Association of anti-oxidized LDL and candidate genes with severity of coronary stenosis in the Women's Ischemia Syndrome Evaluation study

Qi Chen,* Steven E. Reis,† Candace Kammerer,* Wendy Craig,§ Dennis M. McNamara,† Richard Holubkov, Barry L. Sharaf,†† George Sopko,§§ Daniel F. Pauly,*** C. Noel Bairey Merz,††† and M. Ilyas Kamboh[1],* for the WISE study group**

Department of Human Genetics,* and Cardiovascular Institute, Department of Medicine, School of Medicine,† University of Pittsburgh, Pittsburgh, PA;Foundation for Blood Research,§ Scarborough, ME;Intermountain Injury Control Research Center,** Department of Pediatrics, School of Medicine, University of Utah, Salt Lake City, UT;Division of Cardiology,†† Rhode Island Hospital, Providence, RI;Division of Heart and Vascular Diseases,§§ National Heart, Lung, and Blood Institute, Bethesda, MD; Division of Cardiology,*** University of Florida, Gainesville, FL; and Cedars-Sinai Heart Institute,††† Los Angeles, CA

Abstract Atherosclerosis is the major cause of coronary artery disease (CAD), and oxidized LDL (oxLDL) is believed to play a key role in the initiation of the atherosclerotic process. Recent studies show that inflammation and autoimmune reactions are also relevant in atherosclerosis. In this study, we examined the association of antibodies against oxLDL (anti-oxLDL) with the severity of CAD in 558 Women's Ischemia Syndrome Evaluation (WISE) study samples (465 whites; 93 blacks) determined by coronary stenosis (<20%, 20%–49%, >50% stenosis). We also examined the relationship of anti-oxLDL with serum lipid levels and nine candidate genes including *APOE*, *APOH*, *APOA5*, *LPL*, *LRP1*, *HL*, *CETP*, *PON1*, and *OLR1*. IgM anti-oxLDL levels were significantly higher in the >20% stenosis group than in the ∩20% stenosis group in whites (0.69 ± 0.02 vs. 0.64 ± 0.01, respectively; $P = 0.02$). IgM anti-oxLDL levels correlated significantly with total cholesterol ($r^2 = 0.01$; $P = 0.03$) and LDL cholesterol ($r^2 = 0.017$; $P = 0.004$) in whites. Multiple regression analysis revealed a suggestive association of LPL/S447X single-nucleotide polymorphism (SNP) with both IgG anti-oxLDL ($P = 0.02$) and IgM anti-oxLDL ($P = 0.07$), as well as between IgM anti-oxLDL and the OLR1/3′UTR SNP ($P = 0.020$). Our data suggest that higher IgM anti-oxLDL levels may provide protection against coronary stenosis and that genetic variation in some candidate genes are determinants of anti-oxLDL levels.—Chen, Q., S. E. Reis, C. Kammerer, W. Craig, D. M. McNamara, R. Holubkov, B. L. Sharaf, G. Sopko, D. F. Pauly, C. N. B. Merz, and M. Ilyas Kamboh for the WISE study group. **Association of anti-oxidized LDL and candidate genes with severity of coronary stenosis in the WISE study. *J. Lipid Res*. 2011.** 52: **801–807.**

Supplementary key words anti-oxLDL antibodies • genetics • low density lipoprotein

Coronary artery disease (CAD) is a multifactorial chronic disease caused by atherosclerosis. Although the initiation and progression of atherosclerosis depends largely on genetic factors and life style factors, the underlying cellular and molecular mechanism remains unclear. Accumulating data suggest that oxidized low-density lipoprotein (oxLDL) plays an important role in the development and progression of atherosclerosis (1, 2). OxLDL, generated by the action of reactive oxygen species, is taken up by macrophages, which develop into foam cells. Autoantibodies against oxLDL (anti-oxLDL) are found in both atherosclerotic lesions and plasma (3), and thus, the state of oxidative stress might be measured by serum oxLDL antibody levels (4). The serum levels of anti-oxLDL have been reported to predict the progression of carotid and coronary atherosclerosis (5–8). Published studies assessing the relationship between anti-oxLDL antibodies

This work was supported by National Heart, Lung and Blood Institutes contracts R01-HL-54900, R01-HL112883, R01-HL115215, N01-HV-68161, N01-HV-68162, N01-HV-68163, and N01-HV-68164; by GCRC Grant MO1-RR-00425 from the National Center for Research Resources; and by grants from the Gustavus and Louis Pfeiffer Research Foundation, Denville, NJ; the Ladies Hospital Aid Society of Western Pennsylvania, Pittsburgh, PA; and the Women's Guild of Cedars-Sinai Medical Center, the Edythe L. Broad Women's Heart Research Endowment, Cedars-Sinai Medical Center, and the Barbra Streisand Women's Heart Disease Research and Education Program, Cedars-Sinai Medical Center, Los Angeles, CA.

Manuscript received 24 November 2010 and in revised form 10 January 2011.

Published, JLR Papers in Press, January 20, 2011
DOI 10.1194/jlr.M012963

Abbreviations: anti-oxLDL, autoantibodies against oxLDL; CAD, coronary artery disease; MDA-LDL, malondialdehyde-modified LDL; oxLDL, oxidized low-density lipoprotein; SNP, single-nucleotide polymorphism; UTR, untranslated region.

[1]To whom correspondence should be addressed.
e-mail: kamboh@pitt.edu

and atherosclerosis severity by different methods have yielded inconsistent results (6, 9–12) partly because the two antibodies of oxLDL (IgM and IgG) have different mechanistic functions in the atherosclerosis pathway. While IgM antibodies inhibit macrophage uptake of oxLDL (13, 14), oxLDL and IgM immune complexes induce accumulation of macrophages (14). Genetic factors may also affect the association of oxLDL levels with atherosclerosis. While a number of candidate genes have been associated with CAD risk (15), their possible associations with oxLDL parameters have not been studied extensively.

