ALOX5AP gene variants show differential association with coronary artery disease in different populations

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Abstract Coronary artery disease (CAD) is a complex disease with various components, genetic as well as environmental. Previous reports correlating *ALOX5AP* gene variants and CAD showed conflicting results depending on the population studied. In this study, we examined the contribution of *ALOX5AP* genetic predisposition to CAD in a group of CAD patients and controls carefully selected from the Lebanese population. We genotyped SNPs for *ALOX5AP* variants in 289 catheterized patients aged

≤52 years with >50% stenosis in at least one main coronary artery and 227 catheterized control subjects aged 60 years and above with 0% stenosis. Chi-square (χ^2) tests and logistic regression showed no significant difference in the allele and genotype frequencies between the CAD or myocardial infarction (MI) cases and the healthy controls. Haplotype analysis using PHASE showed that the distribution of the risk haplotypes among cases and controls were not significantly different and had no attributable risk to CAD ($P=1.00$ and $P=0.5$, respectively) or MI ($P=0.2$ and $P=0.5$, respectively). Our data revealed that *ALOX5AP* gene variants are not predictors of CAD risk or MI risk among Lebanese patients.

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Introduction

Coronary artery disease (CAD) is a condition characterized by a defect in blood supply to the heart. It includes myocardial infarction (MI) and remains the main cause of fatality in many countries (Mackay et al. 2004). Several clinical and environmental factors have been well established as risk factors for CAD. Clinical risk factors include diabetes mellitus, hypercholesterolemia, and hypertension, whereas environmental risk factors comprise smoking, high-fat diet, and obesity (Mackay et al. 2004; Assmann et al. 1999).

Genome-wide association and linkage studies have identified a large number of CAD susceptibility genes, most of which encode factors involved in the inflammatory pathways associated with CAD (Lusis et al. 2004; Robin et al. 2007; Abchee et al. 2006; Yusuf et al. 2004). These include the lymphotoxin- α (*LTA*), galectin-2 (*LGALS2*),

proteasome subunit α -type 6 gene (*PSMA6*), and leukotriene A4 hydrolase (*LTA4H*) (Ozaki et al. 2002, 2004, 2006; Helgadottir et al. 2004, 2006). The *ALOX5AP* gene encodes the 5-lipoxygenase-activating protein (FLAP) and was found to be significantly associated with CAD and MI (Helgadottir et al. 2004, 2006).

In a study involving 713 MI cases, Helgadottir et al. (2004) identified a haplotype A (HapA), consisting of four SNPs within the *ALOX5AP* gene region, that was associated with twofold increased risk of MI. In the same study, they identified another *ALOX5AP* gene haplotype B (HapB) to be significantly associated with MI in a British population ($P < 0.001$). A replication study on an Italian population revealed an association between HapB and increased risk for CAD (Odds ratio (OR)=1.67; $P = 0.032$). Haplotype analyses in this population additionally revealed significant association between CAD and an *ALOX5AP* haplotype named HapC (OR=2.41; $P = 0.03$), present within the same region than that of HapB. HapC was not considered in previous studies; the frequency of HapC population was low (Girelli et al. 2007). Finally, in a recent study, HapB was found to increase the risk for MI in a German population ($P < 0.0001$) (Linsel-Nitschke et al. 2008).

Other studies, however, showed no correlation between *ALOX5AP* gene variants and CAD. Two US-based studies revealed lack of association of HapB with acute coronary syndrome (OR=1.12; $P = 0.31$) or MI ($P = 0.08$) (Zee et al. 2006; Morgan et al. 2007). A study in a Japanese cohort provided no evidence for an association of *ALOX5AP* gene variants and MI. (Kajimoto et al. 2005) Similar results were seen in a North American cohort indicating a lack of association of HapB with stroke (Lohmussaar et al. 2005; Meschia et al. 2005).

This population-specific association of *ALOX5AP* variants and CAD initiated an investigation of the correlation of these variants with CAD and MI patients in the Lebanese population. Results from this study may help in early identification of subjects at high risk, thus providing greater opportunity for therapeutic applications and prophylaxis in the population.

