Common polymorphisms of *ALOX5* and *ALOX5AP* and risk of coronary artery disease

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Abstract

Recent human genetic studies suggest that allelic variants of leukotriene pathway genes influence the risk of clinical and subclinical atherosclerosis. We sequenced the promoter, exonic, and splice site regions of *ALOX5* and *ALOX5AP* and then genotyped 7 SNPs in *ALOX5* and 6 SNPs in *ALOX5AP* in 1,552 cases with clinically significant coronary artery disease (CAD) and 1,583 controls from Kaiser Permanente including a subset of participants of the coronary artery risk development in young adults study. A nominally significant association was detected between a promoter SNP in *ALOX5* (rs12762303) and CAD in our subset of white/European subjects (adjusted odds ratio per minor allele, log-additive model, 1.32; $P = 0.002$). In this race/ethnic group, rs12762303 has a minor allele frequency of 15% and is tightly linked to variation at the SP1 variable tandem repeat promoter polymorphism. However, the association between CAD and rs12762303 could not be reproduced in the atherosclerosis risk in communities study (hazard rate ratio per minor allele; 1.08, $P = 0.1$). Assuming a recessive mode of inheritance, the association was not significant in either population study but our power to detect modest effects was limited. No significant associations were observed between all other SNPs and the risk of CAD. Overall, our findings do not support a link between common allelic variation in or near *ALOX5* or

ALOX5AP and the risk of CAD. However, additional studies are needed to exclude modest effects of promoter variation in *ALOX5* on the risk of CAD assuming a recessive mode of inheritance.

Introduction

Leukotrienes are short-lived lipid mediators derived from the nuclear membrane of cells that are produced and excreted in response to various immune stimuli (Funk 2001). They have been implicated in a variety of inflammatory responses, including asthma, arthritis, and psoriasis and more recently in the inflammatory component of atherosclerosis and its complications including coronary artery disease (CAD), ischemic stroke, and peripheral arterial disease (PAD) (Funk 2005; Lotzer et al. 2005).

The initial enzymatic step in the leukotriene pathway is the oxidation of arachidonic acid to leukotriene A4 (LTA_4) by 5-lipoxygenase (5-LO, encoded by *ALOX5*) (Funk 2001). A necessary cofactor in this reaction is the 5-LO activating protein (ALOX5AP, encoded by *ALOX5AP*), also known as FLAP (Dixon et al. 1990; Miller et al. 1990). LTA_4 is an unstable intermediate leukotriene that is then metabolized to either leukotriene B4 (LTB_4) or conjugated to produce a series of three related cysteinyl leukotrienes (cysLTs) LTC_4, LTD_4, and LTE_4. These molecules are thought to be the actual mediators of various inflammatory responses, some of which could be relevant to the development of atherosclerosis (Funk 2005; Zhao and Funk 2004). Several proteins in this 5-LO pathway are expressed in both human (Cipollone et al. 2005; Spanbroek et al. 2003) and mouse atherosclerotic plaque (Tabibiazar et al. 2005a, b; Zhao and Funk 2004). Furthermore, genetic studies in mice have suggested a pathophysiologic role of the 5-LO pathway in atherosclerosis (Mehrabian et al. 2002, 2001; Subbarao et al. 2004), and vascular inflammation (Zhao and Funk 2004).

Despite the accumulating evidence linking the 5-LO pathway to atherosclerosis, no human genetic studies have substantiated a relationship between *ALOX5* polymorphisms and clinical complications of atherosclerosis including CAD. A study published in 2004 documented an increase in carotid intima media thickness (IMT) in subjects with two copies of the non-wildtype alleles of a tandem SP1 binding motif polymorphism in the *ALOX5* promoter compared with subjects who had two copies of the wild allele at this site (Dwyer et al. 2004). Carriers of one non-wild type allele had IMT similar to non-carriers and increased dietary arachidonic acid significantly enhanced the apparent atherogenic effect of genotype, whereas increased dietary intake of *n* – 3 fatty [acids eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA)] blunted the effect (Dwyer et al. 2004). However, a more recent small case control study in whites was unable to detect an association between myocardial infarction (MI) and SP1 allelic variants (Gonzalez et al. 2007).

Human genetic association studies to date have also yielded conflicting results for *ALOX5AP*. DeCODE investigators were the first to suggest that common allelic variants of the *ALOX5AP* gene increased the risk of MI (Helgadottir et al. 2005, 2004). However, these findings have not been broadly and convincingly replicated (Morgan et al. 2007; Samani et al. 2007).