The present study was designed to (*1*) investigate the separate associations of IgM and IgG anti-oxLDL with stenosis severity in the well-characterized cohort of Women's Ischemic Syndrome Evaluation (WISE) study, and to (*2*) examine the association between anti-oxLDL levels and genetic variation in selected candidate genes, including the apolipoprotein E (*APOE*), apolipoprotein H (*APOH*), apolipoprotein 5 (*APOA5*), lipoprotein lipase (*LPL*), low-density lipoprotein receptor related protein-1 (*LRP1*), hepatic lipase (*HL*), cholesteryl ester transfer protein (*CETP*), paraoxonase (*PON1* and *PON2*), and oxLDL receptor 1 (*OLR1*) genes. These candidate genes are actively involved in the lipoprotein metabolism pathway, and thus variations in these genes might have significant impact on the levels of anti-oxLDL.

MATERIALS AND METHODS

Subjects

Study subjects were collected as part of the WISE study. Detailed information on the study has been described elsewhere (16, 17). Briefly, female patients were recruited during their clinical examination at one of the four clinical centers (University of Alabama at Birmingham; Allegheny University of the Health Sciences at Pittsburgh; University of Florida at Gainesville; and University of Pittsburgh). Recruitment criteria included (*1*) ≥18 years of age; (*2*) presence of chest pain or other symptoms suggestive of myocardial ischemia; (*3*) clinically indicated coronary angiography; and (*4*) the ability to give informed written consent. Major exclusion criteria were: pregnancy, cardiomyopathy, contraindications to provocative diagnostic testing, New York Heart Association class IV congestive heart failure, recent myocardial infarction, significant valvular or congenital heart disease, and recent coronary angioplasty or coronary bypass surgery.

As previously described (18), patients were divided into three groups based on their angiographic CAD severity. Patients with <20% stenosis in all coronary arteries were labeled the normal group, having minimal stenosis (227 whites, 48 blacks); women with ≥1 stenosis of between 20% and 49% were considered to have mild stenosis (150 whites, 27 blacks); and women with ≥1 stenosis of ≥50% were considered to have severe stenosis (206 whites, 45 blacks). In addition, we also analyzed a measure of overall CAD severity using a score that was developed to account for severity of stenosis, adjusting for partial and complete collaterals, and lesion location (18). Coefficients of variation for the angiographic measurements ranged from 3.8%–6.3% (18). **Table 1** presents a comparison of lipid profile and other parameters among the three stenoses groups in the WISE sample. Informed consent was obtained from each subject, and the study was approved by the Institutional Review Board.

IgG and IgM anti-oxLDL measurement

Sera from 465 white and 93 black women in the WISE study were available to measure IgG and IgM anti-oxLDL. IgG and IgM autoantibodies against native and malondialdehyde-modified LDL (MDA-LDL) were assayed in sera by ELISA, as described elsewhere (19, 20), with the modification that sodium azide was omitted from the wash buffer. Data were calculated as the difference in antibody binding between MDA-LDL and native LDL and then expressed as a percentage of the value of the plasma pool.

Genotyping

The gene fragments containing each genetic polymorphism of a selected candidate gene were amplified by PCR, followed by restriction enzyme digestion for genotyping screening. Detailed methods are described elsewhere for the SNPs *APOA-5*/T−1131C (21) *APOE* E2/E3/E4 polymorphism (22), *APOH*/V247L and W316S (23), *CETP*/*Taq*1B (24), *HL*/C−514T (25), *OLR*1/3′ untranslated region (UTR) C/T (19), *LPL*/S447X and *Hind*III G/T (26), *LRP1*/A216V (27), and *PON1*/Q192R, L55M and *PON2*/S311C (16).

Statistical analysis

All continuous variables, including anti-oxLDL and lipid levels, were tested for distribution normality prior to analysis. To reduce the non-normality, in particular, total cholesterol and LDL cholesterol were transformed using a square root transformation, and triglycerides and HDL cholesterol were transformed using a natural logarithm transformation. All outliers (±4 standard deviations) were removed prior to statistical analyses, and 0–6 values were removed for each variable. All analyses were performed separately for whites and blacks.

Stepwise linear regression analysis was used to identify significant covariates for IgG anti-oxLDL and IgM anti-oxLDL (assuming an overall 10% level of significance). The potential covariates considered included age, body mass index, statins, drug use history, history of using other lipid-lowering agents, smoking, alcohol use, family history of CAD, history of hypertension, history of diabetes, menopause, and serum lipid levels. Due to the small number of blacks (n = 34), the possible association between the antibody levels and genetic variations was tested in whites only. Based on power calculations, with a sample size of 465 individuals, we would have 80% power at a P level of <0.05 to detect genotypic mean differences of ≥0.07 for IgG or IgM anti-oxLDL levels.

Pearson's correlation coefficients were calculated to determine significant relationships between the adjusted anti-oxLDL variables and lipid levels. All analyses were performed using R version 2.0.1 software (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Correlations between the measurements of anti-oxLDL antibodies and various covariates

Table 2 presents the pairwise correlations (r and P values) between the measurements of anti-oxLDL antibodies and all potential covariates available. Each covariate was examined for its association separately. In whites, the IgM antibody levels were positively correlated with total (r^2 = 0.01, P = 0.03) and LDL (r^2 = 0.017, P = 0.004) cholesterol, and cigarette smoking (r^2 = 0.014, P = 0.007), and negatively correlated with age (r^2 = 0.012, P = 0.02). White women with diabetes also had lower IgG antibody levels

TABLE 1. Patient characteristics based upon the stenosis of coronary arteries in the WISE sample

