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Pro-Inflammatory Interleukin-1 Genotypes Potentiate the Risk of Coronary Artery Disease and Cardiovascular Events Mediated by Oxidized Phospholipids and Lipoprotein(a)

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Objectives The aim of this study was to assess the influence of pro-inflammatory interleukin (IL)-1 genotype status on the risk for coronary artery disease (CAD), defined as >50% diameter stenosis, and cardiovascular events mediated by oxidized phospholipids (OxPLs) and lipoprotein (Lp) (a).

Background OxPLs are pro-inflammatory, circulate on Lp(a), and mediate CAD. Genetic variations in the IL-1 region are associated with increased inflammatory mediators.

Methods IL-1 genotypes, OxPL on apolipoprotein B-100 (OxPL/apoB), and Lp(a) levels were measured in 499 patients undergoing coronary angiography. The composite genotype termed *IL-1(+)* was defined by 3 single-nucleotide polymorphisms in the IL-1 gene cluster associated with higher levels of pro-inflammatory cytokines. All other IL-1 genotypes were termed *IL-1(−)*.

Results Among IL-1(+) patients, the highest quartile of OxPL/apoB was significantly associated with a higher risk for CAD compared with the lowest quartile (odds ratio [OR]: 2.84; $p = 0.001$). This effect was accentuated in patients age ≤60 years (OR: 7.03; $p < 0.001$). In IL-1(−) patients, OxPL/apoB levels showed no association with CAD. The interaction was significant for OxPL/apoB (OR: 1.99; $p = 0.004$) and Lp(a) (OR: 1.96; $p < 0.001$) in the IL-1(+) group versus the IL-1(−) group in patients age ≤60 years but not in those age >60 years. In IL-1(+) patients age ≤60 years, after adjustment for established risk factors, high-sensitivity C-reactive protein, and Lp(a), OxPL/apoB remained an independent predictor of CAD. IL-1(+) patients above the median OxPL/apoB presented to the cardiac catheterization laboratory a mean of 3.9 years earlier ($p = 0.002$) and had worse 4-year event-free survival (death, myocardial infarction, stroke, and need for revascularization) compared with other groups ($p = 0.006$).

Conclusions Our study suggests that IL-1 genotype status can stratify population risk for CAD and cardiovascular events mediated by OxPL. These data suggest a clinically relevant biological link between pro-inflammatory *IL-1* genotype, oxidation of phospholipids, Lp(a), and genetic predisposition to CAD and cardiovascular events. (J Am Coll Cardiol 2014;63:1724–34) © 2014 by the American College of Cardiology Foundation

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The presence of chronic arterial inflammation in response to atherogenic stimuli provides a framework in understanding the development and destabilization of atherosclerotic plaques. Oxidized lipids play a central role in mediating a variety of immune, pro-inflammatory, and plaque-destabilizing processes that further amplify inflammatory responses (1). Underlying this inflammatory cascade is the production and secretion of cytokines, growth factors, and metalloproteinases, such as interleukin (IL)-1, tumor necrosis factor-α, and C-reactive protein (CRP) (2). Genetic variations in the IL-1 gene family (chromosome 2q13 region), which include the pro-inflammatory cytokines IL-1α and IL-1β, and the anti-inflammatory IL-1 receptor antagonist (IL-1Ra) (3–5), are commonly found in the human population, affect pro-inflammatory gene regulation (6), and have been associated with elevated levels of pro-inflammatory mediators (7–10). The interplay of various single-nucleotide polymorphisms (SNPs) within this IL-1 family determines the overall net effect on pro- or anti-inflammatory responses.

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The majority of published studies have shown an association of IL-1 and cardiovascular disease, including early myocardial infarction (MI)/acute coronary syndromes (8,11–16), coronary artery disease (CAD) (17–20), acute ischemic stroke (21–23), restenosis following coronary stenting (24), and venous thrombosis (25). CANTOS (the Canakinumab Anti-inflammatory Thrombosis Outcomes Study) (26) will test the hypothesis that treating patients with persistent elevation of CRP post-MI with a human monoclonal antibody that neutralizes IL-1β antibody will reduce cardiovascular events.

Oxidized phospholipids (OxPLs) are pro-inflammatory (27), mediate atherothrombosis, and are abundant in pathologically defined human vulnerable plaques (28). Plasma levels of specific OxPL on apolipoprotein B-100 particles (OxPL/apoB) are elevated in patients with coronary, carotid, and peripheral artery disease (29), as well as in those with acute coronary syndromes (30), and following percutaneous coronary intervention (31). Importantly, they predict the occurrence of cardiac death, MI, and stroke in unselected populations (32–34). Additionally, they reclassify up to one-third of patients in intermediate Framingham risk categories into either higher or lower categories (33). In human plasma, OxPLs are preferentially carried by lipoprotein (Lp) (a) compared with other apoB-100 particles (reviewed in Taleb et al. [35]). OxPLs are also covalently bound by plasminogen, but early data suggest different pathophysiological implications when OxPLs are on Lp(a) versus plasminogen (36).

Because OxPL mediate pro-inflammatory responses on endothelial cells and monocytes/macrophages (27), it is possible that the risk they confer on atherothrombosis is potentiated by genetic predisposition to inflammation. In the present study, we hypothesized that the risk for CAD conferred by OxPL/apoB and Lp(a) may be influenced by IL-1 genotypes known to be associated with enhanced inflammatory responses.

