Intercellular Adhesion Molecule 1 (ICAM1) Lys56Met and Gly241Arg Gene Variants, Plasma-Soluble ICAM1 Concentrations, and Risk of Incident Cardiovascular Events in 23 014 Initially Healthy White Women

Robert Y.L. Zee, PhD; Suzanne Cheng, PhD; Henry A. Erlich, PhD; Klaus Lindpaintner, MD; Nader Rifai, PhD; Julie E. Buring, ScD; Paul M Ridker, MD

Background and Purpose—The objective of this study was to examine the association of 2 nonsynonymous intercellular adhesion molecule 1 (ICAM1) gene variants (Lys56Met and Gly241Arg) with baseline plasma soluble ICAM1 concentrations and with risk of total and selected cardiovascular disease (CVD) events in a prospective cohort of 23 014 apparently healthy white American women followed for 10 years. ICAM1 variations have been associated with plasma soluble ICAM1 concentrations and inflammatory conditions, including atherosclerosis. However, to date, no large prospective, genetic–epidemiological data set is available that would allow evaluation of the degree of association of these gene variants with risk of CVD.

Methods—ICAM1 genotypes and baseline plasma soluble ICAM1 concentrations were determined. The primary outcome measure was a composite CVD end point (incident ischemic stroke, myocardial infarction, or death due to ischemic CVD); other measures were incident ischemic stroke, myocardial infarction, and coronary revascularization. During follow-up, 751 total incident CVD events, 187 incident myocardial infarction cases, 203 incident ischemic stroke cases, and 433 coronary revascularization events occurred.

Results—All observed genotype frequencies were in Hardy-Weinberg equilibrium across the whole sample population. We found baseline plasma soluble ICAM1 concentrations to be significantly reduced among carriers of Met56 allele (P<0.0001) and Arg241 allele (P<0.0001) as compared with the respective noncarriers of these variants. However, the polymorphisms tested and the respective haplotypes were neither associated with overall risk nor with risk for selected CVD events regardless of whether analyses were adjusted for traditional CVD risk factors/confounders (all P values >0.10).

Conclusions—In this large prospective study, we found an association of the nonsynonymous gene variants tested with reduced baseline plasma soluble ICAM1 concentrations. However, no evidence was found for an association of the gene variants tested with the overall or selected CVD end points examined, suggesting that these variants may not add useful aids to current risk predictors for early assessment of cardiovascular events. (Stroke. 2007;38:000-000.)

Key Words: CVD ■ ICAM1 ■ polymorphisms ■ risk factors

Inflammatory and immune responses have consistently been implicated in the pathogenesis of atherosclerosis, the most important cause of cardiovascular disease (CVD). The underlying process is today believed to be predominantly mediated by cell-matrix adhesion molecules expressed on the vascular endothelium and on circulating leukocytes in response to several proinflammatory cytokines. Intercellular adhesion molecule 1 (ICAM1; intercellular adhesion molecule 1 [CD54], human rhinovirus receptor)—a member of the large immunoglobulin superfamily—is widely expressed at a basal level and can be upregulated by proinflammatory cytokines. Furthermore, circulating plasma soluble ICAM1 (sICAM1) concentrations are increased in various inflammatory conditions, including CVD, and have been associated with future CVD risk.

The human ICAM1 gene is located on chromosome 19p13.3 to 13.2 (GeneID 3383; www.ncbi.nlm.nih.gov/entrez/query). Several nonsynonymous polymorphisms have been
previously described; in particular, a Gly241Arg gene variant has been associated with lower plasma sICAM1 concentrations. Although the ICAM1 gene has been implicated as an important candidate for CVD risk, robust demonstration of its association with inflammatory conditions, including atherosclerosis, remains elusive.2,9,10

To date, no large prospective genetic–epidemiological data sets are available to address the relationship of ICAM1 gene variants, plasma sICAM1 concentrations, and incident CVD risk. We, therefore, investigated the possible association of 2 previously described nonsynonymous ICAM1 polymorphisms: a lysine-to-methionine substitution at amino acid position 56 (Lys56Met; exon 2; dbSNP rs5491) and a glycine-to-arginine substitution at amino acid position 241 (Gly241Arg; exon 4; dbSNP rs1799969) with the occurrence of vascular events in a prospective study of apparently healthy white women with 10-year follow-up.

