Association of Variation at the ABO Locus With Circulating Levels of Soluble Intercellular Adhesion Molecule-1, Soluble P-selectin, and Soluble E-selectin
A Meta-Analysis

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Background—Circulating levels of soluble intercellular adhesion molecule-1, soluble P-selectin, and soluble E-selectin have been associated with variation at the ABO locus. To evaluate these associations and the effect sizes, we performed a meta-analysis with new and previously reported data for polymorphism rs579459.

Methods and Results—Compared with major allele homozygotes, heterozygotes and minor allele homozygotes had 4.6% (95% CI, 3.4%–5.8%, \( P = 7.3 \times 10^{-14} \)) and 7.2% (95% CI, 4.7%–9.7%, \( P = 1.5 \times 10^{-8} \)), respectively, lower soluble intercellular adhesion molecule-1 levels (n = 33,671). An allele dose-dependent association also was observed for soluble P-selectin (n = 4921) with heterozygotes and minor allele homozygotes having 11.5% (95% CI, 7.2%–15.8%, \( P = 1.7 \times 10^{-7} \)) and 18.6% (95% CI, 9.1%–28.1%, \( P = 1.2 \times 10^{-4} \)), respectively, lower levels than in major allele homozygotes. A larger effect size, again consistent with an additive genetic model, was seen for soluble E-selectin (n = 2860) whose level was 25.6% (95% CI, 19.0%–32.2%, \( P = 2.1 \times 10^{-14} \)) lower in heterozygotes and 43.3% (95% CI, 36.9%–49.3%, \( P = 4.3 \times 10^{-42} \)) lower in minor allele homozygotes than in major allele homozygotes.

Conclusions—The data support the association of variation at the ABO locus with soluble intercellular adhesion molecule-1, soluble P-selectin, and soluble E-selectin levels. (Circ Cardiovasc Genet. 2011;4:681-686.)

Key Words: cell adhesion molecules ▪ cardiovascular disease ▪ genetics ▪ plasma

Leukocyte recruitment plays an important role in inflammatory diseases.1 It typically begins with leukocyte rolling on the endothelium followed by leukocyte attachment to endothelial cells and subsequently transendothelial migration. Rolling involves the interaction of leukocytes with P-selectin and E-selectin on endothelial cells, whereas leukocyte attachment to endothelial cells is mediated by intercellular adhesion molecule-1 and vascular cell adhesion molecule-1.1

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Blood contains soluble forms of intercellular adhesion molecule-1 (sICAM-1), P-selectin (sP-selectin), and E-selectin (sE-selectin) generated by shedding of ectodo-
they are associated with single nucleotide polymorphisms. sICAM-1, sP-selectin, and sE-selectin levels have shown that heritability estimates of these reported studies range from 0.24 to 0.63, 0.45 to 0.70, and 0.50 to 0.64, respectively.11–14 Interestingly, genomewide association studies of coronary heart disease (CHD) have revealed an association between CHD and variation at the ABO locus.15,16

To more robustly evaluate the associations of sICAM-1, sP-selectin, and sE-selectin with the ABO locus, and more reliably estimate the effect sizes, we performed a meta-analysis. We included new data from the Bruneck Study, data from several reported studies,11,12,14 and additional data from 1 of these reported studies.11

### Methods

To identify association studies of SNPs at the ABO locus in relation to levels of sICAM-1, sP-selectin, and sE-selectin, we performed systematic searches of PubMed, scanned the reference lists of original reports, and communicated with authors of the included studies. The electronic searches combined search terms related to polymorphisms at the ABO locus (eg, ABO, polymorphism, SNP, variation, and variant) and intercellular adhesion molecule-1, P-selectin, or E-selectin. The searches identified 4 publications. In 2 of these publications,12,13 SNP rs579459 showed the strongest association with sP-selectin or sE-selectin levels among all tested SNPs at the ABO locus. In another study (in which rs579459 was not directly typed),11 SNP rs507666 had the most significant association with sP-selectin or sE-selectin levels among all tested SNPs at this locus. In the fourth study (which also did not type rs579459 directly),14 SNP rs651007 was the top SNP at the ABO locus associated with sICAM-1 and sE-selectin levels. An analysis using the SNAP program (www.broadinstitute.org/mpg/snap/) with data from the 1000 Genomes Project showed that rs579459 was in perfect linkage disequilibrium with rs651007 and in near perfect linkage disequilibrium (r^2 = 0.96) with rs507666 in individuals of European ancestry.

