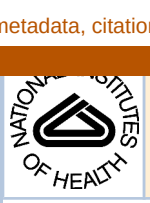


Published in final edited form as:

Pharmacogenet Genomics. 2007 May ; 17(5): 349–358.

CYP2J2 and *CYP2C8* polymorphisms and coronary heart disease risk: the Atherosclerosis Risk in Communities (ARIC) study

Craig R. Lee[a,b], **Kari E. North**[c], **Molly S. Bray**[e], **David J. Couper**[d], **Gerardo Heiss**[c], and **Darryl C. Zeldin**[a]

a *Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina*

b *Division of Pharmacotherapy and Experimental Therapeutics, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*

c *Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*

d *Department of Biostatistics, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*

e *Department of Pediatrics, Children's Nutrition Research Center, Baylor College of Medicine, Houston, Texas*

Abstract

Objective— The cytochromes P450 epoxygenases CYP2J2 and CYP2C8 synthesize epoxyeicosatrienoic acids, which regulate endothelial function. We sought to determine if genetic variation in *CYP2J2* and *CYP2C8* was associated with coronary heart disease risk.

Methods— We genotyped 2065 Atherosclerosis Risk in Communities study participants (1085 incident coronary heart disease cases, 980 noncases) for polymorphisms in *CYP2J2* and *CYP2C8*. Using a case–cohort design, associations between genotype and incident coronary heart disease risk were evaluated using proportional hazards regression. The influence of cigarette smoking on these associations was also evaluated. False discovery rate *q*-values were estimated to minimize the impact of the multiple statistical comparisons completed. All analyses were race stratified.

Results— The *CYP2J2 G-50T* polymorphism variant *–50T* allele was associated with significantly lower risk of incident coronary heart disease in African-Americans (adjusted hazard rate ratio 0.58, 95% confidence interval 0.35–0.96, $P = 0.036$, $q = 0.051$); however, no significant association was observed in Caucasians. Overall, the *I264M*, *I269F*, and *K399R* polymorphisms in *CYP2C8* were not significantly associated with risk of incident coronary heart disease. In Caucasians, the relationship between the *I264M* and *K399R* polymorphisms and incident coronary heart disease risk was significantly modified by cigarette smoking status (P for interaction = 0.008, $q = 0.064$), with the highest risk observed in smokers carrying at least one variant allele.

Conclusions— The *G-50T* polymorphism in *CYP2J2* may be an important risk factor for the development of coronary heart disease events in African-Americans, whereas cigarette smoking may modify the relationship between the *I264M* and *K399R* polymorphisms in *CYP2C8* and coronary heart disease risk in Caucasians. Confirmation of these findings in an independent population is warranted.

Correspondence to Darryl C. Zeldin, National Institute of Environmental Health Sciences, 111 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA Tel: +1 919 541 1169; fax: +1 919 541 4133; e-mail: zeldin@niehs.nih.gov

Keywords

cigarette smoking; coronary heart disease; *CYP2J2*; *CYP2C8*; polymorphism

Introduction

Coronary heart disease (CHD) is a major cause of morbidity and mortality, with 1.2 million Americans estimated to experience a new or recurrent acute coronary syndrome event this year [1]. Endothelial dysfunction is associated with risk of CHD events such as myocardial infarction [2]. Various risk factors including cigarette smoking contribute to this process [3], which has been primarily ascribed to functional impairments in nitric oxide biosynthesis and activity. However, dysfunction, in other endothelial pathways also appears to be important.

Arachidonic acid is oxidatively metabolized to epoxyeicosatrienoic acids (EETs) in endothelial cells and cardiomyocytes by the cytochromes P450 epoxygenases CYP2J2 and CYP2C8 [4]. The EETs have potent vasodilatory [5,6], antiinflammatory [7], fibrinolytic [8], and postischemic cardioprotective [9,10] effects, and are considered one of the primary endothelial-derived hyperpolarizing factors [5,6]. Multiple genetic polymorphisms in *CYP2J2* and *CYP2C8* have been recently discovered [11–14]. In *CYP2J2*, the *G-50T* (*CYP2J2*7*) polymorphism in the proximal promoter disrupts a Sp1 transcription factor binding site and leads to reduced *CYP2J2* transcription [12]. In *CYP2C8*, the *I269F* (*CYP2C8*2*), *R139K/K399R* (*CYP2C8*3*) and *I264M* (*CYP2C8*4*) nonsynonymous variants possess lower CYP2C8 metabolic activity *in vitro* [13,14]. Case–control studies have demonstrated an association between the *CYP2J2 – 50T* variant allele and higher risk of angiographically documented CHD in a German population [12], and between the *CYP2C8*3* variant allele and higher risk of prevalent myocardial infarction in a Swedish cohort [15]. Associations between polymorphisms in these genes and risk of incident CHD clinical events, however, remain to be evaluated.

Our primary aim was to determine if genetic variation in *CYP2J2* and *CYP2C8* was associated with risk of incident CHD events in individuals enrolled in the biethnic Atherosclerosis Risk in Communities (ARIC) study. A secondary aim was to determine if this risk was modified by environmental factors known to impair endothelial function such as cigarette smoking.

Methods

Study population

Participants were selected from the ARIC study, a longitudinal, population-based cohort study of 15 792 men and women aged 45–64 years from four U.S. communities (Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland) enrolled between 1987 and 1989 [16]. Since enrollment, participants have been followed prospectively via annual phone interviews, clinic examinations approximately every 3 years through 1998, and ongoing abstraction of hospital and death certificate records. The study protocol was approved by the Institutional Review Board of each center, and consent was obtained from each participant.

Ascertainment of incident coronary heart disease cases

All incident cases that occurred between baseline and 31 December 1998 were evaluated (median follow-up 9.1 years), excluding participants with a history of a physician-diagnosed CHD or stroke event at baseline. Incident CHD (n = 1085) was defined as (1) definite or probable myocardial infarction (n = 520), (2) electrocardiographic evidence of silent

myocardial infarction (n = 112), (3) definite CHD death (n = 1 10), or (4) coronary revascularization procedure (n = 343). The ascertainment of cases and criteria for classification have been described previously [17]. All potential events were systematically reviewed and adjudicated by the ARIC Morbidity and Mortality Classification Committee [16,17]. Briefly, hospitalized myocardial infarction was classified as definite or probable based on chest pain symptoms, cardiac enzyme levels, and electrocardiographic changes. An unrecognized or silent myocardial infarction was defined by presence of a major Q-wave, a minor Q-wave with ischemic ST-Tchanges or evidence of a myocardial infarction by computerized Novacode criteria [18] via a 12-lead electrocardiogram obtained during an ARIC follow-up examination. Presence of a new finding was confirmed by visual comparison of the baseline and follow-up electrocardiograms by a centralized reader. Definite CHD death was classified based on chest pain symptoms, underlying cause of death, hospitalization records, and medical history. Coronary revascularization procedures included coronary artery bypass grafting and percutaneous coronary interventions.