Abbreviations and Acronyms

apo = apolipoprotein
CAD = coronary artery disease
CI = confidence interval
HDL-C = high-density lipoprotein cholesterol
hs-CRP = high-sensitivity C-reactive protein
IL = interleukin
LDL-C = low-density lipoprotein cholesterol
Lp(a) = lipoprotein(a)
MI = myocardial infarction
OR = odds ratio
OxPL = oxidized phospholipid
SNP = single-nucleotide polymorphism

Methods

Study design. A full description of the methods is available in the Online Appendix. The study was prospectively designed to test the association of CAD with specific IL-1 genotype groups known to be associated with higher inflammatory responses. The study design has been described previously in detail (37). Briefly, 504 eligible, consecutive patients undergoing clinically indicated coronary angiography were recruited. We focused our analyses on angiographically significant disease, defined as diameter stenosis >50%. Two patients had incomplete OxPL/apoB data, and 3 patients had incomplete IL-1 data; therefore, 499 patients were available for the present analysis. Four hundred sixty-six patients (92.5%) were followed for up to 4.0 years (interquartile range: 3.9 to 4.2 years). The follow-up events consisted of 20 deaths (6 cardiac), 14 MIs, 26 coronary revascularizations (15 percutaneous intervention only, 9 coronary artery bypass surgery only, and 2 with both), and 10 strokes.

Genetic analyses. SNPs were genotyped at 1 locus in the gene for IL-1α (*IL1A*[+4845], rs17561, G > T) and at 2 loci in the gene for IL-1β (*IL1B*[+3954], rs1143634, C > T; and *IL1B*[−511], rs16944, C > T]) (38).

IL-1 composite genotype patterns used for association with biochemical and clinical parameters. We designed the study to evaluate the relationship between CAD and IL-1 genotypes that are associated with differential expression of IL-1β. Four SNPs in the promoter region of *IL1B* have been shown to be functional at the molecular level and operate in haplotype context to alter transcriptional activity of IL-1β (6). The functional *IL1B* SNPs define 4 predominant haplotypes that, as pairs observed together, account for significantly different clinical levels of IL-1β protein in tissue fluid samples (10). All possible composite genotype combinations of the 3 SNPs used in the study provided an efficient tagging of the composite genotypes resulting from combinations of the functional *IL1B* promoter haplotypes that define differential expression of IL-1β protein (10). Table 1 shows the composite genotypes in this study that defined the IL-1(+) group, which are associated with overexpression of IL-1β, and the IL-1(−) group, which are the composite genotypes that have not been associated with overexpression of IL-1β. The IL-1(+)

Table 1 Composite Genotypes Used in the Study

Group Classification for Analysis	*IL1A*(+4845), rs17561, G > T	*IL1B*(+3954), rs1143634, C > T	*IL1B*(−511), rs16944, C > T
IL-1(+)	T*	T*	CC
	GG	T*	CC
	†	CC	CC
	T*	T*	CT
IL-1(−)	T*	T*	TT
	GG	T*	TT
	T*	CC	TT
	GG	CC	TT
	T*	CC	CT
	GG	CT	CT
	GG	CC	CT

T* indicates that the second allele in the genotype can be either a G or a T. †Indicates that the genotype at that locus can be GG, GT, or TT.

and IL-1(−) groups were defined and published before data analysis (39).

Results

Baseline characteristics of the study group. Table 2 displays the baseline characteristics of the entire study group and of the IL-1(+) and IL-1(−) groups. IL-1(+) patients represented 59.9% of the population. There were no significant differences in any parameters between the IL-1(+) and IL-1(−) patients, including extent of CAD; there were trends toward greater prevalences of previous MI (18% vs. 12%; p = 0.08) and high-sensitivity (hs) CRP (3.1 vs. 2.3 mg/l; p = 0.057) in the IL-1(+) patients (Table 2). IL-1 status was not different between groups in the extent of CAD (none; mild; or 1-, 2-, or 3-vessel CAD) when analyzed by age ≤60 years (p = 0.88) or >60 years (p = 0.36). Similar results were obtained when IL-1 status was evaluated as >50% diameter stenosis and analyzed by age ≤60 years (p = 0.51) and >60 years (p = 0.47) (data not shown).

CAD risk for OxPL mediation by IL-1 genetic differences. Odds ratios (OR) for CAD in each quartile of OxPL/apoB were calculated in all patients, by IL-1(+) or IL-1(−) genotype, and further by age (all ages, ≤60 years, and >60 years).

In the entire cohort, a significant relationship was present between OxPL/apoB and CAD (>50% diameter stenosis); the OR was 1.96 (95% confidence interval [CI]: 1.18 to 3.26; p = 0.009) for fourth quartile compared with the first quartile, and the OR for trend was 1.25 (95% CI: 1.06 to 1.46; p = 0.007) (Table 3).

On analysis of patients by genotype, a significant association was present between increasing OxPL/apoB levels and risk for CAD in IL-1(+) patients (OR: 2.84; 95% CI: 1.45

Table 2 Baseline Characteristics of the Study Group

	All (N = 499)	IL-1(+) (n = 299)	IL-1(−) (n = 200)	p Value, IL-1 Effect
Age, yrs	60.0 ± 10.9	59.6 ± 11.1	60.6 ± 10.7	0.82
Female	190 (38)	111 (37)	79 (40)	0.59
White	485 (97)	294 (98)	191 (96)	0.51
HTN	230 (46)	140 (47)	90 (45)	0.69
Current smoker	40 (8)	24 (8)	16 (8)	0.99
MI	77 (15)	53 (18)	24 (12)	0.08
CHF	59 (12)	32 (11)	27 (14)	0.35
Family history of CAD	126 (25)	76 (25)	50 (25)	0.92
Extent of CAD				0.79
None	122 (24)	71 (24)	51 (26)	
Mild	109 (22)	68 (23)	41 (21)	
1-vessel	84 (17)	46 (15)	38 (19)	
2-vessel	80 (16)	50 (17)	30 (15)	
3-vessel	104 (21)	64 (21)	40 (20)	
Laboratory values				
TC, mg/dl	207 ± 45	208 ± 46	206 ± 43	0.57
LDL-C, mg/dl	125 ± 37	124 ± 35	125 ± 37	0.81
HDL-C, mg/dl	48 ± 15	48 ± 15	48 ± 14	0.94
Tg, mg/dl	153 (112–207)	154 (113–220)	151 (106–200)	0.48
ApoB-100, mg/dl	98 ± 21	98 ± 20	98 ± 22	0.91
ApoAI, mg/dl	132 ± 26	132 ± 27	131 ± 24	0.48
Lp(a), mg/dl	21.1 (8.7–39.6)	21.5 (9.2–38.4)	20.0 (7.7–42.3)	0.96
OxPL/apoB, RLU	6,268 (3,381–20,829)	6,268 (3,361–20,620)	6,121 (3,414–20,843)	0.96
hsCRP, mg/l	2.9 (1.2–6.7)	3.1 (1.3–7.3)	2.3 (1.0–6.1)	0.057