As part of the overall goals of the *A*therosclerotic *D*isease, *VA*scular FuNction, and Geneti*C E*pidemiology (ADVANCE) study at Stanford University and Kaiser Permanente of Northern California (KPNC), we sought to test whether SNPs in the *ALOX5* or *ALOX5AP* genes confer susceptibility to clinically significant CAD. To this end, we studied the composite endpoint of the various clinical manifestation of CAD, which served as surrogate to the underlying burden of coronary atherosclerosis. We also sought to replicate any putative genotype-phenotype associations in an independent and well characterized cohort as replication is now considered the gold standard method to validate such associations (Chanock et al. 2007). We selected the National, Heart, Lung, and Blood Institute's atherosclerosis risk in communities (ARIC) study to serve as our replication cohort because the primary outcome of interest in that study of incident coronary heart disease (CHD) was the same as that in the ADVANCE study (1989; Volcik et al. 2006; White et al. 1996).

Methods

Study sample ADVANCE

The ADVANCE study is a case-control study among adults (age 18 years) receiving medical care within KPNC. Detailed description of the eligibility criteria and the source population for all study participants have been published previously (Go et al. 2006; Iribarren et al. 2006; Taylor-Piliae et al. 2006). Briefly, between 28 October 2001 and 31 December 2003, we recruited a total of 3,179 case and control subjects. Cases consisted of subjects presenting with clinically significant CAD (MI or angina with angiogram showing at least one coronary artery stenosis of >50%) at a young age (45 years for males, 55 years for females) or subjects presenting with incident stable angina or incident acute myocardial infarction (AMI) at an older age. Controls consisted of young subjects with no history of CAD (30–45 years for males, 30–55 for females) or subjects aged 60–72 with no history of CAD, cerebrovascular accident (CVA), or PAD. Young controls recruited de novo from Kaiser were complemented with a subset of 479 subjects from the coronary artery risk development in young adults (CARDIA) study originally recruited at the Oakland field center and attending the study's year 15 examination in 2000–2001 (Hughes et al. 1987). During recruitment, some race/ethnic and gender strata were over-sampled to maximize the probability that case and control groups were balanced in this respect.

The design of ADVANCE allowed for several primary case-control comparisons. In this study, we compared subjects with early onset CAD ("young cases") with young subjects without CAD ("young controls") and older subjects presenting with stable angina or AMI ("older cases") with older subjects with no history of CAD, CVA, or PAD ("older controls").

Clinical measurements

Through a phone interview, an extensive self administered questionnaire, and the use of the KPNC electronic databases, we documented the presence or absence and age of onset of clinically significant CAD, CVA, and PAD, as well as all traditional risk factors for atherosclerosis. Subjects also provided information on race/ethnicity and were classified into one of nine race/ethnic groups: white/Europeans, black/African Americans, Hispanics,

South Asians, East Asians, Pacific Islanders, Native Americans, admixed Hispanics, and admixed non-Hispanics. At the clinic visit, we measured height, weight and blood pressure of all participants and then collected whole blood for DNA extraction and quantification of various serum markers. For this study, traditional risk factors (smoking, hypertension, high cholesterol, and diabetes) were defined based on self-report. In cases, these risk factors were considered to be present only if subjects reported an age of onset of the risk factor that was younger than the age of onset of clinically significant CAD.

Sequencing and genotyping

Using an automated fluorescent labeling system (Strachan and Read 1999), we sequenced the promoter region, the exons including the 5′ and 3′ untranslated regions (UTRs), and the intron-exon boundaries of *ALOX5* and *ALOX5AP* in 24 ethnically diverse males with a history of CAD (SNP discovery set). A subset of all SNPs identified by sequencing was then genotyped in all participants of the ADVANCE study using the TaqMan® assay (Strachan and Read 1999) (primers available on request). For all SNPs, the reference strand used for the determination of the minor allele was chosen to be consistent with dbSNP convention. Information on all SNPs from the SNP discovery effort has been submitted to dbSNP. *ALOX5* and *ALOX5AP* represent two of approximately 100 candidate genes of CAD sequenced in our SNP discovery set and genotyped in the ADVANCE cohorts to date.

The subset of sequenced SNPs selected for genotyping in all study participants was selected on the basis of the minor allele frequency observed in the SNP discovery set, the predicted functional effect, and the degree of linkage disequilibrium between SNPs. In general, we prioritized for genotyping SNPs with a higher minor allele frequency (to maximize statistical power), SNPs in exonic or promoter regions (to maximize the probability of a functional effect), and SNPs that appeared to be in least linkage disequilibrium with each other (to try to capture as many haplotypes as possible in the region).

