

Large-scale genomic studies reveal central role of ABO in sP-selectin and sICAM-1 levels

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P-selectin and intercellular adhesion molecule-1 (ICAM-1) participate in inflammatory processes by promoting adhesion of leukocytes to vascular wall endothelium. Their soluble levels have been associated with adverse cardiovascular events. To identify loci affecting soluble levels of P-selectin (sP-selectin) and ICAM-1 (sICAM-1), we performed a genome-wide association study in a sample of 4115 (sP-selectin) and 9813 (sICAM-1) individuals of European ancestry as a part of The Cohorts for Heart and Aging Research in Genome Epidemiology consortium. The most significant SNP association for sP-selectin was within the *SELP* gene (rs6136, $P = 4.05 \times 10^{-61}$) and for sICAM-1 levels within the *ICAM-1* gene (rs3093030, $P = 3.53 \times 10^{-23}$). Both sP-selectin and sICAM-1 were associated with *ABO* gene variants (rs579459, $P = 1.86 \times 10^{-41}$ and rs649129, $P = 1.22 \times 10^{-15}$, respectively) and in both cases the observed associations could be accounted for by the A1 allele of the *ABO* blood group. The absence of an association between *ABO*

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blood group and platelet-bound P-selectin levels in an independent subsample ($N = 1088$) from the ARIC study, suggests that the ABO blood group may influence cleavage of the P-selectin protein from the cell surface or clearance from the circulation, rather than its production and cellular presentation. These results provide new insights into adhesion molecule biology.

INTRODUCTION

Cardiovascular diseases (CVD), including coronary heart disease, stroke and the renal and peripheral complications of diabetes are leading causes of morbidity and mortality in the USA and elsewhere (1). Inflammation has a critical role in their pathogenesis (2–4). During vascular inflammation, leukocytes attach to and migrate into the vascular wall. This crucial step is mediated by adhesion molecules, which are expressed on leukocytes and vascular endothelial cells in response to inflammatory stimuli (5,6).

Adhesion molecules are found in two forms, membrane-bound and soluble. The membrane bound forms mediate leukocyte attachment and internalization (7). The soluble form arises as a result of shedding or enzymatic cleavage of the membrane bound form, and in some cases as alternatively spliced molecules lacking the transmembrane domain (8). The precise proteolytic mechanism responsible for cleavage is not fully understood. The soluble form may inhibit additional leukocyte adhesion thus having a modulating effect on leukocyte recruitment (9,10). Although there are limited data available, it is thought that soluble levels of the adhesion molecules reflect differences in cellular levels (11). Soluble levels of multiple adhesion molecules, including P-selectin and intercellular adhesion molecule-1 (sICAM-1), have been associated with coronary heart disease (12,13) and other cardiovascular conditions (14–16).

P-selectin is a member of the selectin family of adhesion molecules and is expressed mainly at the surface of platelets and endothelial cells. It promotes leukocyte rolling and mediates interactions of leukocytes and platelets with the endothelium (17). ICAM-1 is a member of the immunoglobulin gene superfamily of adhesion molecules and is expressed on leukocytes, fibroblasts, epithelial cells and endothelial cells (18). Soluble forms of these two molecules, sP-selectin and sICAM-1 have high heritabilities (45–70 and 34–59%, respectively; 19–23), suggesting a strong genetic influence. Linkage studies have shown evidence of quantitative trait loci for P-selectin levels on chromosome 15 (LOD = 3.8), chromosome 12 (LOD = 2.6) (24) and chromosome 1 (LOD = 1.73) (22). ICAM-1 levels have shown linkage to the ICAM gene cluster on chromosome 19 (25,26). In a large-scale genomic study in 6578 women, multiple SNPs within the ICAM gene and a SNP in the ABO locus were associated with plasma sICAM-1 concentrations (21). No similar studies have been carried out on sP-selectin levels.

The Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) was established as a consortium to identify genetic variants influencing CVD and its risk factors (27). In the framework of CHARGE, we present the results of a genome-wide association study (GWAS) on a full HapMap set of ~2.5 million SNPs for both sICAM-1 and

sP-selectin levels in a sample of 9813 (sICAM-1) and 4115 (sP-selectin) individuals of European ancestry. For significant loci obtained from sP-selectin GWAS, we also examine the association with cellular P-selectin on platelets in an independent sample of 1,088 individuals of European ancestry from Atherosclerosis Risk in Communities (ARIC) study.

RESULTS

The total study sample for the sP-selectin studies consisted of 4115 individuals. Mean age ranged from 56.6 years in ARIC to 69.4 years in Rotterdam Study (RS) and percentage of women from 35.7% in ARIC to 48.8% in RS. Mean sP-selectin levels ranged from 31.6 ng/ml in RS to 43.9 ng/ml in ARIC. Additional characteristics of the three study samples involved in the sP-selectin analysis are provided in Supplementary Material, Table S1a.