Baseline measurements

Detailed demographic, clinical, and biochemical data were obtained from each participant at baseline, as described [19,20]. Briefly, race was self-reported and detailed information on cigarette smoking was obtained through an interview-administered questionnaire.

Cohort random sample

A random sample of all ARIC participants without history of CHD or stroke at baseline was assembled to serve as the reference group for the case–cohort comparisons (n = 1065, 85 of which are also incident CHD cases). Sampling of the cohort was stratified on age (<55 or ≥ 55 years), sex, and race (Caucasian or African-American). Sampling proportions varied across each stratum.

Genotyping

Genomic DNA from all incident CHD cases and the cohort random sample was genotyped for 10 polymorphisms in coding and noncoding regions of *CYP2J2* (Fig. 1) and the nonsynonymous *I269F* (*CYP2C8*2*), *K399R* (*CYP2C8*3*), and *I264M* (*CYP2C8*4*) polymorphisms in *CYP2C8* using either multiplex matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom Inc., San Diego, California) [21] or BeadArray (Illumina Inc., San Diego, California) [22] methods, as described (Supplementary Table 1 available online). Blind replicates were included for quality control.

The *CYP2J2* polymorphisms were identified from our resequencing effort as part of the NIEHS Environmental Genome Single Nucleotide Polymorphism program (*https://dir-apps.niehs.nih.gov/egsnp/home.htm*) [11] and specifically selected based on their known functional relevance *in vitro* and/or haplotype tagging properties. Owing to the very low frequency of nonsynonymous variants in *CYP2J2*, evaluation of potential associations between common *CYP2J2* haplotypes and CHD risk were also considered. Briefly, pairwise linkage disequilibrium (LD) statistics were calculated and haplotypes were reconstructed separately in European/Caucasians (n = 24) and Africans (n = 24) using all polymorphisms in *CYP2J2* identified by resequencing (Haploview 3.2) [11,23]. Polymorphisms tagging haplotypes with >5% frequency in either population were selected for genotyping.

Three nonsynonymous polymorphisms in *CYP2C8* (*CYP2C8*2*, **3* and **4*) were selected since these represent the most frequent variants in Caucasian or African-American populations with known functional relevance [13,14]. The *CYP2C8*3* variant consists of two nonsynonymous polymorphisms in perfect LD (*R139K* and *K399R*) [13]; however, only the *K399R* locus was genotyped in our analysis. *CYP2C8* haplotype tagging polymorphisms were not evaluated.

Data analysis

All incident CHD cases and individuals from the cohort random sample were included in our analysis (n = 2065). Inverse sampling fractions from each stratum were used as weights in estimation of adjusted covariate means and proportions by linear and logistic regression, respectively, in noncases included in the cohort random sample. Hazard rate ratios (HRR) and 95% confidence intervals (CI) for the development of incident CHD in relation to *CYP2J2* or *CYP2C8* genotype were calculated by weighted proportional hazards regression, using Barlow's method to account for the stratified random sampling and case–cohort design [24]. Model 1 included baseline age, sex, and study center as covariates. Model 2 also included current smoking status, diabetes, hypertension, high-density lipoprotein cholesterol, total cholesterol, and body mass index at baseline. Assuming an autosomal-dominant mode of inheritance, individuals with one or two variant alleles were combined for comparison with wild-type individuals. This assumption was based on previous studies demonstrating significant functional effects of *CYP2J2* and *CYP2C8* polymorphisms in individuals carrying one or two variant alleles [12,14]. All analyses were completed separately in Caucasians and African-Americans. Assuming a case–control design, type I error $\alpha = 0.05$ and 10% variant genotype frequency, we had approximately 99 and 80% power to detect an odds ratio of 2.0 in Caucasians and African-Americans, respectively.

Cohort random sample allele frequencies were evaluated for deviation from Hardy–Weinberg equilibrium, separately in Caucasians and African-Americans, and pairwise LD statistics were calculated (Haploview 3.2) [23]. *CYP2J2* haplotypes and their frequencies were estimated using the phase reconstruction method (PHASE 2.1), which assigned the most probable haplotype pair to each individual [25]. Only polymorphisms with >5% frequency and pairwise r^2 values <0.80 were considered for haplotype reconstruction, which included polymorphisms 1 and 7 in Caucasians and 1, 7, 9, and 10 in African-Americans (Fig. 1). Haplotype frequencies were compared across case status by χ^2. Only haplotypes with >5% frequency were considered. Frequency comparisons were repeated using the expectation–maximization algorithm (Haploview 3.2) [23], which accounted for the uncertainty in haplotype reconstruction by weight-adjusting each inferred haplotype according to its estimated posterior haplotype probability. Associations between haplotype (0,1) and risk of incident CHD were also evaluated by modeling each haplotype relative to all other haplotypes using Barlow's method [24]. The association analysis was repeated after excluding individuals with posterior haplotype probabilities <0.75.

Gene–environment interaction testing was completed on a multiplicative scale between selected *CYP2J2* and *CYP2C8* variants and baseline current smoking status (yes/no) using a Wald χ^2 test for significance of the estimated β-coefficient for the interaction term [26]. Because interaction hypothesis testing on a multiplicative scale is underpowered, the critical value for statistical significance was set to $\alpha = 0.15$, two-sided [27]. Stratified weighted proportional hazards regression was also completed to explore further potential interactions.

To minimize the impact of the multiple statistical tests conducted in our analysis, we estimated the false discovery rate (FDR) q-value of our findings, separately for *CYP2J2* and *CYP2C8*, which is defined as the expected proportion of statistical tests deemed significant that are actually false positives (QVALUE) [28]. We considered statistical tests from the unadjusted, model 1 and model 2 association analysis of each polymorphism and reconstructed haplotype as independent, even though each model assessed the same independent variable and certain genotype and haplotype associations are not completely independent. Additional q-value estimates were also calculated for the gene-smoking interaction analysis. Only q-values for significant findings are presented.