Materials and methods

Subjects

From a total of 5,000 individuals recruited between 2007 and 2009, 516 Lebanese subjects were selected. These subjects were referred to the cardiovascular care units to undergo cardiac catheterization due to clinical indications and not for the purposes of the study itself at either the American University of Beirut Medical Center or the Rafic Hariri University Hospital in Beirut. Angiography was

performed to visualize the left main coronary artery, the left anterior descending artery, the left circumflex artery, and the right coronary artery. All coronary arteries were imaged from different angles. The extent of coronary artery stenosis and the number of diseased arteries were documented for each case. Angiogram results were utilized as the main outcome variable.

Trained research assistants discussed informed consent with patients and obtained permission prior to the angiographic procedure. Data concerning life style factors, demographic variables, medical history, family history, therapeutic applications, and clinical information about subjects were documented. The Institutional Review Board at the Lebanese American University approved the study protocol.

Cases and controls selection

Under the assumption that young patients with CAD will likely carry a lower contribution from lifestyle and environmental factors and reflect a higher genetic contribution, 289 young cases with positive family history of CAD were selected (≤ 52 years old). Of these, 288 had all the variables required for adjusted logistic regression estimates of ORs. All 289 were used in unadjusted regressions against the controls.

They have been angiographically confirmed to have a significant coronary artery narrowing ($> 50\%$ stenosis) in at least one main coronary artery. Among these cases, a subgroup of 55 patients was diagnosed with MI based on their documented medical records. For the statistical analysis, the 289 patients were grouped according to the number of diseased vessels into single vessel disease (SVD) patients and multiple vessel disease (MVD) patients. Only the 288 fully annotated samples were used in MVD vs. SVD analysis.

The control group included subjects that have undergone angiography and were confirmed to be CAD-free (0% stenosis). This is a more stringent selection compared to other studies where controls were either population-based (not necessarily confirmed to be CAD-free) or shown by angiography to have non-significant artery stenosis ($< 50\%$) (Ozaki et al. 2002, 2004, 2006; Helgadottir et al. 2004, 2006); however, healthy subjects with minor stenosis may have sub-clinical artery narrowing and may become cases later in life with fully manifested clinical signs (Luo et al. 2007). Taking that into consideration, we have excluded the controls with non-significant coronary stenosis ($< 50\%$ stenosis) from our study. In addition, we chose control subjects with a much higher mean age than that of the cases (≥ 60 years old; the difference between the mean ages was 22 years) (Table 1), proposing that such group has a relatively low genetic load.

Table 1 Characteristics of the study subjects

	Cases (<i>n</i> =289)	Controls (<i>n</i> =227)	<i>P</i> values
Age (years)	46.7	68.65	<0.0001 ^a
Sex (male, %)	84.8	46.7	<0.0001 ^b
BMI (kg/m ²)	29.43	29.2	0.62 ^a
C-smoking (%)	58.9	22	<0.0001 ^b
Hypertension (%)	45.5	64.3	<0.0001 ^b
Diabetes (%)	24	19.4	0.213 ^b
Hyperlipidemia (%)	56.6	43.2	0.02 ^b
In hypertension, blood pressure>140/90 mm Hg. In diabetes, two fasting glucose consecutive readings>125 mg/dl. In hyperlipidemia, LDL>130 mg/dl	t-Chol (mg/dl) HDL (mg/dl) LDL (mg/dl) TG (mg/dl) FBS (mg/dl) Uric acid (mg/dl)	209 38.67 133.3 218.4 109.3 6	<0.0001 ^a <0.0001 ^a <0.0001 ^a <0.0001 ^a 0.025 ^a 0.86 ^a

^a Means compared by Student's *t* test^b Percentages compared by χ^2 test

Blood collection and DNA extraction

Venous blood specimens were collected by cardiologists performing coronary angiography; all patients underwent coronary catheterization by Judkins' technique. A blood sample of 20 ml was collected in a sterile syringe from the femoral artery catheterization site of every patient consenting to enroll in the database. EDTA-treated and plain tubes were placed on ice to be delivered to the laboratory. DNA was extracted from a 5-ml peripheral blood samples using phenol and stored at -70°C until genotyping. Concentrations of DNA samples were determined by ultraviolet spectrophotometry.