Values are mean ± SD, n (%), or median (interquartile range).

Apo = apolipoprotein; CHF = congestive heart failure; HDL-C = high-density lipoprotein cholesterol; hsCRP = high-sensitivity C-reactive protein; HTN = hypertension; IL = interleukin; LDL-C = low-density lipoprotein cholesterol; Lp(a) = lipoprotein(a); MI = myocardial infarction; OxPL/apoB = oxidized phospholipid/apolipoprotein B; RLU = relative light units; TC = total cholesterol; Tg = triglycerides.

Table 3 ORs for CAD (>50% Diameter Stenosis) According to Quartiles of OxPL/ApoB in IL-1(+) and IL-1(–) Patients, by Age Subgroup

	All Patients		IL-1(+)		IL-1(–)	
Subgroup	CAD	OR (95% CI)	CAD	OR (95% CI)	CAD	OR (95% CI)
All ages						
Quartile I	59/125 (47)	1.00	35/77 (45)	1.00	24/48 (50)	1.00
Quartile II	62/125 (50)	1.10 (0.67–1.81)	36/73 (49)	1.17 (0.62–2.22)	26/52 (50)	1.00 (0.46–2.19)
Quartile III	68/125 (54)	1.34 (0.81–2.19)	37/75 (49)	1.17 (0.62–2.21)	31/50 (62)	1.63 (0.73–3.65)
Quartile IV	79/124 (64)	1.96 (1.18–3.26)	52/74 (70)	2.84 (1.45–5.55)	27/50 (54)	1.17 (0.53–2.60)
OR: (95% CI) for trend		1.25 (1.06–1.46)		1.35 (1.10–1.66)		1.10 (0.86–1.42)
p value for trend		0.007		0.004		0.45
Age ≤60 yrs						
Quartile I	19/60 (33)	1.00	9/33 (27)	1.00	12/27 (44)	1.00
Quartile II	17/52 (32)	0.75 (0.34–1.67)	8/32 (25)	0.89 (0.29–2.69)	7/20 (35)	0.67 (0.20–2.22)
Quartile III	27/64 (45)	1.64 (0.80–3.38)	18/43 (42)	1.92 (0.73–5.10)	12/21 (57)	1.67 (0.53–5.27)
Quartile IV	41/63 (60)	2.82 (1.36–5.87)	29/40 (73)	7.03 (2.50–19.77)	9/23 (39)	0.80 (0.26–2.49)
OR: (95% CI) for trend		1.48 (1.17–1.87)		1.99 (1.43–2.78)		1.01 (0.71–1.45)
p value for trend		0.001		<0.001		0.94
Age >60 yrs						
Quartile I	40/65 (59)	1.00	26/44 (59)	1.00	12/21 (57)	1.00
Quartile II	47/73 (65)	1.28 (0.65–2.55)	28/41 (68)	1.49 (0.61–3.64)	19/32 (59)	1.10 (0.36–3.35)
Quartile III	41/61 (62)	1.17 (0.57–2.40)	19/32 (59)	1.01 (0.40–2.56)	19/29 (66)	1.43 (0.45–4.52)
Quartile IV	38/61 (67)	1.46 (0.70–3.02)	23/34 (68)	1.45 (0.57–3.69)	18/27 (67)	1.50 (0.46–4.87)
OR: (95% CI) for trend		1.11 (0.88–1.39)		1.08 (0.80–1.45)		1.16 (0.81–1.68)
p value for trend		0.38		0.61		0.42

Values are n/N (%) unless otherwise indicated. Q1 = <3,382 RLU; Q2 = 3,382 to <6,268 RLU; Q3 = 6,268 to <20,829 RLU; Q4 = >20,829 RLU.
CI = confidence interval; OR: = odds ratio; other abbreviations as in Table 2.

to 5.55; p = 0.001 for fourth quartile compared with first quartile), whereas no significant relationship was present in IL-1(–) patients. This genotype effect was strongly accentuated in IL-1(+) patients age ≤60 years (OR: 7.03; 95% CI: 2.50 to 19.77; p < 0.001) but not in IL-1(–) patients age ≤60 years (OR: 0.80; 95% CI: 0.26 to 2.49; p =

Table 4 ORs for CAD (>50% Diameter Stenosis) According to Quartiles of Lp(a) in IL-1(+) and IL-1(–) Patients, by Age Subgroup

