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A near null variant of 12/15-LOX encoded by a novel SNP in *ALOX15* and the risk of coronary artery disease:

Assimes T560M in *ALOX15* and the risk of CAD

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Abstract

Objective—Murine genetic models suggest that function of the 12/15 LOX enzyme promotes atherosclerosis. We tested the hypothesis that exonic and/or promoter single nucleotide polymorphisms (SNPs) in the human 12/15-LOX gene (*ALOX15*) alter the risk of symptomatic coronary artery disease (CAD).

Methods & Results—We resequenced *ALOX15* and then genotyped a common promoter and a less common novel coding SNP (T560M) in 1809 subjects with CAD and 1734 controls from Kaiser Permanente including a subset of participants of the Coronary Artery Risk Development in Young Adults study. We found no association between the promoter SNP and the risk of CAD. However, heterozygote carriers of the 560M allele had an increased risk of CAD (adjusted OR, 1.62; P=0.02) compared to non-carriers. *In vitro* studies demonstrated a 20-fold reduction in the catalytic activity of 560M when compared to 560T. We then genotyped T560M in 12974 participants of the Atherosclerosis Risk in Communities study and similarly found that heterozygote carriers had an increased risk of CAD compared to non-carriers (adjusted HR, 1.31; P=0.06). In both population studies, homozygote carriers were rare and associated with a non-significant decreased risk of CAD compared to non-carriers (adjusted OR, 0.55; P=0.63 and HR, 0.93; P=0.9).

Conclusions—A coding SNP in *ALOX15* (T560M) results in a near null variant of human 12/15-LOX. Assuming a co-dominant mode of inheritance, this variant does not protect against CAD.

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Assuming a recessive mode of inheritance, the effect of this mutation remains unclear, but is unlikely to provide a protective effect to the degree suggested by mouse knockout studies.

Keywords

Arachidonate 15-Lipoxygenase; polymorphism – single nucleotide; coronary disease

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The 12/15 lipoxygenases (12/15 LOXs) are members of a diverse family of lipid peroxidizing enzymes, which catalyze the stereospecific oxygenation of polyunsaturated fatty acids even if these substrates are incorporated in biomembranes or lipoproteins[1, 2]. The human 12/15-LOX is encoded for by a single gene, *ALOX15*, localized in the LOX gene cluster on chromosome 17 and oxygenates arachidonic acid to 15-S-hydroperoxyeicosatetraenoic acid (15-HpETE)[3]. Although 12/15-LOXs have been previously implicated in the pathogenesis of atherosclerosis, their precise role in this context remains controversial as both *in vitro* and *in vivo* experiments have yielded conflicting results. *In vitro*, 12/15-LOXs may facilitate the oxidation of LDL to a more atherogenic form[4]. In atherosclerotic lesions from rabbits, both the mRNA and the protein have been shown to co-localize with macrophage rich regions and epitopes of oxidized LDL. However, more recent gene expression studies of human atherosclerotic lesions at different stages suggest minimal expression of 12/15-LOX[5]. I*n vivo*, both 12/15-LOX/apoE and the 12/15-LOX/LDL double knockout mice develop significantly less atherosclerosis than the single apoE and LDL knockouts respectively[6]. Furthermore, over expression of a human *ALOX15* transgene in murine endothelium increased the formation of atherosclerotic lesions in LDL-receptor deficient mice[7]. In contrast, transgenic rabbits over-expressing the human 12/15-LOX in monocyte/macrophages or systemically are protected from development of atherosclerosis[8, 9, 10].

To better define the role of 12/15-LOX in human atherosclerosis, we sought to test the hypothesis that common polymorphisms in the human 12/15-LOX gene (*ALOX15*) alter the risk of symptomatic CAD.

Material and Methods

Study Design

The ADVANCE study (*A*therosclerotic *D*isease, *VA*scular FuNction, and Genetic *E*pidemiology) was approved by the Institutional Review Board at both Stanford University and Kaiser Permanente of Northern California (KPNC).