Results of the meta-analysis are shown in Figure 1A. The most significant association was observed in a region on chromosome 1 encompassing the P-selectin precursor gene (*SELP*; Supplementary Material, Fig. S1a). Apart from *SELP*, this region also contains the coagulation factor V precursor gene (*F5*). More than 100 SNPs in this region were above the pre-specified genome-wide significance level (5×10^{-8}). The most statistically significant association was detected at rs6136 located in exon 13 of the *SELP* gene (MAF = 10.3%, $P = 4.05 \times 10^{-61}$) (Table 1). This SNP is a missense variation changing a Threonine to a Proline, and is in complete LD ($r^2 = 1$) with the second most significant SNP, rs9332575 (MAF = 10.3%, $P = 4.23 \times 10^{-61}$), located in intron 5 of the *F5* gene. Rs6136 and rs9332575 are in weak LD ($r^2 < 0.2$) with the remaining genome-wide significant SNPs in the region. The other significant SNPs form two almost perfect LD blocks (Supplementary Material, Fig. S1b). Rs760694 (MAF = 46.7%, $P = 2.60 \times 10^{-19}$) is the most significant well-imputed SNP in the left block (3' end) and rs2235302 (MAF = 48.01%, $P = 3.95 \times 10^{-16}$) is the most significant SNP in the right block (5' end). After including in the same model all three SNPs representing three distinct LD blocks (rs6136, rs760694 and rs2235302), rs6136 and rs2235302 remained significantly associated with sP-selectin levels ($P = 1.26 \times 10^{-41}$, $P = 2.34 \times 10^{-12}$, respectively). The significance of rs760694 did not reach genome-wide threshold ($P = 6.80 \times 10^{-4}$).

The second region with genome-wide significant signals for sP-selectin levels was observed on chromosome 9, where rs579459 (MAF = 21.8%, $P = 1.86 \times 10^{-41}$) achieved the most significant P -value. Rs579459 is located ~4 kb from the 5' end of ABO blood group gene (Fig. 1B). In total, 30 SNPs reached genome-wide significance in this region, 18 of which were located within the ABO gene. After conditioning

