

Genome-wide association study identifies seven novel loci associating with circulating cytokines and cell adhesion molecules in Finns

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Abbreviations: GWAS, genome-wide association study; IL1 α , interleukin 1-alpha; IL1 β , interleukin 1-beta; IL1ra, interleukin 1 receptor antagonist; IL4, interleukin 4; IL6, interleukin 6; IL8, interleukin 8; IL17, interleukin 17; IP10, interferon gamma-induced protein 10; MAF, minor allele frequency; MCP1, monocyte chemoattractant protein 1; NFBC1966, Northern Finland Birth Cohort 1966; PAI-1, plasminogen activator inhibitor 1; sCD40L, soluble CD40 ligand; sE-selectin, soluble E-selectin; sICAM-1, soluble intercellular cell adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; TNF α , tumor necrosis factor alpha; VEGF, vascular endothelial growth factor

49 Abstract

50 **Background:** Inflammatory processes contribute to the pathophysiology of multiple chronic conditions.
51 Genetic factors play a crucial role in modulating the inflammatory load, but the exact mechanisms are
52 incompletely understood.

53 **Methods:** To add understanding to the molecular mechanisms in inflammation, we performed a genome-
54 wide association study (GWAS) on 16 circulating cytokines and cell adhesion molecules (inflammatory
55 phenotypes) in Northern Finland Birth Cohort 1966 (NFBC1966, N=5,284). A subsequent meta-analysis was
56 completed for 10 phenotypes available in a GWAS of three other Finnish population cohorts adding up to
57 13,577 individuals in the study. Complementary association tests were performed to study the effect of the
58 ABO blood types on soluble adhesion molecule levels.

59 **Results:** We identified seven novel and confirmed six previously reported loci associating with at least one of
60 the studied inflammatory phenotypes ($p < 3.1 \times 10^{-9}$). We observed three loci associating with the
61 concentration of soluble vascular cell adhesion molecule-1 (sVCAM-1), one of which is the *ABO* locus that has
62 been previously associated with soluble E-selectin (sE-selectin) and intercellular adhesion molecule-1
63 (sICAM-1) levels. Results from the complementary analyses suggest that the blood type B associates primarily
64 with the concentration of sVCAM-1 while the A1 subtype shows a robust effect on sE-selectin and sICAM-1
65 levels. Furthermore, the genotypes in the *ABO* locus associating with higher soluble adhesion molecule levels
66 tend to associate with lower low-density lipoprotein cholesterol level and lower cardiovascular disease risk.

67 **Conclusion:** The present results extend the knowledge about genetic factors contributing to the
68 inflammatory load. Our findings suggest that two distinct mechanisms contribute to the soluble adhesion
69 molecule levels at the *ABO* locus. The negative correlation between the genetic effects on soluble adhesion
70 molecule levels and cardiovascular traits in this locus further suggests that increased soluble adhesion
71 molecule levels per se may not be a risk factor for cardiovascular disease.

72 Introduction

73 It is currently established that inflammatory load may play a role in the etiology of autoimmune and
 74 infectious diseases, but also in a broad range of other diseases, such as chronic cardio-metabolic disorders
 75 (1), neurodegenerative diseases (2), and cancer (3). The risk for these diseases increases with age (4), and
 76 due to the world's aging population (5), their prevalence is likely to expand. Moreover, these diseases often
 77 co-occur which is likely due to shared inflammation related pathophysiology (6).

78 Inflammation is the body's physiological response to harmful stimuli involving multiple molecular and cellular
 79 interactions attempting to restore disturbances in tissue or systemic homeostasis (7). Circulating cytokines,
 80 growth factors, chemokines, and cell adhesion molecules (hereafter inflammatory phenotypes) are
 81 fundamental mediators of inflammatory responses. Genes encoding these molecules and their receptors play
 82 a crucial role in mediating the related functions. Previous studies have identified loci associating with levels
 83 of inflammatory phenotypes (8–10), but the understanding of the exact regulatory mechanisms is still
 84 incomplete.