Between October 28, 2001 and December 31, 2003, we recruited a total of 3179 subjects into 5 cohorts: a cohort of subjects with clinically significant CAD at a young age (<45 years for males, < 55 years for females), a cohort of subjects with incident stable angina at an older age, a cohort of subjects with incident acute myocardial infarction (AMI) at an older age, a cohort of young subjects with no history of CAD, and a cohort of subjects aged 60 to 72 with no history of CAD, ischemic stroke, or peripheral arterial disease (PAD). Eligible subjects were identified using the KPNC electronic databases and those who agreed to participate were interviewed and examined at one of several clinics in the San Francisco Bay Area. A sixth cohort of young subjects with no history of CAD included 479 participants in the Coronary Artery Risk Development in Young Adults (CARDIA) Study[11] originally recruited through KPNC and attending the study's year 15 exam in 2000-2001. A detailed description of the source population for all cohorts has been published elsewhere[12-14].

The design of the ADVANCE study allowed for several case control comparisons. In this study, we compared subjects with symptomatic 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, a 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, ischemic stroke, and PAD, as well as 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 the height and weight of all participants and collected blood for DNA extraction.

Traditional risk factors (smoking, hypertension, high cholesterol, and diabetes) were defined based on self report and were considered to be present only if subjects reported an age of onset of a risk factor that was younger than the age of onset of clinically significant CAD.

Resequencing and Genotyping

Using an automated fluorescent labeling system[15], we resequenced the promoter region, the exons, and the intron-exon boundaries of *ALOX15* in 24 ethnically diverse males with a history of CAD (SNP discovery set). We then selected a subset of sequenced SNPs to genotype in all participants of the ADVANCE study using the TaqMan® assay. All resequencing and genotyping was performed by the Stanford Human Genome Center.

Statistical Analysis

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

We compared the distributions and frequencies of all non-genetic covariates of interest in cases and controls using standard parametric and non-parametric methods. For each race/ethnic group, we calculated the minor allele frequency (MAF) and bootstrap derived 95% confidence intervals of all SNPs in both sets of controls combined and tested for Hardy-Weinberg equilibrium (HWE) with the permutation version of the exact test[16].

Using Cochran-Mantel-Haenszel (CMH) methods, we derived estimates of the Odds Ratio (OR) and a general association statistic[17, 18] for each case/control comparison stratified by race/ethnic group. We compared the heterozygote carriers of the minor allele to non-carriers and homozygote carriers to non-carriers separately. The effect of other covariates of interest was evaluated through a multivariate unconditional logistic regression. To minimize the probability of confounding due to population substructure in our 'Admixed Hispanic' and 'Admixed Non-Hispanic' race/ethnic groups, we estimated the proportion of our 4 'parent' ancestries (white/European, black/African-America, Hispanic, East Asian) at the individual level[19] for all cases and controls in these groups and used these estimates as covariates in our adjusted analyses.

For each SNP genotyped, we estimated the minimal OR we could detect with a power of 80% given the minor allele frequency observed in controls[20]. For these calculations, we assumed the polymorphism was causal, a population prevalence of symptomatic CAD of 6.2%[21], and a Type 1 error of 0.05.

Transcription Factor Binding studies

We examined the possibility that the minor allele of a SNP potentially associated with CAD lead to either the creation or destruction of a transcription binding site using MatInspector[22]

Mutagenesis studies

For bacterial expression wild-type and mutant 12/15-LOX cDNAs were cloned into the pQE-9 expression plasmid (Qiagen, Hilden, Germany). The enzyme species were expressed as N-terminal his-tag fusion proteins and purified to near homogeneity by Ni-agarose affinity chromatography[32]. For transient eukaryotic expression the coding region of the 12/15-LOX cDNA was cloned into the pcDNA3.1 expression plasmid (Invitrogen, Hilden, Germany) and HEK293 cells were lipofected with this construct. Site-directed mutagenesis was performed using the QuickChange™ Site-Directed Mutagenesis Kit (Stratagene, Amsterdam, The Netherlands). For each mutant, 5-10 clones were selected, screened for 12/15-LOX expression by immunoblotting and activity assays and were finally sequenced to confirm the T560M exchange and the absence of any other change

Arachidonic acid oxygenase activity of wild-type and mutant 12/15-LOX species was assayed by High Pressure Liquid Chromatography (HPLC) quantification of the oxygenation products after incubating the purified enzyme preparations or the transfected cells for 15 min with exogenous arachidonic acid[32]. For immunoblotting, transfected cells were lysed in the presence of 5 mM EDTA and aliquots of the lysis supernatants or of purified enzyme preparations were applied to SDS-PAGE. The blots were probed with a polyclonal rabbit antibody raised in guinea pigs against the pure rabbit 12/15-LOX.