	All Patients		IL-1(+)		IL-1(–)	
Subgroup	CAD	OR (95% CI)	CAD	OR (95% CI)	CAD	OR (95% CI)
All ages						
Quartile I	55/125 (44)	1.00	30/70 (43)	1.00	25/55 (45)	1.00
Quartile II	62/125 (50)	1.25 (0.76–2.06)	37/77 (48)	1.23 (0.64–2.37)	25/48 (52)	1.30 (0.60–2.83)
Quartile III	68/125 (54)	1.52 (0.92–2.50)	42/84 (50)	1.33 (0.70–2.52)	26/41 (63)	2.08 (0.91–4.76)
Quartile IV	83/124 (67)	2.58 (1.54–4.31)	51/68 (75)	4.00 (1.94–8.26)	32/56 (57)	1.60 (0.76–3.39)
OR: (95% CI) for trend		1.35 (1.15–1.59)		1.49 (1.20–1.85)		1.20 (0.94–1.52)
p value for trend		<0.001		<0.001		0.14
Age ≤60 yrs						
Quartile I	18/60 (30)	1.00	8/32 (25)	1.00	10/28 (36)	1.00
Quartile II	19/57 (33)	1.17 (0.54–2.54)	10/36 (28)	1.15 (0.39–3.41)	9/21 (43)	1.35 (0.42–4.30)
Quartile III	28/58 (48)	2.18 (1.02–4.63)	19/44 (43)	2.28 (0.84–6.19)	9/14 (64)	3.24 (0.85–12.4)
Quartile IV	39/64 (61)	3.64 (1.73–7.68)	27/36 (75)	9.00 (3.00–27.03)	12/28 (43)	1.35 (0.46–3.96)
OR: (95% CI) for trend		1.58 (1.24–2.00)		2.11 (1.49–2.98)		1.15 (0.82–1.64)
p value for trend		0.001		<0.001		0.43
Age >60 yrs						
Quartile I	38/65 (58)	1.00	22/38 (58)	1.00	15/27 (56)	1.00
Quartile II	43/68 (62)	1.30 (0.65–2.61)	27/41 (66)	1.40 (0.56–3.49)	16/27 (59)	1.16 (0.40–3.42)
Quartile III	42/67 (61)	1.21 (0.56–2.24)	23/40 (58)	0.98 (0.40–2.42)	17/27 (63)	1.36 (0.46–4.04)
Quartile IV	44/60 (72)	2.08 (0.98–4.42)	24/32 (75)	2.18 (0.78–6.09)	20/28 (71)	2.00 (0.65–6.11)
OR: (95% CI) for trend		1.21 (0.96–1.53)		1.19 (0.87–1.62)		1.25 (0.88–1.77)
p value for trend		0.10		0.27		0.22

Values are n/N (%) unless otherwise indicated.
Abbreviations as in Tables 2 and 3.

0.71). In patients age >60 years, the association between OxPL/apoB levels and risk for CAD was not significant in either IL-1(+) or IL-1(−) patients (Table 3).

Similar to the OxPL/apoB results, the association between Lp(a) and the risk for CAD was observed primarily in IL-1(+) patients, with the strongest genotype effect present in patients ≤60 years of age (OR: 9.00; 95% CI: 3.00 to 27.03; p < 0.001) (Table 4).

Interaction tests were performed comparing IL-1(+) patients to IL-1(−) patients. p Values for the interaction of OxPL/apoB were 0.007 for IL-1(+) versus IL-1(−) patients ≤60 years of age, and 0.70 for IL-1(+) versus IL-1(−) patients >60 years of age. p Values for the interaction of Lp(a) were 0.019 for IL-1(+) versus IL-1(−) patients ≤60 years of age and p = 0.77 for IL-1(+) versus IL-1(−) patients >60 years of age.

Multivariate analysis of CAD risk in the different genetic strata. Multivariate logistic regression analysis was performed to adjust for factors known to affect the risk for CAD. Figure 1 shows ORs for sex, OxPL/apoB, hsCRP, current smoking, low-density lipoprotein cholesterol (LDL-C), hypertension, triglycerides, Lp(a), and high-density lipoprotein cholesterol (HDL-C) when all were included in a single logistic binary regression model. In patients ≤60 years of age and IL-1(+), male sex (p = 0.001), OxPL/apoB (per doubling) (p = 0.010), and hsCRP (log2) (p = 0.004) were