Genotyping

Using the NCBI PubMed Entrez database, we selected the four *ALOX5AP* SNPs (SG13S377, SG13S114, SG13S41, and SG13S35) from which HapB and HapC are derived.

Genotyping was carried out using TaqMan real-time PCR assay. For each reaction, 30 ng of genomic DNA was used, and the end-point PCR was carried out on the ABI primer 7900 HT (Applied Biosystems, Foster City, CA).

Statistical analysis

Statistical analyses reported in Table 1 were performed using SPSS version 16.0 for Windows (Statistical Package for the Social Science, SPSS Inc., Chicago, IL). Discrete variables were compared by the χ^2 test. Continuous variables were expressed as means, and significance of quantitative data was assessed using Student's *t* test. A *P* value of a two-tailed $P<0.05$ was considered significant and $P<0.01$ highly significant.

OR tests were used to compare the Allele frequencies between cases vs. controls. Logistic regressions were performed for all genotype data, treating homozygous and

heterozygous states as distinct categories in order to identify heterozygous advantage, if present. They also estimated ORs adjusted for the effects of gender, smoking, hyperlipidemia, and hypertension. Logistic regression tests were also used to compare the genotype frequency between patient groups with SVD and MVD. Haplotype analysis was performed using PHASE v2.1 to estimate haplotype frequencies among cases vs. controls, with OR tests performed for each of those configurations.

Tests were performed using singular value decomposition to implement linear regression using a logit linkage function with standard variance estimators. OR values with 95% confidence intervals (CIs), *P* values, and statistical test powers were calculated assuming 95% two-tailed CIs to estimate the association between the haplotypes and the clinical outcome (CAD and MI). These computations were performed for unadjusted homozygous and heterozygous states, as well as adjusted to control for significant risk of smoking, gender, hypertension, and hyperlipidemia. ORs with 95% (CIs), *P* values, and powers were also computed based on allele frequencies for each of the CAD and MI categories. Similar analyses were performed for SVD vs. MVD; however, specific assignment of allele frequency to subjects was not performed, excluding the computation of risk factor adjustments. Results for these computations are displayed in Table 2 for CAD, Table 3 for MI, and Table 4 for SVD vs. MVD.

For multiple SNP haplotypes determined by PHASE, we computed ORs with 95% CIs, *P* values, and powers based on the two-tailed 95% CI for CAD, displayed in Table 5, and for MI, displayed in Table 6.

Results

Clinical characteristics and demographic data of the study population are shown in Table 1. Males constitute a

Table 2 Genotype and allele frequencies of *ALOX5AP* SNPs in the study subjects grouped to CAD cases and controls

<i>ALOX5AP</i> genotype (%)	CAD cases (%)	CAD cases counts (<i>n</i> =289)	Controls (%)	Controls counts (<i>n</i> =227)	OR, 95% CI	<i>P</i> value power	Adj. OR, 95% CI	<i>P</i> value power
SG13S377								
GG	73.4	212	69.6	158	0.293 0.06–1.38	0.059 0.343	1.131 0.88–1.45	0.164 0.163
GA	23.5	68	29.5	67	0.219 0.05–1.05	0.028 0.475	0.861 0.63–1.18	0.178 0.149
AA	3.1	9	0.9	2	– –	– –	– –	– –
A allele	14.9		15.7		0.940	0.401	–	–
G allele	85.1		84.3		0.63–1.41	0.044	–	–
SG13S114								
TT	20	58	17.6	40	1.151 0.68–1.94	0.298 0.076	1.153 0.62–2.12	0.324 0.066
TA	50.2	145	52	118	1.021 0.68–1.53	0.460 0.031	1.125 0.67–1.87	0.324 0.366
AA	29.8	86	30.4	69	– –	– –	– –	– –
T allele	45.1		43.6		1.063	0.367	–	–
A allele	54.9		56.4		0.75–1.50	0.053	–	–
SG13S41								
AA	74.4	215	78	177	0.326 0.09–1.19	0.045 0.397	1.484 0.20–11.0	0.350 0.058
AG	21.4	62	20.7	47	0.352 0.09–1.34	0.062 0.336	1.563 0.02–12.1	0.335 0.0063
GG	4.2	12	1.3	3	– –	– –	– –	– –
A allele	85.1		88.3		0.757	0.146	–	–
G allele	14.9		11.7		0.45–1.27	0.182	–	–
SG13S35								
GG	82.3	238	83.3	189	0.245 0.03–2.11	0.101 0.247	1.132 0.86–1.49	0.190 0.140
GA	15.9	46	16.3	37	0.250 0.03–2.24	0.107 0.235	0.887 0.59–1.34	0.283 0.083
AA	1.7	5	0.5	1	– –	– –	– –	– –
G allele	90.3		91.4		0.875	0.334	–	–
A allele	9.7		8.6		0.48–1.60	0.063	–	–