Replication of human genetic associations in the ADVANCE study

We planned to replicate suggestive associations between one or more SNPs in *ALOX15* and CAD by genotyping the same SNPs in the Atherosclerosis Risk in Communities (ARIC) cohort and assessing their effect on incident CAD. The ARIC Study is an ongoing prospective investigation of atherosclerosis and its clinical sequelae involving 15,792 white and black persons aged 45–64 years at recruitment (1987–1989). Study IRBs approved the ARIC Study and all participants provided written informed consent. A detailed description of the ARIC study design, sampling procedures, methods, definitions of cardiovascular outcomes, and approach to statistical analyses is published elsewhere[23 24, 25]. Incident CAD in ARIC is defined as documented AMI, coronary artery bypass surgery, unstable angina, or coronary-related death. Follow-up was available until December 31, 2002. Time-to-event was analyzed using Cox proportional hazards modeling. We adopted the methods of Hsieh and Lavori[26] to calculate post-hoc the minimal detectable hazard ratio.

For SNPs genotyped in both the ADVANCE and ARIC cohorts, we estimated the OR and 95% confidence interval across both population studies using CMH methods for both the comparison of heterozygotes carriers to non carriers and the comparison of homozygote carriers to non carriers. These OR were adjusted for study and race/ethnic group and were calculated only if there was no significant difference between the minimally and fully adjusted risk ratios derived by regression analyses in each of the two population studies.

Results

Non-Genetic Characteristics of the ADVANCE study sample

The ADVANCE study sample consisted of 3546 subjects, whose clinical and other non-genetic characteristics are summarized in Table 1. The older age of controls in the set of older onset cases and controls was a consequence of our stratified sampling design. Stratified sampling

and/or preferential participation also led to differences in the prevalence of certain race/ethnic groups by case/control status and to the low prevalence of young cases that were male.

Resequencing and Genotyping

We identified 27 polymorphisms by resequencing *ALOX15* in the SNP discovery set. The location and minor allele frequencies of these SNPs have been submitted to dbSNP. Based on their presence in putative functional domains, evolutionary conservation, or being a non-synonymous substitution, we selected a previously identified SNP in the promoter region and a novel SNP in exon 13 to genotype in all participants (Table 2).

Association Analyses in ADVANCE

Both SNPs were in Hardy-Weinberg equilibrium in all race/ethnic and case-control strata (details not shown). Across all race/ethnic groups, the minor allele frequency of the coding SNP (ALOX15.18) was considerably lower than the promoter SNP with the highest frequency of ALOX15.18 in Hispanics (Table 2). ALOX15.18 was uncommon in white/Europeans, very rare in black/African Americans, and absent in East Asians. The minor allele of ALOX15.18 was also absent in all East Asian cases (details not shown).

Both the unadjusted and fully adjusted analyses for the promoter SNP revealed no evidence of an association between the minor allele and clinical CAD (details not shown). In contrast, the ORs for ALOX15.18 revealed a significantly increased risk of clinical CAD in heterozygote carriers compared to non-carriers (CMH derived OR, 1.66; P=0.008 and fully adjusted OR, 1.62; P=0.02) (Table 3). This elevated risk in heterozygotes remains significant even after considering a conservative Bonferroni adjusted threshold[27] of 0.025 to account for the number of SNPs tested. For homozygote carriers compared to non-carriers, there was a non significant decreased risk of CAD (CMH derived OR, 0.37; P=0.40 and fully adjusted OR, 0.55; P=0.63).

The minimal detectable ORs for the promoter SNP was 1.2 (or 0.83 assuming a low risk minor allele) for the log additive co-dominant mode of inheritance and 1.8 (0.25) for the recessive mode of inheritance. For ALOX15.18 the minimal detectable OR was 1.7 (or 0.53 assuming a low risk minor allele) and 46.0 (0.00), respectively.

Transcription Factor Binding studies

The minor allele of ALOX15.18 results in a non-synonymous amino acid exchange from threonine at position 560 to methionine (T560M). MatInspector analysis of the DNA sequence in the region of the polymorphism did not find a concensus binding site for known transcriptional regulators with either the major or the minor allele base at the ALOX15.18 polymorphic site.