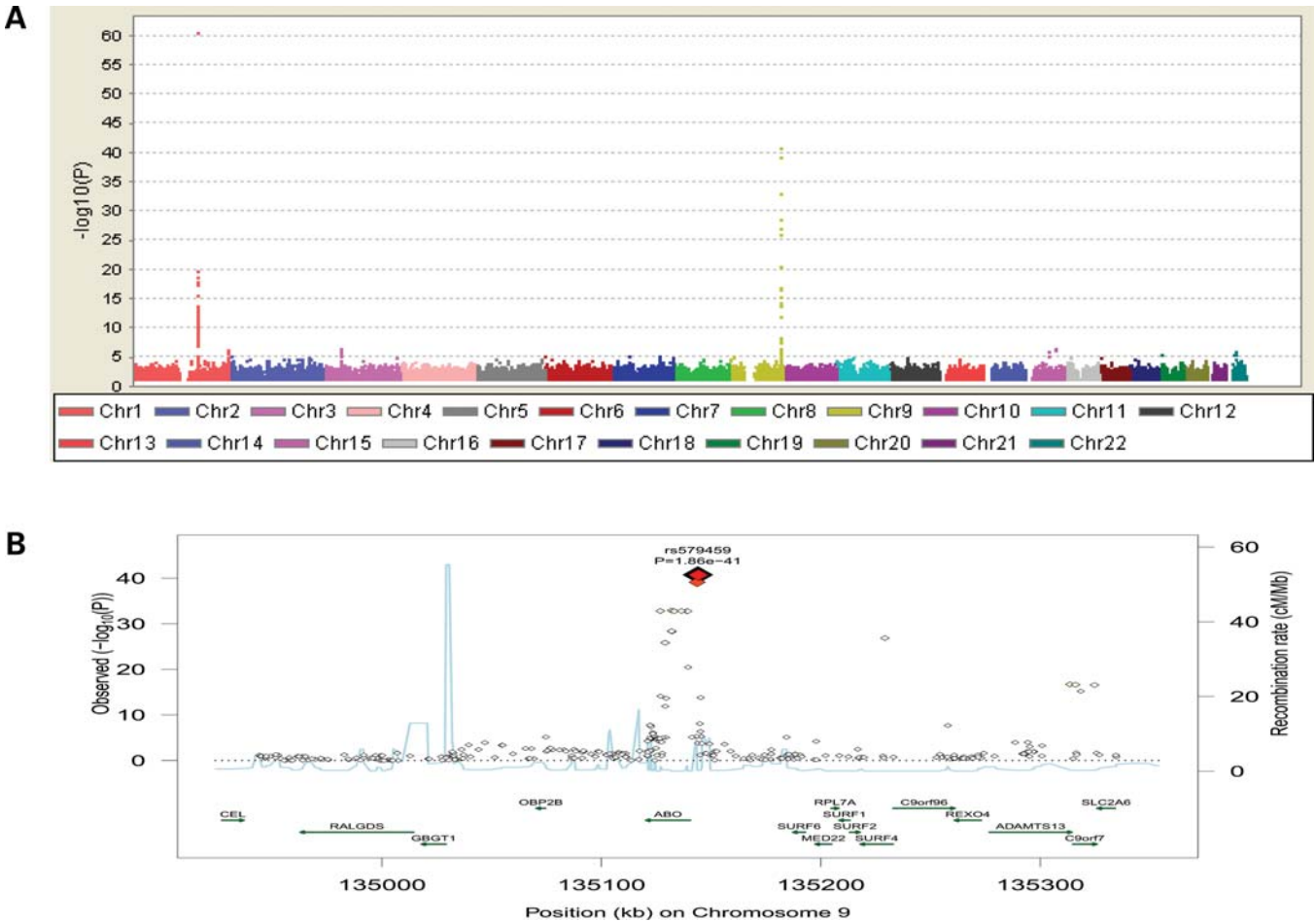


Figure 1. (A) Genome wide association plot for sP-selectin; (B) Regional plot of ABO locus for association with sP-selectin. Top SNP and corresponding P -value are indicated.

on rs579459, none of the variants was significantly associated with sP-selectin.

No other region contained genome-wide significant signals. However, applying the less stringent threshold of $P < 4 \times 10^{-7}$ (expecting one false positive per 2.5 million SNPs), one additional region on chromosome 3 emerged (Supplementary Material, Fig. S1c). In this region the most significant SNP, rs11711824 (MAF = 41.37%, $P = 3.72 \times 10^{-7}$), is located in intron 6 of *SCAP*—a gene encoding a protein involved in regulation of sterol biosynthesis. However, SNPs with very similar level of significance ($P < 10^{-6}$) are dispersed across a broad region containing several genes—*PTPN23* (protein-tyrosine phosphatase), *KIF9* (kinesin family member 9) and *TMEM103* (transmembrane protein) (Supplementary Material, Fig. S1c).

The above GWAS results reflect an association with sP-selectin levels that are readily measurable in large cohort studies. However, the more biologically active molecule is thought to be the membrane bound form (7). Therefore, we examined four SNPs from the two regions significantly associated with sP-selectin (rs6136, rs760694, rs2235302, rs579459) in relation to membrane-bound platelet P-selectin (the proportion of cells expressing P-selectin and the average

fluorescence of P-selectin on platelets) as measured by flow cytometry in the ARIC carotid MRI substudy ($N = 1088$, see Materials and Methods). Out of three *SELP* gene SNPs (rs6136, rs760694 and rs2235302) that were significantly associated with sP-selectin, rs6136 was also associated with platelet P-selectin ($P = 0.01$ with average fluorescence of P-selectin on platelets and $P = 0.02$ with the proportion of cells expressing P-selectin). The ABO SNP, rs579459, was not associated with P-selectin on platelets (Table 2).

Four of the CHARGE cohorts measured plasma sICAM-1 levels, providing a total sample of 9813 individuals. Mean age ranged from 49.4 years in Framingham Heart Study (FHS) to 72.8 years in Cardiovascular Health Study (CHS), and the percent of women ranged from 38.4% in ARIC to 53.3% in RS. sICAM-1 levels ranged from 222.7 ng/ml in RS to 329.1 ng/ml in CHS. Additional sample characteristics are presented in Supplementary Material, Table S1b.

The meta-analysis for the genome-wide association analysis of sICAM-1 levels are shown in Figure 2A. The strongest signal was on chromosome 19 in the region of the *ICAM* genes cluster (Table 3 and Supplementary Material, Fig. S2). The most significant association was at SNP rs3093030 (MAF = 41.72%, $P = 3.53 \times 10^{-23}$), located ~100 bp from

Table 1. GWAS results for sP-selectin

SNP	Chr	Position	Gene	Coded allele		Meta-analysis	ARIC ($N = 673$)	FHS ($N = 3036$)	RS ($N = 406$)
rs6136	1	167830575	SELP	T	<i>P</i> -value	4.05×10^{-61}	2.7×10^{-3}	2.33×10^{-59}	0.002
					β^a	0.226	0.358	0.242	0.117
					SE	0.014	0.119	0.015	0.037
					R^{2b}		0.8	7.9	2.2
rs760694	1	167835322	SELP	T	<i>P</i> -value	2.60×10^{-19}	0.001	3.24×10^{-17}	0.054
					β^a	0.077	0.143	0.08	0.043
					SE	0.009	0.044	0.01	0.022
					R^{2b}		1.2	2.2	0.9
rs2235302	1	rs2235302	SELP	T	<i>P</i> -value	3.95×10^{-16}	0.020	8.01×10^{-14}	0.01
					β^a	-0.073	-0.109	-0.074	-0.058
					SE	0.009	0.047	0.01	0.024
					R^{2b}		0.9	1.8	1.3
rs579459	9	135143989	ABO	T	<i>P</i> -value	1.86×10^{-41}	0.00001	3.29×10^{-33}	3.61×10^{-6}
					β^a	0.140	0.223	0.139	0.125
					SE	0.010	0.051	0.012	0.027
					R^{2b}		2.7	4.6	4.7

^a β calculated using log-transformed sP-selectin values.