In addition to the SNPs described above, we attempted to genotype three other SNPs that we did not sequence in ALOX5AP (rs17222814, rs17216473 and rs10507391). These three intronic SNPs define the 5′ end of deCODE's "Hap A" and "Hap B" ALOX5AP haplotypes (Helgadottir et al. 2005, 2004). We specifically selected these deCODE SNPs because they were all located in the haplotype block immediately 5′ to the recombination site between exon 1 and 2 (The International HapMap Consortium 2003; Helgadottir et al. 2004), a region covered by only one of our sequenced SNPs selected for genotyping.

One of the ALOX5 SNPs genotyped in all participants (rs12762303) was noted to be within 500 base pairs to the previously described SP1 copy number variation in the ALOX5 promoter found previously to be associated with carotid IMT (Dwyer et al. 2004). Variant alleles at the SP1 variation site involve deletions (one or two) or additions (one, two, or three) of motifs to the five tandem motifs observed in the common allele. We genotyped the SP1 repeat polymorphism in a sub sample of 167 ADVANCE subjects using previously published primers and methods (Dwyer et al. 2004; In et al. 1997) to determine the degree of linkage disequilibrium between rs12762303 and the SP1 repeat. The sample was selected to include individuals of all races except "Admixed Hispanic" and "Admixed Non-Hispanic" and all three rs12762303 genotypes regardless of case-controls status. All homozygous

minor carriers of rs12762303 were included because of the low frequency of this genotype. For non-white/Europeans, all heterozygote carriers were included because of the small numbers of subjects in these race/ethnic groups. Finally, we selected a random sample of heterozygote white/European and homozygote major carriers for all race/ethnic groups. Genotyping calls at this site were made blinded to all study parameters including case/control status, race/ethnic group, and SNP genotyping results.

Statistical analysis

We excluded from analysis subjects who did not provide blood for DNA extraction ($n = 40$) and who did not Wll out the study questionnaire ($n = 9$). We also excluded South Asians ($n = 55$) because of a lack of young controls. Lastly, we excluded Pacific Islanders ($n = 9$) and Native Americans ($n = 2$) because of small numbers.

We first compared the distributions and frequencies of all non-genetic covariates of interest, using standard parametric and non-parametric methods stratified by the two sets of cases and controls. Next, we calculated minor allele frequencies of all SNPs genotyped in all participants and tested for Hardy–Weinberg equilibrium (HWE) with the permutation version of the exact test (Guo and Thompson 1992) stratified by race/ethnic group. For rs12762303 and the SP1 repeat polymorphisms, we calculated pair wise linkage disequilibrium statistics (D' and r^2) (Devlin and Risch 1995) and determined haplotype structure using the EM algorithm (Excoffier and Slatkin 1995).

Using multivariate unconditional logistic regression, we then calculated odds ratios (ORs) for the risk of symptomatic CAD between carriers and non carriers of the minor allele. All ORs were first adjusted for age, gender, self-reported race/ethic group, and source of cases and controls (young onset set vs. older onset set) and then further adjusted for BMI, smoking status (ever vs. never), hypertension, diabetes and hyperlipidemia. To minimize the probability of confounding due to population substructure in our "Admixed Hispanic" and "Admixed Non-Hispanic" race/ethnic groups, we used STRUCTURE (Pritchard et al. 2000) to estimate the proportion of white/European, black/African–American, Hispanic and East Asian ancestry at the individual level for all cases and controls in these groups and then allowed a stepwise selection algorithm to enter one or more of these covariates into the multivariate analysis. Only the covariate representing the proportion of white ancestry was selected. For each SNP, we also tested for the presence of heterogeneity among the ORs across all race/ethnic groups and both set of cases and controls using appropriate interaction terms. Unadjusted P values produced by our regression analyses were considered significant if they were lower than a Bonferroni adjusted threshold for the number of SNPs tested in the same gene and race/ethnic group assuming an overall Type 1 error of 0.05. Lastly, we inferred haplotypes using the EM algorithm (Excoffier and Slatkin 1995) and tested these haplotypes for association with case control status (Zaykin et al. 2002; Zhao et al. 2000) stratified by race/ethnic group.