significantly higher percentage among cases. Using χ^2 test for categorical variable and student t test for continuous variables, the cases and controls groups were comparable for diabetes (OR, 1.31), BMI, and uric acid.

Cases showed significantly higher levels of total cholesterol, LDL cholesterol, triglyceride (all highly significant), and fasting blood sugar (FBS; significant). In contrast, lower levels of HDL cholesterol (highly significant) were detected, hence reflecting a role of dyslipidemia as a risk factor for CAD in our population.

Both χ^2 and logistic regression tests are constructed assuming sufficiently large samples so that asymptotic approximations hold. In Tables 1–3, for SG13S377, SG13S41, and SG13S35, the hypothesized non-pathogenic state was very rare in our populations. In these cases, error bars on the hypothesized base condition for logistic regression are large. In several such cases, the inversion of the poorly conditioned matrix resulted in the loss of one component, indicating that the weight of the logistic regression came entirely from the heterozygous state vs. the

Table 3 Genotype and allele frequencies of *ALOX5AP* SNPs in the study subjects grouped to MI cases and controls

<i>ALOX5AP</i> genotype (%)	MI cases (%)	MI cases counts (<i>n</i> =55)	Controls(%)	Controls counts (<i>n</i> =222)	OR, 95% CI	<i>P</i> value power	Adj. OR, 95% CI	<i>P</i> value power
SG13S377								
GG	69.1	38	69.4	154	0.247 0.03–1.81	0.084 0.280	0.650 0.41–1.02	0.032 0.459
GA	27.3	15	29.7	66	0.227 0.03–1.74	0.077 0.296	0.598 0.33–1.09	0.046 0.391
AA	3.6	2	0.9	2	– –	– –	– –	– –
G allele	82.75	45.5	84.25	187	0.896	0.393	–	–
A allele	17.25	9.5	30.15	35	0.41–1.97	0.046	–	–
SG13S114								
TT	21.8	12	17.6	39	1.249 0.54–2.88	0.301 0.075	0.908 0.32–2.55	0.428 0.038
TA	47.3	26	51.4	114	0.926 0.47–1.83	0.412 0.041	0.731 0.28–1.90	0.261 0.094
AA	30.9	17	31.1	69	– –	– –	– –	– –
T allele	45.4	25	43.3	96	1.094	0.384	–	–
A allele	54.6	30	56.8	126	0.60–1.98	0.048	–	–
SG13S41								
AA	65.5	36	78	174	0.207 0.04–1.07	0.030 0.469	0.527 0.34–0.82	0.002 0.812
AG	29.1	16	20.3	45	0.356 0.07–1.94	0.116 0.221	0.724 0.41–1.27	0.130 0.202
GG	5.5	3	1.4	3	– –	– –	– –	– –
A allele	80	44	88.5	196.5	0.519	0.049	–	–
G allele	20	11	11.5	25.5	0.24–1.13	0.378	–	–
SG13S35								
GG	89.1	49	83.3	185	0.749 0.52, 0.107	0.056 0.354	0.582 0.30–1.12	0.052 0.370
GA	10.9	6	16.2	36	0.471 0.26–0.48	0.006 0.710	0.659 0.26–1.70	0.195 0.136
AA	0	0	0.5	1	– –	– –	– –	– –
G allele	94.5	52	91.4	203	1.622	0.225	–	–
A allele	5.5	3	8.6	19	0.46–5.69	0.114	–	–

most common homozygous state. In the most extreme regressions, the allele frequency ORs deviated dramatically from the logistic regression estimates of the heterozygous and homozygous ORs. Also, it is clear that sample sizes impacted test resolution when rather dramatic ORs resulted in non-significant results. In Table 4, the estimates of haplotype frequency were often so low that the error bars exceeded the estimates of the population fractions. Also, category sizes were often too small for probabilities to be meaningfully obtained for those ORs. ORs were not computed in those conditions. For Table 4, the population

fractions were applied to the number of samples in disease vs. control groups to get numbers for the OR calculations.