^bPercentage of sP-selectin variance explained by the SNP reported.

Table 2. Association of platelet P-selectin (proportion of cells expressing P-selectin and P-selectin expression assessed by MFI) with three SNPs in SELP and one in ABO associated with sP-selectin

SNP	Gene	Proportion of cells expressing P-selectin			P-selectin expression assessed by MFI		
		β	SE	<i>P</i>	β	SE	<i>P</i>
rs6136	SELP	4.55	2.01	0.02	1.31	0.53	0.01
rs760694	SELP	0.13	0.73	0.86	-0.01	0.22	0.95
rs2235302	SELP	0.82	0.74	0.27	-0.08	0.20	0.69
rs579459	ABO	-1.18	0.78	0.13	-0.01	0.22	0.98

MFI, median fluorescence intensity.

the 3' end of *ICAM-1* gene. It is located near to previously described non-synonymous coding SNPs, rs5498 (Lys469Glu) and rs1799969 (Gly241Arg). Rs3093030 is in high LD with rs5498 ($r^2 = 0.98$) and in weak LD with rs1799969 ($r^2 = 0.18$). rs5498 and rs1799969 were less significantly associated with sICAM-1 levels than rs3093030 (rs5498, $P = 2.5 \times 10^{-21}$; rs1799969, $P = 2.8 \times 10^{-6}$). In order to determine if rs1799969 is independently associated with sICAM-1 levels from rs3093030, we performed an analysis including both rs1799969 and rs3093030 in the model. Results showed strong independent association of both SNPs ($\beta = 0.126$; $P = 1.344 \times 10^{-43}$ for rs1799969 and $\beta = 0.079$; $P = 1.038 \times 10^{-56}$ for rs3093030).

The second region reaching genome-wide significance was in the *ABO* gene region (Fig. 2B). Rs649129, a SNP in complete LD with rs579459, the most associated one with sP-selectin levels (see above), provided the highest significance level for sICAM-1 levels ($P = 1.22 \times 10^{-15}$). In total, 20 SNPs within this region were above the genome-wide significance level. When viewed together, the association plots for sICAM-1 and sP-selectin in the *ABO* region overlap, with the only difference being the level of statistical significance (Figs 1B and 2B). The correlation between sICAM-1 and sP-selectin levels was 0.19 in FHS. After adjusting

sP-selectin levels for sICAM-1, the ABO SNP rs579459 remained significantly associated with sP-selectin levels ($P = 1.62 \times 10^{-28}$; proportional change in effect size = 7%). After adjusting sICAM-1 for sP-selectin, the same SNP is associated with sICAM with $P = 7.98 \times 10^{-4}$ (with proportional change in effect size of 31%).

We next sought to investigate whether the observed association of the ABO locus with sICAM and sP-selectin could be accounted for by the well-known ABO blood group variants (A1, A2, B and O). The ABO blood group alleles in ARIC and FHS were estimated using haplotypes formed out of 3 ABO SNPs—rs8176749, rs8176704 and rs687289 (Table 4). None of these three SNPs are in high LD with the significant GWAS ABO SNP—rs579459 ($r^2 = 0.02$; 0.02 and 0.40, respectively). The estimated ABO blood group allele frequencies are shown in Table 4 and reflect their expected frequencies in populations of European descent. The A1 allele was negatively associated with both traits (meta-analysis of FHS and ARIC results: $\beta = -0.20$, $P = 3.88 \times 10^{-46}$ for sP-selectin; $\beta = -0.06$, $P = 2.89 \times 10^{-18}$ for sICAM-1). The β estimates were higher in models with A1 allele than in those with GWAS ABO hit SNP (rs579459) for both sP-selectin and sICAM. Moreover, the significant association of sP-selectin and sICAM-1 levels with the GWAS SNP rs579459 was no longer significant after inclusion in the model with the ABO blood group alleles. On the other hand, the A1 allele remained significant (Table 4, P-selectin and ICAM). The A2 allele showed the same (negative) direction of effect on sP-selectin and sICAM-1, but the association was only significant for sP-selectin levels. In contrast, none of the ABO blood group alleles were associated with P-selectin on platelets (Table 4, Platelet P-selectin).