We genotyped the Bruneck cohort17 for SNP rs579459 using the KASPar method. sICAM-1, sP-selectin, and sE-selectin levels in the Bruneck cohort had been measured by an enzyme-linked immunosorbent assay. SLPA, SearchLight Proteome Array.

### Table. Summary of Participating Studies for the Meta-Analysis

<table>
<thead>
<tr>
<th>Participating Studies</th>
<th>Subjects</th>
<th>Age, y</th>
<th>Female, %</th>
<th>Sample</th>
<th>Method</th>
<th>SNP</th>
<th>A/A Genotype</th>
<th>A/A Genotype</th>
<th>a/a Genotype</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>sICAM-1</td>
<td>Bruneck 17</td>
<td>Population-based</td>
<td>63±11</td>
<td>51.1</td>
<td>Plasma</td>
<td>ELISA</td>
<td>5.1%</td>
<td>6.9%</td>
<td>rs579459</td>
<td>440</td>
</tr>
<tr>
<td>HFS12</td>
<td>Community-based</td>
<td>49±14</td>
<td>45.9</td>
<td>Serum</td>
<td>ELISA</td>
<td>3.9%</td>
<td>3.9%</td>
<td>rs579459</td>
<td>4176</td>
<td>2340</td>
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<tr>
<td>ARIC12</td>
<td>Community-based</td>
<td>56±5</td>
<td>38.4</td>
<td>Plasma</td>
<td>ELISA</td>
<td>4.0%</td>
<td>5.1%</td>
<td>rs579459</td>
<td>495</td>
<td>287</td>
</tr>
<tr>
<td>RS12</td>
<td>Community-based</td>
<td>70±9</td>
<td>53.3</td>
<td>Plasma</td>
<td>ELISA</td>
<td>6.9%</td>
<td>rs579459</td>
<td>351</td>
<td>214</td>
<td>35</td>
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<tr>
<td>CHS12</td>
<td>Population-based</td>
<td>73±6</td>
<td>42.8</td>
<td>Plasma</td>
<td>ELISA</td>
<td>5.0%</td>
<td>rs579459</td>
<td>855</td>
<td>556</td>
<td>69</td>
</tr>
<tr>
<td>WGH51</td>
<td>Population-based</td>
<td>55±7</td>
<td>100</td>
<td>Plasma</td>
<td>ELISA</td>
<td>6.7%</td>
<td>rs507666†</td>
<td>14391</td>
<td>6857</td>
<td>936</td>
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<tr>
<td>NHS14</td>
<td>Type 2 diabetes</td>
<td>56±7</td>
<td>100</td>
<td>Plasma</td>
<td>ELISA</td>
<td>3.3–4.8%</td>
<td>rs651007‡</td>
<td>612</td>
<td>337</td>
<td>47</td>
</tr>
<tr>
<td>sP-selectin</td>
<td>Bruneck 17</td>
<td>Population-based</td>
<td>63±11</td>
<td>51.1</td>
<td>Plasma</td>
<td>ELISA</td>
<td>5.5%</td>
<td>6.9%</td>
<td>rs579459</td>
<td>440</td>
</tr>
<tr>
<td>HFS12</td>
<td>Community-based</td>
<td>61±10</td>
<td>45.6</td>
<td>Plasma</td>
<td>ELISA</td>
<td>3.2%</td>
<td>rs579459</td>
<td>1872</td>
<td>1000</td>
<td>164</td>
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<tr>
<td>ARIC12</td>
<td>Community-based</td>
<td>57±5</td>
<td>35.7</td>
<td>Plasma</td>
<td>ELISA</td>
<td>3.9%</td>
<td>5.8%</td>
<td>rs579459</td>
<td>432</td>
<td>265</td>
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<tr>
<td>RS12</td>
<td>Community-based</td>
<td>69±9</td>
<td>48.8</td>
<td>Plasma</td>
<td>ELISA</td>
<td>&lt;5%</td>
<td>rs579459</td>
<td>253</td>
<td>135</td>
<td>18</td>
</tr>
<tr>
<td>sE-selectin</td>
<td>Bruneck 17</td>
<td>Population-based</td>
<td>63±11</td>
<td>51.1</td>
<td>Plasma</td>
<td>ELISA</td>
<td>4.8%</td>
<td>7.4%</td>
<td>rs579459</td>
<td>440</td>
</tr>
<tr>
<td>FHS12</td>
<td>Type 1 diabetes</td>
<td>39±7</td>
<td>46</td>
<td>Serum</td>
<td>SLPA</td>
<td>&lt;2%</td>
<td>5%</td>
<td>rs579459</td>
<td>452</td>
<td>209</td>
</tr>
<tr>
<td>DCCT/EDIC13</td>
<td>Type 1 diabetes</td>
<td>39±7</td>
<td>46</td>
<td>Serum</td>
<td>SLPA</td>
<td>&lt;2%</td>
<td>5%</td>
<td>rs579459</td>
<td>280</td>
<td>143</td>
</tr>
<tr>
<td>DCCT siblings17</td>
<td>Type 2 diabetes</td>
<td>56±7</td>
<td>100</td>
<td>Plasma</td>
<td>ELISA</td>
<td>4.5%–6.2%</td>
<td>rs651007‡</td>
<td>612</td>
<td>337</td>
<td>47</td>
</tr>
</tbody>
</table>