85 To add insights to the genetic mechanisms contributing to the inflammatory load, we performed a genome-
 86 wide association study (GWAS) of 16 circulating inflammatory phenotypes in 5,284 individuals from Northern
 87 Finland Birth Cohort 1966 (NFBC1966), and a subsequent meta-analysis of 10 phenotypes in three other
 88 Finnish population cohorts (8) adding up to a total of 13,577 individuals in the study. We report identification
 89 of seven novel and replication of six loci associating with levels of the circulating inflammatory markers.

90 Methods

91 Study populations, genotyping and inflammatory phenotype quantification

92 *Northern Finland Birth Cohort 1966*

93 The Northern Finland Birth Cohort 1966 (NFBC1966) was initiated to study factors affecting preterm birth,
 94 low birth weight, and subsequent morbidity and mortality (www.oulu.fi/nfbc). It comprises 96% of all births
 95 during 1966 in the two northernmost provinces in Finland; altogether 12,058 children were live-born into
 96 the cohort, and the follow-ups occurred at the ages of 1, 14, 31, and 46 years (11, 12). The data analyzed in
 97 the present study is from the 31-years follow-up when clinical examinations and blood sampling was
 98 completed for altogether 6,033 individuals, 5,284 of whom had body mass index, inflammatory phenotypes
 99 and genotype data available (a maximum number of individuals per inflammatory marker 5,100). Genotyping
 100 of the samples was completed using 370k Illumina HumanHap arrays (Illumina Inc., CA, USA) and subsequent
 101 imputation was performed based on the 1000 Genome reference panel. A total of 16 inflammatory
 102 phenotypes were quantified from overnight fasting plasma samples using Bio-Rad's Bio-Plex 200 system (Bio-
 103 Rad Laboratories Inc., CA, USA) with Milliplex human chemokine/cytokine and CVD/cytokine kits (Cat#
 104 HCYTOMAG-60K-12 and Cat# SPR349; Millipore, St Charles, MO, USA) and Bio-Plex Manager Software version
 105 4.3 as previously described (13). The 16 inflammatory phenotypes studied in the NFBC1966 were interleukin
 106 (IL) 1-alpha (IL1 α), IL1-beta (IL1 β), IL4, IL6, IL8, IL17, IL1 receptor antagonist (IL1ra), interferon gamma-
 107 induced protein 10 (IP10), monocyte chemoattractant protein 1 (MCP1), tumor necrosis factor alpha (TNF α),
 108 vascular endothelial growth factor (VEGF), plasminogen activator inhibitor 1 (PAI-1), soluble CD40 ligand
 109 (sCD40L), soluble E-selectin (sE-selectin), soluble intercellular adhesion molecule 1 (sICAM-1) and soluble
 110 vascular cell adhesion molecule 1 (sVCAM-1).

111 *GWAS summary statistics from three Finnish population cohorts*

112 Meta-analyses were conducted for 10 phenotypes available in a previous GWAS (8). The study included up
 113 to 8,293 Finnish individuals from The Cardiovascular Risk in Young Finns Study (YFS) (14) and FINRISK
 114 (www.thl.fi/finriski) (15) adding up to 13,577 individuals studied in the present meta-analyses. Shortly, YFS is

a population-based follow-up study started in 1980 comprising randomly chosen individuals from Finnish cities Helsinki, Kuopio, Tampere, Oulu, and Turku. The YFS data included in the previous GWAS is from 2,019 individuals who participated in the follow-up in 2007 and who had both inflammatory phenotype and genotype data available. FINRISK is a Finnish population survey conducted every five years to monitor chronic diseases and their risk factors. The surveys use independent, random, and representative samples from different geographical areas of Finland. The data included in the present meta-analyses were from participants of the 1997 and 2002 surveys. Genotypes were obtained using 670k Illumina HumanHap arrays (Illumina Inc., CA, USA) and imputed based on 1000 Genome reference panel. Inflammatory markers were quantified using Bio-Rad's premixed Bio-Plex Pro Human Cytokine 27-plex Assay and 21-plex Assay, and Bio-Plex 200 reader with Bio-Plex 6.0 software (Bio-Rad Laboratories Inc., CA, USA) as previously described (16, 17). Samples were serum in YFS, EDTA plasma in FINRISK1997, and heparin plasma in FINRISK2002.