We carried out post-hoc power calculations for individual SNPs using the online genetic power calculator (Purcell et al. 2003). For these calculations, we assumed the polymorphism was causal, a population prevalence of symptomatic CAD of 6.2% (Holtby et al. 2004), and a Type 1 error of 0.01. We used SAS v 9.1.3 (SAS Institute Inc., Cary, USA) including the

SAS genetics module v. 2.2 and STRUCTURE (Pritchard et al. 2000) to carry out all other analyses.

Replication in the ARIC study

The ARIC study is a prospective investigation of atherosclerosis and its clinical sequelae involving 15,792 individuals from four US communities aged 45–64 years at recruitment (1987–1989). A detailed description of the design, sampling procedures, methods, definitions of cardiovascular outcomes, and approach to statistical analyses is published elsewhere (The ARIC Investigators 1989; Volcik et al. 2006; White et al. 1996). Genotyping was performed using the TaqMan Assay. Incident CHD cases were defined as definite or probable MI, a silent MI between examinations by ECG, a definite CHD death or coronary revascularization. ARIC participants with the following characteristics were excluded: a positive or unknown history of prevalent CHD, TIA or stroke at baseline, a race/ethnic background other than white or African–American, African–Americans not from Jackson or Forsyth, and lack of consent for use of DNA. Follow-up was available until 31 December 2002 and time-to-CHD event was analyzed using Cox proportional hazards modeling. The hazard ratio (HR) of a prospective study is closely related to the OR of a case control study examining the same outcome assuming a common true HR across all populations of less than two (the norm for complex traits) and an event rate of less than 15% (Symons and Moore 2002) (in ARIC the event rate was 12.5%). In a post-hoc analysis, we adopted the methods of Hsieh and Lavori (Hsieh and Lavori 2000) to calculate the minimal detectable HR assuming a power of 90%. We used STATA (StataCorp LP, College Station, USA) and PASS (Hintze 2006) to carry out all analyses.

Consent and Institutional Review Board approval

The ADVANCE study was approved by the Institutional Review Boards (IRBs) at Stanford, the Kaiser Foundation Research Institute and the Palo Alto Veterans Administration Hospital. The ARIC study was approved by all participating institutional IRBs. All study participants gave written informed consent.

Results

Non-genetic covariates of interest in ADVANCE

Table 1 summarizes the baseline characteristics of the 1,809 cases and 1,734 controls in the ADVANCE study. As expected, the prevalence of most traditional risk factors of atherosclerosis was significantly greater in cases than in controls. The lower prevalence of males in the younger case/control set and the younger age of cases in the older case/control set were the result of stratified sampling. Stratified sampling and/or preferential participation also led to differences in the prevalence of certain race/ethnic groups.

Sequencing and genotyping

We identified 27 polymorphisms in ALOX5 and 10 polymorphisms in ALOX5AP by sequencing these genes in our SNP discovery set. We then genotyped seven of the SNPs sequenced in ALOX5 and six of the SNPs sequenced in ALOX5P in all participants of the ADVANCE study (Table 2). As a result of nearby repetitive sequences, we were successful

in genotyping only one of the three deCODE SNPs in ALOX5AP (rs10507391). We excluded rs41323349 from further analysis because of a MAF significantly less than 1%. All remaining SNPs were in HWE in both sets of controls combined when stratified by race/ ethnic group (lowest exact P value = 0.014, further details not shown).

Linkage disequilibrium statistics between rs12762303 and the SP1 repeat polymorphism in ALOX5 are summarized in Table 3. The overall failure rate for this assay was 6.3%. In whites and Hispanics, the correlation between the common allele at SP1 (five repeats) and the T allele at rs12762303 was very high (r^2 of 0.94 and 1, respectively). The correlation between SP1 repeat number and allele at rs12762303 was significantly lower in African Americans and East Asians. In these latter two race/ethnic groups, the T allele was seen in significant numbers not only with the wild type SP1 of five repeats but also with the minor SP1 allele of three repeats in African Americans and four, six, and seven repeats in East Asians. Thus, for whites and Hispanics, the T allele at rs12762303 is an excellent surrogate for the presence of the major SP1 allele of five repeats.

SNP association analysis

For all SNPs, we noted no significant differences in the point estimate and the variance of the ORs between unadjusted, minimally adjusted (age, gender, and case/control status) and fully adjusted models (details not shown). For all SNPs, we also could not detect significant differences in the ORs between the two sets of cases and controls (details not shown). Therefore, we present only the fully adjusted ORs combining both case/control sets.