All participants were of European ancestry. Genotype distributions in the various cohorts were all consistent with Hardy-Weinberg equilibrium except for WGH5 (sICAM-1, P=0.001) and FHS (sP-selectin, P=0.046).

CV indicates coefficient of variation; SNP, single nucleotide polymorphism; A/A genotype, major allele homozygotes; A/a genotype, heterozygotes; a/a genotype, minor allele homozygotes; sICAM-1, soluble intercellular adhesion molecule-1; sP-selectin, soluble P-selectin; sE-selectin, soluble E-selectin; FHS, Framingham Heart Study; ARIC, Atherosclerosis Risk in Communities; RS, Rotterdam Study; CHS, Cardiovascular Health Study; WGH5, Women’s Genome Health Study; NHS, Nurses’ Health Study; DCCT, Diabetes Control and Complications Trial; EDIC, Epidemiology of Diabetes Intervention and Complications; ELISA, enzyme-linked immunosorbent assay; SLPA, SearchLight Proteome Array.

*Mean±SD.
†In nearly complete linkage disequilibrium with SNP rs579459 (r^2 = 0.96) based on data from the 1000 Genomes Project.
‡In complete linkage disequilibrium with SNP rs579459 (r^2 = 1) based on data from the 1000 Genomes Project.
standardized effect size (estimator $d$) for each adhesion molecule comparing minor allele homozygotes with heterozygotes and separately minor allele homozygotes with major allele homozygotes. The StatsDirect software provided the pooled mean effect size estimate (weighted mean difference or $d$) with a 95% CI, a $\chi^2$ statistic, and probability of this pooled effect size being equal to zero. Consistency of findings across studies was assessed by the $I^2$ statistic. Evidence of publication bias was assessed using funnel plots and the Egger test. Possible reasons for heterogeneity were investigated by metaregression analysis.

**Results and Discussion**

The characteristics of study subjects are summarized in the Table. A total of 33,671 subjects were available for the meta-analysis of sICAM-1, 4,921 for sP-selectin, and 2,860 for sE-selectin.