Characteristic	% of stenosis⩾1 in the epicardial coronary artery <20%	20%–49%	⩾50%	P
White women	(n = 185)	(n = 114)	(n = 166)	
Mean age (years) ± SEM	53.9 ± 0.76	58.5 ± 1.00	63.1 ± 0.92	<0.0001
Mean BMI (kg/m^2) ± SEM	29.5 ± 0.50	28.8 ± 0.56	28.6 ± 0.46	0.19
Former smoker N (%)	53 (28.8)	35 (30.7)	66 (39.7)	—
Current smoker N (%)	28 (15.2)	28 (24.5)	27 (16.3)	0.03
Alcohol use within previous 6 months N (%)	28 (15.1)	24 (21.0)	19 (11.7)	0.11
Lipid-lowering drug intake				
Statin N (%)	20 (10.8)	44 (38.6)	67 (40.4)	<0.0001
Other N (%)	2 (1.0)	8 (7.0)	9 (5.4)	0.02
Family history of CAD N (%)	117 (63.9)	76 (67.8)	110 (67.5)	0.71
History of hypertension N (%)	79 (42.7)	65 (57.0)	98 (59.7)	0.003
History of diabetes N (%)	19 (10.3)	19 (16.7)	61 (36.7)	<0.0001
Menopause N (%)	136 (73.9)	99 (87.6)	148 (90.2)	<0.0001
Mean total cholesterol (mg/dl) ± SEM	193.5 ± 2.93	196.2 ± 4.09	188.0 ± 3.22	0.21
Mean LDL cholesterol (mg/dl) ± SEM	111.1 ± 2.51	110.9 ± 3.39	105.2 ± 3.00	0.09
Mean triglycerides (mg/dl) ± SEM	144.3 ± 6.87	152.2 ± 9.31	162.8 ± 6.43	0.006
Mean HDL cholesterol (mg/dl) ± SEM	54.8 ± 0.88	53.4 ± 1.24	50.6 ± 0.78	0.002
Black women	(n = 39)	(n = 21)	(n = 34)	
Mean age (years) ± SEM	50 ± 2.00	57.3 ± 2.00	56.8 ± 1.99	0.01
Mean BMI (kg/m^2) ± SEM	31.4 ± 1.01	32.3 ± 1.19	31.5 ± 1.06	0.91
Former smoker N (%)	12 (30.7)	7 (33.3)	10 (29.4)	—
Current smoker N (%)	9 (23.1)	4 (19.0)	12 (35.3)	0.67
Alcohol use within the previous 6 months N (%)	5 (12.8)	4 (19.0)	4 (11.8)	0.73
Lipid-lowering drug intake				
Statin N (%)	6 (15.4)	8 (38.1)	12 (35.3)	0.08
Other N (%)	0 (0)	1 (4.8)	0 (0)	0.17
Family history of CAD N (%)	25 (65.8)	11 (57.9)	20 (64.5)	0.84
History of hypertension N (%)	27 (69.2)	17 (80.9)	31 (91.2)	0.06
History of diabetes N (%)	11 (28.2)	8 (38.1)	17 (51.5)	0.13
Menopause N (%)	26 (66.7)	17 (80.9)	27 (79.4)	0.34
Mean total cholesterol (mg/dl) ± SEM	175.3 ± 7.36	187.2 ± 6.04	197.6 ± 7.96	0.02
Mean LDL cholesterol (mg/dl) ± SEM	100.6 ± 5.17	104.9 ± 5.93	114.9 ± 7.44	0.14
Mean triglycerides (mg/dl) ± SEM	87.8 ± 9.46	106.6 ± 11.4	147.9 ± 15.7	<0.0001
Mean HDL cholesterol (mg/dl) ± SEM	54.4 ± 2.13	61.0 ± 2.75	51.7 ± 1.99	0.45

BMI, body mass index; SEM, standard error of the mean.

than nondiabetic women ($P = 0.04$). Among black women, only cigarette smoking was found to be significantly associated with IgG anti-oxLDL ($r^2 = 0.044$, $P = 0.04$). These significant covariates were included in the subsequent general linear regression analysis models to test the association between anti-oxLDL antibody levels and CAD severity (measured categorically as CAD stenosis groups and by a severity score), as well as the association between the antibody levels and genotypic variations.

Association between the anti-oxLDL antibody levels and the severity of stenosis

The relationship between the anti-oxLDL antibody levels and the severity of stenosis is presented in **Table 3**. Because the IgM anti-oxLDL antibody levels were similar in the 20%–49% and >50% stenosis groups, for the purpose of statistical analysis, we combined these two groups to compare with the <20% stenosis group. After adjusting for the effects of age, smoking, and total and LDL

TABLE 2. Correlation between anti-oxLDL measures and various potential covariates in the WISE sample

Covariate	White women (n = 465) IgG		White women (n = 465) IgM		Black women (n = 93) IgG		Black women (n = 93) IgM	
Potential covariates	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
Total cholesterol	0.02	0.62	0.10	**0.03**	0.09	0.36	0.07	0.48
Triglycerides	0.01	0.81	−0.03	0.48	0.13	0.21	0.06	0.57
HDL cholesterol	−0.04	0.43	0.007	0.88	−0.04	0.69	−0.05	0.66
LDL cholesterol	0.03	0.53	0.13	**0.004**	0.07	0.51	0.09	0.36
Statins drug use	0.07	0.12	0.0009	0.98	−0.02	0.86	−0.03	0.79
Other lipid lowering agents	−0.03	0.55	0.01	0.75	−0.14	0.18	−0.15	0.15
Cigarette smoking	0.05	0.26	0.12	**0.007**	0.21	**0.04**	0.19	0.05
Alcohol use	0.06	0.20	0.03	0.46	0.02	0.87	−0.009	0.93
Family history of CAD	0.02	0.65	0.008	0.86	0.01	0.92	−0.10	0.36
History of hypertension	0.01	0.74	0.005	0.90	0.06	0.59	−0.02	0.81
History of diabetes	−0.09	**0.04**	−0.06	0.17	−0.14	0.18	−0.15	0.15
Body mass index	0.004	0.93	0.02	0.67	−0.13	0.22	−0.10	0.35
Age	0.009	0.85	−0.11	**0.02**	−0.04	0.70	−0.08	0.41
Menopause	−0.005	0.91	−0.04	0.35	−0.09	0.40	−0.15	0.14

cholesterol levels, IgM anti-oxLDL antibody levels remained slightly but significantly higher in the <20% stenosis group than in the >20% stenosis groups (0.69 ± 0.02 vs. 0.64 ± 0.02, respectively; $P = 0.03$). After adjusting for history of diabetes, no significant association was found between IgG anti-oxLDL levels and stenosis severity. Finally, no significant association was observed between IgM or IgG anti-oxLDL level and severity of stenosis in black subjects. In contrast, we found no significant relationship between the angiographic severity score and IgM or IgG anti-oxLDL antibody levels ($P = 0.41$ and 0.88, respectively).