Results

Study population

Significant baseline differences in various risk factors were observed between incident CHD cases and noncases included in the cohort random sample, as described previously [19,20]. Participants were significantly older and more likely to be male, cigarette smokers, diabetic, hypertensive, and have abnormal fasting lipid panels compared with noncases.

CYP2J2/CYP2C8 genotype

The observed race-specific allele frequencies of the 10 *CYP2J2* polymorphisms are presented in Fig. 1. The *R158C* (*CYP2J2*3*), *I192N* (*CYP2J2*4*), and *N404Y* (*CYP2J2*6*) polymorphisms were monomorphic in both Caucasians and African-Americans. Significant LD was observed between certain *CYP2J2* polymorphisms, particularly in Caucasians (Fig. 2). The minor allele frequencies of the *I264M* (0.041 versus 0.011), *I269F* (0.002 versus 0.141), and *K399R* (0.108 versus 0.019) polymorphisms in *CYP2C8* were significantly different in Caucasians and African-Americans, respectively, in the cohort random sample ($P<0.001$ for each comparison). Significant LD was not observed in either racial group ($r^2<0.01$). The distribution of all evaluated polymorphisms were in Hardy–Weinberg equilibrium in both Caucasians and African-Americans ($P>0.05$).

CYP2J2 polymorphisms and coronary heart disease risk

In African-Americans, the variant – *50T* allele was significantly less common among CHD cases compared with cohort random sample noncases (20.9 versus 29.3%, respectively, $P = 0.043$) (Table 1). Moreover, presence of at least one variant – *50T* allele was associated with significantly lower risk of incident CHD events relative to – *50G* homozygotes (model 2, HRR 0.58, 95% CI 0.35– 0.96, $P = 0.036$, $q = 0.051$) (Table 2). In Caucasians, no significant difference in *G-50T* genotype frequency was observed across CHD case status ($P = 0.719$) (Table 1), and no significant association between presence of the variant – *50T* allele and CHD risk was observed (model 2, HRR 1.15, 95% CI 0.78–1.70, $P = 0.472$) (Table 2). The rare *R49S* variant allele appeared to be less common in CHD cases compared with noncases in African-Americans (0.5 versus 2.5%, respectively) and Caucasians (0.1 versus 0.6%, respectively) (Table 1).

Haplotype reconstruction identified five common haplotypes in African-Americans and three in Caucasians, which accounted for 94.7 and 98.1% of all haplotypes, respectively. In African-Americans, the overall haplotype distribution tended to be different in individuals with and without CHD ($P = 0.086$); however, this difference was not statistically significant (Table 3). No difference in haplotype distribution was observed in Caucasians ($P = 0.731$) (Table 3). In African-Americans, haplotype ***TGT****A* (tagged by the polymorphism 1 (*G-50T*), seven and nine variant alleles) was less frequent (8.1 versus 12.3%, $P = 0.052$) in CHD cases compared with noncases, respectively (Table 3). Similar differences in haplotype ***TGT****A* frequency were also observed using the expectation–maximization algorithm ($P = 0.027$). Moreover, presence of at least one haplotype ***TGT****A* copy was significantly less common in CHD cases compared with noncases (14.5 versus 22.9%, respectively, $P = 0.027$), and was associated with significantly lower risk of incident CHD (model 2, HRR 0.51, 95% CI 0.29–0.91, $P = 0.023$, $q = 0.041$) (Table 3). Significant associations were also observed after excluding the 1.5% of individuals with a posterior haplotype probability <0.75 from the analysis ($P = 0.020$). In Caucasians, presence of haplotype ***TG*** (tagged by the polymorphism 1 (*G-50T*) and seven variant alleles) was not significantly associated with risk of incident CHD after covariate adjustment (model 2, HRR 1.33, 95% CI 0.87–2.02, $P = 0.184$) (Table 3).

CYP2C8 polymorphisms and coronary heart disease risk

No significant differences in the genotype frequency of the *I264M*, *I269F* or *K399R* polymorphisms in *CYP2C8* were observed across CHD case status in either African-Americans or Caucasians (Table 1). Moreover, presence of the *I264M*, *I269F*, or *K399R* variant alleles were not significantly associated with risk of incident CHD after covariate adjustment (Table 2).

Genotype by smoking interaction

In Caucasians, cigarette smoking status at baseline appeared to modify the association between CHD risk and both the *I264M* (*P* for interaction = 0.104, *q* = 0.166) and *K399R* (*P* for interaction = 0.060, *q* = 0.160) polymorphisms in *CYP2C8* (Table 4). A significant interaction was identified when individuals carrying at least one variant allele in either the *I264M* or *K399R* polymorphisms were compared with those wild-type for both (*P* for interaction = 0.008, *q* = 0.064) (Table 4). When stratified by smoking status, smokers carrying at least one variant allele were at significantly higher risk of CHD compared with smokers wild type at both loci (model 2, HRR 1.89, 95% CI 1.05–3.38, *P* = 0.033). Smoking status did not modify risk associated with the *CYP2C8* variant *I269F* allele in African-Americans (*P* for interaction = 0.746), or the *CYP2J2* variant – *50T* allele in either African-Americans (*P* for interaction = 0.605) or Caucasians (*P* for interaction = 0.690).

Discussion

Our analysis demonstrated a statistically significant association between the variant – *50T* allele in *CYP2J2* and lower risk of incident CHD events in African-Americans enrolled in the ARIC study. A common *CYP2J2* haplotype that carried the – *50T* allele was also associated with significantly lower CHD risk in African-Americans. The *I264M*, *I269F*, and *K399R* polymorphisms in *CYP2C8* were not associated with risk of CHD in either racial group. In Caucasians, however, cigarette smokers carrying either the variant *I264M* or *K399R* allele were at significantly higher risk of incident CHD.

P450 epoxygenase pathway

Endothelial dysfunction is associated with increased risk of acute cardiovascular events [2], and is typically manifested by impairment in endothelial-dependent vasodilation [3]. The vasodilatory and antiinflammatory properties of CYP2J2 and CYP2C8-derived EETs are important mediators of this process [4,29]. Soluble epoxide hydrolase (*EPHX2*) rapidly hydrolyzes EETs and is integrally involved in regulation of their cellular levels and vascular effects [30]. We recently reported that the *K55R* variant allele in *EPHX2* was associated with significantly higher soluble epoxide hydrolase activity *in vivo* and higher risk of incident CHD events in Caucasians [20]. Although regulation of EET levels by soluble epoxide hydrolase *in vivo* is well documented, variation in CYP2J2 and/or CYP2C8-mediated EET biosynthesis may also be critical. The relative contribution of each P450 epoxygenase to the regulation of cellular and systemic EET levels, however, has not been extensively characterized, particularly in humans.