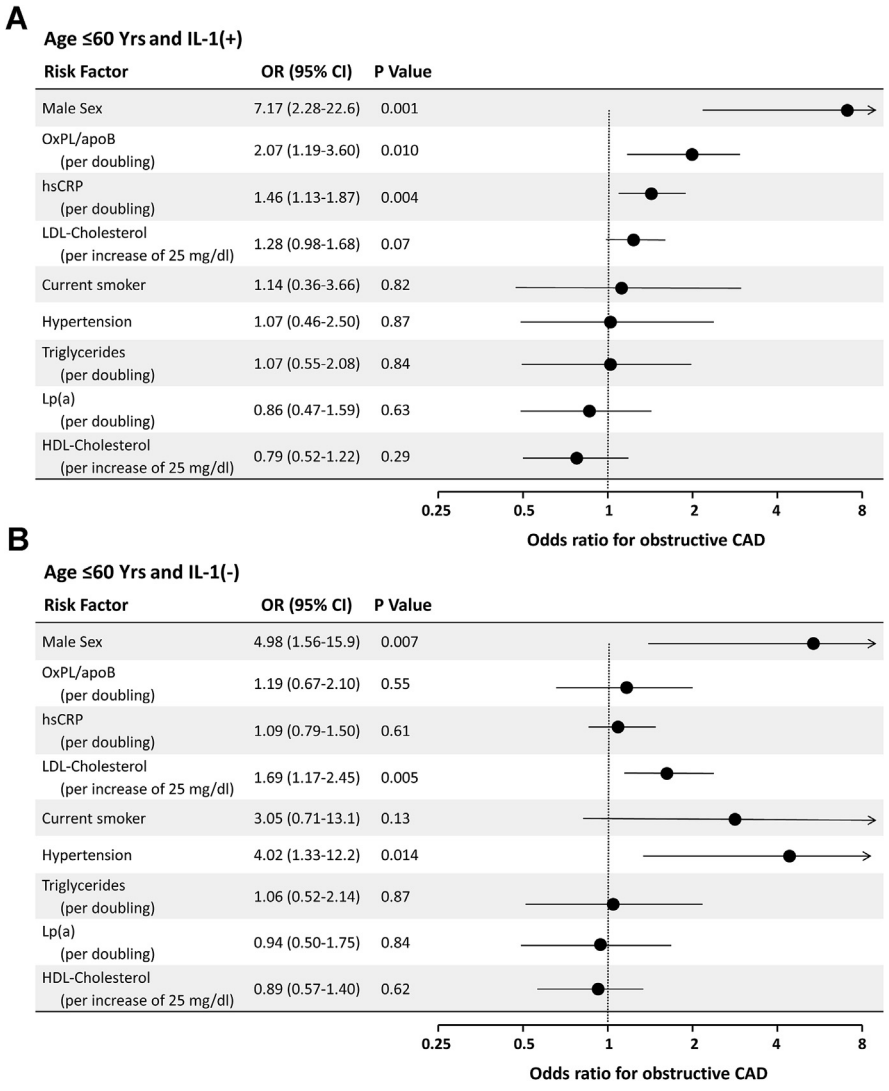

Figure 1 Selected Risk Factors for CAD Among Patients Age ≤60 Years, Stratified by Genotype

Multivariate analysis–derived odds ratios (ORs) for coronary artery disease (CAD) associated with selected risk factors among patients age ≤60 years and with interleukin (IL)-1(+) genotype **(A)** or IL-1(−) **(B)** genotype. CI = confidence interval; HDL = high-density lipoprotein (per increase of 10 mg/dl); hsCRP = high-sensitivity C-reactive protein (per doubling); IL = interleukin; LDL = low-density lipoprotein (per increase of 25 mg/dl); Lp(a) = lipoprotein a (per doubling); ORs = odds ratios; OxPL/apoB = oxidized phospholipid/apolipoprotein B (per doubling).

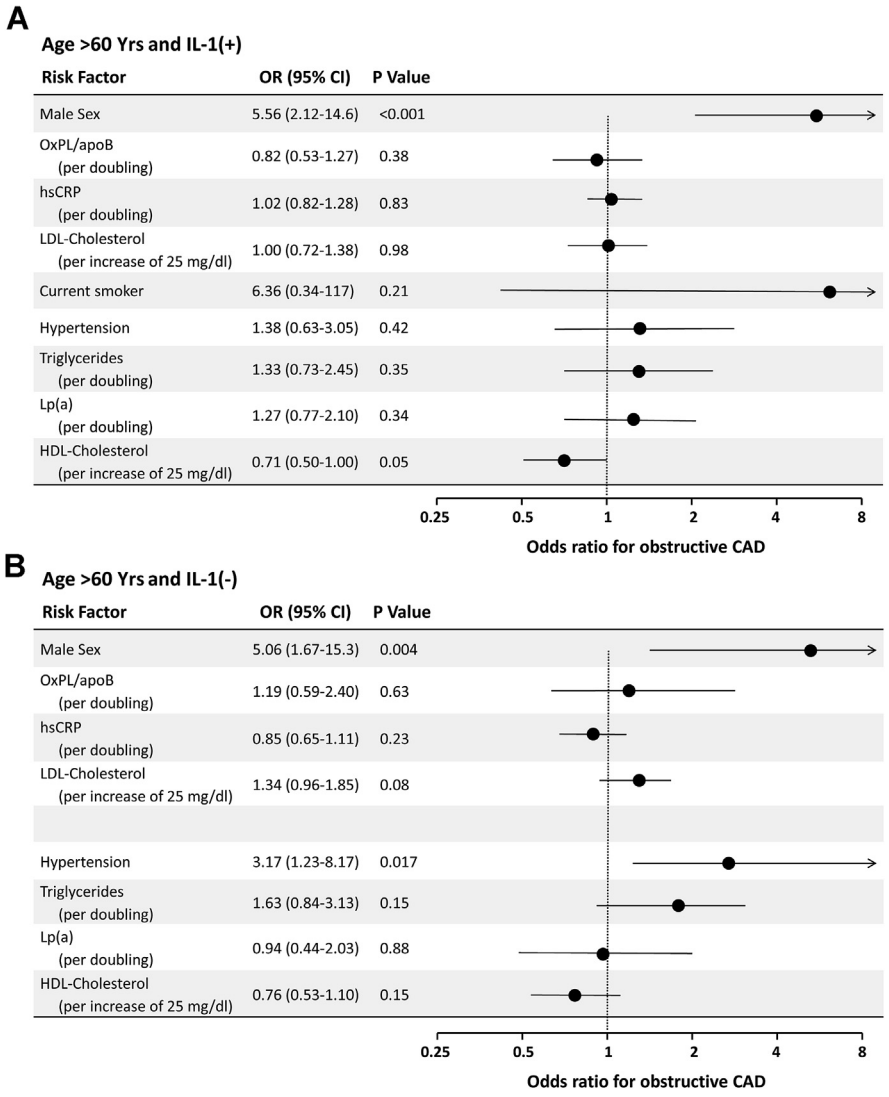

Figure 2 **Selected Risk Factors for CAD Among Patients Age >60 Years, Stratified by Genotype**

Multivariate analysis–derived ORs for CAD associated with selected risk factors among patients age >60 years and with interleukin (IL)-1(+) genotype **(A)** or IL-1(–) **(B)** genotype. Age is measured per decade. Current smoking was omitted as a factor for those age >60 years because of negligible sample size of smokers in this category. Abbreviations as in Figure 1.

independent predictors of CAD, whereas Lp(a) was not a significant predictor (Fig. 1A). The association of OxPL/apoB (OR: 1.83; 95% CI: 1.10 to 3.05; p = 0.02) to CAD remained similar without hsCRP in the model. When the 44 patients with MI within 60 days before coronary angiography were excluded, the data remained qualitatively similar, except that hsCRP was no longer a predictor of CAD (OR: 1.23; 95% CI: 0.89 to 1.68; p = 0.21). Baseline hsCRP levels in these patients were significantly elevated compared with those in patients without MI, as described previously (33). In patients ≤60 years of age and IL-1(–), male sex (p = 0.007), LDL-C (p = 0.005), and hypertension (p = 0.014) were independent predictors (Fig. 1B).