Association between the anti-oxLDL antibody levels and candidate genes

The results of association analyses between adjusted anti-oxLDL antibody levels and various candidate gene polymorphisms are summarized in **Table 4**. A significant association ($P = 0.02$) was observed for IgM anti-oxLDL levels and *OLR1* genotypes. The *OLR1*/3′UTR single-nucleotide polymorphism (SNP) showed gene dosage effects on the IgM antibody levels, with the lowest value in the TT genotype (mean = 0.64 ± 0.02), the highest value in the CC genotype (mean = 0.65 ± 0.02), and an intermediate value in the TC genotype (mean = 0.71 ± 0.02). As the XX genotype of the *LPL*/S447X SNP was uncommon among our subjects (n = 5), we combined XX and SX genotypes to compare with the SS wild-type genotype. The *LPL*/S447X SNP showed significant association with IgG anti-oxLDL ($P = 0.02$) and borderline association with IgM anti-oxLDL ($P = 0.07$) (Table 4). While 447X allele carriers had higher IgM antibody levels than SS homozygotes (0.72 ± 0.03 and 0.65 ± 0.01, respectively), the reverse trend was observed for the IgG antibody level (0.71 ± 0.02 vs. 0.93 ± 0.01). Association analyses were also carried out to determine whether these polymorphisms were significantly correlated with stenosis severity; however, no significant results were discovered (data not shown).

DISCUSSION

It has been suggested that progression of atherosclerosis is modified by an immune reaction trigged by different immunogens (3, 28–32), with oxLDL as the major immunogen for such reaction (3, 31). In animal studies, immunization with modified LDL results in an increased titer of antibodies against MDA-LDL and suppression of atherosclerosis (33). Following the initial report of a significant association between anti-oxLDL antibodies and the progression of carotid intima-media thickness in 30 healthy Finnish men (7), subsequent studies have shown inconsistent associations between anti-oxLDL antibodies and cardiovascular disease or related risk factors, most probably due to methodological variations in the anti-oxLDL assay (34). The novel contribution of the present study is the examination of the impact of genetic variation in candidate genes on both IgM and IgG anti-oxLDL antibody levels while testing the association between anti-oxLDL antibodies and CAD severity in women. The WISE study participants are well characterized with detailed angiographic and ischemic assessment, allowing patient categorization by the severity of stenosis into <20% stenosis, 20%–49% stenosis, and >50% stenosis groups.

Age and gender are two major physiological factors related to the individuals' anti-oxLDL levels in the general population (34–36). Since only women are included in our study, gender-specific effects on anti-oxLDL antibodies were not evaluated. However, we found that age is inversely correlated with IgM anti-oxLDL levels ($r = -0.11$, $P = 0.02$), indicating that women of a younger age have higher levels of IgM antibody than older women. Consistent with this finding, Tinahones et al. (35) reported that the levels of anti-oxLDL antibodies were significantly higher in women 16–35 years old, with a significant decrease after 36 years old. Lower levels of anti-oxLDL antibodies have also been reported in elderly persons with high cardiovascular risk factors (37). In addition to the inverse relationship with age, we also found in whites a significant association between smoking and higher levels of both IgM and IgG anti-oxLDL, which is consistent with earlier reports (38). Given that higher levels of IgM anti-oxLDL are associated with less severe stenosis, this relationship with smoking appears to be counterintuitive; it may, however, reflect the coexistence of additional immunologic processes involving anti-oxLDL. For example, cigarette smoke increases leukocyte, platelet, and monocyte adhesion to endothelial cells and platelet aggregation, which might expand a local inflammatory response (38).

The major finding in this study is the inverse association between IgM anti-oxLDL and the severity of steno-

TABLE 3. Mean anti-oxLDL antibody levels among coronary stenosis groups

	Stenosis of ⩾1 in epicardial coronary artery			
Antibody	<20%	20–49%	⩾50%	P
White women	(n = 185)	(n = 114)	(n = 166)	
IgG anti-oxLDL	0.65 ± 0.01	0.64 ± 0.02	0.65 ± 0.02	0.84
IgM anti-oxLDL	0.69 ± 0.02	0.64 ± 0.02	0.64 ± 0.02	**0.02**
Black women	(n = 39)	(n = 21)	(n = 34)	
IgG anti-oxLDL	0.69 ± 0.03	0.65 ± 0.03	0.75 ± 0.04	0.15
IgM anti-oxLDL	0.65 ± 0.03	0.61 ± 0.05	0.70 ± 0.04	0.41

Data show adjusted means ± standard deviations (SD) of anti-oxLDL antibody levels (%M-L) among coronary stenosis groups, where %M-L is the difference in antibody binding between MDA-LDL and native LDL expressed as a percentage of the value of the plasma pool.

ANOVA *P* values adjusting for total and LDL cholesterol, history of smoking, and age.