Methods

Study Design

Study participants were enrolled in the Women’s Health Study, a recently completed randomized, double-blind, placebo-controlled clinical trial of low-dose aspirin and vitamin E aimed at assessing the hypothesis of primary prevention of CVD and cancer in US female healthcare professionals through this intervention.6,11 In brief, eligible participants were apparently healthy women, ages 45 years or older, who were free of self-reported CVD or cancer at study entry (1992 to 1995) with follow-up for incident CVD through February 2005. At the time of enrollment, participants gave written informed consent; completed questionnaires on racial/ethnic status, demographic variables, medical history, medication use, dietary and lifestyle variables; and were asked to provide a blood sample. In total, 23,014 white women in whom both baseline plasma sICAM1 concentrations and ICAM1 genotypes could be determined comprised the study population for the present investigation. The study was approved by the Institutional Review Board of Brigham and Women’s Hospital (Boston, Mass).

Plasma Soluble Intercellular Adhesion Molecule 1 Concentrations and Laboratory Measurements

EDTA-anticoagulated blood samples were obtained at the time of enrollment and stored in vapor phase liquid nitrogen (−170°C). The concentrations of sICAM1 were determined using a quantitative sandwich enzyme immunoassay technique (R&D Systems, Minneapolis, Minn).6 The assay has a sensitivity of 0.35 ng/mL, and day-to-day variabilities at concentrations of 64.2, 117, 290, and 453 ng/mL are 10.1%, 7.4%, 6.0% and 6.1%, respectively.12 High-sensitivity C-reactive protein was measured using a validated, latex-enhanced immunoturbidimetric assay (Denka Seiken).6

Intercellular Adhesion Molecule 1 Genotype Determination

Genotype analysis was performed using an immobilized probe approach, essentially as previously described (Roche Molecular Systems, Alameda, Calif).13 In brief, each DNA sample was amplified in a multiplex polymerase chain reaction using biotinylated primers. Each polymerase chain reaction product pool was then hybridized to a panel of sequence-specific oligonucleotide probes immobilized on a linear array. The colorimetric detection method was based on the use of streptavidin–horseradish peroxidase conjugates with hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine as substrates. To confirm genotype assignment, scoring was carried out by 2 independent observers. Discordant results (<1% of all scoring results) were resolved by a joint reading and when necessary, a repeat genotyping. In addition, 5% randomly selected samples as duplicates were genotyped for quality control, and we obtained 100% concordance.

Ascertainment of Incident Cardiovascular Events

Participants were followed for the composite end point of incident CVD (nonfatal myocardial infarction, nonfatal ischemic stroke, coronary revascularization, or cardiovascular death) and the individual end points of nonfatal myocardial infarction, nonfatal ischemic stroke, and coronary revascularization. Medical records were obtained and reviewed for confirmation of events as previously described.14 Deaths from cardiovascular causes were identified by reports from family members, postal authorities, and a search of the National Death Index and were confirmed by autopsy reports, death certificates, and medical records.

| Table 1. Baseline Characteristics of the Study Population by Genotype Status |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Lys56Met | Gly241Arg | Polymorphism | KK | KM | P | KK | KM | P | KK | KM | P | KK | KM |
| Age, years | 52.9 (48.9–59.0) | 53.3 (49.8–59.6) | 0.28 | 52.9 (48.9–59.0) | 53.0 (48.9–59.6) | 0.0001 | 53.5 (50.1–59.0) | 0.10 |
| C-reactive protein, mg/L | 343.7 (302.3–395.7) | 274.1 (185.8–342.2) | <0.0001 | 347.9 (306.0–400.4) | 329.3 (289.8–377.0) | 0.0001 |
| Aspirin, % | 50.0 | 45.4 | 0.28 | 49.9 | 50.2 | 0.02 |
| Body mass index, kg/m² | 24.8 (22.4–28.3) | 24.2 (22.6–28.7) | 0.95 | 24.8 (22.4–28.3) | 24.8 (22.3–28.3) | 0.67 |
| Hormone replacement therapy use, % | 43.8 | 41.3 | 0.54 | 44.2 | 42.0 | 0.01 |

Values shown for continuous variables are median with interquartile ranges in parentheses. *P* values for categorical variables were obtained from *χ*² tests.