Mutagenesis studies

Expression of the wild type (wt) 12/15-LOX in E. coli resulted in large amounts of enzyme protein. In contrast, expression of the 560M mutant was strongly reduced (Fig. 1, inset A). To directly compare the specific catalytic activities, the two enzyme species were purified by affinity chromatography and equal amounts of 12/15-LOX protein were subjected to activity assays, in which the 560M mutant exhibited a residual catalytic activity of about 5% compared to the wt (Fig 1, Table 4). To exclude possible artefacts that might be related to the prokaryotic expression system we transiently transfected HEK792 (human kidney carcinoma) cells with mammalian expression plasmids containing either the wt or the mutant 12/15-LO cDNA. Transfection efficiency was normalized to a control plasmid (luciferase) and 12/15-LOX protein expression was quantified by immunoblotting. In this system we did not find major

differences in the level of protein expression. Again the 560M mutant exhibited a residual catalytic activity of about 7% when compared with the wt enzyme (Table 4).

We repeated the HPLC quantification of oxygenation products using an internal loading control an obtained essentially identical results (details not shown).

Association Analysis in the ARIC study

The frequency of ALOX15.18 (T560M) in the ARIC study controls was similar to that of the ADVANCE controls in both whites (1.8%, 95% CI 1.6%-2.0%) and blacks (0.3%, 95% CI 0.16%-0.44%). The minor allele was in HWE in both race/ethnic strata (details not shown).

Table 5 summarizes the association analyses from the ARIC study. Compared to non-carriers, unadjusted analyses revealed that neither heterozygote nor homozygote carriers were at increased risk of incident CAD. However, both unadjusted estimates of the relative risk were greater than one (RR 1.23, P=0.18 and RR 1.3, P=0.81). Cox proportional hazards ratios adjusting for all traditional risk factors demonstrated a trend towards an increased risk in heterozygote carriers (HR 1.31, P=0.06) and a non significant slight decrease risk in homozygote carriers (HR 0.93, P=0.9). The minimal detectable hazard rate ratio was 1.6 (0.63 assuming a low risk minor allele) for the log additive co-dominant mode of inheritance and 27 (0.04) for the recessive mode of inheritance.

The CMH derived OR across both population studies for the effect of the heterozygotes carriers to non-carriers was 1.4 with 95% CI of 1.1 to 1.8 (p = 0.004). For the comparison of homozygous carriers to non carriers, the OR was 0.8 with 95% CI of 0.2 to 3.9 (p = 0.7).

Discussion

We investigated the effect of specific targeted genetic variation in *ALOX15* on the risk of CAD. We found no association between a previously identified SNP in the promoter region and clinical CAD. We also identified a novel SNP in exon 13, ALOX15.18, which produces a near null variant of 12/15-LOX (T560M). In the race/ethnic groups we studied, the minor allele of ALOX15.18 was most common in Hispanics followed by white/Europeans. The minor allele was exceedingly rare in black/African Americans and absent in East Asians. Two independent cohort studies reveal interesting trends on the risk of CAD in carriers of the ALOX15.18 minor allele that warrant further investigation.

The mutagenesis data indicate an important role of Thr560 for the catalytic activity of the 12/15-LOX. In the 3D-structure of the rabbit enzyme, the corresponding amino acid is located in proximity to residues that are critical for catalysis[28, 29] Using the DNA-Strider software package, multiple sequence alignments of mammalian LOX isoforms reveal a high level of conservation of this residue (details not shown). The human and murine 12R-LOXs as well as the human epidermis-type 15-LOX (15-LOX2) have a Ser at this position. However, both amino acids (Thr and Ser) contain a hydroxyl group in their side chains suggesting that such OH-groups may play a role in the catalytic activity. In fact, when we mutated Thr560 to a Ser in the human 12/15-LOX we obtained an active enzyme species (data not shown). Modeling studies suggest that this amino acid may not directly interact with fatty acid substrates but appears to contact the primary determinants for the positional specificity (Phe353, Ile418, Ile593) located at the bottom of the substrate binding pocket[29]. Mutation of these residues alters the catalytic activity of the enzyme and impacts reaction specificity[29]. A larger Met residue at this position may lead to conformational changes within this critical region impairing catalytic activity.