We observed a nominally significant association between rs12762303 and CAD in white/ European subjects (Table 4). In fully adjusted logistic regression analyses assuming an additive model, the presence of each additional copy of the minor allele at rs12762303 was associated with an elevated risk of clinical coronary disease (adjusted OR: 1.32, P = 0.002). The OR observed in the young set of cases and controls was slightly higher than the OR observed in the older set of cases and controls (1.38, P = 0.08 vs. 1.30, P = 0.01) but the difference was not significant (P for interaction term: 0.97). The point estimates of the ORs for rs12762303 in Hispanics, mixed Hispanics, and mixed other groups were similar to whites but not statistically significant. Only one other SNPs in ALOX5 demonstrated nominally significant protective effects but only in one of the smaller non white race/ethnic groups. Two SNPs in ALOX5 and one SNP in ALOX5AP demonstrated significant heterogeneity of the OR across race/ethnic groups (Table 4).

We found no strong evidence of dominance effects (i.e. evidence that the recessive or dominant model of inheritance was more appropriate than the additive model). Therefore, we present only the additive model for all SNPs in the tables. However, we calculated the OR for the recessive model of inheritance for rs12762303 because the recessive model appears to be most relevant in the association between minor alleles at the SP1 site in the ALOX5 promoter region and carotid IMT (Dwyer et al. 2004). The adjusted OR for this SNP for the recessive model was 1.18 (P = 0.56) in whites and 0.94 (P = 0.96) in Hispanics.

Haplotype association analyses were no more revealing than the SNP by SNP analysis. For ALOX5, all of the most common haplotypes carrying the high-risk allele at rs12762303 had

a higher frequency in cases compared to controls among whites but a lower frequency among East Asians. Similarly, all of the most common haplotypes carrying the high-risk allele at rs2029253 had a higher frequency in cases compared to controls in East Asians. For ALOX5AP, none of the common haplotypes were associated with case control status.

Replication in the ARIC cohort

We genotyped rs12762303 in 9,800 white and 3,352 African American subjects from the ARIC Study, including 1,154 and 255 cases of incident CHD, respectively. The mean number of years to incident CHD was 8.2 for whites and 8.0 for African Americans. In fully adjusted models we found no association with the "C" allele with disease status. The hazard rate ratios per copy of the "C" allele assuming a log additive mode of inheritance was 1.08 ($P = 0.2$) in whites and 1.07 ($P = 0.5$) in blacks (Table 5). The HRs assuming a recessive model of inheritance was 1.27 ($P = 0.1$) in whites and 1.06 ($P = 0.9$) in blacks.

Post hoc calculations of power and minimal detectable risk ratios for clinical CAD

The power to detect an OR of 1.3 was 87% in the ADVANCE study assuming a MAF of 10% across all race/ethnic groups and an additive model. Assuming a MAF of 15% (the frequency of rs12762303) and a recessive model of inheritance, the minimal detectable OR with a power of 90% was 3.2 in whites and Hispanics combined. In ARIC, the minimal detectable HR for rs12762303 was 1.25 in whites assuming the log additive model and 2.1 assuming a recessive model of inheritance.

Additional association analyses

Despite our inability to replicate the "main effect" association between rs12762303 and CAD observed in the ADVANCE study whites, the very high correlation between rs12762303 and the SP1 repeat site in whites (Table 3) combined with the findings of Dwyer et al. (2004) motivated us to perform three additional analysis. We first tested for the presence of an interaction between rs12762303 genotype and dietary intake of various fatty acids (including arachidonic acid, linoleic acid, EPA plus DHA, monounsaturated fatty acids, and saturated fatty acids) on the risk of CHD. Next, we tested for a main effect association between rs12762303 genotype and the mean degree of carotid IMT using multivariate linear regression ($n = 6,548$ TT, 2,502 CT, and 241 CC genotype). Lastly, we tested for the presence of a significant interaction between rs12762303 genotype and dietary intake of various fatty acids on the mean degree of carotid IMT. All of these additional analyses were carried out in the ARIC study alone as it was not possible to do these analyses in the ADVANCE study. Intake of each of these fatty acids was estimated as a percentage of total fat intake measured at baseline with a semi-quantitative food frequency questionnaire (Ma et al. 1995) while carotid IMT was determined by high resolution B-mode ultrasound, as described previously (The ARIC Study Group 1991; Stevens et al. 1998) None of these additional analyses yielded statistically significant associations regardless of the mode of inheritance assumed (all unadjusted Ps   0.05, details not shown).