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During a mean follow-up period of 9.9 ± 1.3 years, there were 751 total incident CVD events (including 187 myocardial infarctions and 203 ischemic strokes) and 433 coronary revascularization. The total person-years of follow-up were 227 569.80.

Statistical Analysis
Allele and genotype frequencies in the whole sample population were compared with values predicted by Hardy-Weinberg equilibrium using the \( \chi^2 \) test. The relationship between the polymorphisms evaluated and baseline plasma sICAM1 concentrations was examined using the Wilcoxon rank sum test or the Kruskal-Wallis analysis of variance.

Hazard ratios (ie, the incidence rate ratios) of total CVD, myocardial infarction, ischemic stroke, or coronary revascularization associated with each polymorphism were calculated separately by Cox proportional hazards analysis with adjustment for age and smoking status and further adjustment for traditional CVD risk factors or potential confounders (randomized treatment assignment, body mass index, history of hypertension [\( \geq \) 140/90 mm Hg or on antihypertensive medications], presence or absence of diabetes, hyperlipidemia [\( \geq \) 240 mg/DL], and the use of hormone replacement therapy) assuming an additive, dominant, or recessive mode of inheritance. Because no Arg-Arg-homozygote was observed, analysis assuming a recessive model was not performed for the Gly241Arg variant. Pairwise linkage disequilibrium was examined as described by Devlin and Risch.\(^{15}\) Haplotype inference and frequency estimation were performed using PHASE v2.1.1.\(^{16,17}\) In addition, the relationship between haplotypes and each specific clinical end point was examined separately using a haplotype-based Cox proportional hazards analysis with baseline parameterization assuming additivity\(^{18}\) and adjusting for the same potential risk factors/confounders. As discussed by Wallenstein and coauthors, this approach can be applied to cohort studies.\(^{18}\) A likelihood ratio test statistic, comparing the model with genetic data with the model without genetic data using the likelihood ratio test statistic, was performed to check the overall association of haplotypes with the clinical end points evaluated. For each hazard ratio, we calculated 95% CIs. All analyses were carried out using SAS/Genetics 9.1 package (SAS Institute Inc).

Results
Baseline characteristics of the study participants were similar across ICAM1 genotypes (Table 1). Furthermore, the baseline characteristics of the present cohort were virtually demonstrating its appropriate representation (supplemental Table I, available online at http://stroke.ahajournals.org). Genotype distributions were in Hardy-Weinberg equilibrium (Lys56Met, \( P = 0.64; \) Gly241Arg, \( P = 0.73 \)) with allele frequencies of 99.7% and 0.3% for Lys56 and Met56 alleles, respectively, and 88.4% and 11.6% for Gly241 and Arg241 alleles, respectively. As shown in Table 1, the median baseline sICAM1 concentrations were significantly lower among heterozygotes carrying the minor Met56 allele as compared with the wild-type homozygotes (Lys-Lys) (\( P < 0.0001 \)); similar significant association with lower baseline median sICAM1 concentrations were also observed for the Gly241Arg variant (\( P < 0.0001 \)). These associations remained statistically significant in prespecified subgroup analyses stratified by current smoking status, body mass index (\( < 25, \geq 25 \text{ kg/m}^2 \)), presence or absence of hypertension, diabetes, hyperlipidemia, and use of hormone replacement therapy (Lys56Met: all \( P \) values \( < 0.001 \); Gly241Arg: all \( P \) values \( \leq 0.01 \)), like in a linear regression analysis (using log-transformed baseline plasma sICAM1 concentrations) with adjustment for the same traditional CVD risk factors/confounders (Lys56Met: all \( P \) values \( < 0.0001 \); Gly241Arg: all \( P \) values \( < 0.0001 \)).