TABLE 4. *P* values for associations between adjusted anti-oxLDL antibody levels and genetic polymorphisms in white women

Genetic polymorphism	IgG % M-L	IgM % M-L
APOH/V247L	0.96	0.12
PON2/S311C	0.21	0.66
PON1/Q192R	0.22	0.44
PON1/L55M	0.23	0.17
APOE/E2/E3/E4	0.85	0.14
APOH/W316S	0.31	0.41
OLR1/3′UTR C/T	0.58	**0.02**
LRP1/A216V	0.62	0.85
LPL/S447X	**0.02**	0.07
LPL/*Hind* III G/T	0.58	0.69
CETP/*Taq*1B	0.34	0.14
HL/C−514T	0.37	0.62
APOA5/T−1131C	0.30	0.87

%M-L is the difference in antibody binding between MDA-LDL and native LDL expressed as a percentage of the value of the plasma pool.

sis. IgM anti-oxLDL levels were significantly lower in the moderate and severe stenosis groups than in the minimal stenosis group (0.64 ± 0.01 vs. 0.69 ± 0.02, respectively; $P = 0.02$). A similar relationship between anti-oxLDL levels and CAD stenosis has been shown by Sherer et al. (11), with anti-oxLDL levels higher in the insignificant CAD stenosis group than in the significant CAD stenosis group, but the difference was not statistically significant (311 ± 163 vs. 290 ± 142, respectively; $P = 0.68$). Tornvall et al. (12) observed a negative correlation between autoantibody titers of modified LDL and coronary atherosclerosis score. However, those studies did not differentiate between the two antibodies IgM and IgG in their analyses. On the other hand, those studies that examined IgG and IgM antibodies separately reported an inverse relationship of IgM anti-oxLDL antibody titer with coronary (39) and carotid (40–42) artery atherosclerosis and with hypertension (43). Our data are also consistent with those of van de Vijver et al. (10), who reported no association between IgG anti-oxLDL and angiographic atherosclerosis severity. Taken together, these studies are consistent in that they suggestion that oxLDL antibody has a protective role in atherosclerosis and indicate that IgM anti-oxLDL antibodies may be particularly important. It has been shown that IgM anti-oxLDL antibodies have the capacity to block the uptake of oxLDL by macrophages, and thus, they could modulate atherosclerotic processes by preventing foam cell formation and by removing minimally oxLDL from plasma (44). Furthermore, Craig et al. (45) demonstrated that IgM anti-oxLDL antibodies are higher in the presence of an atheroma lipid composition associated with greater plaque stability. Some earlier findings also support this hypothesis. The immunization of WHHL rabbits with MDA-LDL has been shown to inhibit the progression of atherogenesis (33), which has been confirmed in several other animal models (46–49). Zhou et al. (49) showed that immunization of mice with MDA-LDL not only provided protection against atherosclerosis, the increased titers of antibodies also negatively correlated with the degree of lesion formation (49). The fact that IgM anti-oxLDL antibodies appear to be protective in atherosclerosis suggests a new immunologic paradigm that the immune recognition of oxidation-specific epitopes contributes to physiologic homeostasis of lipoproteins that have undergone oxidative changes that could possibly slow down atherosclerosis (50). Due to the complexity of the underlying mechanism, further molecular and cellular level characterizations of IgM anti-oxLDL antibodies are needed. In addition to the observed association between anti-oxLDL antibodies and severity of coronary stenosis, it would also be useful to determine the association between oxLDL levels and degree of stenosis. Although oxLDL levels were not available in this study, recently Tsimikas et al. (51) reported a significant association of oxidized phospholipids on apolipoprotein B-100 particles and Lp(a) with an increased risk of CAD events in a prospective case-control study, indicating the value of oxidation-specific biomarkers in CAD.

In our study, we also investigated the impact of 13 polymorphisms in 10 candidate genes on anti-oxLDL levels. The chosen candidate genes, including *APOE*, *APOH*, *APOA5*, *LPL*, *LRP1*, *HL*, *CETP*, *PON1*, *PON2*, and *OLR1* are involved in lipid metabolism, and genetic variations in these genes have been found to be associated with CAD and/or plasma lipid profile (52–57). We found one significant association with anti-IgG, and one with anti-IgM. The *LPL*/S447X polymorphism significantly correlated with anti-IgG ($P = 0.02$) and had borderline significant association with anti-IgM ($P = 0.07$). The unadjusted anti-IgG levels were higher in the wild-type SS genotype than in X allele carriers (0.93 ± 0.01 vs. 0.71 ± 0.02). On the other hand, X allele carriers had higher anti-IgM levels than SS homozygotes (0.72 ± 0.03 vs. 0.65 ± 0.01, respectively). The *OLR1*/3′UTR polymorphism was significantly associated with anti-IgM. There was a gene-dosage effect such that the highest levels were observed in CC homozygotes (mean, 0.71 ± 0.02), intermediate in CT heterozygotes (mean, 0.65 ± 0.02), and lowest in TT homozygotes (mean, 0.64 ± 0.02). The *OLR1* gene is a receptor for oxLDL, and it could affect the risk of CAD through its direct effect on the metabolism of oxLDL. Previously we have shown that the *OLR1*/3′UTR C allele, which is associated with higher anti-IgM levels, is protective against coronary stenosis (19). LPL has both anti-atherogenic and proatherogenic roles (58). The majority of the total LPL is located in the capillary endothelium where it hydrolyzes triglycerides and thus acts as an anti-atherogenic. However, a small fraction of LPL is also located in the arterial wall where it can act as a proatherogenic by retaining LDL in arterial intima and binding to oxLDL. A number of studies have suggested that the *LPL*/447X allele is associated with a favorable lipid profile and lower CAD risk (59). In this study, the 447X allele carriers had higher anti-IgM levels, which are protective against coronary stenosis. However, additional studies in independent samples are needed to confirm these genetic association data because of the modest observed *P* values, which will not stand if corrected for multiple testing.

CONCLUSION

In conclusion, our study shows that levels of IgM anti-oxLDL are significantly lower in patients with severe CAD than in patients with minimal stenosis. These data, in conjunction with previous studies, suggest that IgM anti-oxLDL may be protective in atherosclerosis. Our data also suggest that genetic variations in selected genes associated with atherosclerosis risk can influence serum IgM or IgG anti-oxLDL levels.