In patients >60 years of age and IL-1(+), male sex (p < 0.001) and HDL-C (p = 0.05) were independent predictors of CAD, but not OxPL/apoB (Fig. 2A). In patients age >60 years and IL-1(–), male sex (p = 0.004) and hypertension (p = 0.017) were associated with higher risk (Fig. 2B).

To further explore the relationship between OxPL/apoB or Lp(a) levels and IL-1 genotype relative to the risk for CAD, we stratified patients by IL-1 genotype and developed regression models to assess the relationship of OxPL/apoB and Lp(a) levels to CAD risk. The relationships of the OR values for CAD are expressed as functions of the magnitudes of differences in OxPL/apoB and Lp(a) levels in IL-1(+)

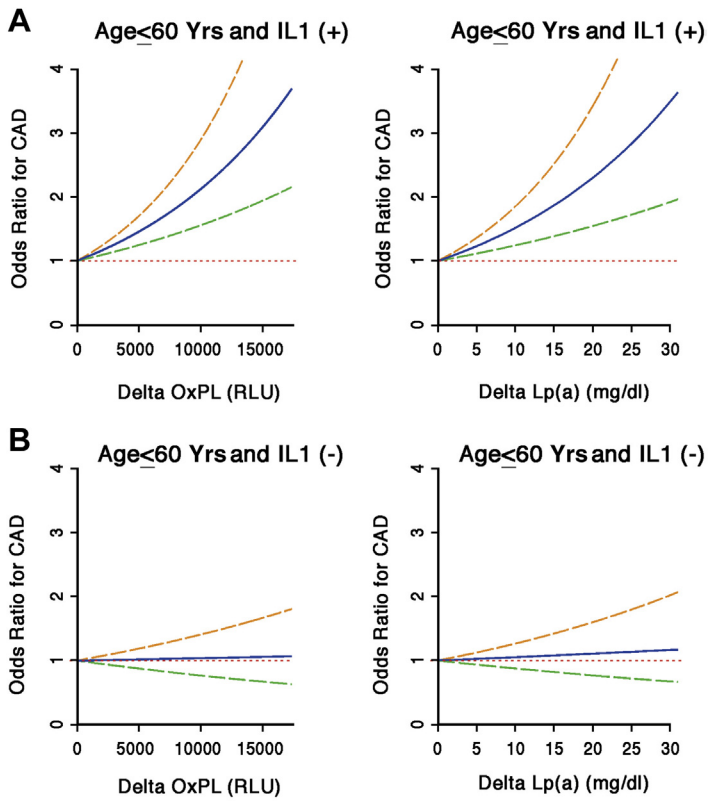

Figure 3 OR for CAD, Calculated in a Logistic Regression Model

Risk associated with an incremental increase in each risk factor, ranging from 0 (i.e., an OR: **[solid lines]** of 1) to the value equal to the difference between the 75th and 25th percentiles of the risk factors. The analysis was performed in patients ≤60 years of age, stratified by IL-1(+) or IL-1(–) genotype. **Dashed lines** represent confidence intervals. RLU = relative light units; other abbreviations as in Figure 1.

and IL-1(–) patients. The OR for CAD was highly sensitive to differences in levels of both OxPL/apoB and Lp(a) in IL-1(+) patients, but no association was present in IL-1(–) patients (Fig. 3).

IL-1 genotype effect on OxPL risk for CAD and CRP levels. Because some of the IL-1 genetic variations included in the genetic patterns used in this study have been previously associated with elevated CRP (7,10), we evaluated whether the IL-1 genotype influence on the OxPL association with CAD was influenced by hsCRP levels. The relationship of OxPL/apoB to CAD in IL-1(+) patients ≤60 years of age was analyzed in the multivariate logistic regression framework in patients with hsCRP above and below the median hsCRP level in this study population (2.86 mg/l). The OR for the OxPL/apoB association with CAD in IL-1(+) patients with hsCRP >2.86 mg/l was 3.36 (95% CI: 1.21 to 9.40; p = 0.02), and in those with hsCRP <2.86 mg/l, the OR was 1.43 (95% CI: 0.62 to 3.31; p = 0.40). Removing the 44 patients with recent MI yielded similar results, with ORs of 4.57 (95% CI: 1.06 to 19.67; p = 0.042) for the OxPL/apoB association with CAD in IL-1(+) patients with hsCRP above the median, and 1.21 (95% CI: 0.50 to 2.90; p = 0.68) in those below the median.

Relationship of IL-1 genotype to age at presentation to the cardiac catheterization laboratory. Having established the relationship of OxPL/apoB and Lp(a) with CAD in IL-1(+), but not IL-1(–), patients, particularly those at a younger age, we evaluated whether age at the time of cardiac catheterization was related to IL-1 composite genotype. IL-1(+) patients above the median of OxPL/apoB presented to the cardiac catheterization laboratory a mean of 3.9 years earlier than did IL-1(–) patients (mean age at presentation 58.0 vs. 61.9 years; p = 0.006). Similarly, IL-1(+) patients above the median of Lp(a) presented a mean of 3.5 years earlier than did IL-1(–) patients (mean: 58.8 vs. 62.1 years; p = 0.019). In contrast, there was no significant IL-1 genotype effect on age at presentation to the cardiac catheterization laboratory in patients below the median of OxPL/apoB (mean: 58.9 vs. 60.7 years; p = 0.18) or Lp(a) (mean: 58.7 vs. 59.9; p = 0.40).