Statistical analyses

GWAS and meta-analysis

To allow meta-analysis between the present results and the previous GWAS, the data processing and analysis model were done according to Ahola-Olli *et al.* (8): Preceding the analyses, linear regression models were fitted to adjust the inflammatory phenotypes for age, sex, BMI, and ten first genetic principal components to control for population stratification. The resulting residuals were normalized with inverse-rank based transformation, and the adjusted and transformed residuals were used as phenotypes in the analyses. Genome-wide association tests were performed using snptest_v2.5.1 software (18, 19). Allele effects were estimated using an additive model (-frequentist 1) and the option to center and scale the phenotypes was disabled (-use_raw_phenotypes). The GWAS results were filtered by including markers with model fit info > 0.8 and minor allele count > 10. Filtered data was used to perform meta-analyses by METAL software (v.2011-03-25) (20) for the 10 phenotypes (IL1 β , IL1ra, IL17, IL4, IL6, IL8, IP10, MCP1, TNF α , and VEGF) available in the previous GWAS (8). Genomic control correction was enabled to account for population stratification and cryptic relatedness.

140 *Supplemental genome-wide tests in NFBC1966*

141 Individuals showing symptoms of an acute infection were omitted from the supplemental genome-wide tests
142 performed in the NFBC1966 population. Here, individuals reported having fever at the time of the blood
143 sampling and individuals having C-reactive protein (CRP) level > 10 mg/l were excluded. Otherwise the
144 analysis models were as above.

145 *Conditional analyses and variance explained*

146 To assess whether the identified loci harbor multiple independent association signals, we conducted
147 conditional analyses by further adjusting the models with the locus-specific lead variants. The association
148 tests were repeated within a 2Mb window around the lead SNP for the phenotypes studied in the NFBC1966
149 population only. For the meta-analyzed phenotypes, we applied a method proposed by Yang *et al.* that
150 enables conditional analyses of GWAS summary statistics (21, 22). NFBC1966 was used as a reference sample
151 to estimate linkage disequilibrium (LD) corrections in these analyses. The proportion of variance explained
152 was calculated using all independent variants using the following formula:

$$153 \quad \text{Variance explained} = (\beta \times \sqrt{2 \times \text{MAF}(1 - \text{MAF})})^2$$

154 Here β is the variant's effect estimate on the inflammatory phenotype and MAF denotes minor allele
155 frequency.

156 *Complementary association tests on soluble adhesion molecule levels*

157 Complementary association tests within a 2Mb window were conducted to better evaluate the effect of the
158 ABO blood type on the association with soluble adhesion molecule levels at the ABO locus. For sE-selectin,
159 sICAM-1 and sVCAM-1 levels, linear models were fitted by further adjusting for the ABO blood type or
160 rs507666 genotype tagging the A1 subtype (23).

161 In addition, we determined the effect estimates of ABO blood types and ABO blood types stratified by
162 rs507666 genotype on sE-selectin, sICAM-1 and sVCAM-1 levels in linear models. Here, adjusted and
163 transformed soluble adhesion molecule concentrations were as outcomes and ABO blood types as

164 categorical variables (blood type A versus non-A, etc.); corresponding models were fitted for the stratified
165 blood types (blood type A with rs507666 G/G versus others, etc.)

166 *Correlations of the genetic effects*

167 As previous evidence suggests that the elevated concentrations of circulating markers of inflammation
168 increase the risk of cardiovascular diseases (CVD) (24, 25), we further evaluated how variants at the loci
169 associating with inflammatory phenotypes may relate to other cardiovascular traits. We extracted SNP
170 effects on coronary artery disease (CAD) risk, stroke risk, and low-density lipoprotein cholesterol (LDL-C) or
171 high-density lipoprotein cholesterol (HDL-C) levels from open-access data provided by CARDIoGRAM (26),
172 Stroke Consortia (27), and a metabolomics GWAS (28). First, data were filtered to include only the SNPs
173 available in all the three data sets within a 1Mb window around each lead variant. Next, subsets of
174 representative SNPs at the each of the significant loci were extracted using clumping function in PLINK
175 1.90b4.1 (29). Here, NFBC1966 was used as the reference sample to construct LD structures and $r^2=0.2$ was
176 used as the LD threshold while other parameters were as by default. The subsets of SNPs were used to
177 determine the linear relationships of the genetic effects (Z-scores) on inflammatory phenotypes versus other
178 traits for each significant loci identified in the present GWAS.