CYP2J2 polymorphisms

The *R158C*, *I192N*, and *N404Y* polymorphisms possess significantly lower CYP2J2-mediated epoxygenase activity *in vitro* [11]. These variants are, however, extremely infrequent and were found to be monomorphic in the 2065 individuals genotyped in our analysis. The *G-50T* polymorphism disrupts a Sp1 transcription factor binding site in the proximal promoter, leads to reduced *CYP2J2* transcription *in vitro* and represents the only common, functionally relevant polymorphism in *CYP2J2* identified to date [12]. The variant – *50T* allele was associated with

significantly lower plasma dihydroxyeicosatrienoic acid (DHET) levels and higher risk of prevalent angiographically documented CHD in a recently characterized German population [12]. In our analysis, the variant – *50T* allele was, associated with significantly lower risk of incident CHD clinical events in African-Americans at both the polymorphism and haplotype levels, in contrast to these previous findings and our current hypotheses related to the beneficial effects of EETs in the vasculature. The rare *R49S* variant allele also appeared to be less frequent in Caucasian and African-American CHD cases; however, the metabolic relevance of this infrequent variant has not been characterized. Interestingly, we recently reported an association between the – *50T* allele and lower risk of prevalent hypertension in Caucasians from a Vanderbilt University cohort [31]. The – *50T* allele was not significantly associated with risk of incident CHD events in Caucasians from the ARIC study, inconsistent with the previously described German population [12]. The primary analysis of this investigation by Spiecker *et al.* [12], defined CHD angiographically, not by clinical event incidence as defined in the ARIC study. A relationship between the – *50T* allele and risk of prevalent acute coronary syndrome events (adjusted odds ratio 1.38, 95% CI 0.85–2.25, P=0.19), which was similar to the relationship we observed was also reported in Caucasians between a common haplotype carrying the – *50T* allele and risk of incident CHD events in the ARIC study (HRR 1.33, 95% CI 0.87–2.02, P=0.184); however, neither relationship was statistically significant.

The relationship between the *G-50T* polymorphism and CHD risk appears to differ across the Caucasian and African-American subsets of the ARIC cohort. In particular, the statistically significant association observed in African-Americans existed in the opposite direction of both preclinical findings and previous genetic epidemiological data in a Caucasian population [12]. Additional metabolic studies evaluating the influence of the *G-50T* polymorphism on EET biosynthesis in both Caucasians and African-Americans will be necessary to confirm the functional relevance of this polymorphism *in vivo*. Significant associations, however, between variants in other important endothelial genes (*EPHX2*, *NOS3*, *ALOX5*) and cardiovascular disease risk have been reported in contrast to established hypotheses [32–34]. Although the mechanism underlying the association between the – *50T* allele and lower CHD risk in African-Americans remains unclear, these data suggest that reduced P450-mediated EET biosynthesis could have protective effects against the development of CHD clinical events. Despite their well-characterized vasodilatory and antiinflammatory effects, preclinical evidence has demonstrated that increased EET generation also significantly increases matrix metalloproteinase (MMP) enzyme activity in endothelial cells [35]. The MMPs are potent stimulators of vascular remodeling and atherosclerotic plaque destabilization, and are integrally involved in the precipitation of plaque rupture and acute CHD events [36,37]. Perhaps the role of EET-mediated regulation of MMP activity is particularly important in certain populations and genetic determinants of reduced EET generation, such as the *G-50T* polymorphism in *CYP2J2*, can significantly reduce the risk of experiencing an acute CHD clinical event via its downregulation of MMP activity. Interestingly, racial differences in endothelial-mediated vasodilatory responses are well documented, with African-American populations typically demonstrating more pronounced endothelial dysfunction compared with Caucasian populations [38]. Enhanced impairment in endothelial nitric oxide synthase-mediated nitric oxide biosynthesis has been implicated as one potential mechanism [39]. Potential population-specific differences in P450 epoxygenase-derived EET biosynthesis and its effect on endothelial function, vascular inflammation, and MMP activity in humans remain to be evaluated.

CYP2C8 polymorphisms

The *I264M* (**4*), *I269F* (**2*), and *R139K/K399R* (**3*) polymorphisms possess significantly lower CYP2C8 metabolic activity *in vitro*; however, only the *R139K/K399R* variant has been associated with significantly reduced CYP2C8-mediated EET biosynthesis [13,14] and higher

risk of prevalent myocardial infarction in a Swedish cohort [15]. The *I264M* and *R139K/K399R* polymorphisms are more frequent in Caucasians, whereas the *I269F* polymorphism is more frequent in African-Americans. Overall, these variants were not significantly associated with risk of incident CHD events in the ARIC population. Similarly, we previously observed no association between the *R139K/K399R* variant and risk of prevalent hypertension in a cohort from Vanderbilt University [31]. In Caucasians, however, cigarette smoking history significantly modified the association between the variant *I264M* and *K399R* alleles and CHD incidence, such that smokers carrying either of these variant alleles were at the greatest risk of developing CHD. Smoking did not modify the association between the *G-50T* polymorphism in *CYP2J2* and CHD risk, suggesting such an interaction may be isolated to CYP2C8-mediated epoxygenase activity. Cigarette smoking is a well-characterized environmental risk factor that substantially impairs endothelial function [3]. We have reported previously that cigarette smoking modifies the association between each of the endothelial nitric oxide synthase (*NOS3*) *E298D* and *EPHX2 K55R* polymorphisms and risk of incident CHD in Caucasians, with the highest risk observed in smokers carrying these variant alleles [19,20]. Perhaps the presence of established underlying endothelial dysfunction, as observed in cigarette smokers, may be necessary for genetic variation in *CYP2C8*, *NOS3*, and *EPHX2* to influence significantly endothelial function and cardiovascular disease risk. Future studies will be required to further characterize this potential gene–environment interaction and the mechanistic influence of cigarette smoking on P450-mediated EET biosynthesis.

