressive risk modification or drug therapy. Therefore, we assessed whether common variants of *HNF1A* are associated with various atherosclerotic and metabolic risk factors, as well as the more complex phenotype of subclinical coronary atherosclerosis, in a large, population-based study of apparently healthy young European-American (EA) and African-American (AA) adults. To further assess the clinical relevance of *HNF1A* polymorphisms, we replicated our association findings with multiple CVD-related phenotypes in a U.S. population-based sample of older adults who are at higher global risk for developing clinical coronary heart disease.

METHODS

CARDIA study participants and phenotype measurements

The Coronary Artery Risk Development in Young Adults (CARDIA) Study is a prospective cohort study of the development of cardiovascular risk factors in young adults [16]. In 1985-86, 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 study subjects were 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. Those eligible for the current study were 4,304 consenting participants who had DNA aliquots available for genotyping.

Measurement of the plasma CVD biomarkers CRP, fibrinogen, cholesterol, apolipoprotein B, GGT, aspartate aminotransferase (AST), creatinine, glucose, and insulin, were performed at various CARDIA examination time points, as described under Supplemental Methods. Coronary artery calcium (CAC), a measure of subclinical coronary atherosclerosis, was determined at the year 20 CARDIA exam by electron beam or multi-detector computerized tomography scanning using methods that have been previously described [17]. For each CVD biomarker, multiple measurements were available on the majority of participants, but each biomarker was measured at different time points (Supplemental Table 1). Therefore, for each biomarker, covariate (age, sex, BMI, and smoking)-adjusted Z-score values derived at each time point were averaged to obtain a composite standardized value for each participant (see Supplemental Methods for further details). This final averaged value was used as the phenotype (dependent variable) in regression models assessing association with *HNF1A* genotype. The approach of averaging multiple phenotypic measures over time can help to reduce the effects of measurement error and environmental variation, thereby providing a more stable estimate of the phenotype and enhancing power to detect true genetic signals [11].

***HNF1A* SNP selection and genotyping**

Six SNPs in *HNF1A* (*rs1169288*, *rs2071190*, *rs2259820*, *rs2464196*, *rs3999413*, and *rs1882149*) were typed in 4,304 CARDIA participants (2,129 AA and 2,175 EA). The *rs1169288*, *rs2259820*, and *rs2464196* polymorphisms were selected because they encode previously known non-synonymous *HNF1A* exonic substitutions that alter the coding sequence. The remaining 3 tagSNPs were selected on the basis of having a minor allele frequency of >10% in EA and providing non-redundant coverage of linkage disequilibrium (LD) patterns across *HNF1A*. It should be noted that these SNPs were selected using sequence variation data from the Perlegen database in 2003, prior to the availability of more comprehensive sequence variation databases such as the current version of the HapMap. When assessed against the current release of the HapMap, using an allele frequency threshold of >5% and a multi-marker LD tagging strategy of $r^2 > 0.7$ [18], the selected *HNF1A* SNPs tag 6 of 12 common LD bins (50%) present in Europeans and 4 of 14 LD bins (29%) in Africans.

Polymorphisms were genotyped using the TaqMan assay (Applied Biosystems, Foster City, CA) as previously described [19]. Primer and probes are available from the authors upon request. Polymorphism genotyping in the CARDIA study adheres to a rigorous quality control (QC) program, which includes barcode identification of samples, robotic sample handling, and blind replicate genotype assessment on 5% of the total sample.

After excluding 67 participants with missing genotype data at 2 or more SNPs, the total missing genotype rate was 1.5% and ranged from 0.5% to 2.6% per SNP. Genotypes at *rs2259820* and *rs2464196* were highly correlated (pair-wise $r^2 = 0.997$ in EA and 0.962 in AA); therefore *rs2259820* was excluded from further analysis. The minor allele frequency of *rs3999413* was <5% in AA, and therefore was excluded from analysis among the AA cohort.

Validation cohort (the Cardiovascular Health Study)

The Cardiovascular Health Study (CHS) is a prospective population-based cohort study of 5,888 men and women aged 65 and older recruited from four U.S. field centers: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania [20]. The original CHS cohort (n=5,201) was recruited from 1989 to 1990, and included 4,925 self-identified EA and 246 AA. A second AA cohort (n= 687) was recruited between 1992 and 1993. At study entry, CHS participants underwent assessment of

CVD risk factors and fasting blood collection [21]. Blood CVD biomarker measurements from the baseline examination (year 0 for the original cohort or year 3 for the minority cohort) were performed at the Central CHS Laboratory at the University of Vermont, as described under Supplemental Methods. In addition to serum creatinine, cystatin C concentration was available as an alternative measure of renal function. The final CHS sample for the current study was restricted to 4,352 European-American and 790 African-American men and women who consented to DNA testing. Several *HNFA* tagSNPs, including the two non-coding variants *rs1169288* and *rs2464196*, were typed in CHS as previously described [11].