179 **Results**

180 Basic characteristics of the NFBC1966 study population is provided in Table 1. Inflammatory phenotype
181 distributions are tabulated in Table S1 and their correlation structure is shown in Figure S1. Using a threshold
182 of $p<3.1\times10^{-9}$ for statistical significance (standard genome-wide significance level $p<5\times10^{-8}$ corrected for 16
183 phenotypes tested), we identified seven novel and six previously reported loci associating with one or more
184 of the inflammatory phenotypes. The results are summarized in Table 1 and combined Manhattan plots are
185 shown in Figure 1. Manhattan plots and Q-Q plots for each inflammatory phenotype are provided in the
186 supplement (Figure S2 A-Z). Genomic inflation factor values range between 0.99-1.02 suggesting no inflation
187 in the test statistics (Table S2). Table S3 lists traits associated previously with the loci showing novel
188 associations with inflammatory phenotypes in the present study.

189

190 Cell adhesion molecules

191 *The ABO locus shows large effects on sE-selectin, sICAM-1, and sVCAM-1 levels*

192 We observed a novel effect on sVCAM-1 concentration in 9q34.2 near *ABO* (*ABO*, alpha 1-3-N-
193 acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase) in the NFBC1966 population. This locus
194 showed a robust association also with sE-selectin and sICAM-1 concentrations as previously reported (23, 30,
195 31). Noteworthy, the lead variant for sE-selectin and sICAM-1 associations (rs2519093) was different from
196 the lead variant for sVCAM-1 association (rs8176746). The former variant is in LD ($r^2=1$ in NFBC1966) with
197 rs507666 tagging the *ABO* blood type A subtype A1 whereas the latter variant tags the blood type B (23).

198 To better evaluate if the *ABO* blood types constitute the molecular mechanism explaining the association
199 between the *ABO* locus and soluble adhesion molecule levels, we completed supplementary association tests
200 further adjusted for *ABO* blood type or rs507666 genotype indicative of the A1 subtype. The results of the
201 supplementary tests suggested that the association of the rs8176746 with sVCAM-1 concentration is
202 independent of the A1 subtype ($p=4.98 \times 10^{-15}$ for the rs507666-adjusted association). On the contrary, the
203 associations of the rs2519093 with concentrations of sE-selectin and sVCAM-1 remained highly significant
204 when adjusted for *ABO* blood type ($p=3.40 \times 10^{-123}$ and $p=3.43 \times 10^{-17}$, respectively). Statistical significances
205 were abolished when rs8176746 association with sVCAM-1 was adjusted for *ABO* blood type and rs2519093
206 association with sE-selectin or sICAM-1 was adjusted for rs507666.

207 We further determined the effect estimates of the *ABO* blood types and *ABO* blood types stratified by
208 rs507666 genotype on soluble adhesion molecule levels. The blood type A showed negative associations with
209 the levels of all the three adhesion molecules and the effect was the most robust on the sE-selectin level
210 (Figure 2, Panel 1). However, major discrepancies in the effect directions were seen when the analyses were
211 stratified by the rs507666 genotype (Figure 2, Panel 2). Congruent with previous reports (23, 30), the present
212 results suggest that the A1 subtype/rs507666 genotype influences sE-selectin or sVCAM-1 levels. In contrast,

the blood type B seems to attribute predominantly to sVCAM-1 level while the A1 subtype/rs507666 shows only a modest effect on sVCAM-1.

HSP90B1 and ABCA8 loci associate with sVCAM-1 levels

We identified two other novel loci for sVCAM-1 (12q23.3 and 17q24.2) in the NFBC1966 population. In chr12 the lead variant rs117238625 is in LD ($r^2=1$ in NFBC1966) with rs117468318 locating in the 5' UTR region of *HSP90B1* (heat shock protein 90kDa beta member 1) and, according to RegulomeDB (32), is likely to affect transcription factor binding providing evidence for a possible regulatory mechanism. Variants in this locus have been previously associated with stem cell growth factor beta levels (8) and corneal structure (33). The association signal in chr17 locates near *ABCA8* (ATP binding cassette subfamily A member 8) encoding one of the ATP binding cassette transporters. Other studies have identified associations in this locus with HDL and LDL cholesterol levels (34–37), breast cancer risk (38), heart's electrical cycle related traits (QT interval, QRS duration) (39–41).