Limitations

First, although we are unable to elucidate mechanisms underlying the observed race-specific associations, additional genetic and/or environmental factors not accounted for in our analysis could be contributing to population differences in the likely complex relationship between the P450 epoxygenase pathway, endothelial function, and cardiovascular disease risk. The potential contribution of population stratification was not quantified or adjusted for in our analysis. Consequently, all analyses were stratified by self-reported race, and comparison of gene–disease associations across the Caucasian and African-American subsets of the ARIC population should be interpreted with caution. Second, therapeutic interventions initiated throughout follow-up could have impacted the observed relationships between *CYP2J2* and *CYP2C8* polymorphisms and CHD risk. Inclusion of variables reflecting treatment with blood pressure-lowering medications, cholesterol lowering medications or aspirin at baseline into our modeling did not significantly alter any of the reported associations. For instance, the risk of CHD associated with the *CYP2J2 – 50T* variant allele in African-Americans (adjusted HRR 0.50, 95% CI 0.30–0.84, P=0.009) and the reported interaction between the *CYP2C8 I264M* and *K399R* variant alleles and cigarette smoking (P for interaction=0.003) remained statistically significant when including these treatment variables in the model. Third, because haplotypes are inferred, some uncertainty exists in the *CYP2J2* haplotype assigned to each individual. Similar results were, observed using the expectation–maximization algorithm and after excluding individuals with posterior haplotype probabilities <0.75. Fourth, although we specifically selected polymorphisms with known functional relevance and/or haplotype tagging properties and utilized a hypothesis-driven approach in our analysis, we also acknowledge it may be difficult to gauge the statistical significance of these findings considering the number of comparisons completed. Consequently, we assessed the FDR across all completed tests in our genotype and haplotype association analysis. Since all q-values related to the association between CHD risk and both the *G-50T* polymorphism and ***TGT****A* haplotype in African-Americans were estimated to be 0.051 or less, we have a high level of confidence in these findings. Similarly, the FDR analysis enhanced our confidence in the *CYP2C8*–smoking interaction findings. Our observations of significant gene–smoking interactions with the *I264M* and *K399R* polymorphisms in *CYP2C8* in the absence of significant main effects, however, suggest these findings should be interpreted with caution.

Conclusions

Genetic variation in *CYP2J2* and *CYP2C8* may be important risk factors for the development of CHD clinical events. Future studies across multiple populations will undoubtedly be required to validate our findings, in addition to molecular and physiological studies evaluating the mechanistic relationship between the P450 epoxygenase pathway, endothelial function, and cardiovascular disease risk.

Acknowledgements

The authors gratefully acknowledge the staff and participants of the ARIC study for their important contributions.

This publication was made possible by grant ES012856 to Dr Lee, HL073366 to Dr Bray, and funds from the Intramural Research Program of the NIH, NIEHS to Dr Zeldin. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH. The ARIC study is carried out as a collaborative study supported by NHLBI contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022.

References

1. American Heart Association. Heart Disease and Stroke Statistics-2004 Update. TX: Dallas; 2003.
2. Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, et al. Prognostic value of coronary vascular endothelial dysfunction. Circulation 2002;6:653–658. [PubMed: 12163423]
3. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. J Am Coll Cardiol 2004;10:1731–1737. [PubMed: 15145091]
4. Zeldin DC. Epoxygenase pathways of arachidonic acid metabolism. J Biol Chem 2001;39:36059–36062. [PubMed: 11451964]
5. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circ Res 1996;3:415–423. [PubMed: 8593700]
6. Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, et al. Cytochrome P450 2C is an EDHF synthase in coronary arteries. Nature 1999;6752:493–497. [PubMed: 10519554]
7. Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, et al. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. Science 1999;5431:1276–1279. [PubMed: 10455056]
8. Node K, Ruan XL, Dai J, Yang SX, Graham L, Zeldin DC, et al. Activation of Galpha s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. J Biol Chem 2001;19:15983–15989. [PubMed: 11279071]
9. Wu S, Chen W, Murphy E, Gabel S, Tomer KB, Foley J, et al. Molecular cloning, expression, and functional significance of a cytochrome P450 highly expressed in rat heart myocytes. J Biol Chem 1997;19:12551–12559. [PubMed: 9139707]
10. Seubert J, Yang B, Bradbury JA, Graves J, Degraff LM, Gabel S, et al. Enhanced postischemic functional recovery in CYP2J2 transgenic hearts involves mitochondrial ATP-sensitive K + channels and p42/p44 MAPK pathway. Circ Res 2004;5:506–514. [PubMed: 15256482]
11. King LM, Ma J, Srettabunjong S, Graves J, Bradbury JA, Li L, et al. Cloning of CYP2J2 gene and identification of functional polymorphisms. Mol Pharmacol 2002;4:840–852. [PubMed: 11901223]
12. Spiecker M, Darius H, Hankeln T, Soufi M, Sattler AM, Schaefer JR, et al. Risk of coronary artery disease associated with polymorphism of the cytochrome P450 epoxygenase CYP2J2. Circulation 2004;15:2132–2136. [PubMed: 15466638]
13. Dai D, Zeldin DC, Blaisdell JA, Chanas B, Coulter SJ, Ghanayem BI, et al. Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. Pharmacogenetics 2001;7:597–607. [PubMed: 11668219]
14. Bahadur N, Leathart JB, Mutch E, Steimel-Crespi D, Dunn SA, Gilissen R, et al. CYP2C8 polymorphisms in Caucasians and their relationship with paclitaxel 6alpha-hydroxylase activity in human liver microsomes. Biochem Pharmacol 2002;11:1579–1589. [PubMed: 12429347]