Discussion

The most significant association detected in our discovery sample set was between a SNP in the promoter region of ALOX5 (rs12762303) and CAD in white/European subjects assuming a log-additive model of inheritance. The point estimate of the OR of this SNP was similar in Hispanics. The rs12762303 SNP was found to be nearly perfectly correlated with variation (wild type vs. non wild type) at the nearby SP1 transcription factor consensus binding sequence in these same two race/ethnic groups. Furthermore, whites and Hispanics constituted a large majority of participants (85%) in the single study to date that has linked allelic variation at the SP1 site with subclinical atherosclerosis (Dwyer et al. 2004). We hypothesized that the lack of an association in blacks and East Asians may have been a consequence of a lack of power not only due to smaller numbers in these race/ethnic groups but also poorer correlations with the SP1 site polymorphism. To rule out a false positive finding, we proceeded with a replication study in an independent cohort. Unfortunately, we were unable to replicate this association in the ARIC cohort despite adequate power to detect a HR in the same range as the OR observed in the ADVANCE study. Furthermore, in the ARIC cohort, we found no evidence of an association between rs12762303 genotypes and carotid IMT or that the association between rs12762303 and either clinical CAD or IMT was modified by intake of various dietary fats including those fats found to modify the association between SP1 allelic variants and carotid IMT in a previous study (Dwyer et al. 2004).

We found no convincing association between SNPs in *ALOX5AP* and CAD. Previous human genetic studies concerning the relationship of polymorphisms in this gene with CAD have yielded inconsistent results. DeCODE investigators were the first to suggest that common allelic variants of the *ALOX5AP* gene influence the risk of MI (Helgadottir et al. 2005, 2004). In an Icelandic cohort, they reported a relative risk of 1.8 for MI in carriers of a haplotype, named "Hap A", defined by four SNPs and spanning 33 kb across four out of the five *ALOX5AP* exons. This finding could not be replicated in an independent British cohort but another common haplotype, named "Hap B" (defined by different SNPs than in Hap A) almost doubled the risk of MI. Both haplotypes were defined by intronic SNPs only. To date, these findings have not been broadly replicated (Girelli et al. 2007; Morgan et al. 2007; Samani et al. 2007).

Our study has several limitations. First, we did not have adequate power to detect an OR either in the lower range of expected for complex trait genotype-phenotype associations (i.e. OR < 1.3 or >0.77) assuming a log additive model of inheritance or in the upper or lower range expected (i.e. OR 1.3–2.0 or 0.5–0.77) assuming a recessive mode of inheritance. The latter limitation in power is important because an increase in carotid IMT was seen only in homozygote carriers of minor alleles at the SP1 site in the ALOX5 promoter (Dwyer et al. 2004). However, the complete lack of association between carotid IMT and rs12762303 genotype in the same cohort assuming a recessive mode of inheritance does not support the possibility that an association between clinical CAD and rs12762303 was missed. Second, we did not enroll cases that either died or were too ill to participate at any time after their incident event and prior to the clinic visit. It is difficult to predict what effect this selection bias may have had on the observed ORs in the ADVANCE study. However, this bias was

not present in the ARIC cohort study, which captured incident fatal events. Finally, we cannot be certain that other common allelic variants of this gene are not associated with CAD because we did not genotyped all common variants in the region or a maximally informative subset of tagSNPs (Carlson et al. 2004).

In summary, while the 5LO pathway may play a role in the development of atherosclerosis, in our two population studies there was no consistent evidence of an association between clinically significant CAD and several common variants in or near *ALOX5* and *ALOX5AP*. In CAD alone, many initially positive reports have not withstood the test of replication in other cohorts (Morgan et al. 2007). Our results underscore this observation and the need to robustly replicate initially promising genotype–phenotype associations (Chanock et al. 2007). To facilitate this process, we report, for the first time, a SNP (rs12762303) that can be used as an excellent surrogate for the presence or absence of the common allele at the SP1 site of the *ALOX5* promoter in whites and Hispanics. Genotyping this SNP in other cohorts to explore associations between allelic variation at the SP1 site and CAD or other phenotypes may prove to be easier than genotyping the GC rich SP1 site.