Relationship of IL-1 genotype to CAD events. In the overall group, cardiovascular events were not significantly different between IL-1(+) and IL-1(–) patients (p = 0.56).

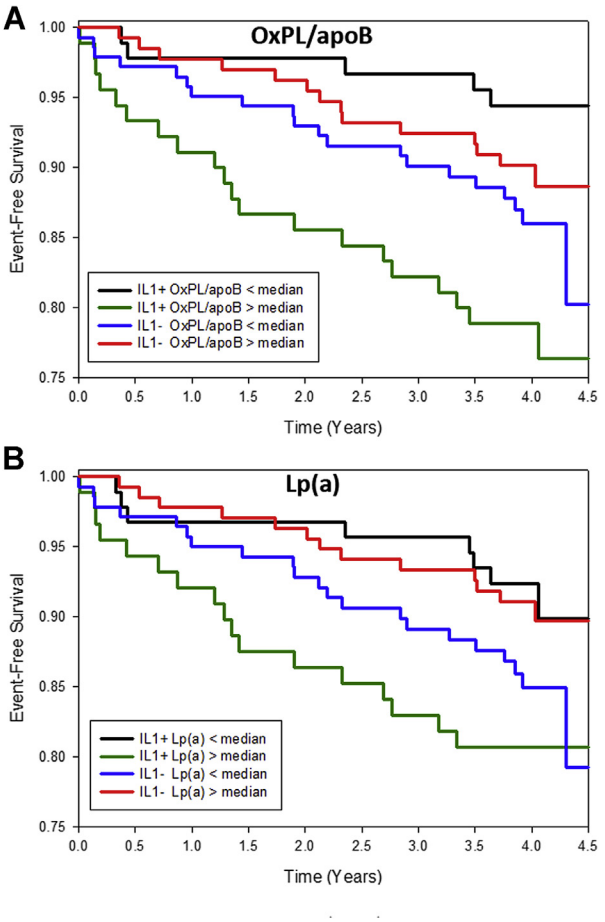

Figure 4 **Event-Free Survival**

Event-free survival (composite of death, myocardial infarction, stroke, and need for revascularization) plotted in 4 groups based on IL-1 genotype and OxPL/apoB **(A)** and Lp(a) **(B)** median, split by the Kaplan-Meier method and compared using a log-rank test. Abbreviations as in Figure 1.

However, Kaplan-Meier curves revealed that IL-1(+) patients with OxPL/apoB above the median had the worst 4-year event-free survival (composite of death, MI, stroke, and need for revascularization) ($p = 0.002$ compared with the other 3 groups) (Fig. 4A). The p value among the 4 groups for death was 0.069 and for death/MI was 0.016. The interaction test for IL-1 group by OxPL/apoB group was $p = 0.002$ for event-free survival. For Lp(a), IL-1(+) patients with Lp(a) above the median exhibited worst 4-year event-free survival ($p = 0.034$ compared with the other 3 groups) (Fig. 4B). The p value among the 4 groups for death was 0.054 and for death/MI was 0.011. The interaction test for IL-1 group by Lp(a) group was $p = 0.014$ for event-free survival. There were not enough events to analyze by age cutoffs.

Discussion

This study demonstrates that genetic differences in the IL-1 gene cluster, known to be associated with inflammatory responsiveness, strongly influence the presence of angiographically determined CAD and CAD events mediated by OxPL/apoB and Lp(a). Patients with pro-inflammatory IL-1(+) genotypes were at a continuum of risk for the presence of CAD, whereas patients with IL-1(−) genotypes seemed to be insensitive to the risk for CAD mediated by increasing OxPL/apoB or Lp(a) levels. These findings were accentuated in subjects with elevated hsCRP levels. This study provides evidence of a biological link between genetic predisposition to inflammation, oxidation of phospholipids, and genetically mediated elevated Lp(a) levels. It also highlights a possible effect of specific genetic factors in accelerating atherogenesis, development of CAD on angiography, and mediation of cardiovascular events.

The genes encoding the pro-inflammatory cytokines IL-1α and IL-1β are among the first to be activated in the course of an inflammatory response and play a major role in both acute and chronic inflammation (4). Plasma levels of IL-1α and IL-1β show reproducible interindividual differences. Furthermore, IL-1 genetic patterns that are highly prevalent in the population, 60% of the white population, as noted in this study, have been associated with variations in the levels or expression of IL-1α (40), IL-1β (8), and the endogenous antagonist, IL-1Ra (41). The IL-1 composite genotypes used in this study were derived from combinations of the predominant functional haplotypes in the promoter region of the gene for IL-1β (6) and other SNPs in the IL-1α and β genes that have been associated with pro-inflammatory responses (10,42). IL-1β haplotypes exhibit allele-specific differences in nuclear protein binding and transcription rates (6). IL-1(+) genotypes are associated with enhanced generation of IL-1β when mononuclear cells are stimulated (8) and have been associated with higher IL-1β levels in plasma (41). Some of the 3 composite genotypes that compose the IL-1(+) pattern for this study have been associated with significantly elevated hsCRP levels in plasma compared with the IL-1(−) pattern (7,8,10,43,44). It should be noted that although the IL-1 genotype association with elevated IL-1β expression is also significant in gastric mucosa, the genotypes associated with elevated expression appear to be different from those reported for peripheral blood mononuclear cells (45,46).

IL-1Ra is an important component of the net IL-1 biological activity of this system, as signaled through the IL-1 receptor type 1, and has been implicated in atherosclerotic cardiovascular diseases (3). Variants in the gene for IL-1Ra (*IL1RN*) have been associated with lower expression and circulating levels of IL-1Ra (9,47,48) and with cardiovascular disease outcomes in some, but not all, studies (14,20,49). Several variants in *IL1RN* and other genes in the IL-1 cluster on chromosome 2q13-14 are in linkage disequilibrium with the specific *IL1A* and *IL1B* markers used in this study. For example, in a population cohort of 839 unrelated white patients who tested positive

for the IL-1 genotype patterns used in the current study, 64.5% were also homozygous for the T allele of *IL1RN* (+2018, rs419598) compared with 35.0% of those who tested negative. Future studies with larger datasets may allow for the analysis of contributions by other variants in the IL-1 region, in light of the strong linkage disequilibrium.