The meta-analysis showed that sICAM-1 levels were 4.6% (95% CI, 3.4%–5.8%) lower in heterozygotes and 7.2% (4.7%–9.7%) lower in minor allele homozygotes than in major allele homozygotes ($P=7.3\times10^{-14}$ and $P=1.5\times10^{-8}$; Figure A). Similarly, an allele dose-dependent association was observed for sP-selectin with heterozygotes and minor allele homozygotes having 11.5% (7.2%–15.8%) and 18.6% (9.1%–28.1%), respectively, lower levels than in major allele homozygotes ($P=1.7\times10^{-7}$ and $P=1.2\times10^{-4}$; Figure B). An allele dose-dependent association also was seen for...
sE-selectin whose level was 25.6% (19.0%–32.2%) lower in heterozygotes and 43.3% (36.9%–49.3%) lower in minor allele homozygotes than in major allele homozygotes ($P=2.1 \times 10^{-14}$ and $P=4.3 \times 10^{-42}$; Figure C). Standardized effect size was larger for sE-selectin than for sICAM-1 and sP-selectin (Supplemental Figures I–III; http://circ.ahajournals.org). We noted heterogeneity (Supplemental Table I) which a metaregression analysis indicated was not attributed to differences among individual studies in age, sex, type of subjects (population-based or diabetics), number of subjects (>1000 or <1000), type of blood sample used (plasma or serum), or which SNP studied, although the metaregression analysis had low power due to the relatively small numbers of individual studies. There was no evidence of publication bias. We observed correlations among sICAM-1, sP-selectin, and sE-selectin levels (Supplemental Table II).

SNP rs507666 is located within the ABO gene, and SNP rs579459 and rs651007 are in its proximity. The ABO gene encodes a glycosyltransferase that transfers sugar residues to the H antigen and determines the ABO blood group. Group A has 3 subtypes, that is, A1 and A2, respectively. It has been shown that the A1 subtype has >30-fold higher transferase activity than the A2 subtype. The A1 allele is perfectly tagged by the minor allele of SNP rs507666, while SNP rs507666 is in near perfect linkage disequilibrium ($r^2=0.96$) with rs579459 and rs651007. Thus, the associations of these SNPs with sICAM-1, sP-selectin, and sE-selectin levels may represent an effect of the ABO group A1 subtype. It has been suggested that the increased glycosyltransferase activity in individuals carrying the A1 allele might have an effect on the shedding, clearance, or secretion of adhesion molecules, thereby influencing their levels in the circulation.

Adhesion molecules are crucial to platelet leukocyte interaction and leukocyte migration into the vessel wall and thus important players in the atherosclerosis process underlying CHD.2,22 In a number of previous studies, increased CHD risk has been associated with high sICAM-1, sP-selectin, and sE-selectin levels.5,5,6 Unexpectedly, variants at the ABO locus conferring elevated CHD risk,15,16,23 like the minor allele of SNP rs579459, were associated with decreased levels of soluble adhesion molecules in our meta-analysis. One possible explanation for this seeming paradox may be that soluble adhesion molecules, although elevated in the case of endothelial dysfunction, actually compete with leukocyte adhesion to the endothelium (competition to cell surface adhesion molecules). Another possibility may be that the lower levels of soluble adhesion molecules might arise because of lower shedding of ectodomains, potentially leaving higher levels of intact cell surface adhesion molecules to recruit leukocytes to the blood vessel wall. To date, it is not known whether elevated levels of soluble adhesion molecules in vascular high-risk patients represent an epiphenomenon of vessel wall pathology, a true risk factor, or a counterregulatory per se protective mechanism as indicated by preliminary experimental data.16 Experimental studies are required to further elaborate the pathophysiological role of soluble adhesion molecules and to clarify whether the prominent alterations in sICAM-1, sP-selectin, and sE-selectin observed in this study are relevant to the recently discovered association between ABO SNPs and CHD risk.