Logistic regression analyses resolved no significant difference in the allele and genotype frequencies of the four *ALOX5AP* SNPs among CAD cases and controls (Table 2). When considering a subgroup of cases with MI and comparing the allele and genotype frequencies between the MI patients and controls, no significant difference was observed (Table 3). There was no significant association between allele frequencies and the various subgroups. The GG and GA genotypes of the SG13S35 SNP and the GG

Table 4 Genotype frequencies of *ALOX5AP* SNPs in CAD cases grouped to SVD cases and MVD cases

<i>ALOX5AP</i> genotype	SVD (%)	SVD counts (n=40)	MVD (%)	MVD counts (n=248)	OR 95% CI	P value power	Adj. OR, 95% CI	P value power
SG13S377								
GG	80	32	72.2	179	0.699 0.08–5.78	0.370 0.052	1.514 0.97–3.26	0.034 0.446
GA	17.5	7	24.6	61	1.089 0.12–10.0	0.470 0.030	1.715 0.90–3.26	0.050 0.376
AA	2.5 ^c	1	3.2 ^c	8	– –	– –	– –	– –
G allele	88.75	35.5	84.5	209.5	0.690	0.242		
A Allele	11.25	4.5	15.5	38.5	0.24–1.95	0.104		
SG13S114								
TT	12.5	5	21.0	52	2.562 0.89–7.40	0.041 0.412	1.197 0.35–4.04	0.386 0.047
TA	45.0	18	51.2	127	1.738 0.84–3.59	0.067 0.321	1.869 0.83–4.21	0.065 0.326
AA	42.5	17	27.8	69	– –	– –	– –	– –
T allele	35	14	46.6	115.5	1.619	0.087		
A Allele	65	26	53.4	132.5	0.81–3.25	0.273		
SG13S41								
AA	72.5	29	75.0	186	1.425 0.29–6.93	0.330 0.064	4.663 0.72–30.0	0.052 0.367
AG	22.5	9	21.4	53	1.309 0.24–7.07	0.377 0.050	3.256 0.44–23.8	0.122 0.213
GG	5 ^c	2	3.6 ^c	9	– –	– –	– –	– –
A allele	83.75	33.5	85.7	212.5	1.161	0.374		
G allele	16.25	6.5	14.3	35.5	0.47–2.89	0.051		
SG13S35								
GG	95	38	80.2	199	1.076 0.64–1.82	0.392 0.046	2.076 1.02–4.23	0.022 0.520
GA	5	2	17.7	44	4.521 1.75–11.7	0.001 0.874	1.059 0.32–3.37	0.461 0.031
AA	0 ^c	0	2.1 ^c	5	– –	– –	– –	– –
G allele	97.5	39	89.1	221	0.210	0.065		
A allele	2.5	1	10.9	27	0.03–1.59	0.327		

genotype of the SG13S377 SNP appear to be nearly significantly protective against both MI in general, and among MI subjects, in SVD vs. MVD; however, the uncertainty due to small samples among MI AA genotypes for both SNPs may not be ruled out as a possible source of artifact. Subgrouping the CAD cases into SVD cases and MVD cases did not resolve a significant variation in the individual polymorphisms comprising HapB between the two groups prior to adjustment (Table 4), though a trend at the 90% two-tailed level was notable for SG13S377, SG13S114, and SG13S41 after adjustment; however, this

result is also vulnerable to uncertainty due to small sample sizes at this level of stratification.