Studies in mice show that loss of both functional *ALOX15* alleles significantly decreases the formation of aortic atherosclerosis[30, 31]. While an intermediate protective effect of single functional allele loss has not been definitively established it cannot be ruled out[30]. In the apo $E^{-/-}$/L-12$LO^{-/-}$ double-knockout mice fed normal chow, a marked 98.7% reduction in the lesion area of aortic atherosclerosis was witnessed compared with the apo $E^{-/-}$/L-12$LO^{+/+}$ mice at 15 weeks[30]. Reduction of aortic atherosclerosis was also observed in the LDL receptor knockout model, LDL-$R^{-/-}$/L-12$LO^{-/-}$ mice compared with LDL-$R^{-/-}$/L-12$LO^{+/+}$ mice showed approximately 94% reduction in the percent surface coverage of the aorta at 9 weeks and 50% reduction at 12 and 18 weeks[31]. In our genetic association studies in humans, our estimated lower 95% confidence interval for the OR between heterozygote carriers of 560M and non carriers across both population studies argues strongly against any protective effect of a near null variant of this enzyme when assuming a co-dominant mode of inheritance. In fact, assuming this mode of inheritance, 560M may increase the risk of CAD, a situation more consistent with experimental findings in transgenic rabbits [8 , 9, 10]. The lack of an elevated risk in our very small number of homozygote carriers is inconsistent with this possibility, although the low allele frequencies of 560M found in all race/ethnic groups studied limited our ability to examine the risks associated with homozygosity of the 560M allele in a meaningful way. Importantly, our estimated lower 95% confidence interval of 0.2 across both population studies argues against the presence of a protective effect to the degree suggested by the authors of the two mouse knockout studies to date[30, 31].

In conclusion, we identified a SNP in *ALOX15* that considerably impairs the enzymatic activity of 12/15-LOX, leading to a near null variant of this enzyme. Among the race/ethnic groups we studied, the frequency of the 560M allele was highest in Hispanic subjects. Assuming a co-dominant mode of inheritance, this variant does not protect against clinically significant CAD and may actually increase risk. Assuming a recessive mode of inheritance, the effect of this mutation remains unclear, but a protective effect to the degree noted in mouse knockouts appears unlikely. Additional genetic studies are required before firm conclusions can be made on whether this mutation alters the risk of CAD.

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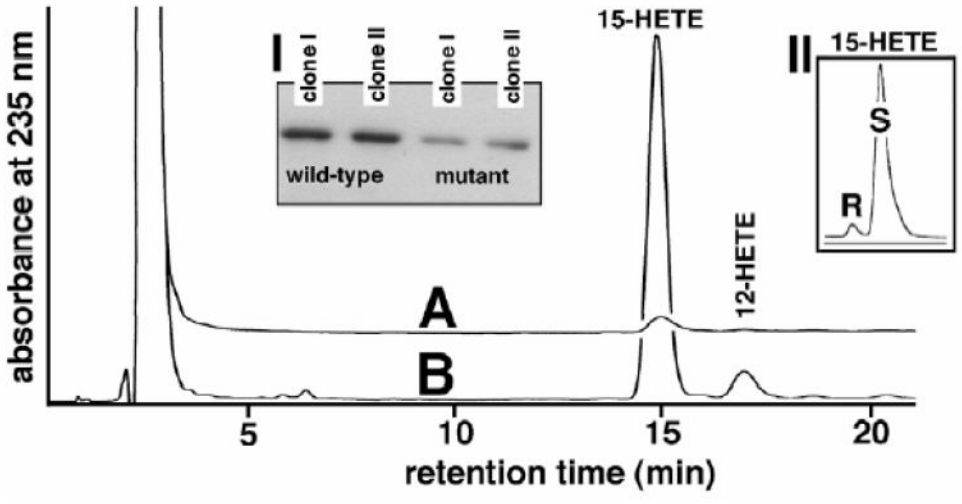

Fig. 1. Expression of wt-human 12/15-LOX and its T560 M mutant in *E. coli*

The two enzyme species were expressed in *E. coli*, purified on a Ni-agarose column and used in activity assays and immunoblotting (see Material and methods). A: T560M mutant, B: wild-type enzyme, Inset I: Comparison of the absolute expression levels of the two enzyme species (immunoblotting of lysis supernatant). Inset II: Enantiomer composition 15-HETE formed by the mutant enzyme.