Variations in sialyltransferase encoding genes show an effect on sE-selectin level

For sE-selectin level, we identified a novel association in 11q24.2 in the region of *ST3GAL4* (ST3 beta-galactoside alpha-2,3-sialyltransferase 4). Other studies have associated other variants in this region with mean platelet volume and platelet count (42), LDL cholesterol levels (34, 36) or pleiotropic associations with LDL cholesterol and C-reactive protein (43), blood protein levels (44) and liver enzyme levels (45). We identified a suggestive signal with sE-selectin level also in 3q12.1 near *ST3GAL6* (ST3 beta-galactoside alpha-2,3-sialyltransferase 6), but the association was not significant after multiple correction ($p=1.75 \times 10^{-08}$). Both of the sialyltransferase genes have been implicated in the production of functional E-, P-, and L-selectin ligands in mice (46).

Two independent association signals on sICAM-1 level near ICAM1

We replicated the previously reported association for sICAM-1 level in 19p13.2 near *ICAM1* (intracellular adhesion molecule 1) (23, 44). When the primary association test was conditioned for the lead variant

rs117960796, another significant association was detected (rs74428614, $p=1.14 \times 10^{-16}$) indicative of more than one independent variant contributing to sICAM-1 level in this locus.

Vascular endothelial growth factor

In the meta-analyses, we identified a novel locus 4p16.2 with a large effect on VEGF ($\beta=-2.38$ SD). This locus harbours genes *EVC* (EvC ciliary complex subunit 1), *EVC2* (EvC ciliary complex subunit 2), and *STK32B* (serine/threonine kinase 32B). Mutations in this locus have been associated previously with Celiac disease (47), coronary heart disease (48), essential tremor (49), and Ellis van Creveld syndrome (50, 51). In addition, we replicated two previously reported loci associating with VEGF levels in 6p21.1 near *VEGFA* (vascular endothelial growth factor A) and in 9p24.2 near *VLDLR* (very-low-density lipoprotein receptor) (8).

Pro-inflammatory cytokines

Locus near DLEU1 shows a large effect on TNF α

We identified a novel variant with a large effect on TNF α levels ($\beta=2.13$ SD) in 13q14.3 near *DLEU1* and *DLEU7* (deleted in lymphocytic leukemia 1 and 7) in the meta-analyses. Previous GWAS findings in this locus include associations with reticulocyte related traits (42) and tooth development (52).

The HLA locus shows a small effect on IL-1 β

A novel variant at 6p22.1 in the human leukocyte antigen locus associating with IL-1 β level was identified in the meta-analyses. In the conditional analyses, we observed two independent association signals at this locus (Table 1, Figure S2 J). The same locus and the same lead variant rs6917603 showed also a suggestive effect on IL4 level (Figure S2 L), but the meta-analysed result was not significant after multiple correction ($p=5.56 \times 10^{-9}$). Variants in this region have been associated previously with multiple immune system related traits such as white blood cell count (42), but also with several other traits, including lipid metabolism phenotypes (53), schizophrenia (54) and lung cancer (55).

259 Chemokines

260 We replicated previously reported loci near *CXCL10* (C-X-C motif chemokine ligand 10) and *ACKR1* (atypical
261 chemokine receptor 1) associating with IP10 levels and with MCP1 levels, respectively (8).

262 Supplemental genome-wide tests in NFBC1966

263 Altogether 236 individuals having fever or CRP>10 mg/l were excluded from the supplemental genome-wide
264 tests performed in the NFBC1966 population. The results of the supplementary analyses were congruent
265 with the original findings (Table S4).