Acknowledgments

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Table 1

Characteristics of the study sample according to case/control status (symptomatic coronary artery disease) stratified by two primary pre-defined comparisons

	Young cases (n = 373)	Young controls (n = 681)	P	Older cases (n = 1174)	Older controls (n = 902)	P
	Mean (SD)	Mean (SD)	T test	Mean (SD)	Mean (SD)	T test
Age (years)	45.8 (6.5)	43.9 (5.3)	<0.001	62.2 (8.4)	65.8 (2.9)	<0.001
	Median (range)	Median (range)	Wilcoxon	Median (range)	Median (range)	Wilcoxon
Body mass index	30.9 (17.3–61.1)	26.7 (15.8–66.2)	<0.001	28.2 (16.9–66.1)	27.3 (17.3–52.9)	<0.001
Months from coronary event to study visit	18.0 (3.3–60.8)*	–	–	3.5 (1.3–26.3)*	–	–
	Count (%)	Count (%)	χ^2	Count (%)	Count (%)	χ^2
Male	157 (42.1)	316 (46.4)	0.178	879 (74.9)	566 (62.7)	<0.001
Current/former smoker	219 (58.7)	235 (34.5)	<0.001	733 (62.4)	519 (57.5)	0.024
Hypertension	133 (35.7)	130 (19.1)	<0.001	553 (47.1)	360 (39.9)	0.001
Diabetes mellitus	90 (24.1)	33 (4.8)	<0.001	251 (21.4)	123 (13.6)	<0.001
Dyslipidemia	84 (22.5)	27 (4)	<0.001	332 (28.3)	194 (21.5)	<0.001
AMI at first presentation	230 (61.7)	–	–	781 (66.5)	–	–
Ancestry						
White/European	257 (68.9)	369 (54.2)	<0.001	959 (81.7)	690 (76.5)	0.004
Black/African–American	47 (12.6)	254 (37.3)	<0.001	54 (4.6)	83 (9.2)	<0.001
Hispanic	25 (6.7)	22 (3.2)	0.009	87 (7.4)	61 (6.8)	0.570
East Asian	44 (11.8)	36 (5.3)	<0.001	74 (6.3)	68 (7.5)	0.269

* P < 0.001 for Wilcoxon test. Younger cases may have had qualifying coronary events as early as 1 January 1999, while all older cases had qualifying coronary events at anytime after the start of recruitment in October 2001

Table 2

Summary of *ALOX5* and *ALOX5AP* polymorphisms genotyped in the study sample (ordered 5′–3′)

Gene/SNP public name	Major → minor allele[a]	Details	MAF (%)			
			W	AA	H	EA
ALOX5						
rsl2762303	T → C	Promoter	15	16	13	17
rs2228064	G → A	Thr → Thr in exon 2	1	26	2	9
rs41526545	A → G	Intronic	16	4	8	0.5
rs2029253	A → G	Intronic	38	26	53	61
rs28395866	T → C	Intronic	4	25	3	1
rs2228065	G → A	Glu → Lys in exon 6	0	10	1	5
rs2229136	A → G	3′ UTR	6	15	5	4
ALOX5AP						
rs4769055	A → C	Intronic	69	27	46	48
rs10507391	A → T	Intronic	67	23	47	64
rs3803277	C → A	Intronic	44	42	52	64
rs3803278	T → C	Intronic	23	13	29	44
rs12721458	G → C	Intronic	31	6	20	23
rs41323349	T → C	Exon 5 (Tyr–His)	0.1	0	0.2	0
rs1132340	A → G	3′ UTR	5	14	7	1

MAF minor allele frequency, *W* white, *AA* African–American, *H* Hispanic, *EA* East Asian

[a] Minor allele is defined as the least prevalent base for a given SNP across all race/ethnic strata in all controls combined. For rs2029253, the minor allele is more common than the major allele in Hispanics and Asians

Table 3

Linkage disequilibrium statistics and observed haplotypes between the Sp1 repeat polymorphism and rs12762303 in the promoter region of *ALOX5*, by race/ethnic group

Haplotypes		Whites (*n* = 98)			African–American (*n* = 28)			Hispanic (*n* = 6)			E. Asian (*n* = 35)		
Sp1 repeat #	Allele	Freq. (%)	r^2	D'	Freq. (%)	r^2	D'	Freq. (%)	r^2	D'	Freq. (%)	r^2	D'
3	C	0.5	0.08	1	–	–	–	–	–	–	1	0.15	1
3	T	–	–	–	27	0.45	1	–	–	–	–	–	–
4	C	41	0.95	1	32	0.88	0.92	66	1	1	33	0.69	0.69
4	T	–	–	–	2	-0.88	-0.92	–	–	–	7	-0.69	-0.69
5	C	2	-0.94	-0.96	–	–	–	–	–	–	7	-0.47	-0.63
5	T	55	0.94	0.96	37	0.58	1	33	1	1	39	0.47	0.63
6	C	–	–	–	–	–	–	–	–	–	–	–	–
6	T	1	0.09	1	–	–	–	–	–	–	10	0.27	1
7	C	–	–	–	–	–	–	–	–	–	–	–	–
7	T	–	–	–	2	0.1	1	–	–	–	3	0.14	1