15. Yasar U, Bennet AM, Eliasson E, Lundgren S, Wiman B, De Faire U, et al. Allelic variants of cytochromes P450 2C modify the risk for acute myocardial infarction. Pharmacogenetics 2003;12:715–720. [PubMed: 14646690]
16. The ARIC investigators. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. Am J Epidemiol 1989;4:687–702.
17. White AD, Folsom AR, Chambless LE, Sharret AR, Yang K, Conwill D, et al. Community surveillance of coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) Study: methods and initial two years' experience. J Clin Epidemiol 1996;2:223–233. [PubMed: 8606324]
18. Rautaharju PM, Warren JW, Jain U, Wolf HK, Nielsen CL. Cardiac infarction injury score: an electrocardiographic coding scheme for ischemic heart disease. Circulation 1981;2:249–256. [PubMed: 7249294]
19. Lee CR, North KE, Bray MS, Avery CL, Mosher MJ, Couper DJ, et al. *NOS3* polymorphisms, cigarette smoking, and cardiovascular disease risk: the Atherosclerosis Risk in Communities study. Pharmacogenet Genomics 2006;12:891–899. [PubMed: 17108813]
20. Lee CR, North KE, Bray MS, Fornage M, Seubert JM, Newman JW, et al. Genetic variation in soluble epoxide hydrolase (*EPHX2*) and risk of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. Hum Mol Genet 2006;10:1640–1649. [PubMed: 16595607]
21. Bray MS, Boerwinkle E, Doris PA. High-throughput multiplex SNP genotyping with MALDI-TOF mass spectrometry: practice, problems and promise. Hum Mutat 2001;4:296–304. [PubMed: 11295828]
22. Shen R, Fan JB, Campbell D, Chang W, Chen J, Doucet D, et al. Highthroughput SNP genotyping on universal bead arrays. Mutat Res 2005;1–2:70–82.
23. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;2:263–265. [PubMed: 15297300]
24. Barlow WE, Ichikawa L, Rosner D, Izumi S. Analysis of case–cohort designs. J Clin Epidemiol 1999;12:1165–1172. [PubMed: 10580779]
25. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;5:1162–1169. [PubMed: 14574645]
26. Li R, Folsom AR, Sharrett AR, Couper D, Bray M, Tyroler HA. Interaction of the glutathione S-transferase genes and cigarette smoking on risk of lower extremity arterial disease: the Atherosclerosis Risk in Communities (ARIC) study. Atherosclerosis 2001;3:729–738. [PubMed: 11257276]
27. Hosmer, D.; Lemeshow, S. Applied logistic regression. New York: Wiley; 1989.
28. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 2003;16:9440–9445. [PubMed: 12883005]
29. Spiecker M, Liao JK. Vascular protective effects of cytochrome p450 epoxygenase-derived eicosanoids. Arch Biochem Biophys 2005;2:413–420. [PubMed: 15581597]
30. Yu Z, Xu F, Huse LM, Morisseau C, Draper AJ, Newman JW, et al. Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. Circ Res 2000;11:992–998. [PubMed: 11090543]
31. King LM, Gainer JV, David GL, Dai D, Goldstein JA, Brown NJ, et al. Single nucleotide polymorphisms in the *CYP2J2* and *CYP2C8* genes and the risk of hypertension. Pharmacogenet Genomics 2005;1:7–13. [PubMed: 15864120]
32. Fornage M, Boerwinkle E, Doris PA, Jacobs D, Liu K, Wong ND. Polymorphism of the soluble epoxide hydrolase is associated with coronary artery calcification in African-American subjects: the Coronary Artery Risk Development in Young Adults (CARDIA) study. Circulation 2004;3:335–339. [PubMed: 14732757]
33. Rossi GP, Maiolino G, Zanchetta M, Sticchi D, Pedon L, Cesari M, et al. The T(– 786)C endothelial nitric oxide synthase genotype predicts cardiovascular mortality in high-risk patients. J Am Coll Cardiol 2006;6:1166–1174. [PubMed: 16979000]
34. Dwyer JH, Allayee H, Dwyer KM, Fan J, Wu H, Mar R, et al. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. N Engl J Med 2004;1:29–37. [PubMed: 14702425]

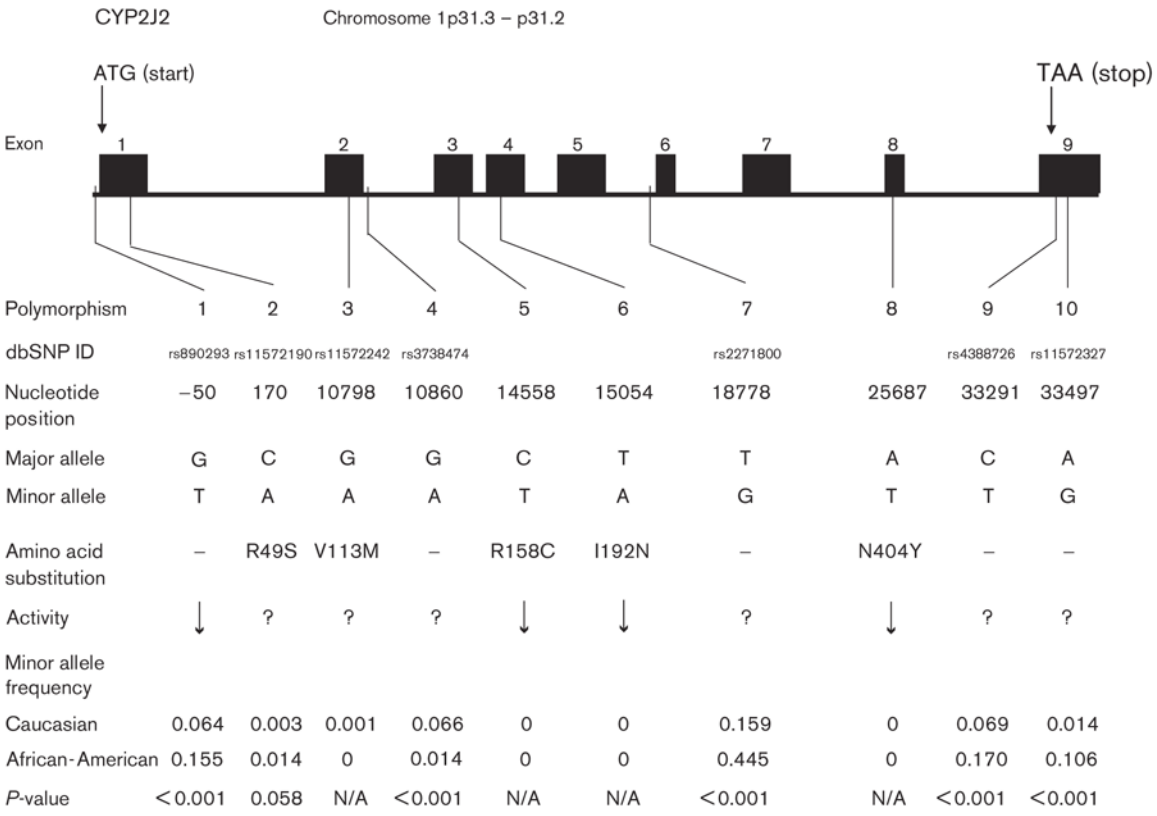
35. Michaelis UR, Fisslthaler B, Barbosa-Sicard E, Falck JR, Fleming I, Busse R. Cytochrome P450 epoxygenases 2C8 and 2C9 are implicated in hypoxiainduced endothelial cell migration and angiogenesis. J Cell Sci 2005;(Pt 23):5489–5498. [PubMed: 16291720]