266 Comparisons of SNP effects on inflammatory markers versus other traits

267 Elevated circulating concentrations of inflammatory markers is a risk factor for cardiovascular diseases (24,
268 25). In order to add insights how genetic variants associating with inflammatory phenotypes contribute to
269 other cardiovascular health-related traits, we compared the SNP effects (Z-scores) at each significant locus
270 versus corresponding SNP effects obtained from open-access data (26–28). The list of SNPs used to
271 determine the correlations of the SNP effects on inflammatory phenotypes versus other traits is provided in
272 Table S5. At the *ABO* locus, we observed a negative linear relationship between the SNP effects on sE-selectin
273 and sICAM-1 levels and CAD risk, stroke risk as well as LDL-C and HDL-C levels (Figure 3A-B, Figure S3 A-B).
274 For sVCAM-1 at the same locus, the overall trend was similar, but correlations did not reach statistical
275 significance due to a low number of representative SNPs obtained in clumping (Figure 3C, Figure S3 C). The
276 results at the other loci are provided in Figures S3 D-I.

277 Discussion

278 The present study examines genetic determinants of 16 circulating inflammatory phenotypes in 5,284
279 individuals from Northern Finland with a subsequent meta-analysis of 10 phenotypes in three other Finnish
280 populations adding up to a total of 13,577 participants. We report seven novel and replication of six
281 previously published genetic associations.

282 We detected a novel association for sVCAM-1 concentration at the *ABO* locus. This locus showed robust
283 associations also with sE-selectin and sICAM-1 concentrations congruent with previous studies (23, 30, 31).
284 The present GWAS findings suggested two distinct association signals at the *ABO* locus for the sE-selectin and
285 sICAM-1 levels versus sVCAM-1 level. The results of the supplementary tests supported the perception that
286 genetic variants in this locus may regulate the circulating concentration of adhesion molecules by at least
287 two different mechanisms. The first mechanism comprises the blood type A subtype A1, tagged by
288 rs507666-A, that has a robust lowering effect on sE-selectin and sICAM-1 levels (23, 30). The second
289 mechanism involves the blood type B that seems to have an increasing effect on sVCAM-1 level. Others have
290 suggested that the lowering effect of the A1 subtype on sE-selectin and sICAM-1 could arise from increased
291 glycosyltransferase activity that possibly modifies the shedding of the adhesion molecules from the
292 endothelium and/or their clearance rate from circulation (23, 30, 56). To the best of our knowledge, the
293 underlying mechanism explaining the association between the blood type B and the higher sVCAM-1
294 concentration remains unknown and warrants research. VCAM-1-mediated adhesion involves interaction
295 with galectin-3, a protein that has a specificity for galactosides (57, 58). As the B antigen holds an additional
296 galactose monomer compared with the A and O antigens, and galectins are known to recognize blood type
297 antigens (59), it raises the speculation that the amount of unbound sVCAM-1 in the circulation could be
298 influenced by a possible competitive binding of galectin-3 with sVCAM-1 and the B antigen.

299 To evaluate how variants in the *ABO* locus may relate to other health outcomes, we compared the
300 correspondence of genetic effects on soluble adhesion molecule levels versus cardiovascular health-related
301 traits. We observed that the genetic effects on adhesion molecule levels were inclined to show a negative
302 correlation with the genetic effects on LDL-C and HDL-C levels as well as lower risk for CAD and stroke. This
303 denotes that the genotypes at the *ABO* locus associating with increased levels of soluble adhesion molecules
304 tended to associate with lower circulating cholesterol level as well as lower risk of cardiovascular outcomes.
305 This was unexpected since according to previous evidence increased soluble adhesion molecule levels are
306 linked with atherosclerosis progression and vascular outcomes (25, 60, 61). Possible explanations unravelling
307 the negative correlation advocate that soluble adhesion molecules may compete with leukocyte adhesion to

the endothelial molecules or that enhanced ectodomain shedding may contribute to the reduced recruitment of leukocytes to the subendothelial space thereby promoting cardioprotective effects (62). Additionally, the observed negative relationship between the genetic effects on soluble adhesion molecule and LDL-C levels suggests that altered cholesterol metabolism could contribute to the CAD risk associated with the *ABO* locus; the genetic effects of the same SNPs on LDL-C versus CAD risk showed a positive correlation (Figure 3). Nevertheless, further studies are warranted to understand the exact mechanisms.