Details of clinical CHD events ascertainment during CHS follow-up have been published [22]. Clinical CHD events were adjudicated by physician review panel according to medical records, death certificates, and supplemented by Medicare utilization data. Adjudicated events occurring through June 30, 2005 were available, which allowed for a maximum of 16 years of follow-up. For purposes of the current study, we analyzed all incident coronary heart disease events (angina, myocardial infarction, fatal CHD, coronary re-vascularization, or coronary bypass surgery) as a composite clinical CHD endpoint. Subjects with a history of CHD prior to baseline (847 EA and 152 AA) were excluded from the analysis of incident CHD events.

Statistical analysis

Consistency of observed genotype frequencies with Hardy-Weinberg equilibrium (HWE) within each self-reported race/ethnicity group was assessed by performing Pearson's chi-squared test. Linkage disequilibrium as a measure of allelic correlation between pairs of polymorphic sites was calculated as r^2 . Pair-wise LD calculations and haplotype estimation was performed using Haploview program version 4.1 (<http://www.broad.mit.edu/mpg/haploview/download.php>).

Associations between individual SNP genotypes and biomarker phenotypes were assessed using linear regression, and adjusted for age-, sex-, BMI-, and smoking. We assessed the association of *HNFA* SNPs with subclinical coronary atherosclerotic disease defined as the presence of CAC at the year 20 CARDIA exam using logistic regression, initially adjusted for age, sex, and clinic. In CHS, associations with time to clinical CHD event were assessed using Cox proportional hazards regression, and adjusted for age, sex, and major risk factors (smoking, diabetes, hypertension, LDL-cholesterol, BMI, and CRP) at baseline. All regression analyses were performed using the statistical package Stata/SE8.2 (Stata Corp., College Station, TX, USA). For our primary analysis, we assessed phenotypic associations with *HNFA* SNP genotypes using an additive genetic model, assuming a constant, linear effect size for each additional copy of the minor allele. Covariate-adjusted SNP-specific change in estimated mean plasma biomarker level, odds ratio of CAC, or relative risk of CHD was estimated from the regression coefficients (β). To assess whether a particular *HNFA* genotype – CVD phenotype association was influenced by the presence of other risk factors, we repeated each analyses adjusting for additional CVD biomarkers, atherosclerotic and metabolic risk factors. To combine *HNFA* genotype – CVD biomarker phenotype association results across CARDIA and CHS, we used a variance-weighted meta-analysis approach that allows for heterogeneity of results between studies [23]. Procedures for adjustment of population stratification, correction for multiple hypothesis testing, and haplotype association analysis are described under Supplemental Methods.

RESULTS

CARDIA study participant characteristics, phenotype and genotype distributions

Descriptive characteristics of eligible CARDIA participants at the baseline (n=4,304) and year 20 follow-up (n=3,504) exams are shown in Table 1. The mean age at study entry was 25 years,

and 55% were women. Current smoking, BMI, GGT, blood pressure, CRP, fibrinogen, and insulin resistance were higher among EA than AA. CAC scores at year 20 ranged between 0 and 6058, but the distribution was highly skewed, with a mean score of 24. The prevalence of coronary artery calcium (score >0) was higher among EA (21%) than AA (16%). Several of the age-, sex-, BMI-, and smoking-adjusted averaged phenotype values (in S.D. units) had pair-wise correlation coefficients greater than 0.25: LDL and apolipoprotein B ($r^2=0.72$); CRP and fibrinogen ($r^2=0.42$), and GGT and AST ($r^2=0.28$).

HNF1A SNP genomic locations and allele frequencies are shown in Table 2, by race. The minor alleles of *rs1169288* and *rs2464196* were nearly 3 times as common among EA compared to AA. Within each race/ethnicity, each SNP was in Hardy-Weinberg equilibrium, except for *rs2464196* in EA ($p=0.005$). In pair-wise linkage disequilibrium analysis (supplemental Figure 1), the r-squared between *rs1169288* and *rs2464196* was 0.69 among EA and 0.41 among AA. The r-squared between *rs2071190* and *rs1882149* was 0.42 among EA and 0.35 among AA. All other pair-wise SNP r-squared values were <0.15.

Associations between *HNF1A* genotype and CVD-related biomarkers in CARDIA

Using the averaged, standardized biomarker values as the phenotype to reduce measurement error, each additional copy of the minor alleles of the two non-synonymous coding SNPs, Ile27Leu (*rs1169288*) and Ser486Asn (*rs2464196*), were significantly associated with 0.15 S.D. units (95% CI 0.09 – 0.21 S.D. units) lower mean log(CRP) and with 0.10 S.D. units (95% CI 0.05 – 0.15) S.D. units) lower mean GGT in the EA cohort (Table 3). There was a non-significant trend toward lower log(CRP) and GGT among AA. At a nominal alpha level of 0.05, the *HNF1A* *rs1169288* or *rs2464196* coding variant alleles were also associated with higher levels of total and LDL cholesterol, apolipoprotein B, and creatinine in EA, and with higher fasting glucose in AA. Except for the log(CRP), GGT, and creatinine phenotype associations in EA, the *p*-values became non-significant (<0.05) following correction for multiple testing.