Some limitations to our study warrant mentioning. First, the mechanism underlying the association of SNPs at the ABO locus with sICAM-1, sP-selectin, and sE-selectin levels has remained unclear. Second, because SNP rs579459 is in strong linkage disequilibrium with a number of other SNPs at this locus, it remains unknown which SNP is the causal variant. Third, because this study was conducted in individuals of European ancestry, the findings may not be generalizable to other races/ethnicities.

In conclusion, our study provides compelling evidence of an allele dose-dependent association of variation at the ABO locus with circulating sICAM-1, sP-selectin, and sE-selectin levels. These results contribute to the knowledge of genetic influences on these adhesion molecules, which play important roles in many inflammatory diseases.

**Sources of Funding**

This work forms part of the research themes contributing to the translational research portfolio of Barts and the London Cardiovascular Biomedical Research Unit, which is supported and funded by the National Institutes of Health Research. The Framingham Heart Study (FHS) was supported by grants from the Boston University (N01-HC-25195) and the National Institutes of Health (RO1HL064753, R01HL076784, and R01AG028321). The analyses reflect intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource (SHARE) project. This work was partially supported by a contract with Affymetrix, Inc for genotyping services (Contract No. N02-HL-6-4278). A portion of this research used the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center. The Atherosclerosis Risk in Communities Study (ARIC) is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL095367, and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The infrastructure was partly supported by Grant No. UL1RR025055, a component of the National Institutes of Health (NIH) and NIH Roadmap for Medical Research. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, the Netherlands Organisation for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The genomewide association study was funded by the Netherlands Organisation of Scientific Research NWO Investments (No. 175.010.2005.011, 911-03-012), the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the
Netherlands Genomics Initiative (NGI)/Netherlands Consortium for Healthy Ageing (NCHA) project No. 050-060-810. A.D. is supported by Netherlands Organisation for Scientific Research (NOW) grant (vici, 918-76-619). The Women’s Genome Health Study (WGHS) is supported by HL 043851 and HL69757 from the National Heart, Lung, and Blood Institute and CA 047988 from the National Cancer Institute, the Donald W. Reynolds Foundation, and the Fondation Leducq, with collaborative scientific support and funding for genotyping provided by Amgen.

Disclosures

S.K. received research grants: TRP188-B12 FWF. L.Q. received research grants from the National Institutes of Health (NIH). R.P.T. received other research grants from AstraZeneca, Novartis, Merck, the National Heart, Lung, and Blood Institute (NHLBI), and the National Cancer Institute. He received other support from Amgen and Celera as well as honorarium from several universities. He has ownership/interest in a patent related to inflammatory biomarkers. Also, he is a consultant or serves on the advisory board for ISIS, Merck, Vascular Institute. He received other support from Amgen and Celera. P.M.R. received research grants R01HL09257, RCHL101056, RO1HL102214, and RO1AG028231. She also consults or is on advisory committees for the NIH and NHLBI.

References


Adhesion molecules play important roles in the recruitment of leukocytes into inflamed tissues. Blood contains soluble forms of intercellular adhesion molecule-1, P-selectin, and E-selectin generated by shedding of ectodomains of the membrane-bound forms of these molecules or produced from transcript variants lacking the transmembrane domain. Recently genomewide association studies showed evidence of associations of circulating levels of soluble intercellular adhesion molecule-1, soluble P-selectin, and soluble E-selectin with single nucleotide polymorphisms within or near the ABO gene, which encodes a glycosyltransferase that determines the ABO blood group. Our present study involving a meta-analysis of data from a number of cohorts further supports these associations. Interestingly, the same ABO single nucleotide polymorphisms have also been associated with risk of coronary heart disease. The vast majority of coronary heart disease is caused by atherosclerosis whose pathogenesis involves leukocyte recruitment into the vascular wall. Intriguingly, the ABO genotypes related to increased coronary heart disease risk are associated with lower, rather than higher, levels of circulating soluble intercellular adhesion molecule-1, soluble P-selectin, and soluble E-selectin. Further studies will be needed to investigate if the association between variants at the ABO locus and coronary heart disease risk is related to changes in these adhesion molecules and, if so, by what mechanisms. One possibility could be that soluble adhesion molecules could compete with and thus reduce the effect of full-length adhesion molecules in leukocyte recruitment. Another possibility could be that lower levels of soluble adhesion molecules might arise because of lower shedding of ectodomains, potentially leaving higher levels of intact adhesion molecules on the cell surface.
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Circ Cardiovasc Genet. 2011;4:681-686; originally published online October 18, 2011; doi: 10.1161/CIRCGENETICS.111.960682

Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

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SUPPLEMENTAL MATERIAL

Supplemental Material for

Association of variation at the ABO locus with circulating levels of sICAM-1, sP-selectin and sE-selectin: a meta-analysis analysis

Stefan Kiechl, Guillaume Paré, Maja Barbalic, Lu Qi, Josée Dupuis, Abbas Dehghan, Joshua C. Bis, Ross C. Laxton, Qingzhong Xiao, Enzo Bonora, Johann Willeit, Qingbo Xu, Jacqueline C.M. Witteman, Daniel Chasman, Russell P. Tracy, Christie M. Ballantyne, Paul M. Ridker, Emelia J. Benjamin, Shu Ye
Description of study cohorts

Bruneck Study: \(^1\) The study is a prospective population-based survey of the epidemiology and pathogenesis of atherosclerosis. At the 1990 base-line evaluation, the study population was recruited as a random sample, stratified according to sex and age, of all inhabitants of Bruneck, Italy (125 women and 125 men in each of the following age groups: 40 to 49 years, 50 to 59 years, 60 to 69 years, and 70 to 79 years). A total of 93.6 percent of those recruited participated, and data assessment was completed for 919 subjects. Between 1990 and the reevaluation in the summer of 1995 (the first five-year period), 63 subjects died or moved away. During the second period, 810 were followed up. Circulating ICAM-1, sP-selectin and sE-selectin levels were measured by ELISA (R&D Systems).

FHS (Framingham Heart Study): \(^2\) The FHS started in 1948 with 5,209 participants from Framingham, Massachusetts, US, who have undergone biannual examinations to investigate CVD and its risk factors. In 1971, the Offspring cohort (comprised of 5,124 children of the Original cohort, and the children’s spouses) and in 2002, the Third Generation (consisting of 4,095 children of the Offspring cohort), were recruited. Included in this study were sICAM-1 data for 6,845 individuals of the Offspring and Third Generation, and sP-selectin data for 3,036 individuals of Offspring Generation. Serum ICAM-1 was measured by quantitative ELISA (R&D Systems, Cat. No. BBE 1B). P-selectin was determined from EDTA plasma by quantitative ELISA (R&D Systems, Cat. No. BBE 6).

ARIC (Atherosclerosis Risk in Communities): \(^2\) The ARIC study is a population-based prospective cohort study of cardiovascular disease and its risk factors. ARIC includes 15,792 persons aged 45-64 years at baseline between 1987 and 89, randomly selected from four US communities. Cohort members completed four clinic examinations, conducted approximately every three years between 1987 and 1998. Circulating sICAM-1 and sP-selectin were measured in nested case-cohorts samples. sICAM-1 levels were determined by quantitative sandwich ELISA (R&D Systems). sP-selectin concentrations were determined by sandwich ELISA (Amersham Pharmacia Biotech).

RS (Rotterdam Study): \(^2\) The RS is a prospective, community-based cohort study of determinants of several chronic diseases in older adults. The study comprised 7,983 inhabitants of Ommoord, a district of Rotterdam in the Netherlands, who were 55 years or over. The baseline examination took place between 1990 and 1993. Included in this study were sICAM-1 data from a random subsample of 600 individuals and sP-selectin data from 406 individuals consisting of 162 prevalent atrial fibrilation cases and 324 age (within 5 years strata) and sex adjusted controls. sICAM-1 and sP-selectin levels were determined by ELISA kits (R&D Systems).