REFERENCES

1. Witztum, J. L., and D. Steinberg. 1991. Role of oxidized low density lipoprotein in atherogenesis. *J. Clin. Invest.* **88:** 1785–1792.
2. Ohashi, R., H. Mu, Q. Yao, and C. Chen. 2004. Atherosclerosis: immunopathogenesis and immunotherapy. *Med. Sci. Monit.* **10:** RA255–RA260.
3. Witztum, J. L. 1994. The oxidation hypothesis of atherosclerosis. *Lancet.* **344:** 793–795.
4. Suzuki, K., Y. Ito, K. Wakai, M. Kawado, S. Hashimoto, H. Toyoshima, M. Kojima, S. Tokudome, N. Hayakawa, Y. Watanabe, et al. 2004. Serum oxidized low-density lipoprotein levels and risk of colorectal cancer: a case-control study nested in the Japan Collaborative Cohort Study. *Cancer Epidemiol. Biomarkers Prev.* **13:** 1781–1787.
5. Lehtimaki, T., S. Lehtinen, T. Solakivi, M. Nikkila, O. Jaakkola, H. Jokela, S. Yla-Herttuala, J. S. Luoma, T. Koivula, and T. Nikkari. 1999. Autoantibodies against oxidized low density lipoprotein in patients with angiographically verified coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **19:** 23–27.
6. Inoue, T., T. Uchida, H. Kamishirado, K. Takayanagi, T. Hayashi, and S. Morooka. 2001. Clinical significance of antibody against oxidized low density lipoprotein in patients with atherosclerotic coronary artery disease. *J. Am. Coll. Cardiol.* **37:** 775–779.
7. Salonen, J. T., S. Yla-Herttuala, R. Yamamoto, S. Butler, H. Korpela, R. Salonen, K. Nyyssonen, W. Palinski, and J. L. Witztum. 1992. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet.* **339:** 883–887.
8. Puurunen, M., M. Manttari, V. Manninen, L. Tenkanen, G. Alfthan, C. Ehnholm, O. Vaarala, K. Aho, and T. Palosuo. 1994. Antibody against oxidized low-density lipoprotein predicting myocardial infarction. *Arch. Intern. Med.* **154:** 2605–2609.
9. Rossi, G. P., M. Cesari, R. De Toni, M. Zanchetta, G. Maiolino, L. Pedon, C. Ganzaroli, P. Maiolino, and A. C. Pessina. 2003. Antibodies to oxidized low-density lipoproteins and angiographically assessed coronary artery disease in white patients. *Circulation.* **108:** 2467–2472.
10. van de Vijver, L. P., R. Steyger, G. van Poppel, J. M. Boer, D. A. Kruijssen, J. C. Seidell, and H. M. Princen. 1996. Autoantibodies against MDA-LDL in subjects with severe and minor atherosclerosis and healthy population controls. *Atherosclerosis.* **122:** 245–253.
11. Sherer, Y., A. Tenenbaum, S. Praprotnik, J. Shemesh, M. Blank, E. Z. Fisman, D. Harats, J. George, Y. Levy, J. B. Peter, et al. 2001. Coronary artery disease but not coronary calcification is associated with elevated levels of cardiolipin, beta-2-glycoprotein-I, and oxidized LDL antibodies. *Cardiology.* **95:** 20–24.
12. Tornvall, P., G. Waeg, J. Nilsson, A. Hamsten, and J. Regnstrom. 2003. Autoantibodies against modified low-density lipoproteins in coronary artery disease. *Atherosclerosis.* **167:** 347–353.
13. Horkko, S., D. A. Bird, E. Miller, H. Itabe, N. Leitinger, G. Subbanagounder, J. A. Berliner, P. Friedman, E. A. Dennis, L. K. Curtiss, et al. 1999. Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. *J. Clin. Invest.* **103:** 117–128.
14. Oksjoki, R., P. T. Kovanen, K. A. Lindstedt, B. Jansson, and M. O. Pentikainen. 2006. OxLDL-IgG immune complexes induce survival of human monocytes. *Arterioscler. Thromb. Vasc. Biol.* **26:** 576–583.
15. Winkelmann, B. R., and J. Hager. 2000. Genetic variation in coronary heart disease and myocardial infarction: methodological overview and clinical evidence. *Pharmacogenomics.* **1:** 73–94.
16. Chen, Q., S. E. Reis, C. M. Kammerer, D. M. McNamara, R. Holubkov, B. L. Sharaf, G. Sopko, D. F. Pauly, C. N. Merz, and M. I. Kamboh. 2003. Association between the severity of angiographic coronary artery disease and paraoxonase gene polymorphisms in the National Heart, Lung, and Blood Institute-sponsored Women's Ischemia Syndrome Evaluation (WISE) study. *Am. J. Hum. Genet.* **72:** 13–22.