There was no association between *HNF1A* genotype and fibrinogen, HDL cholesterol, insulin, or AST levels in models adjusted for age, sex, clinic, BMI, and smoking. When these analyses were additionally adjusted for other risk factors including CRP, the minor alleles of *rs1169288* ($\beta=0.11 \pm 0.03$; $p=0.0002$) and *rs2464196* ($\beta=0.13 \pm 0.03$; $p=1 \times 10^{-5}$) became strongly associated with higher fibrinogen levels in EA.

Haplotype analyses for selected phenotypes are shown in Supplemental Table 2. *HNF1A* haplotypes were associated with CRP in EA (global $p=4 \times 10^{-6}$) but not in AA (global $p=0.45$) (Supplemental Table 2). A common haplotype tagged by the minor Val27 allele of *rs1169288* and minor Asn486 allele of *rs2464196* (frequency =0.28% among EA and 0.08 among AA) was associated lower CRP in EA ($p=3 \times 10^{-7}$) but not in AA ($p=0.71$). By performing a conditional haplotype analysis in which the effect of each SNP is stratified according to haplotypic background, an independent association with CRP levels in EA was observed for *rs1169288* ($p=0.01$) but not for *rs2464196* ($p=0.25$). Similar haplotype analysis results were obtained for GGT, apolipoprotein B, and creatinine (Supplemental Table 2).

Replication of *HNF1A* genotype-CVD biomarker associations in CHS

The CHS participant characteristics are shown in Table 1, stratified by race. The mean age was 73 years, 60% were female, and 15% were African-American. Compared to CARDIA participants, CHS participants were older and at greater cardiovascular risk (19% had prevalent CHD at baseline). As shown in Table 4, when the results for the most recent CARDIA exam (year 20; mean age 45) were compared to the results from the CHS baseline exam (mean age 73), the effect sizes and magnitude of the *HNF1A* genotype - biomarker associations were

similar in EA for *rs1169288* and *rs2464196*. The association between *rs1169288*, *rs2464196* and CRP has been previously reported in CHS EA [11]. Here, we confirm that the variant alleles of *HNF1A* *rs1169288* and *rs2464196* coding SNPs are associated with higher total and LDL cholesterol and decreased renal function (as assessed by higher plasma cystatin C levels) in older EA adults from CHS. We also replicated the fibrinogen association, which again was only demonstrable upon adjustment for CRP. Among 790 AA participants from CHS, there were no significant associations with *HNF1A* genotype, except *rs1169288* was associated with decreased renal function, as indicated by increased serum creatinine and cystatin C levels (Supplemental Table 3). In a pooled stratified analysis of 2,873 AA subjects across CARDIA and CHS, none of the CVD biomarker phenotype associations were statistically significant (pooled p-values <0.05).

Association of *HNF1A* genotype with subclinical atherosclerosis in CARDIA and clinical coronary heart disease in CHS

In age-, sex-, and clinic- adjusted models, the minor allele of the *rs2464196* Ser486Asn polymorphism was associated with 1.3-fold increased risk of CAC (nominal $p=0.006$; multiple test corrected $p = 0.23$) at the year 20 CARDIA exam (Table 5). In contrast, there was little evidence of association between *HNF1A* genotype and risk of coronary atherosclerosis in CARDIA AA. Conditional haplotype analysis in EA confirmed an independent association between CAC and *rs2464196* ($p=0.02$) but not *rs1169288* ($p=0.12$). Additional multivariable adjustment for other atherosclerotic risk factors ascertained at the year 20 examination (smoking, BMI, diabetes, hypertension, lipids, CRP, and fibrinogen levels) did not alter the *rs2464196* - CAC association (odds ratio = 1.27; 95% CI 1.04 – 1.56). When CAC score was analyzed as a quantitative variable, the age-, sex-, and clinic-adjusted p-values were 0.08 for *rs1169288* and 0.009 for *rs2464196* in EA and 0.76 and 0.51 in AA. When the CAC association results for the *rs2464196* Ser486Asn polymorphism were stratified by sex, there were no appreciable differences between men and women.

In CHS, there were 1,492 incident CHD events (MI, angina, coronary bypass surgery or revascularization) during a median follow-up of 11.4 years. When adjusted for age, sex, race, smoking, BMI, diabetes, hypertension, lipids, CRP, and fibrinogen levels, the hazard ratio for CHD events associated with each additional copy of the minor allele of *rs1169288* and *rs2464196* were 1.12 (95% confidence interval 1.03 – 1.22; $p=0.008$) and 1.10 (95% confidence interval 1.01 – 1.20; $p=0.026$), respectively.