DISCUSSION

We present here the results of meta-analyses of GWAS results, performed in the CHARGE consortium, that identified significant and replicated associations of sICAM-1 and sP-selectin

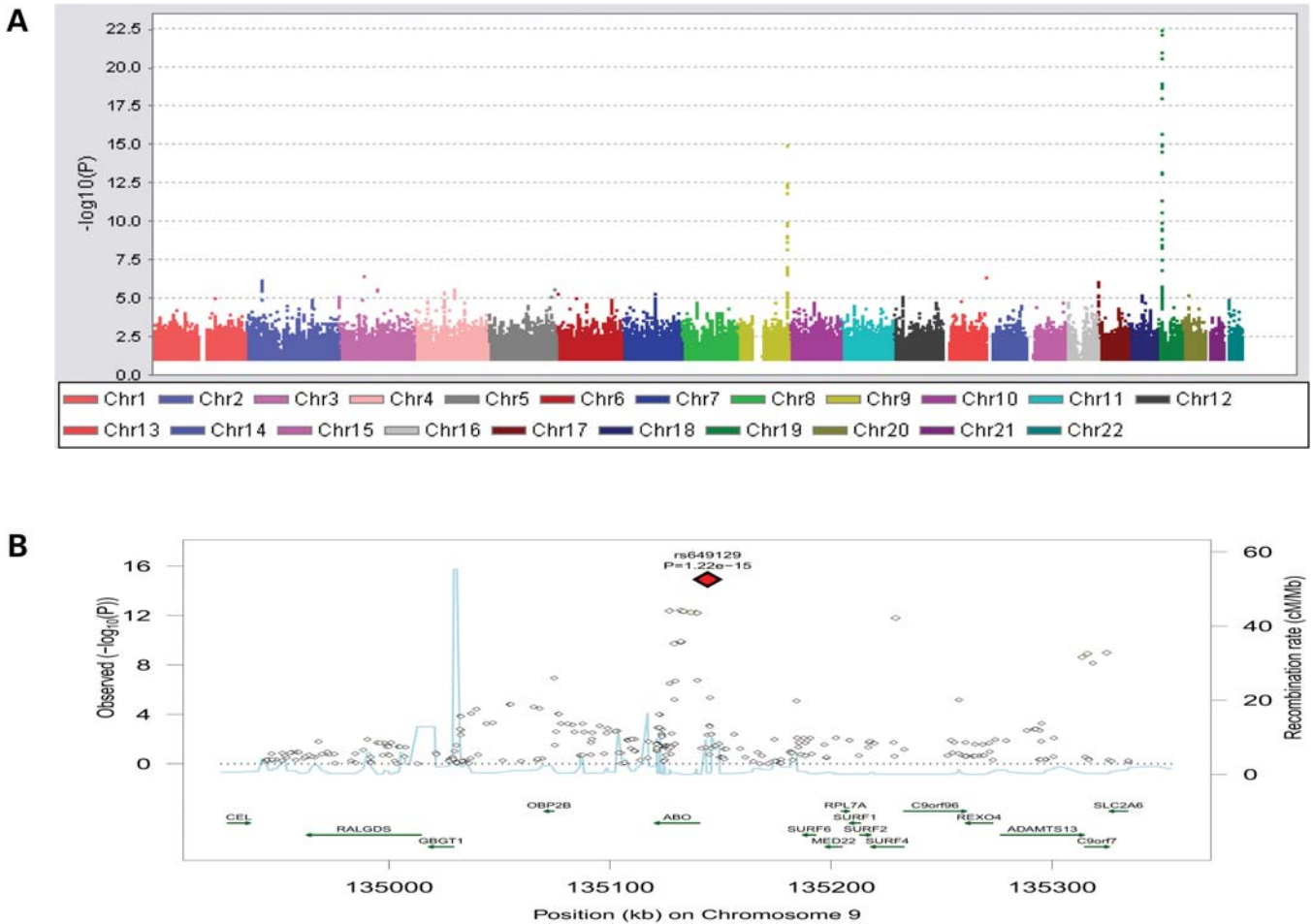


Figure 2. (A) Genome wide association plot for sICAM-1; (B) Regional plot of ABO locus for association with sICAM-1. Top SNP and corresponding P -value are indicated.

Table 3. GWAS results for sICAM-1

SNP	Chr	Position	Gene	Coded allele		Meta-analysis	ARIC ($N = 829$)	FHS ($N = 6845$)	CHS ($N = 1487$)	RS ($N = 600$)
rs3093030	19	10258403	ICAM	T	P	3.53×10^{-23}	0.011	6.24×10^{-19}	0.00046	0.107
					β^a	0.0415	0.031	0.045	0.042	0.027
					SE	0.0042	0.012	0.005	0.012	0.017
					R^{2b}		0.8	1.2	1.1	0.3
rs649129	9	135144125	ABO	T	P	1.22×10^{-15}	0.032	2.24×10^{-12}	0.010	0.040
					β^a	-0.0395	-0.030	-0.041	-0.042	-0.039
					SE	0.0049	0.014	0.006	0.016	0.019
					R^{2b}		0.5	0.8	0.6	0.5

^a β calculated using log-transformed sICAM-1 values.