Despite these significant associations between genotypes and plasma sICAM1 concentrations, we found no evidence of a univariable association between the variants tested and risk of subsequent CVD events using an additive, dominant, or recessive mode of inheritance (Table 2). Additional adjustment for traditional CVD risk factors/confounders showed similar null findings (Table 2). The variants tested were in modest linkage disequilibrium (\( D' = 0.57 \)), which is similar to that observed by Li and coauthors (\( D' = 0.36 \)).\(^{19}\) Three haplotypes were inferred and estimated in our cohort with frequencies of 0.8809, 0.1160, and 0.0030 for haplotype Lys56-Gly, Lys56-Arg, and Met56, respectively. Because haplotype Met-Gly was extremely rare, it was not analyzed further. Results from a haplotype-based Cox proportional hazards analysis with baseline parameterization showed no evidence for an association with future CVD risk (Table 3). Further adjustment for the same traditional CVD risk factors/
confounders again showed similar null findings (Table 3). Because 2 haplotypes were analyzed (which only differ at 241 variant position), in essence, results from the haplotype-based analysis were similar to those from the single-marker analysis assuming an additive model.

The ICAM1 gene variants tested and the covariables adjusted in the regression models were in agreement with the Cox proportionality assumption (all \( P \) values >0.05).

### Discussion

In this large, prospective study of 23,014 initially healthy US white women, we found significant associations of the nonsynonymous variants (Lys56Met and Gly241Arg) tested with baseline plasma sICAM1 concentrations. Despite these associations, we found no evidence for an association of the 2 nonsynonymous variants tested with incident CVD events. Because the original investigation previously shown to be in linkage disequilibrium with the Gly241Arg variant and associated with polymorphic site.

Only haplotypes with frequency \( >1\% \) were shown.

HR indicates hazard ratio; MI, myocardial infarction; IsST, ischemic stroke; RVAS, coronary revascularization.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Crude HR, 95% CI</th>
<th>( P^* )</th>
<th>Adjusted HR, 95% CI</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( P )</td>
<td></td>
<td>( P )</td>
<td></td>
</tr>
<tr>
<td>CVD</td>
<td></td>
<td></td>
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<tr>
<td>Lys-Gly (1-1)</td>
<td>Reference</td>
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<td>0.54</td>
<td>...</td>
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<td>Lys-Arg (1-2)</td>
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<td>0.99, 0.84–1.16</td>
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<tr>
<td>Lys-Gly (1-1)</td>
<td>Reference</td>
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<td>0.24</td>
<td>...</td>
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<tr>
<td>Lys-Arg (1-2)</td>
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<td>0.61</td>
<td>1.14, 0.83–1.56</td>
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<td>IsST</td>
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<td></td>
</tr>
<tr>
<td>Lys-Gly (1-1)</td>
<td>Reference</td>
<td>...</td>
<td>0.54</td>
<td>...</td>
</tr>
<tr>
<td>Lys-Arg (1-2)</td>
<td>1.13, 0.84–1.52</td>
<td>0.41</td>
<td>1.14, 0.85–1.54</td>
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<td>RVAS</td>
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<tr>
<td>Lys-Gly (1-1)</td>
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<tr>
<td>Lys-Arg (1-2)</td>
<td>0.92, 0.74–1.14</td>
<td>0.45</td>
<td>0.98, 0.78–1.21</td>
<td>0.82</td>
</tr>
</tbody>
</table>

\(^*\) \( P \) values for likelihood ratio test comparing model with haplotypes to model without.

Crude=age and smoking-adjusted.

Adjusted=further adjusted for randomized treatment assignment, body mass index, history of hypertension, presence or absence of diabetes, and hormone replacement therapy use.

Confidence level for haplotype estimation and inference was \( \geq 95\% \). 1 indicates major allele; 2, minor allele at each polymorphic site.

Table 3. Haplotype-Based Cox Proportional Hazard Regression Analysis Assuming Additivity

Human rhinoviruses. The Gly241Arg variant is located in the third immunoglobulin domain of the protein. This domain is of importance for binding to the leukocyte integrin Mac-1.20 It has been hypothesized that a combination of Lys56Met and Gly241Arg variants affecting the immunoglobulin domains 1 and 3, respectively, of the ICAM1 protein molecule may influence the inflammatory/immune response to inflammatory conditions, including CVD. However, our present study provides no support for this hypothesis from the standpoint of clinically observable associations between genotype and cardiovascular outcome.