DISCUSSION

Our findings from the population-based CARDIA cohort confirm results from recent genome-wide association studies [11-13] that common coding sequence variants of the transcription factor gene *HNF1A* [*rs1169288* (Ile27Leu) and *rs2464196* (Ser486Asn)] are associated with lower plasma CRP and GGT levels in EA adults. These *HNF1A* coding variants also showed evidence of association with several other blood biomarker phenotypes related to CVD risk, including higher LDL cholesterol, apolipoprotein B, and fibrinogen levels and reduced renal function, in younger and older EA adults from CARDIA and CHS, respectively. Finally, there was some evidence that the *HNF1A* *rs2464196* coding variant were associated with increased risk of subclinical coronary atherosclerosis and with incident clinical CHD. While this manuscript was under review, two large-scale GWAS studies (involving tens of thousands of subjects) were published mapping common *HNF1A* risk alleles to higher plasma LDL levels [24] and increased risk of CHD [25] in European-Americans, providing further validation for the association between *HNF1A* genotype and atherosclerosis outcomes. Together with the known role of *HNF1A* mutations in familial monogenic diabetes (MODY) [9], the pleiotropic effects of *HNF1A* variants on multiple CVD and metabolic phenotypes highlight the role of

HNF-1 α as both a positive and negative transcriptional regulator of a large network of hepatic, renal, and pancreatic genes involved in inflammation, blood coagulation, insulin secretion, cholesterol synthesis, lipid transport, cellular detoxification, and renal function [1-8], which in concert may act to influence overall susceptibility to coronary atherosclerotic disease.

The lack of statistically significant associations with lower CRP or GGT in AA might reflect reduced statistical power due to the 3-fold lower frequency of Ile27Leu and Ser486Asn allele in AA compared to EA. Nonetheless, we were still unable to observe significant observations by performing a combined analysis of n=2,873 AA participants from CARDIA and CHS. Therefore possible reasons for the lack of observed associations in AA include differences in genetic or environmental background or differential linkage disequilibrium between SNPs in EA versus AA populations due to greater nucleotide diversity among Africans. It is becoming increasingly apparent that genetic differences exist between the determinants of CVD phenotypes in African and European Americans [27]. Moreover, it is important to note that SNPs typed in the current study covered only a fraction of the known genetic diversity of the *HNF1A* locus in Africans.

While rare, heterozygous mutations in *HNF1A* are responsible for familial monogenic forms of diabetes (MODY3), the role of common *HNF1A* polymorphisms in type 2 diabetes in the general population is less clear. The region harboring *HNF1A* on chromosome 12q24 has shown genetic linkage to diabetes in EA pedigrees [28], but European and North American case-control studies of common *HNF1A* variants in type 2 diabetes have yielded conflicting results [29-31]. Recently, the Ile27Val variant was associated with increased risk of type 2 diabetes in a population-based cohort study from Scandinavia [32]. These data again suggest the possible importance of genetic or environmental background on the association between common *HNF1A* polymorphisms and CVD- and metabolism-related traits in the general population.

HNF-1 α has a complex role in coordinating hepatocyte-specific gene expression. Promoter or enhancer HNF-1 α binding sites are present in the structural genes encoding hepatic synthesis of many plasma proteins such as CRP [33,34], fibrinogen [35,36], and apolipoprotein B [37]. In addition to positively regulating a large number of downstream target genes, HNF-1 α can also modulate transcription indirectly by HNF-1 α -mediated negative regulation of genes activated by HNF-4 α [38,39]. CRP and fibrinogen are both acute phase reactants that tend to be positively correlated, but they are not regulated in an identical manner [40,41]. CRP is unique among acute phase reactants in that estrogen appears to have a major first-pass liver effect and up-regulates CRP while down regulating other acute phase reactants such as fibrinogen [42]. Together these observations support the complex genetic regulatory relationships that likely underlie the direction of some of the observed *HNF1A* genotype – CVD phenotypic associations, as well as the observation that by adjusting our fibrinogen analysis for CRP, we were able to uncover an *HNF1A* genotype-fibrinogen relationship that was distinct from the observed effect of *HNF1A* genotype on CRP. While multiple phenotypic associations may suggest a common genetic cause (pleiotropy), it is also important to point out that such a scenario may also represent indirect genetic effects primarily with a subset of these phenotypes and/or complex non-genetic/environmental correlations between CVD traits [43]. Ultimately, the application of more complex multivariate statistical methods along with molecular functional studies will be required distinguish among these possibilities.