The distribution of HapB between CAD cases and CAD-free controls showed too few samples in this category for standard OR normal-variance approximations to apply. The most frequent haplotype was G-A-A-G (Table 5). The same results appeared in estimation to the association of HapB with MI compared to the control group (Table 6). When CAD cases were compared to controls with no family history of CAD ($n=121$), the SG13S377 genotype showed nearly significant association ($P=0.032$), where HapB

Table 5 Comparison of haplotypes percentages among control group and cases

Haplotype	Population (%±SD)	Control (%±SD)	CAD cases (%±SD)	Odds ratio, 95% CI	<i>P</i> value power
G-T-A-G	18.23±0.32	19.15±0.45	17.84±0.38	0.917 0.59–1.43	0.648 0.057
G-T-A-A (HapC)	0.84±0.19	0.81±0.25	0.85±0.21	1.050 0.15–7.16 ^a	0.480 ^a 0.028
G-T-G-G	10.101±0.32	6.81±0.45	11.48±0.36	1.775 0.94–3.33	0.0372 0.430
G-T-G-A	0.11±0.14	0.11±0.21	0.12±0.14	— —	— —
G-A-A-G	49.55±0.41	51.14±0.60	48.88±0.47	0.914 0.65–1.29	0.694 0.073
G-A-A-A	2.98±0.29	1.44±0.49	3.62±0.33	2.571 0.73–9.01 ^a	0.070 ^a 0.314
G-A-G-G	2.25±0.35	2.35±0.47	2.21±0.39	0.939 0.29–3.00 ^a	0.294 0.032
G-A-G-A	0.10±0.096	0.02±0.09	0.13±0.13	— —	— —
A-T-A-G	9.761±0.36	11.67±0.63	8.96±0.41	0.745 0.42–1.32	0.843 0.171
A-T-A-A	5.10±0.28	5.45±0.54	4.95±0.29	0.903 0.41–1.97	0.600 0.044
A-T-G-G	0.96±0.25	1.03±0.41	0.93±0.29	0.902 0.15–5.25 ^a	0.546 ^a 0.033
A-T-G-A	0.02±0.09	0.02±0.08	0.02±0.10	— —	— —
A-A-A-G (HapB)	0.01±0.037	0.0001±0.01	0.012±0.06	— —	— —
A-A-A-A	0.003±0.03	0.01±0.07	0.0017±0.03	— —	— —
A-A-G-G	0.001±0.01	0.0041±0.04	0±0.00	— —	— —

^a These values are inaccurate for small sample sizes

frequency was significantly higher in the controls with no FxCAD ($P=0.021$) (Data not shown).

The association of HapC with CAD was also under-represented in our population, but risk was also seen to be insignificant, and therefore population-specific (Girelli et al. 2007), and its correlation with CAD in this study population was examined. Table 5 shows that HapC SNPs are not significantly correlated with CAD (OR=1.050 and P value=0.480), but we lacked sufficient samples to draw conclusions about MI.

Discussion

Inflammation plays an essential role in the pathogenesis of CAD. Several studies reported the contribution of the

leukotriene pathway to atherosclerosis and CAD (Lotzer et al. 2005). Consequently, we have chosen *ALOX5AP*, which has been previously implicated in the inflammatory pathway, to examine in the Lebanese population.

The difference in allele, genotype, and HapB frequencies for the variants under study between the various populations studied may be attributed to the differences in ethnic background leading to different SNP linkage disequilibrium and haplotype structures. This difference was clearly observed in the Japanese population where a number of HapB frequencies were too low for performing an association analysis, and the authors relied on other haplotypes to detect the association of *ALOX5AP* gene with MI (Kajimoto et al. 2005). The same situation exists in our population as well, as was noted extensively in the “Results” section.