The observed allele and genotype frequencies for the Gly241Arg variant in the present study are similar to those previously reported in other sample populations,19–22 and the observed association with reduced plasma sICAM1 concentrations are in concordance with those previously reported elsewhere.20,22,23 To the best of our knowledge, the present study is the first to demonstrate a significant association of the Lys56Met variant with reduced plasma sICAM1 concentrations. However, as noted by others,20 the observed association of the variants tested with reduced plasma sICAM1 concentrations may be due to linkage disequilibrium with other (nonsynonymous or functional) genetic polymorphism(s) that affect sICAM1 concentrations. Of note, the Lys469Glu variant in exon 6 (not tested in the present investigation) was previously shown to be in linkage disequilibrium with the Gly241Arg variant and associated with reduced plasma sICAM1 concentrations; however, the potential involvement of the Lys469Glu variant in vascular disease remains unclear. Although this variant was identified as risk factor for coronary heart disease and myocardial infarction in a German sample population,24 and for peripheral arterial occlusive disease in an Italian sample popula-
tion, it was found not to be associated with ischemic heart disease in a family-based study.

Prior studies have included simultaneous measurements of ICAM1 genotypes and plasma sICAM1 concentrations in relation to inflammatory conditions, but no study has as yet evaluated associations with actual atherothrombotic events, and none was conducted in a large, prospective setting. The prospective nature of the Women’s Health Study and the use of a closed population sampling scheme in which subsequent case status was determined solely by the development of disease strongly reduce the possibility that our findings are due to bias or unrecognized confounding. Also, the current study includes a large number of healthy women participants with simultaneous assessment and detailed information on CVD risk factors as well as an intermediate “biomarker” phenotype (baseline plasma sICAM1 concentrations) allowing adjustment of potential confounding of these variables.

Nonetheless, our study has several potential limitations. Our sample population was limited to white female health-care professionals from the United States. Thus, our results may not be generalizable to other racial/ethnic or socioeconomic groups, geographical regions, to males or to individuals with preexisting CVD. The single measurement of (baseline) plasma sICAM1 concentrations may underestimate the magnitude of its association with CVD, making it difficult to establish whether elevated concentrations of sICAM1 precede clinical CVD or vice versa. It is also important to recognize that association studies like the present one only examine the possible association between phenotype(s) and the actually tested polymorphism(s); such studies cannot exclude the possibility that examination of a different polymorphism(s) of the same gene(s)—not in linkage disequilibrium with the ones tested—might lead to different observations. Because the ICAM1 gene sits in a cluster of ICAM genes, including ICAM4 and ICAM5, further examination of the potential contributions of this gene cluster (with the haplotype tagSNP approach) in the pathogenesis of CVD is warranted. In our study, we had the ability to detect, based on the present cohort size, the observed allele frequencies, and assuming 80% power and an α value of 0.05, a risk ratio (for total incident CVD events) of equal or greater than 2.6, and of 1.4 for Lys56Met and Gly241Arg, respectively, assuming an additive model. Power will be less for other individual clinical end points. Thus, we cannot rule out a modest risk of CVD associated with the polymorphisms/haplotypes tested.

In conclusion, we found no association of the nonsynonymous ICAM1 (Lys56Met and Gly241Arg) genetic variants tested and CVD risk despite a significant association with plasma sICAM1 concentrations observed. This would suggest that these polymorphisms are not useful markers for risk assessment for future vascular disorders.

Acknowledgments

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Disclosures

None.

References

Table I. Comparison of Baseline Characteristics Between the Whole Cohort as Reported Previously* and the Current Study Population

<table>
<thead>
<tr>
<th>Variables</th>
<th>*Whole Cohort (N=39,876)</th>
<th>Current Study Participants (N=23,014)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>54.6 ± 7.0</td>
<td>54.7 ± 7.1</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>13.1</td>
<td>11.5</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>25.9</td>
<td>24.5</td>
</tr>
<tr>
<td>Hyperlipidemia, %</td>
<td>29.5</td>
<td>29.5</td>
</tr>
<tr>
<td>Postmenopausal and current hormone replacement therapy, %</td>
<td>30.0</td>
<td>31.9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.0 ± 5.1</td>
<td>25.9 ± 5.0</td>
</tr>
<tr>
<td>Aspirin, %</td>
<td>50.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>


Values shown for continuous variables are mean and standard deviation. There were no significant differences in the distribution of the baseline variables between the whole cohort and the current study participants.
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