The association between the *HNF1A* Ser486Asn variant and *increased* risk of coronary atherosclerosis and CHD, despite the strong association of the Ser486Asn and Ile27Val alleles with *lower* levels of CRP and GGT, may have several possible explanations. Given the central but complex role of HNF-1 α in transcriptional regulation, it is likely that the same *HNF1A* polymorphisms, besides their influence on higher LDL cholesterol and fibrinogen, affect the

activity or expression of additional (unmeasured) phenotypes that influence initiation or progression of atherosclerosis. Supporting this possibility, the *HNF1A* genotype – CHD associations in CARDIA and CHS persisted despite adjustment for other traditional CVD risk factors, such as blood pressure, BMI, lipids, and other CVD biomarkers. Moreover, the *HNF1A* *rs2259816* variant (which in strong LD with our *rs2464196* typed SNP) associated with increased CHD risk in a recent GWAS showed no significant associations with other traditional CVD risk factors [25]. Such associations can be indicative of pleiotropy (i.e. common genetic causes), of indirect genetic effects via one of these phenotypes, or can be solely attributable to non-genetic/environmental links between the traits. To identify the phenotypes with the inducing genetic association, statistical methodology is needed that is able to distinguish between the different causes of the genetic associations.

HNF1A variants may influence multiple atherosclerosis-related genes or their plasma products through distinct effects on HNF-1 α structure or function (i.e., allelic heterogeneity). Ile27Leu and Ser486Asn are in linkage disequilibrium with one another as well as with a number of other SNPs across the ~24 kb *HNF1A* region on chromosome 12q24 [11,12]. For some phenotypes (CRP, lipids), the evidence has been stronger for association with *rs1169288* (Ile27Leu), while for other phenotypes (GGT, creatinine, fibrinogen, CAC), the association appeared stronger for *rs2464196* (Ser486Asn). Ile27Leu is located within the HNF-1 α dimerization domain and has been associated with decreased *in vitro* transcriptional activity of downstream target gene promoters [44]. The Ser486Asn variant is located in the C-terminal trans-activation domain of HNF-1 α in a specific region implicated in target-gene specific recruitment and interactions with transcriptional co-activators [45]. By assessing typed SNPs and also imputing genotypes at untyped *HNF1A* polymorphisms from the HapMap, a cluster of 7 variants (*rs7979473*, *rs7979478*, *rs2393791*, *rs2393775*, *rs7310409*, *rs10774579*, and *rs7953249*) within a putative regulatory region of intron 1 showed the strongest evidence of association with CRP phenotype [11]. Differential splicing represents another potential level of functional influence of common *HNF1A* sequence variants [46,47]. For example, *rs2464196*, *rs2259820*, and *rs2464195* are all predicted to alter exonic splice enhancer elements [48].

In summary, common variants of *HNF1A* are associated with CRP and other atherosclerosis-related traits in young EA adults. There are multiple mechanisms by which common *HNF1A* variants may alter gene and protein function. The identity of the functional variant(s) responsible for the observed phenotypic associations remains to be determined through additional molecular studies. Finally, our findings suggest that assessment of genetic variants within additional genes encoding transcription factors involved in the complex regulatory network that govern liver-specific gene expression (such as HNF-4, HNF-3, C/EBP and their co-activators) [1-8,38,39,45-47] might provide further insights into the heritability of complex human traits and/or disease such as atherosclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

A full list of principal CHS investigators and institutions can be found at <http://www.chsnhlbi.org/pi.htm>.

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Table 1

CARDIA participant characteristics at study entry and at most recent follow-up (year 20 examination) and CHS participant characteristics at study entry, by race

Characteristic	CARDIA				CHS	
	Year 0		Year 20		Baseline	
	European-Americans	African-Americans	European-Americans	African-Americans	European-Americans	African-Americans
Number	2,175	2,129	1,876	1,628	4,352	790
Mean age, years [range]	25.5 [17 – 32]	24.4 [17 – 35]	45.6 [37 – 52]	44.5 [37 – 54]	72.7 [65 – 98]	72.9 [65 – 93]
Female sex (%)	1,157 (53)	1,238 (58)	994 (53)	995 (61)	2478 (57)	499 (63)
Current smokers (%)	554 (26)	689 (33)	274 (15)	399 (25)	474 (11)	122 (16)
Body mass index (kg/m ²)	23.7 ± 4.1	25.4 ± 5.8	27.9 ± 6.5	31.3 ± 7.6	26.4 ± 4.5	28.5 ± 5.5
Systolic blood pressure (mm Hg)	109 ± 11	111 ± 11	113 ± 13	120 ± 16	135 ± 21	143 ± 23
Diastolic blood pressure (mm Hg)	68 ± 9	69 ± 10	70 ± 10	76 ± 11	70 ± 12	76 ± 12
Total cholesterol (mg/dL)	176 ± 32	178 ± 34	187 ± 34	184 ± 36	212 ± 39	210 ± 39
LDL cholesterol (mg/dL)	109 ± 30	110 ± 32	110 ± 31	110 ± 33	130 ± 36	129 ± 36
HDL cholesterol (mg/dL)	52 ± 13	54 ± 13	54 ± 17	54 ± 16	54 ± 16	58 ± 16
Glucose (mg/dL)	83 ± 12	82 ± 15	96 ± 22	100 ± 30	109 ± 32	119 ± 48
Insulin (mg/dL)	9.3 ± 6.4	12.3 ± 9.0	15.0 ± 10.1	18.3 ± 12.1	16.9 ± 23.8	20.3 ± 42.3
Apo B (mg/dL)	91 ± 24	91 ± 25	ND	ND	ND	ND
Diabetes (%)	22 (1.0)	19 (0.9)	107 (6)	162 (10)	625 (14)	196 (25)
C-reactive protein (mg/L)	ND	ND	2.09 ± 4.14	3.63 ± 5.36	4.47 ± 7.74	6.14 ± 8.42
Fibrinogen (mg/dL)*	ND	ND	386 ± 83	430 ± 95	319 ± 65	343 ± 74
Aspartate aminotransferase (U/L)	25.6 ± 23.2	25.9 ± 17.8	ND	ND	ND	ND
Gamma-glutamyl transferase (U/L)	8.3 ± 8.9	13.6 ± 23.7	27.4 ± 35.4	39.6 ± 34.0	ND	ND
Serum creatinine (mg/dL)	0.88 ± 0.24	0.94 ± 0.55	1.03 ± 0.37	1.04 ± 0.20	1.05 ± 0.34	1.13 ± 0.58
Coronary artery calcium (%)	ND	ND	356 (21)	220 (16)	ND	ND
Prevalent CHD**	0 (0)	5 (0.2)	23 (1.1)	15 (0.7)	847 (19)	152 (19)