36. Cipollone F, Toniato E, Martinotti S, Fazia M, Iezzi A, Cuccurullo C, et al. A polymorphism in the cyclooxygenase 2 gene as an inherited protective factor against myocardial infarction and stroke. JAMA 2004;18:2221–2228. [PubMed: 15138244]

37. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002;3:251–262. [PubMed: 11861412]

38. Lang CC, Stein CM, Brown RM, Deegan R, Nelson R, He HB, et al. Attenuation of isoproterenol-mediated vasodilatation in blacks. N Engl J Med 1995;3:155–160. [PubMed: 7791817]

39. Kalinowski L, Dobrucki IT, Malinski T. Race-specific differences in endothelial function: predisposition of African Americans to vascular diseases. Circulation 2004;21:2511–2517. [PubMed: 15159296]



Polymorphism	1	2	3	4	5	6	7	8	9	10
dbSNP ID	rs890293	rs11572190	rs11572242	rs3738474			rs2271800		rs4388726	rs11572327
Nucleotide position	−50	170	10798	10860	14558	15054	18778	25687	33291	33497
Major allele	G	C	G	G	C	T	T	A	C	A
Minor allele	T	A	A	A	T	A	G	T	T	G
Amino acid substitution	–	R49S	V113M	–	R158C	I192N	–	N404Y	–	–
Activity	↓	?	?	?	↓	↓	?	↓	?	?
Minor allele frequency										
Caucasian	0.064	0.003	0.001	0.066	0	0	0.159	0	0.069	0.014
African-American	0.155	0.014	0	0.014	0	0	0.445	0	0.170	0.106
P-value	<0.001	0.058	N/A	<0.001	N/A	N/A	<0.001	N/A	<0.001	<0.001

Fig 1.

Nucleotide positions of the 10 *CYP2J2* polymorphisms evaluated are given relative to the *CYP2J2* transcriptional start site (Genbank accession number AF272142). Activity is reported as lower (↓) or unknown (?) relative to wild-type enzyme based on previous studies [11,12]. Cohort random sample minor allele frequencies are presented separately by race, and *P*-values for the comparison of allele frequency across race by χ^2 are reported. N/A, not applicable.

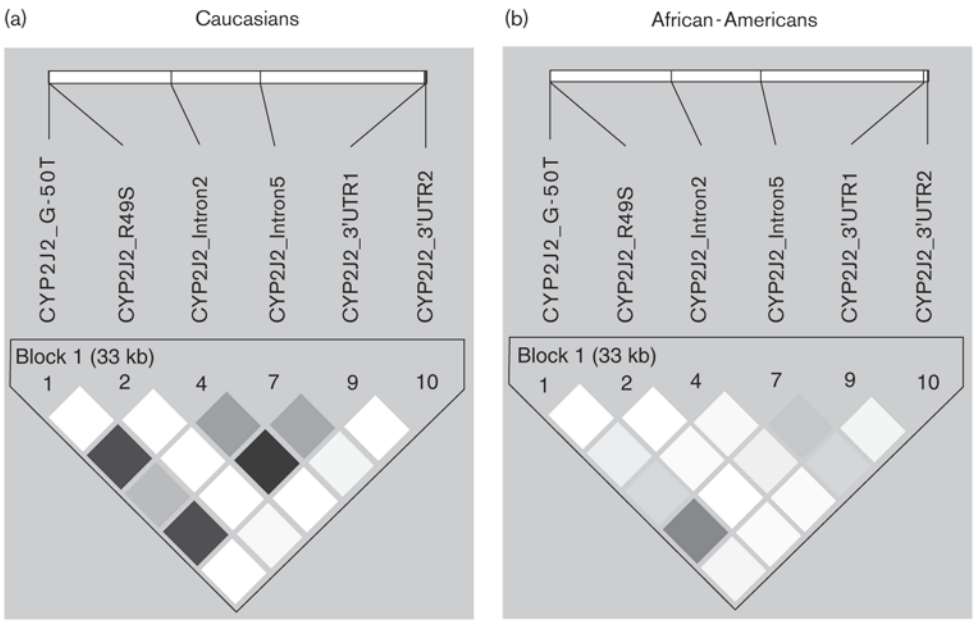


Fig 2.

Pairwise estimates of linkage disequilibrium (LD) between each *CYP2J2* polymorphism are plotted for (a) Caucasians and (b) African-Americans in the cohort random sample using Haploview 3.2. Each polymorphism is numbered according to its position in the *CYP2J2* gene as presented in Fig. 1. Black squares indicate perfect LD ($r^2 = 1.0$), white squares indicate zero LD ($r^2 = 0.0$), and increasing intensity of grey indicates increasing degrees of LD.

Table 1

CYP2J2 and CYP2C8 polymorphism genotype frequency by incident CHD case status

Genotype[a]	Caucasian			African-American		
	Noncases	CHD cases	*P*-value	Noncases	CHD cases	*P*-value
CYP2J2						
G-50 T(*CYP2J2*7*)						
G/G	501 (88.0%)	648 (88.7%)		189 (70.7%)	167 (79.2%)	
G/T + T/T	65 (12.1%)	83 (11.4%)	0.719	79 (29.3%)	44 (20.9%)	0.043
R49S						
C/C	611 (99.5%)	774 (99.9%)		280 (97.5%)	223 (99.6%)	
C/A+ A/A	4 (0.6%)	1 (0.1%)	N/A	8 (2.5%)	1 (0.5%)	N/A
CYP2C8						
I264M(*CYP2C8*4*)						
C/C	512 (91.9%)	696 (90.0%)		299 (97.7%)	221 (97.7%)	
C/G+ G/G	44 (8.1%)	77 (10.0%)	0.261	6 (2.3%)	5 (2.2%)	0.950
I269F (*CYP2C8*2*)						
A/A	570 (99.7%)	759 (99.5%)		216 (73.8%)	163 (74.1%)	
A/T + T/T	2 (0.3%)	4 (0.5%)	N/A	80 (26.2%)	57 (25.9%)	0.940
K399R(*CYP2C8*3*)						
A/A	461 (81.3%)	618 (80.0%)		299 (96.6%)	213 (95.1%)	
A/G+ G/G	116 (18.7%)	155 (20.1%)	0.552	10 (3.4%)	11 (4.9%)	0.424

[a]Genotype data presented as absolute (percent) genotype frequency. Frequencies are weighted according to the sampling fraction. The *R158C* (*CYP2J2*3*), *I192N* (*CYP2J2*4*), and *N404Y* (*CYP2J2*6*) polymorphisms in *CYP2J2* were monomorphic (minor allele frequency 0.0), and the *V113M* (rs11572242) variant allele was identified in only one Caucasian noncase.