Table 1

Non-genetic characteristics of the ADVANCE study according to case/control status (symptomatic coronary artery disease) stratified by two primary predefined comparisons

	Young Cases (n=472)	Young Controls (n=742)	*P*	Older Cases (n=1337)	Older Controls (n=992)	*P*
	Mean(SD)	**Mean(SD)**	**T test**	**Mean(SD)**	**Mean(SD)**	**T test**
Age(years) at first ever event for cases, study visit date for controls*	45.3(6.5)	44.3(5.5)	0.005	62(8.4)	65.8(2.9)	<0.001
	Median(Range)	Median(Range)	Wilcoxon	Median(Range)	Median(Range)	Wilcoxon
Body Mass Index	31.1(17.3-61.2)	26.8(15.8-66.2)	<0.001	28.4(16.9-66.1)	27.5(17.3-52.9)	<0.001
C-Reactive Protein	2.3(0.1-76.4)	1.2(0.1-76.8)	<0.001	1.7(0.1-77.4)	1.6(0.1-73.9)	0.163
Months from first ever event to study visit	21.3(2.7-222.1)†	--	--	3.5(1.4-26.4)†	--	--
	Count(%)	Count(%)	Chi2	Count(%)	Count(%)	Chi2
Risk Factors (Self Report)						
Male	184(39)	328(44.2)	0.072	975(72.9)	618(62.3)	<0.001
Current/ Former smoker	276(58.5)	266(35.8)	<0.001	842(63)	569(57.4)	0.006
Hypertension	154(32.6)	143(19.3)	<0.001	641(47.9)	407(41)	<0.001
Diabetes Mellitus	101(21.4)	51(6.9)	<0.001	274(20.5)	147(14.8)	<0.001
High Cholesterol	129(27.3)	148(19.9)	0.003	604(45.2)	356(35.9)	<0.001
Ancestry			<0.001			<0.001
White/European	254(53.8)	369(49.7)		947(70.8)	677(68.2)	
Black/ African American	46(9.7)	254(34.2)		50(3.7)	79(8)	
Hispanic	25(5.3)	22(3)		83(6.2)	60(6)	
East Asian	44(9.3)	35(4.7)		73(5.5)	68(6.9)	
Admixed Hispanic	32(6.8)	23(3.1)		52(3.9)	31(3.1)	
Admixed Non-Hispanics	71(15)	39(5.3)		132(9.9)	77(7.8)	

*The age cutoff for a "young case" was not the same for males (<45 years) and females (<55 years).

†P < 0.001 for Wilcoxon test. Young cases may have had their qualifying coronary event as early as Jan 1, 1999, while all older cases had their qualifying coronary event after the start of recruitment in October, 2001.

Table 2

Summary of *ALOX15* single nucleotide polymorphisms genotyped in the ADVANCE study sample (ordered 5′ to 3′)

SNP Alias	Public Name	Details	Major→ Minor Allele*	Minor Allele Frequency (95% Confidence intervals)					
				White/ European	Black/African American	Hispanic	East Asian	Admixed Hispanic	Admixed Non-Hispanics
ALOX15.7	rs7220870	261bp 5′ transc start	T→G	22.8 (21.2-24.8)	13.6(11-16.2)	23.2 (16.5-29.3)	16.3 (11.4-21.6)	22.6 (14.2-31.1)	20.9(15.7-26.1)
ALOX15.18	rs34210653	Thr→Met in exon 13	T→C	1.2(0.7-1.7)	0.5(0-1.1)	8(4.3-12.7)	0(0-0)	3.7(0.9-7.4)	1.3(0-3)

bp = base pairs, transc = transcription

*Minor allele is defined as the least prevalent base for a given SNP across both sets of controls in all race/ethnic strata combined.

Table 3

Genotypes counts and Odds Ratios for the ALOX15.18 SNP in the ADVANCE study, stratified by case/control set and race/ethnic group