17. Merz, C. N., S. F. Kelsey, C. J. Pepine, N. Reichek, S. E. Reis, W. J. Rogers, B. L. Sharaf, and G. Sopko. 1999. The Women's Ischemia Syndrome Evaluation (WISE) study: protocol design, methodology and feasibility report. *J. Am. Coll. Cardiol.* **33:** 1453–1461.
18. Sharaf, B. L., C. J. Pepine, R. A. Kerensky, S. E. Reis, N. Reichek, W. J. Rogers, G. Sopko, S. F. Kelsey, R. Holubkov, M. Olson, et al.for the WISE Study Group. 2001. Detailed angiographic analysis of women with suspected ischemic chest pain (pilot phase data from the NHLBI-sponsored Women's Ischemia Syndrome Evaluation [WISE] Study Angiographic Core Laboratory). *Am. J. Cardiol.* **87:** 937–941.
19. Chen, Q., S. E. Reis, C. Kammerer, W. Y. Craig, S. E. LaPierre, E. L. Zimmer, D. M. McNamara, D. F. Pauly, B. Sharaf, R. Holubkov, et al. 2003. Genetic variation in lectin-like oxidized low-density lipoprotein receptor 1 (LOX1) gene and the risk of coronary artery disease. *Circulation.* **107:** 3146–3151.
20. Craig, W. Y., S. E. Poulin, C. P. Nelson, and R. F. Ritchie. 1994. ELISA of IgG antibody to oxidized low-density lipoprotein: effects of blocking buffer and method of data expression. *Clin. Chem.* **40:** 882–888.
21. Pennacchio, L. A., M. Olivier, J. A. Hubacek, J. C. Cohen, D. R. Cox, J. C. Fruchart, R. M. Krauss, and E. M. Rubin. 2001. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science.* **294:** 169–173.
22. Kamboh, M. I., C. E. Aston, and R. F. Hamman. 1995. The relationship of APOE polymorphism and cholesterol levels in normoglycemic and diabetic subjects in a biethnic population from the San Luis Valley, Colorado. *Atherosclerosis.* **112:** 145–159.
23. Kamboh, M. I., S. Manzi, H. Mehdi, S. Fitzgerald, D. K. Sanghera, L. H. Kuller, and C. E. Atson. 1999. Genetic variation in apolipoprotein H (beta2-glycoprotein I) affects the occurrence of antiphospholipid antibodies and apolipoprotein H concentrations in systemic lupus erythematosus. *Lupus.* **8:** 742–750.
24. Boekholdt, S. M., F. M. Sacks, J. W. Jukema, J. Shepherd, D. J. Freeman, A. D. McMahon, F. Cambien, V. Nicaud, G. J. de Grooth, P. J. Talmud, et al. 2005. Cholesteryl ester transfer protein TaqIB variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient meta-analysis of 13,677 subjects. *Circulation.* **111:** 278–287.
25. Zhang, C., R. Lopez-Ridaura, E. B. Rimm, N. Rifai, D. J. Hunter, and F. B. Hu. 2005. Interactions between the −514C->T polymorphism of the hepatic lipase gene and lifestyle factors in relation to HDL concentrations among US diabetic men. *Am. J. Clin. Nutr.* **81:** 1429–1435.
26. Liu, A., L. Li, W. Cao, S. Shan, J. Lu, X. Guo, and Y. Hu. 2005. [The association of S447X and Hind III polymorphism in the lipoprotein lipase gene with dyslipidemia of the metabolic syndrome in patients with essential hypertension]. *Zhonghua yixue yichuanxue zazhi.* **22:** 151–157.
27. Schweer, D., M. Jacobsen, A. Ziegler, S. Jakel, W. H. Oertel, N. Sommer, and B. Hemmer. 2001. No association of three polymorphisms in the alpha-2-macroglobulin and lipoprotein related receptor genes with multiple sclerosis. *J. Neuroimmunol.* **118:** 300–303.
28. Wick, G., G. Schett, A. Amberger, R. Kleindienst, and Q. Xu. 1995. Is atherosclerosis an immunologically mediated disease? *Immunol. Today.* **16:** 27–33.
29. Libby, P., and G. K. Hansson. 1991. Involvement of the immune system in human atherogenesis: current knowledge and unanswered questions. *Lab. Invest.* **64:** 5–15.
30. George, J., D. Harats, B. Gilburd, and Y. Shoenfeld. 1996. Emerging cross-regulatory roles of immunity and autoimmunity in atherosclerosis. *Immunol. Res.* **15:** 315–322.
31. Steinberg, D., S. Parthasarathy, T. E. Carew, J. C. Khoo, and J. L. Witztum. 1989. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **320:** 915–924.
32. Xu, Q., and G. Wick. 1996. The role of heat shock proteins in protection and pathophysiology of the arterial wall. *Mol. Med. Today.* **2:** 372–379.
33. Palinski, W., E. Miller, and J. L. Witztum. 1995. Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with ho-