Table 4

Multivariate logistic regression analysis of *ALOX5* and *ALOX5AP* variants associated with symptomatic CAD assuming a log additive mode of inheritance

SNP	Whites		Blacks		Hispanics		E. Asians		Mixed Hispanic		Mixed other		Combined		P interaction[c]
	OR[a]	P	OR[a]	P	OR[a]	P	OR[a]	P	OR[a]	P	OR[a]	P	OR[b]	P	
ALOX5															
rs12762303	1.32	0.002*	0.98	0.94	1.46	0.31	0.52	0.02	1.29	0.49	1.22	0.44	1.19	0.02	0.06
rs2228064	(–)	(–)	1.24	0.28	0.24	0.09	0.99	0.98	(–)	(–)	0.53	0.04	0.89	0.42	0.001*
rs41526545	1.1	0.33	0.92	0.87	0.68	0.37	(–)	(–)	1.27	0.59	1.56	0.15	1.10	0.22	0.53
rs2029253	0.91	0.18	0.90	0.64	1.35	0.24	1.84	0.003*	1.04	0.87	1.31	0.17	1.03	0.61	0.003*
rs28395866	1.27	0.17	1.21	0.33	1.04	0.95	(–)	(–)	(–)	(–)	1.03	0.93	1.21	0.10	0.45
rs2228065	(–)	(–)	0.79	0.49	(–)	(–)	2.62	0.07	(–)	(–)	1.36	0.57	1.17	0.49	0.13
rs2229136	1.12	0.40	0.89	0.67	1.32	0.59	0.74	0.53	3.5	0.08	0.75	0.37	1.03	0.76	0.43
ALOX5AP															
rs4769055	0.96	0.51	1.34	0.15	1.18	0.49	1.00	0.98	0.99	0.98	0.81	0.29	0.98	0.70	0.10
rs10507391	0.98	0.73	1.17	0.48	1.28	0.31	0.94	0.77	1.03	0.92	0.94	0.75	0.99	0.84	0.29
rs3803277	1.05	0.41	1.13	0.51	0.96	0.85	0.96	0.84	1.38	0.25	0.75	0.11	1.02	0.69	0.36
rs3803278	1.05	0.51	1.32	0.30	0.80	0.40	1.07	0.76	1.26	0.45	0.83	0.39	1.04	0.50	0.86
rs12721458	1.00	0.97	0.39	0.03	0.73	0.26	1.08	0.74	0.56	0.07	1.14	0.57	0.97	0.56	0.008*
rs1132340	0.83	0.23	1.00	0.99	1.48	0.43	0.66	0.64	0.99	0.99	1.03	0.92	0.92	0.48	0.74

OR odds ratio

*
Below Bonferroni adjusted threshold of significance for the number of SNPs tested in the same gene and race/ethnic group assuming an overall Type 1 error of 0.05

[a] Adjusted for case/control set, age, sex, BMI, smoking status, hypertension, diabetes, high cholesterol. "Admixed" strata further adjusted for individual proportion of white, black, hispanic, and east asian ancestry derived by the program STRUCTURE

[b] Further adjusted for race/ethnic group and the interaction term for study*race/ethnic group was entered into the model

[c] Test of heterogeneity of odds ratios across race/ethnic groups (P value of race/ethnic group*SNP interaction in combined analysis)

Table 5

Allele frequencies and hazard rate ratios for incident CAD for minor alleles of rs12762303 in the ARIC cohort assuming a log additive mode of inheritance

	Genotype	Cases Counts (%)	Non-cases Counts (%)	Fully adjusted model[a] Hazard ratio	P
Whites	TT	795 (69)	6,110 (71)		
	CT	319 (28)	2,325 (27)	1.08	0.2
	CC	40 (3)	211 (2)		
	Total	1154	8,646		
African American	TT	201 (65)	2,080 (68)		
	CT	98 (32)	872 (29)	1.07	0.5
	CC	10 (3)	91 (3)		
	Total	309	3043		
Overall				1.08	0.1

[a] Adjusted for age, gender, center, HDL and total cholesterol, BMI, smoking, diabetes and hypertension status (and race in non-stratified analyses)