Data are presented as number (%) or mean ± standard deviation, unless otherwise indicated. ND = not determined.

* Fibrinogen was measured in CARDIA using an immunologic method (nephelometric assay); while fibrinogen was measured in CHS using a clotting rate method (modified Clauss assay).

** Coronary heart disease (CHD) defined as history of angina, myocardial infarction, or coronary re-vascularization.

Table 2

HNF1A SNPs typed in CARDIA (n=4,237)*

SNP		African-Americans (n=2,083)			European-Americans (n=2,154)				
dbSNP reference	Chromosome 12 coordinate (NCBI 36.1)	Location	Alleles	# with non-missing genotypes	Genotype counts	MAF	# with non-missing genotypes	Genotype counts	MAF
rs1169288	119901033	Exon 1 (Ile27Leu)	T/G	2034	1574/434/26	0.119	2106	954/896/256	0.334
rs2071190	119915655	Intron 2	T/A	2061	1161/794/106	0.244	2111	1232/752/127	0.238
rs2464196	119919810	Exon 7 (Ser486Asn)	C/T	2066	1590/440/36	0.124	2136	1050/854/232	0.309
rs3999413	119922321	Intron 9	C/T				2126	1470/595/61	0.169
rs1882149	119922525	Intron 9	C/T	2071	1661/393/17	0.103	2143	1666/439/38	0.120

* 67 of the original 4,304 participants were excluded from further analysis because of missing genotype data at 2 or more SNPs. For each population, data are shown for SNPs with minor allele frequency (MAF) ≥ 0.05 .

Table 3
Association between *HNF1A* genotype and CVD and metabolic biomarkers in CARDIA, by race

CARDIA European-Americans (n=2,154)						CARDIA African-Americans (n=2,083)					
SNP rs#	N	beta	SE	Nominal P	Adjusted P*	SNP rs#	N	beta	SE	Nominal P	Adjusted P*
Log (C-reactive protein)						Log (C-reactive protein)					
rs1169288	2062	-0.153	0.029	2.01E-07	1.8 × E-05	rs1169288	1937	-0.043	0.053	0.414	NS
rs2071190	2065	0.042	0.033	0.203	NS	rs2071190	1960	-0.005	0.041	0.898	NS
rs2464196	2063	-0.149	0.030	5.28E-07	5.5 × E-05	rs2464196	1959	-0.024	0.051	0.640	NS
rs3999413	2050	0.034	0.038	0.369	NS						
rs1882149	2065	0.079	0.043	0.064	NS	rs1882149	1963	-0.077	0.057	0.171	NS
Fibrinogen **						Fibrinogen					
rs1169288	1885	0.046	0.031	0.137	NS	rs1169288	1891	0.039	0.055	0.476	NS
rs2071190	1890	-0.025	0.035	0.474	NS	rs2071190	1911	-0.051	0.042	0.228	NS
rs2464196	1894	0.072	0.031	0.022	NS	rs2464196	1910	0.036	0.053	0.486	NS
rs3999413	1882	-0.048	0.040	0.226	NS						
rs1882149	1899	-0.024	0.045	0.582	NS	rs1882149	1915	-0.071	0.058	0.226	NS
Total cholesterol						Total cholesterol					
rs1169288	2087	0.063	0.030	0.036	NS	rs1169288	2011	0.088	0.049	0.076	NS
rs2071190	2090	-0.032	0.034	0.335	NS	rs2071190	2035	0.025	0.038	0.503	NS
rs2464196	2088	0.039	0.030	0.205	NS	rs2464196	2034	0.002	0.047	0.971	NS
rs3999413	2075	-0.027	0.039	0.485	NS						
rs1882149	2090	-0.002	0.044	0.968	NS	rs1882149	2038	-0.003	0.052	0.960	NS
HDL cholesterol						HDL cholesterol					
rs1169288	2087	1.41E-05	0.032	1.000	NS	rs1169288	2011	0.036	0.047	0.437	NS
rs2071190	2090	0.001	0.036	0.983	NS	rs2071190	2035	-0.031	0.036	0.385	NS
rs2464196	2088	0.002	0.032	0.942	NS	rs2464196	2034	0.013	0.045	0.778	NS
rs3999413	2075	-0.039	0.041	0.341	NS						
rs1882149	2090	-0.020	0.046	0.670	NS	rs1882149	2038	-0.051	0.050	0.308	NS
LDL cholesterol						LDL cholesterol					
rs1169288	2087	0.074	0.030	0.012	NS	rs1169288	2011	0.058	0.050	0.246	NS