mologous malondialdehyde-modified LDL reduces atherogenesis. *Proc. Natl. Acad. Sci. U S A.* **92:** 821–825.
34. Craig, W. Y. 1995. Autoantibodies against oxidized low-density lipoprotein: a review of clinical findings and assay methodology. *J. Clin. Lab. Anal.* **9:** 70–74.
35. Tinahones, F. J., J. M. Gomez-Zumaquero, L. Garrido-Sanchez, E. Garcia-Fuentes, G. Rojo-Martinez, I. Esteva, M. S. de Adana, F. Cardona, and F. Soriguer. 2005. Influence of age and sex on levels of anti-oxidized LDL antibodies and anti-LDL immune complexes in the general population. *J. Lipid Res.* **46:** 452–457.
36. Block, G., M. Dietrich, E. P. Norkus, J. D. Morrow, M. Hudes, B. Caan, and L. Packer. 2002. Factors associated with oxidative stress in human populations. *Am. J. Epidemiol.* **156:** 274–285.
37. Balada, E., J. Ordi-Ros, L. Matas, M. Mauri, S. Bujan, and M. Vilardell-Tarres. 2002. [Atherosclerosis and anti-oxidized low density lipoprotein antibodies in an elderly population]. *Med. Clin. (Barc.).* **119:** 161–165.
38. Wu, R., G. Shen, R. Morris, M. Patnaik, and J. B. Peter. 2005. Elevated autoantibodies against oxidized palmitoyl arachidonoyl phosphocholine in patients with hypertension and myocardial infarction. *J. Autoimmun.* **24:** 353–360.
39. Garrido-Sanchez, L., P. Chinchurreta, E. Garcia-Fuentes, M. Mora, and F. J. Tinahones. 2010. A higher level of IgM anti-oxidized LDL antibodies is associated with a lower severity of coronary atherosclerosis in patients on statins. *Int. J. Cardiol.* 145: 263–264.
40. Karvonen, J., M. Paivansalo, Y. A. Kesaniemi, and S. Horkko. 2003. Immunoglobulin M type of autoantibodies to oxidized low-density lipoprotein has an inverse relation to carotid artery atherosclerosis. *Circulation.* **108:** 2107–2112.
41. Fukumoto, M., T. Shoji, M. Emoto, T. Kawagishi, Y. Okuno, and Y. Nishizawa. 2000. Antibodies against oxidized LDL and carotid artery intima-media thickness in a healthy population. *Arterioscler. Thromb. Vasc. Biol.* **20:** 703–707.
42. Hulthe, J., L. Bokemark, and B. Fagerberg. 2001. Antibodies to oxidized LDL in relation to intima-media thickness in carotid and femoral arteries in 58-year-old subjectively clinically healthy men. *Arterioscler. Thromb. Vasc. Biol.* **21:** 101–107.
43. Wu, R., U. de Faire, C. Lemne, J. L. Witztum, and J. Frostegard. 1999. Autoantibodies to OxLDL are decreased in individuals with borderline hypertension. *Hypertension.* **33:** 53–59.
44. Chang, M. K., C. Bergmark, A. Laurila, S. Horkko, K. H. Han, P. Friedman, E. A. Dennis, and J. L. Witztum. 1999. Monoclonal antibodies against oxidized low-density lipoprotein bind to apoptotic cells and inhibit their phagocytosis by elicited macrophages: evidence that oxidation-specific epitopes mediate macrophage recognition. *Proc. Natl. Acad. Sci. U S A.* **96:** 6353–6358.
45. Craig, W. Y., M. W. Rawstron, C. A. Rundell, E. Robinson, S. E. Poulin, L. M. Neveux, P. M. Nishina, and L. M. Keilson. 1999. Relationship between lipoprotein- and oxidation-related variables and atheroma lipid composition in subjects undergoing coronary artery bypass graft surgery. *Arterioscler. Thromb. Vasc. Biol.* **19:** 1512–1517.
46. Ameli, S., A. Hultgardh-Nilsson, J. Regnstrom, F. Calara, J. Yano, B. Cercek, P. K. Shah, and J. Nilsson. 1996. Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arterioscler. Thromb. Vasc. Biol.* **16:** 1074–1079.
47. Freigang, S., S. Horkko, E. Miller, J. L. Witztum, and W. Palinski. 1998. Immunization of LDL receptor-deficient mice with homologous malondialdehyde-modified and native LDL reduces progression of atherosclerosis by mechanisms other than induction of high titers of antibodies to oxidative neoepitopes. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1972–1982.
48. George, J., A. Afek, B. Gilburd, H. Levkovitz, A. Shaish, I. Goldberg, Y. Kopolovic, G. Wick, Y. Shoenfeld, and D. Harats. 1998. Hyperimmunization of apo-E-deficient mice with homologous malondialdehyde low-density lipoprotein suppresses early atherogenesis. *Atherosclerosis.* **138:** 147–152.
49. Zhou, X., G. Caligiuri, A. Hamsten, A. K. Lefvert, and G. K. Hansson. 2001. LDL immunization induces T-cell-dependent antibody formation and protection against atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **21:** 108–114.
50. Shaw, P. X., S. Horkko, M. K. Chang, L. K. Curtiss, W. Palinski, G. J. Silverman, and J. L. Witztum. 2000. Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. *J. Clin. Invest.* **105:** 1731–1740.
51. Tsimikas, S., Z. Mallat, P. J. Talmud, J. J. Kastelein, N. J. Wareham, M. S. Sandhu, E. R. Miller, J. Benessiano, A. Tedgili, J. L. Witztum, et al. 2010. Oxidation-specific biomarkers, lipoprotein(a), and risk of fatal and nonfatal coronary artery events. *J. Am. Coll. Cardiol.* **56:** 946–955.
52. Kamboh, M. I., and R. E. Ferrell. 1991. Apolipoprotein H polymorphism and its role in lipid metabolism. *Adv. Lipid Res.* **1:** 9–18.
53. Harris, M. R., C. H. Bunker, R. F. Hamman, D. K. Sanghera, C. E. Aston, and M. I. Kamboh. 1998. Racial differences in the distribution of a low density lipoprotein receptor related protein (LRP) polymorphism and its association with serum lipid and apolipoprotein levels. *Atherosclerosis.* **137:** 187–195.
54. Razzaghi, H., C. E. Aston, R. F. Hamman, and M. I. Kamboh. 2000. Genetic screening of the lipoprotein lipase gene for mutations associated with high triglyceride/low HDL-Cholesterol levels. *Hum. Genet.* **107:** 257–267.
55. Hokanson, J. E., M. I. Kamboh, S. Scarboro, R. H. Eckel, and R. F. Hamman. 2003. Vigorous physical activity protects against the increase in coronary disease associated with the hepatic lipase gene: a case of behavior modifying genetic susceptibility. *Am. J. Epidemiol.* **158:** 836–843.
56. Klos, K. L., S. Hamon, A. G. Clark, E. Boerwinkle, K. Liu, and C. F. Sing. 2005. APOA5 polymorphisms influence plasma triglycerides in young, healthy African Americans and whites of the CARDIA Study. *J. Lipid Res.* **46:** 564–571.
57. Chen, Q., S. E. Reis, C. M. Kammerer, D. M. McNamara, R. Holubkov, B. L. Sharaf, G. Sopko, D. F. Pauley, C. N. B. Merz, and M. I. Kamboh. 2003. for the WISE study group, APOE polymorphism in angiographic coronary artery disease severity in the Women's Ischemia Syndrome Evaluation (WISE) study. *Atherosclerosis.* **169:** 159–167.
58. Pentikainen, M. O., R. Oksjoki, K. Oorni, and P. T. Kovanen. 2002. Lipoprotein lipase in the arterial wall: linking LDL to the arterial extracellular matrix and much more. *Arterioscler. Thromb. Vasc. Biol.* **22:** 211–217.
59. Rip, J., M. C. Nierman, C. J. Ross, J. W. Jukema, M. R. Hayden, J. J. Kastelein, E. S. Stroes, and J. A. Kuivenhoven. 2006. Lipoprotein lipase S447X: a naturally occurring gain-of-function mutation. *Arterioscler. Thromb. Vasc. Biol.* **26:** 1236–1245.