Vascular wall cells, such as endothelial cells and smooth muscle cells, as well as macrophages and monocytes, can produce IL-1β and IL-1Ra, and these cytokines are also present in human atherosclerotic lesions (3,17,19,50). It has been shown that IL-1β promoter haplotype pairs are associated with higher levels of IL-1β in plasma and from stimulated peripheral blood monocytes from patients, as well as elevated levels of CRP (10). In addition, the IL-1(+) genotype patterns used in the current study tag haplotypes that include the T allele of *IL1A*(−889, rs1800587), which has been shown to alter transcription factor binding sites in the *IL1A* gene (51) and was associated with increased levels of IL-1α protein in human gingival fluid samples (40). Transgenic mouse models with variations in IL-1 genotypes further support the causal role of IL-1 in atherogenesis (reviewed by Fearon and Fearon [3] and Ridker et al. [26]). In *IL1RA* knockout mice, unopposed IL-1 biological activity resulted in spontaneous arterial inflammation with massive infiltration of macrophages and $CD4^+$, interferon γ^+ T cells at branch points in intermediate and large arteries (52,53). Decreases in IL-1 biological activity in apoE-deficient mice decreased the rate and extent of atherosclerosis formation (54,55). By contrast, increases in IL-1 activity increased atherosclerotic lesion size with more macrophages within lesions (56). Furthermore, anakinra, a recombinant form of human IL-1Ra, improves vascular function in patients with rheumatoid arthritis (57).

In experimental studies, oxidized phospholipids interact with cells in the vessel wall and promote pro-inflammatory and pro-atherogenic properties. For example, in a large-scale gene-expression analysis involving 9,600 complementary deoxyribonucleic acid targets, *IL1B* was one of the differentially overexpressed genes when macrophages were loaded with oxidized low-density lipoprotein (OxLDL), which is known to be enriched in OxPL detected by E06, compared with acetylated-LDL loading (58). OxLDL stimulation of coronary artery smooth muscle cells also led to significant overexpression of IL-1β (59). More specifically, stimulation of endothelial cells and macrophages with OxPL leads to prominent expression of IL-1α and IL-1β (60,61), and in turn, stimulation of endothelial cells with IL-1β leads to the generation of such OxPLs (62). Thus, it is reasonable to hypothesize that the polymorphisms of the IL-1 family might influence the expression of inflammatory responses to OxPL. Indeed, supporting data show that IL-1 genetic variations have been associated with acute coronary events, CAD, and stroke (11–16,20–23,25,57,63,64).

In clinical studies, elevated OxPL/apoB levels have predicted new CVD events (29,33,35,65). This study has expanded our understanding of the underlying mechanisms behind this risk by showing that the enhanced risk for CAD and CAD events mediated by OxPL/apoB and Lp(a) is particularly potent in IL-1(+) patients. Interestingly, this risk for CAD persisted despite Lp(a) in the present model, suggesting that in certain patient populations, such as patients <60 years of age, OxPL/apoB may be a better predictor than Lp(a). Patients with an underlying genetic predisposition to inflammation and dyslipidemia have exposure to cardiovascular risk from birth, which may explain why IL-1(+) patients ≤60 years of age with elevated Lp(a) and OxPL/apoB levels are at a particularly elevated risk for premature CAD. Consistent with the role of lifelong exposure to a genetic predisposition to inflammation and genetically determined Lp(a) levels, it was demonstrated in this study that IL-1(+) patients with Lp(a) or OxPL/apoB levels above the median presented for coronary angiography several years earlier than did those in the lowest quartiles.

It is noteworthy that in this population, the IL-1 genotype effect on the risk for CAD was more pronounced in patients above the median of hsCRP, a biomarker of inflammation generated secondary to cytokines such as IL-6 and IL-1. Similarly, the *LPA* gene contains an IL-6 response element (66), and patients with inflammatory disorders such as rheumatoid arthritis have elevated Lp(a) levels, which are reduced on treatment with the IL-6Ra antibody tocilizumab (67,68). The CANTOS trial will be instrumental in testing whether inhibition of inflammatory responses leads to a lower rate of cardiovascular events. The data from this study suggest that patients with the IL-1(+) genotype and elevated OxPL/apoB and/or Lp(a) levels are a particularly high-risk subset that may maximally benefit from therapies aimed at inhibiting IL-1β and IL-1α responses.

Study limitations. Limitations of this study included that patients were selected from a population referred for coronary angiography for clinical indications and thus the data may not be generalizable to broader populations. This study also included predominantly white patients whose IL-1 genotypes and genetic associations may differ from those of other ethnic groups, and it will be important to study these associations in other populations.

Conclusions

This study suggests that the previously demonstrated contribution of OxPL/apoB and Lp(a) on angiographically documented CAD and CAD events is conditional on pro-inflammatory IL-1 genotypes. This novel paradigm links the etiology of atherogenesis attributed to OxPL and Lp(a) from genetics to clinical expression of CAD. If confirmed and validated in prospective populations, these findings may

facilitate our understanding of atherogenesis and provide enhanced tools for the diagnosis and treatment of cardiovascular disease.

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Key Words: atherosclerosis ■ genetic risk stratification ■ haplotype ■ IL-1 ■ inflammation ■ lipoprotein(a) ■ lipoproteins ■ oxidation ■ oxidized phospholipids ■ polymorphism.

APPENDIX

For an expanded Methods section, please see the online version of this article.