CHD, coronary heart disease; N/A, not applicable.

Table 2

Hazard rate ratio between CYP2J2 and CYP2C8 polymorphisms and risk of incident CHD

Polymorphism	Caucasian			African-American		
	HRR	**95% CI**	***P*-value**	**HRR**	**95% CI**	***P*-value**
CYP2J2						
G-50T (*CYP2J2*7*)	*G/T* + *T/T* versus *G/G*			*G/T* + *T/T* versus *G/G*		
Model 1[a]	1.08	0.75–1.54	0.692	0.58	0.37–0.89	0.014
Model 2[b]	1.15	0.78–1.70	0.472	0.58	0.35–0.96	0.036
CYP2C8						
I264M (*CYP2C8*4*)	*C/G* + *G/G* versus *C/C*					
Model 1[a]	1.29	0.86–1.92	0.222	N/A		
Model 2[b]	0.98	0.58–1.65	0.926	N/A		
I269F (*CYP2C8*2*)	*A/T* + *T/T* versus *A/A*					
Model 1[a]	N/A			0.90	0.60–1.37	0.634
Model 2[b]	N/A			0.99	0.62–1.56	0.951
K399R (*CYP2C8*3*)	*A/G* + *G/G* versus A/A			*A/G* + *G/G* versus *A/A*		
Model 1[a]	0.97	0.73–1.28	0.816	1.22	0.55–2.73	0.627
Model 2[b]	0.97	0.70–1.34	0.848	1.20	0.51–2.83	0.669

CHD, coronary heart disease; CI, confidence interval; HRR, hazard rate ratio; N/A, modeling not reported since variant allele frequency ≤ 1% in the incident CHD case and/or noncase group.

[a] Adjusted for age, sex, and study center.

[b] Adjusted for age, sex, study center, current smoker, diabetes, hypertension, high-density lipoprotein cholesterol, total cholesterol, and body mass index.

Table 3

CYP2J2 haplotypes and risk of incident CHD

Haplotype,[ab]	Distribution analysis			Association analysis		
	Noncases	CHD cases	*P*-value[c]	HRR[d]	95% CI	*P*-value
African-American[e]	*n* = 260	*n* = 200				
GTCA	56.3%	56.9%		1.14	0.65–2.00	0.653
*G**G**CA*	16.1%	13.6%		0.82	0.51–1.31	0.402
*G**G**C**G***	10.5%	14.9%		1.42	0.83–2.42	0.201
*T**G**TA*	12.3%	8.1%		0.51	0.29–0.91	0.023
*G**G**TA*	4.8%	6.5%	0.086	0.93	0.41–2.10	0.858
Caucasian[f]	*n* = 493	*n* = 692				
GT	85.7%	86.2%		1.15	0.53–2.47	0.728
*G**G***	8.5%	7.6%		0.96	0.66–1.40	0.831
*T**G***	5.8%	6.2%	0.731	1.33	0.87–2.02	0.184

[a] Bold nucleotides represent haplotype tagging polymorphisms.

[b] Estimated copy frequency weighted according to the sampling fraction.

[c] χ^2 *P*-value for the overall distribution of haplotypes by CHD case status.

[d] Adjusted for age, gender, study center, current smoker, diabetes, hypertension, high-density lipoprotein cholesterol, total cholesterol, and body mass index.

[e] Haplotype includes polymorphisms 1, 7, 9, and 10.

[f] Haplotype includes polymorphisms 1 and 7.

CHD, coronary heart disease; HRR, hazard rate ratios

Table 4

Gene–environment interaction between *CYP2C8* polymorphisms, cigarette smoking and risk of incident CHD in Caucasians

Smoking exposure	*CYP2C8* genotype		Interaction	*P*-value
I264M (*CYP2C8*4*)	*C/C*	*C/G + G/G*		
Noncurrent smokers	*n* = 477/400[a]	*n* = 47/34[a]		
Model 1[b]	1 (referent)	1.13 (0.70–1.83)		
Model 2[c]	1 (referent)	0.72 (0.36–1.45)		
Current smokers	*n* = 219/111[a]	*n* = 30/10[a]		
Model 1[b]	1.74 (1.32–2.31)	3.18 (1.47–6.90)	1.61 (0.64–4.09)	0.314
Model 2[c]	1.62 (1.18–2.21)	2.76 (1.28–5.95)	2.38 (0.84–6.78)	0.104
K399R (*CYP2C8*3*)	*A/A*	*A/G + G/G*		
Noncurrent smokers	*n* = 422/362[a]	*n* = 100/93[a]		
Model 1[b]	1 (referent)	0.87 (0.62–1.20)		
Model 2[c]	1 (referent)	0.79 (0.53–1.16)		
Current smokers	*n* = 196/99[a]	*n* = 55/23[a]		
Model 1[b]	1.75 (1.30–2.36)	2.73 (1.58–4.75)	1.80 (0.91–3.54)	0.089
Model 2[c]	1.64 (1.18–2.29)	2.63 (1.46–4.74)	2.03 (0.97–4.25)	0.060
I264M or *K399R*[d]	*Wild-type*	*Variant*		
Noncurrent smokers	*n* = 383/330[a]	*n* = 139/125[a]		
Model 1[b]	1 (referent)	0.90 (0.67–1.21)		
Model 2[c]	1 (referent)	0.73 (0.51–1.05)		
Current smokers	*n* = 169/90[a]	*n* = 82/32[a]		
Model 1[b]	1.63 (1.19–2.24)	2.88 (1.80–4.62)	1.97 (1.08–3.61)	0.028
Model 2[c]	1.48 (1.04–2.11)	2.65 (1.61–4.39)	2.45 (1.26–4.75)	0.008

Data presented as hazard rate ratio (95% confidence interval).

[a] Number of CHD cases/noncases in each cell.

[b] Adjusted for age, sex, and study center.

[c] Adjusted for age, sex, study center, diabetes, hypertension, high-density lipoprotein cholesterol, total cholesterol, and body mass index.

[d] Individuals carrying at least one variant allele for either the *I264M* (*C/G + G/G*) or *K399R* (*A/G + G/G*) polymorphism were compared with those wild-type for both (*C/C* and *A/A*, respectively).

CHD, coronary heart disease.