CHS (Cardiovascular Health Study): \(^2\) The CHS is a population-based, observational study of risk factors for clinical and subclinical cardiovascular disease. The study recruited 5,201 participants 65 years of age and older of European and African ancestry from four US communities in 1989-1990 and an additional 678 African-ancestry participants from 3 communities in 1992-1993. Included in this study were sICAM-1 data for 1,487 individuals of European ancestry. sICAM-1 was determined by quantitative ELISA (R&D Systems).

WGHS (Women’s Genome Health Study): \(^3\) Participants in the WGHS include American women from the Women's Health Study (WHS) with no prior history of cardiovascular disease, diabetes, cancer, or other major chronic illness who also provided a baseline blood
sample at the time of study enrollment. The WHS is a recently completed 2×2 randomized clinical trial of low-dose aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer. For all WGHS participants, EDTA anticoagulated plasma samples were collected at baseline and stored in vapor phase liquid nitrogen (−170°C). Circulating plasma sICAM-1 concentrations were determined using a commercial ELISA assay (R&D Systems).

NHS (Nurses’ Health Study): The NHS was established in 1976 when 121,700 female registered nurses aged 30–55 years and residing in 11 large US states completed a mailed questionnaire on their medical history and lifestyle. A total of 32,826 women provided blood samples between 1989 and 1990. Included in this study were sICAM-1 and sE-selectin data from 996 women included in a nested case–control study of type 2 diabetes. sICAM-1 and sE-selectin levels were measured by ELISA (R&D Systems).

DCCT (Diabetes Control and Complications Trial) and EDIC (Epidemiology of Diabetes Intervention and Complications): In DCCT, the study subjects were patients with type 1 diabetes, aged 13–39 years, recruited between 1983 and 1989. In 1993, DCCT subjects were invited to participate in EDIC to follow-up the long-term effects of glycemic control. Serum sE-selectin levels were measured in 752 EDIC participants and non-diabetic siblings of DCCT probands. Data from one sibling per family were selected for analyses. sE-selectin levels were determined using a SearchLight™ Proteome Array (Pierce Biotechnology).

References
Figure S1. Standardized effect size by genotype for soluble intercellular adhesion molecule-1 (sICAM-1) level. Data shown are standardized effect size ± 95% confidence interval for circulating levels of sICAM-1, comparing heterozygotes or minor allele homozygotes, to major allele homozygotes, in a random-effects model.
Figure S2. Standardized effect size by genotype for soluble P-selectin (sP-selectin) level. Data shown are standardized effect size ± 95% confidence interval for circulating levels of sP-selectin, comparing heterozygotes or minor allele homozygotes, to major allele homozygotes, in a random-effects model.
Figure S3. Standardized effect size by genotype for soluble E-selectin (sE-selectin) level. Data shown are standardized effect size ± 95% confidence interval for circulating levels of sE-selectin, comparing heterozygotes or minor allele homozygotes, to major allele homozygotes, in a random-effects model.
### Supplemental Table 1. $I^2$ values

<table>
<thead>
<tr>
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<th>Analysis comparing heterozygotes with major allele homozygotes</th>
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<tr>
<td>Weighted mean difference analysis</td>
<td>$I^2=45.5%$ (95% CI 0-75.2%)</td>
<td>$I^2=49.9%$ (95% CI 0-76.9%)</td>
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<td>sICAM-1</td>
<td>$I^2=69.1%$ (95% CI 0-87.1%)</td>
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<td>sP-selectin</td>
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<td>sE-selectin</td>
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<tr>
<td>Standardized effect size ($d$) analysis</td>
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<tr>
<td>sE-selectin</td>
<td>$I^2=0%$ (95% CI 0-67.9%)</td>
<td>$I^2=0%$ (95% CI 0-67.9%)</td>
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$I^2$ statistic indicates percentage of total variation across studies that is due to heterogeneity.
Supplemental Table 2. Correlations between sICAM-1, sP-selectin and sE-selectin levels in the Bruneck study

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<th>P-Selectin</th>
<th>ICAM-1</th>
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