Table 6 Comparison of haplotypes percentages among control group and the MI cases

Haplotype	Population (%±SD)	Control (%±SD)	MI cases (%±SD)	OR, 95% CI	P value power
G-T-A-G	16.90±0.52	17.73±0.59	13.47±0.88	0.722 0.32–1.68	0.225 0.114
G-T-A-A (HapC)	0.84±0.32	1.00±0.38	0.15±0.33	– –	– –
G-T-G-G	10.21±0.45	9.22±0.47	14.30±0.96	1.643 0.68–3.96	0.134 0.197
G-T-G-A	0.10±0.15	0.05±0.11	0.33±0.44	– –	– –
G-A-A-G	50.80±0.58	51.24±0.61	48.98±1.06	0.914 0.51–1.65	0.381 0.049
G-A-A-A	3.56±0.42	3.76±0.52	2.73±0.00	0.718 0.12–4.18 ^a	0.356 ^a 0.056
G-A-G-G	1.50±0.43	1.33±0.39	2.20±1.09	1.669 0.20–14.04 ^a	0.319 ^a 0.068
G-A-G-A	0.14±0.11	0.03±0.07	0.58±0.44	– –	– –
A-T-A-G	11.26±0.51	10.83±0.55	12.99±0.85	1.229 0.50–3.00	0.325 0.066
A-T-A-A	3.32±0.41	3.72±0.48	1.63±0.41	– –	– –
A-T-G-G	1.32±0.37	1.02±0.37	2.55±0.79	2.539 0.31–21.12 ^a	0.194 ^a 0.136
A-T-G-A	0.03±0.08	0.02±0.07	0.05±0.20	– –	– –
A-A-A-G (HapB)	0.03±0.10	0.03±0.10	0.05±0.22	– –	– –
A-A-A-A	0.01±0.04	0.01±0.05	0.00±0.00	– –	– –
A-A-G-G	0.00±0.02	0.00±0.02	0.00±0.01	– –	– –

^a These values are inaccurate for small sample sizes

Evidence to the effect of ethnic differences on the genetic susceptibility to CAD is also found in the *LTA4H* gene (encoding the LTA4 hydrolase), which increases the risk for MI depending on the ethnic background of the subjects under study (Helgadottir et al. 2006). Moreover, the previously published reports included different study subjects with different categorization criteria and different phenotypes (MI, CAD, or stroke), which complicated the comparison between different studies.

In our study population, we resolved no difference in the genotype frequency of the polymorphisms comprising HapB when we compared SVD cases and MVD cases. This result again provides no evidence for the role of HapB in the disease severity. While the accumulating evidence on the role of inflammatory mediators in the initiation, progression, and severity of the disease builds (Laxton et

al. 2005; Lusis et al. 2004), the impact of the inflammatory mediator FLAP is difficult to assess due to the strong nearly monoallelic structure of our population.

Our selection for the subjects was based on stringent inclusion criteria based on measured clinical outcomes (angiography results) for the purpose of genetic enrichment. Therefore, the results variability between our study and others may have been due either to less stringent selection of cases and/or improper categorization of the controls.

The primary indicator of disease was catheterization. Subject recruitment was therefore among those for whom the procedure had been ordered for other indications, rather than by random selection of volunteers since the procedure is invasive. Therefore, proportions among interactive variables may be distorted by selection bias, which constitutes a limitation to this study. Specifically, the selection criteria for

ordering the procedure includes variables that may themselves be indicators of inflammatory response, which would render the control and disease groups to be artificially homogeneous and enriched for inflammatory alleles. Furthermore, history of conditions identified as risk factors included neither age of onset and detailed clinical history nor details of medication, treatment, and other factors; however, blood panels measuring FBS and lipids, as well as BMI and other measures, were obtained. We sought to limit the impact of the above-sited factors by selecting patients with earlier onset (younger than 52 years of age); however, this also limits the probe to genetic factors that promotes disease process at earlier ages, rather than those that may inhibit CAD among some older patients. This also implies that the set of patients selected would be from among those whose risk factors may have identified the subject as being at sufficient risk to justify catheterization at a younger age (e.g., family history) that may also impact sampling bias by genetic association.

Finally, the susceptibility HapB is constructed from polymorphisms of no clear effect on the function or the synthesis of the FLAP. Moreover, these variants might be associated with disease-causal SNPs in *ALOX5AP* gene, and this association might vary between populations according to the genetic structure of each race. Thus, other haplotypes in *ALOX5AP* gene may be associated with the disease.

Our study is the first genetic study on the association of the inflammatory gene *ALOX5AP* with CAD that includes cases and controls with detailed clinical and epidemiological criteria of categorization. This is also the first study on the *ALOX5AP* gene in Middle Eastern populations.

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