CARDIA European-Americans (n=2,154)							CARDIA African-Americans (n=2,083)						
SNP rs#	N	beta	SE	Nominal P	Adjusted P*		SNP rs#	N	beta	SE	Nominal P	Adjusted P*	
rs2071190	2090	-0.035	0.034	0.298	NS		rs2071190	2035	0.032	0.038	0.394	NS	
rs2464196	2088	0.049	0.030	0.106	NS		rs2464196	2034	-0.007	0.048	0.886	NS	
rs3999413	2075	-0.015	0.039	0.694	NS								
rs1882149	2090	0.001	0.044	0.984	NS		rs1882149	2038	0.009	0.053	0.861	NS	
Apolipoprotein B						Apolipoprotein B							
rs1169288	2084	0.071	0.031	0.022	NS		rs1169288	2000	0.056	0.050	0.261	NS	
rs2071190	2087	-0.013	0.035	0.705	NS		rs2071190	2023	0.026	0.038	0.486	NS	
rs2464196	2085	0.068	0.031	0.030	NS		rs2464196	2022	-0.062	0.048	0.193	NS	
rs3999413	2072	-0.016	0.040	0.688	NS								
rs1882149	2087	0.051	0.045	0.266	NS		rs1882149	2026	-0.022	0.053	0.680	NS	
Glucose						Glucose							
rs1169288	2087	-0.007	0.021	0.723	NS		rs1169288	2010	0.091	0.046	0.048	NS	
rs2071190	2090	0.004	0.023	0.872	NS		rs2071190	2034	0.009	0.035	0.806	NS	
rs2464196	2088	-0.018	0.021	0.392	NS		rs2464196	2033	0.082	0.044	0.064	NS	
rs3999413	2075	-0.026	0.027	0.340	NS								
rs1882149	2090	0.004	0.030	0.899	NS		rs1882149	2037	0.011	0.049	0.815	NS	
Insulin						Insulin							
rs1169288	2087	0.003	0.024	0.918	NS		rs1169288	2010	0.043	0.054	0.426	NS	
rs2071190	2090	-0.003	0.027	0.904	NS		rs2071190	2034	-0.028	0.041	0.506	NS	
rs2464196	2088	0.008	0.024	0.759	NS		rs2464196	2033	-0.005	0.052	0.917	NS	
rs3999413	2075	-0.023	0.031	0.471	NS								
rs1882149	2090	0.003	0.035	0.934	NS		rs1882149	2037	0.012	0.058	0.835	NS	
Log (Aspartate aminotransferase)						Log (Aspartate aminotransferase)							
rs1169288	2046	0.059	0.032	0.067	NS		rs1169288	1949	0.020	0.049	0.676	NS	
rs2071190	2049	0.012	0.036	0.730	NS		rs2071190	1972	-0.006	0.037	0.861	NS	
rs2464196	2047	0.034	0.032	0.295	NS		rs2464196	1971	0.027	0.046	0.561	NS	
rs3999413	2034	-0.069	0.041	0.093	NS								
rs1882149	2049	0.017	0.046	0.716	NS		rs1882149	1975	-0.036	0.052	0.491	NS	