		Set of Young Onset Cases & Controls						Set of Older Onset Cases & Controls						Both Sets of Cases and Controls					
Race/Ethnic group	Genotype	Cases	Controls	CMH OR*	*P*	Fully Adjusted OR‡	*P*	Cases	Controls	CMH OR*	*P*	Fully Adjusted OR‡	*P*	Cases	Controls	CMH OR†	*P*	Fully Adjusted OR‡	*P*
White	CC	240	357	ref.		ref.		905	655	ref.		ref.		1145	1012	ref.		ref.	
	CT	11	5	**3.27**	**0.022**	**3.74**	**0.031**	38	18	1.53	0.142	1.58	0.137	49	23	**1.81**	**0.018**	**1.96**	**0.014**
	TT	0	0	--	--	--	--	1	1	0.72	0.819	1.00	0.999	1	1	0.72	0.819	1.00	0.997
Black/African American	CC	46	249	ref.		ref.		48	77	ref.		ref.		94	326	ref.		ref.	
	CT	0	3	--	0.458	--	0.987	2	0	--	0.078	--	0.988	2	3	2.62	0.420	0.53	0.655
	TT	0	0	--	--	--	--	0	0	--	--	--	--	0	0	--	--	--	--
Hispanic	CC	19	18	ref.		ref.		66	51	ref.		ref.		85	69	ref.		ref.	
	CT	6	4	1.42	0.630	1.41	0.688	17	7	1.88	0.192	2.25	0.174	23	11	1.73	0.175	1.83	0.201
	TT	0	0	--	--	--	--	0	1	--	0.260	--	0.991	0	1	--	0.260	--	0.991
Admixed Hispanic	CC	30	23	ref.		ref.		48	27	ref.		ref.		78	50	ref.		ref.	
	CT	2	0	--	0.226	--	0.982	4	4	0.56	0.439	0.66	0.269	6	4	0.92	0.906	0.83	0.814
	TT	0	0	--	--	--	--	0	0	--	--	--	--	0	0	--	--	--	--
Admixed Non-Hispanic	CC	69	38	ref.		ref.		128	75	ref.		ref.		197	113	ref.		ref.	
	CT	2	1	1.10	0.938	0.56	0.733	4	2	1.17	0.857	0.67	0.692	6	3	1.15	0.848	0.63	0.562
	TT	0	0	--	--	--	--	0	0	--	--	--	--	0	0	--	--	--	--
All	CC	404	685	ref.		ref.		1195	885	ref.		ref.		1599	1570	ref.		ref.	
	CT	21	13	2.19	0.040	1.84	0.150	65	31	1.51	0.064	1.49	0.098	86	44	**1.66**	**0.008**	**1.62**	**0.020**
	TT	0	0	--	--	--	--	1	2	0.37	0.401	0.53	0.612	1	2	0.37	0.401	0.55	0.633

CMH = Cochran-Mantel-Haenszel, OR = Odds Ratio, ref. = reference group, -- = unable to compute, OR with p values < 0.05 in bold

*Combined analyses adjusted for Race/ethnic group.

†Combined analyses adjusted for race/ethnic group, case-control set, or both.

*,† For all combined analyses, Breslow-Day test of homogeneity of ORs across strata was not significant.

‡Adjusted for 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. Non stratified analyses further adjusted for race/ethnic group, case/control set, or both.

Table 4

Relative expression levels and specific activities* of 12/15-LOX species expressed in pro- and eukaryotic expression systems

Expression system	rel. expression level		rel. specific activity	
	wt enzyme	T560M	wt enzyme	T560M
E. coli	100	31	100	5
HEK 297 cells	100	91	100	7

* The expression level and specific activities observed with the wild-type enzyme in the different expression systems was set 100. The expression levels were quantified by immunoblotting and the specific activities by activity assays (see Material and methods)

Table 5

Genotypes counts and Risk Ratios for the ALOX15.18 SNP in 1407 incident CHD cases and 11567 non-cases from the ARIC study, stratified by race

Race	Genotype	CHD cases	Non-cases	RR*	*P*	Fully Adjusted HR†	*P*
White	CC	1057	8267	ref.	--		
	CT	47	304	1.21	0.23	1.29	0.09
	TT	1	6	1.30	0.81	0.90	0.90
Black	CC	299	2972	ref.	--		
	CT	3	18	1.66	0.42	1.83	0.30
	TT	0	0	--	--	--	--
All	CC	1356	11239	ref.	--		
	CT	50	322	1.23	0.18	1.31	0.06
	TT	1	6	1.30	0.81	0.93	0.90

CHD = Coronary Heart Disease, CMH = Cochran-Mantel-Haenszel, RR = Relative Risk, ref. = reference group, -- = unable to compute due to cells with zero counts

*Adjusted for race in non stratified analyses.

†Adjusted for age, gender, center, smoking, diabetes and hypertension status (and race in non-stratified analyses)