^bPercentage of sICAM-1 variance explained by the SNP reported.

levels with variants in the genes encoding these proteins, *ICAM-1* and *SELP*, respectively. In addition, both markers were associated with variants in the well-known *ABO* blood group locus, which encodes glycosyltransferase enzymes that transfer sugar residues to the H antigen and determine an individual's blood group (28).

The observed association of sICAM-1 and sP-selectin with ABO variants could be explained by the A1 blood group

allele, that determines A blood group. The other A blood group allele, A2, was also associated with decreased levels of sP-selectin, however, with much lower level of significance. Both A1 and A2 alleles encode for the same form of glycosyltransferase that adds the *N*-acetylgalactosamine to the H antigen, however, with lower activity in case of A2 allele (29). These findings suggest a possible role of glycosylation in ABO's association with sP-selectin and sICAM levels.

Table 4. Estimated ABO allele frequencies in ARIC and FHS cohorts; the association results of ABO alleles with sP-selectin; sICAM and P-selectin bound on platelets (all analyses are adjusted for age and sex)

ABO frequencies									
ABO allele	Haplotype			ARIC	FHS				
	rs8176749	rs8176704	rs687289						
O	C	G	G	0.650	0.673				
A1	C	G	A	0.209	0.196				
B	T	G	A	0.073	0.077				
A2	C	A	A	0.068	0.054				
sP-selectin									
	ARIC (N = 653)			FHS (N = 3048)			Meta-analysis		
	β	SE	P	β	SE	P	β	SE	P
A1	-0.358	0.127	0.005	-0.170	0.027	2.7E-10	-0.178	0.026	1.8E-11
A2	-0.032	0.092	0.73	-0.062	0.022	0.005	-0.060	0.021	0.005
B	0.042	0.090	0.64	-0.015	0.019	0.421	-0.013	0.019	0.499
rs579459	-0.029	0.102	0.775	-0.019	0.023	0.388	-0.019	0.022	0.385
sICAM-1									
	ARIC (N = 829)			FHS (N = 6897)			Meta-analysis		
	β	SE	P	β	SE	P	β	SE	P
A1	-0.080	0.033	0.018	-0.056	0.013	1.6E-05	-0.059	0.012	9.9E-07
A2	-0.007	0.023	0.75	-0.017	0.011	0.12	-0.015	0.010	0.127
B	0.042	0.025	0.10	0.0003	0.009	0.97	0.005	0.008	0.548
rs579459	-0.029	0.027	0.29	-0.0006	0.011	0.96	-0.004	0.010	0.649
Platelet P-selectin									
	ARIC (N = 1088)			P-selectin expression assessed by MFI					
	Proportion of cells expressing P-selectin			β	SE	P			
	β	SE	P	β	SE	P			
A1	-0.355	2.152	0.87	0.64	0.98	0.52			
A2	0.831	1.509	0.58	-0.32	0.41	0.43			
B	1.337	1.445	0.36	0.61	0.86	0.45			
rs579459	-1.621	1.540	0.29	-0.15	0.65	0.82			

There was no observed association between the *ABO* locus with platelet-bound P-selectin although the flow cytometry analysis was limited to P-selectin on platelets, and does not rule out the possibility that endothelial P-selectin levels may be influenced by the *ABO* locus. Soluble forms of P-selectin arise in part from shedding or active proteolytic cleavage of membrane molecules (8,15,30). It is known that both soluble and cellular forms of ICAM-1 (31) and probably of P-selectin are glycosylated. The glycosylation is also critical for the binding activity of the P-selectin receptor, P-selectin glycoprotein ligand-1 (32). The observed association of ABO blood group markers with sP-selectin and sICAM-1 and not platelet-bound P-selectin suggests that the *ABO* gene product related glycosylation influences shedding/cleavage of these markers from the endothelium, probably by glycosylation of P-selectin and ICAM-1. Glycosylation could also affect the clearance rate of sP-selectin and sICAM-1 from blood as has been suggested for von Willebrand factor (vWF), a large circulating glycoprotein involved in hemostasis (33,34), whose levels are also influenced by the *ABO* locus (34). It is interesting that both P-selectin and vWF are stored in the same granules, Weibel-Palade bodies, prior to being released on endothelial cells (35,36). Moreover, the vWF receptor, glycoprotein (GP)Ib α , has been shown to act as a counter-receptor for P-selectin (37). vWF carries N-linked ABO antigens, and it has been suggested that vWF levels vary according to vWF glycosylation that influence its secretion and/or clearance rate (34). The similarity in P-selectin and vWF physiology underscores the inter-related nature of hemostatic and inflammation responses, both of related to CVD risk and now reported to be influenced by the *ABO* blood group locus. In

a recent GWA study, variation in *ABO* locus was also associated with serum soluble E-selectin levels (38)—another member of selectin family of adhesion molecules.