CARDIA European-Americans (n=2,154)						CARDIA African-Americans (n=2,083)					
SNP rs#	N	beta	SE	Nominal P	Adjusted P*	SNP rs#	N	beta	SE	Nominal P	Adjusted P*
Log (Gamma glutamyl transferase)											
rs1169288	2086	-0.101	0.027	0.0002	0.003	rs1169288	2007	-0.031	0.052	0.554	NS
rs2071190	2089	0.040	0.030	0.190	NS	rs2071190	2031	0.030	0.040	0.447	NS
rs2464196	2087	-0.113	0.027	4.00E-05	0.002	rs2464196	2030	-0.006	0.050	0.898	NS
rs3999413	2074	0.023	0.035	0.503	NS						
rs1882149	2089	0.016	0.039	0.690	NS	rs1882149	2034	0.023	0.055	0.675	NS
Creatinine											
rs1169288	2084	0.059	0.019	0.0016	0.07	rs1169288	2010	0.013	0.070	0.847	NS
rs2071190	2087	-0.003	0.021	0.8739	NS	rs2071190	2034	-0.058	0.053	0.268	NS
rs2464196	2085	0.063	0.019	0.0008	0.02	rs2464196	2033	-0.017	0.066	0.794	NS
rs3999413	2072	-0.028	0.024	0.2503	NS						
rs1882149	2090	0.010	0.027	0.6988	NS	rs1882149	2037	-0.042	0.074	0.572	NS

Associations are shown for individual SNP genotypes regressed on age-, sex-, BMI-, and smoking- adjusted Z-score values averaged across all CARDIA exam time points available for each biomarker, assuming an additive genetic model with constant effect size for each additional copy of the minor allele. Beta coefficients, standard errors (SE), and nominal p-values correspond to the covariate-adjusted SNP-specific change in estimated mean plasma biomarker level in standard deviation units. Adjusted p-values are shown corrected for performing multiple hypothesis testing. NS = not significant (p<0.05).

* adjusted for multiple testing (as described under Supplement Methods).

** upon additional adjustment for log(CRP), the associations rs1169288, rs2464196, and fibrinogen became statistically significant (see Results).

Table 4

Association between *HNF1A* coding SNP genotypes and CVD and metabolic biomarkers in European-American participants from CARDIA the year 20 exam and from CHS at the baseline exam

CARDIA				CHS				P for CARDIA+CHS combined		
SNP rs#	N	Beta coefficient	Standard Error	P-value	SNP rs#	N	Beta coefficient		Standard Error	P-value
Log (C-reactive protein)										
rs1169288	1787	-0.14	0.036	0.0001	rs1169288	4305	-0.11	0.022	8 × E-07	<0.0001
rs2464196	1815	-0.13	0.036	0.0002	rs2464196	2063	-0.11	0.022	5 × E-07	<0.0001
Fibrinogen										
rs1169288	1761	2.47	2.60	0.34	rs1169288	4294	-0.043	1.43	0.98	0.67
rs2464196	1789	0.92	2.61	0.72	rs2464196	4295	1.13	1.46	0.44	0.40
Fibrinogen additionally adjusted for CRP										
rs1169288	1760	7.45	2.30	0.001	rs1169288	4276	3.39	1.26	0.007	<0.0001
rs2464196	1788	5.54	2.30	0.016	rs2464196	4277	4.58	1.29	0.0004	<0.0001
Total cholesterol										
rs1169288	1789	2.91	1.17	0.01	rs1169288	4324	1.95	0.84	0.02	0.001
rs2464196	1817	1.36	1.18	0.25	rs2464196	4324	1.31	0.86	0.13	0.06
LDL cholesterol										
rs1169288	1762	2.48	1.05	0.02	rs1169288	4261	1.98	0.79	0.01	0.001
rs2464196	1789	1.19	1.06	0.26	rs2464196	4261	1.48	0.81	0.07	0.03
Serum creatinine										
rs1169288	1786	0.010	0.008	0.18	rs1169288	3532	0.002	0.007	0.74	0.30
rs2464196	1815	0.016	0.008	0.03	rs2464196	3533	0.004	0.007	0.58	0.08
Cystatin C										
rs1169288			Not determined		rs1169288	3555	0.008	0.005	0.13	
rs2464196			Not determined		rs2464196	3556	0.012	0.005	0.01	

Adjusted for age, sex, body mass index, and smoking, unless otherwise indicated.

Table 5

Association between *HNF1A* genotype and risk of coronary atherosclerosis at year 20 in CARDIA, by race

CARDIA European-Americans					CARDIA African-Americans						
SNP rs#	N	Odds ratio	SE	95% CI	P	SNP rs#	N	Odds ratio	SE	95% CI	P
rs1169288	1486	1.14	0.10	0.93 – 1.39	0.201	rs1169288	1136	1.16	0.18	0.81 – 1.65	0.430
rs2071190	1489	0.80	0.11	0.64 – 1.00	0.051	rs2071190	1147	1.00	0.14	0.77 – 1.31	0.998
rs2464196	1488	1.32	0.10	1.08 – 1.60	0.006	rs2464196	1147	1.00	0.18	0.71 – 1.42	0.983
rs3999413	1475	0.88	0.13	0.67 – 1.13	0.311						
rs1882149	1488	0.68	0.15	0.51 – 0.92	0.012	rs1882149	1148	1.27	0.18	0.88 – 1.81	0.196

Odds ratio adjusted for age, sex, and clinic