Additional insights may come from ABO's association with malaria infection susceptibility and severity (39,40). Recent studies of *Plasmodium falciparum* infection show that the infected erythrocytes mimic the leukocytes' attachment to endothelium by binding to ICAM-1 and P-selectin on endothelium (41). Erythrocytes also bind to the A and B antigens (but not O) on endothelial cells as an additional contributor to cytoadherence (42). This functional homology suggests the hypothesis that leukocytes might also interact with ABO antigens on endothelium. The A antigen might promote stronger (longer) binding of leukocytes to P-selectin and ICAM-1 on the vascular wall, thereby protecting the protein from enzymatic cleavage which in turn would lead to decreased levels in circulation. Decreased cleavage of adhesion molecules from endothelial cells associated with A allele would mean more adhesion molecules on the endothelial cells, increased adhesion and inflammation (21). This corresponds well with the findings of studies relating *ABO* blood group alleles with CVD that have been of interest for many years (43–45) and most of which suggested A allele association with increased risk of CVD (46). However, multiple epidemiologic studies have reported that increased levels of sP-selectin and sICAM-1 are associated with increased risk of CVD (12,15). Consequently, the A allele's association with decreased levels of sICAM-1 and sP-selectin but increased risk of CVD seems as a paradox and underscores the complex nature of CVD, its risk factors and their interrelationships (21).

Apart from ABO, sP-selectin and sICAM-1 levels are largely determined by variants within the genes encoding the two biomarker proteins, *SELP* and *ICAM-1*. The strong association of rs6136 with sP-selectin has been previously reported by ourselves (22,47) and others (13,20,47). Although heterogeneity test for any of the top SNPs in our sP-selectin and sICAM-1 meta-analyses was not statistically significant after accounting for multiple comparison, the I^2 statistic (percentage of total variation across studies that is due to heterogeneity) for rs6136 was high (82%). This high level of heterogeneity in our meta-analysis for this SNP should be investigated further. rs6136 (Thr715Pro) has a functional effect of changing the conformation of P-selectin near the cleavage site (22). Besides Thr715Pro, the P-selectin gene is characterized by 2 LD blocks that are additionally associated with sP-selectin levels. Another *SELP* variant, Val599Leu (rs6133), reported to be associated with sP-selectin in previous studies (13,20), was not in high LD with any of the 3 LD blocks and did not reach genome-wide significance in our study. In total, the three *SELP* regions accounted for 9.3% of the variance of sP-selectin levels in the FHS sample, the largest in our study. However, only Thr715Pro remained significantly associated with platelets P-selectin, a result that has already been reported (48). The lack of association of the other two SNPs could be explained by smaller sample size and insufficient power to detect the association for platelet P-selectin. Alternatively, it is also possible that different variants contribute to cellular and soluble P-selectin levels.

We report here suggestive evidence for a novel locus influencing soluble P-selectin levels on chromosome 3. The region contains *SCAP* (SREBP cleavage-activating protein), *PTPN23* (protein-tyrosine phosphatase), *KIF9* (kinesin family member 9) and *TMEM103* (transmembrane protein). *SCAP* cleaves the active domain of SREBP (sterol regulatory element binding protein) which acts as a transcription factor and participates in cellular cholesterol homeostasis (49,50). Given *SCAP* variants association with sP-selectin, it could be hypothesized that *SCAP* is also capable of cleaving P-selectin from cell membrane. However, *SCAP* is only known for its intracellular activities and its extracellular presence is doubtful.

The top SNP associated with ICAM-1 levels, rs3093030, is in the 3' end of the *ICAM-1* gene and is in high LD with rs5498 (Lys469Glu) that causes an amino-acid change from glutamic acid to lysine and is located in the fifth Ig-like domain of ICAM-1 (51). Lys469Glu has previously been reported to influence sICAM-1 levels (21,51,52). This variant influences the dimerization of the protein which promotes enhanced binding to leukocytes (53). Another variant in ICAM-1 gene (rs1799969 or Gly241Arg) is independently associated with ICAM-1 levels. This variant changes the amino-acid in the third immunoglobulin domain of ICAM-1 protein and has been shown to be of importance in binding to the leukocyte integrin Mac-1 (52,54).

Although this study was well-suited to identify loci with common variation associated with sP-selectin and sICAM-1 levels detectable by GWAS in large samples, it was limited to European ancestry population and its generalizability to other ethnic groups remains to be investigated. As with other GWAS, we were able to narrow down the association of sP-selectin and sICAM-1 to a particular genomic region,

but further analyses, beginning with DNA resequencing in large well-phenotyped cohorts, will be necessary to detect functional variants within those genes. Future projects with larger data sets will be required to examine any gene–gene and gene–environment interactions and the identification of additional variants that may have been undetected in this study. Finally, the possible heterogeneity of the effect of rs6136 on sP-selectin levels is a limitation and deserves further attention.

In summary, this study suggests the importance of the ABO blood group in influencing circulating sP-selectin and sICAM-1 levels. The probable mechanism involves glycosylation that interacts with P-selectin/ICAM-1 shedding from the cell membrane. These results contribute to the knowledge of the adhesion molecules physiology that is necessary to understand the link between the inflammation and atherosclerotic process and the development of new anti-inflammatory therapies for cardiovascular complications.

MATERIALS AND METHODS

The CHARGE consortium cohorts (27) included in this study are the ARIC study, CHS, FHS and RS. All participants gave written informed consent for study participation and for use of DNA for genetic research. The Institutional Review Board of each cohort approved the study protocols. All participants in this study are of European ancestry. Detailed descriptions of the participating cohorts are provided in the Supplemental Methods.

Laboratory measurements

sP-selectin and sICAM-1 concentrations were determined by standard ELISA methods. EDTA plasma was used for sP-Selectin in ARIC, FHS and RS, and for sICAM-1 in ARIC, CHS and RS; serum was used for sICAM-1 in FHS. Detailed laboratory methods for each cohort are provided in the Supplemental Methods.

Platelet P-selectin analysis

Flow cytometry was performed on a subsample of the ARIC study, as part of the ARIC Carotid MRI ancillary study that was conducted between 2004 and 2005. Participants were selected from the ARIC cohort based on results of their last carotid artery ultrasound examinations (Visits 3 and 4, 1993–1998) (55). For this study we used the data for the proportion of cells expressing P-selectin and the relative level of P-selectin expression assessed by median fluorescence intensity (MFI). The procedures have been described previously elsewhere in more details (56) and in the Supplemental Methods. We included 1088 individuals with platelet P-selectin measures and genotypes in this study. All individuals were of European ancestry.

ABO blood group allele determination and analysis

The ABO blood group alleles were estimated using the genetic data available in ARIC and FHS cohorts. We assessed the

ABO blood group alleles by constructing haplotypes out of three SNPs (rs8176749, rs8176704, rs687289) within the ABO locus (21). Four common haplotypes corresponded to four ABO blood group alleles (A1, A2, B and O). The association analyses of sP-selectin and sICAM with ABO blood group alleles (O is taken as a reference) were performed in ARIC and FHS and the results were combined using inverse-variance weighted meta-analysis.

Genotyping

Genotyping was done in each cohort separately using high-density SNP marker platforms (ARIC—Affymetrix 6.0, CHS—Illumina 370, FHS—Affymetrix 500K, RS—Illumina Infinium HumanHap550) and then imputed to ~2.5 million HapMap SNPs by using either MACH (57) (ARIC, FHS and RS) or BIMBAM (58) (CHS) software. Detailed information on genotyping and imputation in each cohort are provided in the Supplemental Methods.

Statistical analysis

Association studies of sP-selectin and sICAM-1 concentrations were done separately for each cohort. Concentration values were natural log-transformed before analyses. For the primary analysis, linear regression of concentration on (additive) allele dosage adjusted for age and sex was performed. In CHS, sICAM-1 was measured on a case-cohort subset and the appropriate sampling weights were used in the primary analysis to account for the sampling design. In ARIC, sP-selectin and sICAM-1 were measured in multiple nested case-cohorts samples and for those SNPs reaching genome-wide significance in the meta-analysis, the analyses were repeated with appropriate weights accounting for the sampling design. The results were similar to the unweighted analyses and therefore the results of unweighted analyses are shown throughout. In FHS, which consists of extended families, a linear mixed effects model was employed with a fixed additive effect for the SNP genotype, fixed covariate effects and random family specific additive residual polygenic effects to account for within family correlations. The cohort-specific results were combined using inverse-variance weighted meta-analysis with fixed effect models, as implemented in METAL (<http://www.sph.umich.edu/csg/abecasis/Metal/index.html>). Prior to meta-analysis, imputed SNPs with quality score (ratio of observed to expected variance) less than 0.3 were excluded. To test for heterogeneity between the samples used in the meta-analysis, we performed the Cochran's Q statistic using METAL and calculated I^2 statistic that describes the percentage of total variation across studies that is due to heterogeneity (59). Finally, we report as 'genome-wide significant' all associations with $P < 5 \times 10^{-8}$. As an alternative criterion, we also applied a threshold of $P < 4 \times 10^{-7}$, that represents an expectation of one false positive result per 2.5 million tests. All studies had a genomic control parameter ≤ 1.05 . For follow-up studies of platelet P-selectin, weighted analysis was performed on the inverse of the sampling fractions in the sampling strata used in the ARIC carotid MRI substudy. Sex and age were added

as covariates in the model. The analysis was done using the R software's 'survey' package (60).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

Conflict of Interest statement. None declared.

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