Another novel association with sVCAM-1 level was detected in chr12 near *HSP90B1* encoding heat shock protein gp96, a chaperone that is essential for assembly of 14 of 17 integrin pairs in the hematopoietic system (63). Integrin $\alpha 4\beta 1$, also known as very late antigen (VLA)-4, is an important ligand of VCAM-1 (64). The lead SNP of this locus is in LD with rs117468318 ($r^2=1$ in NFBC1966) that locates in the 5'UTR region of *HSP90B1* and, according to RegulomeDB (32), is likely to affect transcription factor binding suggesting a possible regulatory mechanism for the detected association. If altered transcription of *HSP90B1* had a downstream effect on integrin $\alpha 4\beta 1$ level, this could further modify the level of unbound sVCAM-1 in circulation.

The 3rd novel locus showing association with sVCAM-1 level was identified in chr17 near *ABCA8*. The lead SNP rs112001035 is an eQTL for *ABCA8* in multiple tissue types (65). *ABCA8* has been shown to regulate levels of HDL-cholesterol with a mechanism that likely involves interaction with ABCA1 (66). If *ABCA8* is involved in regulation of HDL level (66) and if plasma HDL levels contribute to VCAM-1 expression (67, 68), then altered expression of the *ABCA8* could influence circulating levels of sVCAM-1 via modulating HDL particle concentration. However, this hypothesis is not supported by the fact that the effect of the lead SNP on HDL particle concentration is negligible in a large metabolomics GWAS ($\beta=-0.043$ SD, $p=0.049$) (28). There is evidence suggesting that *ABCA8* may be involved in sphingolipid metabolism (69) and it has been hypothesized that *ABCA8* may be involved in the formation of specific membrane domains during ApoA-I lipidation (66). Thus, one could speculate that the association between the *ABCA8* locus and sVCAM-1 level could be related to altered HDL composition rather than absolute particle concentration, which could contribute to altered endothelial homeostasis. However, more evidence is needed to draw conclusions.

333 We detected a novel effect of rs11220471 in chr11 near *ST3GAL4* on sE-selectin levels in the NFBC1966
334 population. *ST3GAL4* encodes a member of the glycosyltransferase 29 family of enzymes involved in protein
335 glycosylation. In mice, St3Gal4 is needed for synthesis of functional selectin ligands (46) and it has been
336 shown to participate to chemokine C-C motif ligand 5 (Ccl5)-dependent myeloid cell recruitment to inflamed
337 endothelium (70). The altered levels or structure of selectin ligands due to variation in *ST3GAL4* could
338 contribute to the levels of unbound sE-selectin in circulation, providing a biologically rational mechanism for
339 the detected association.

340 In the meta-analyses, we detected a novel large effect locus for VEGF in chr4 ($\beta=-2.38$ SD) near genes *EVC*
341 (*EvC* ciliary complex subunit 1), *EVC2* (*EvC* ciliary complex subunit 2) and *STK32B* (serine/threonine kinase
342 32B). Mutations in this locus have been associated previously with celiac disease (47), coronary artery disease
343 (48), and Ellis-van Creveld syndrome, a rare recessive disorder characterized with congenital defects such as
344 short ribs, postaxial polydactyly, growth retardation, and ectodermal and cardiac defects (50, 51). The
345 expression level of *STK32B* has been associated with clinicopathological features of oral cavity cell carcinoma
346 including peritumoral inflammatory infiltration, metastatic spread to the cervical lymph nodes, and tumour
347 size (71). *STK32B* may play a role in the hedgehog signalling pathway, which has been implicated in metastasis
348 and angiogenesis in cancer (71) and downregulated in celiac disease (72). The hedgehog signalling has shown
349 to be involved in the regulation of VEGF expression during developmental angiogenesis in avian embryo (73).
350 Thus, previous literature and our results advocate that *STK32B* may be involved in the regulation of VEGF
351 levels possibly via hedgehog signalling-related mechanism.

352 The other novel findings obtained in meta-analysis include a large effect locus on $\text{TNF}\alpha$ level in chr13 ($\beta=2.13$
353 SD). The locus in 13q14.3 associating with $\text{TNF}\alpha$ locates near *DLEU1* and *DLEU7* (Deleted in Lymphocytic
354 Leukemia 1 and 7). This region is recurrently deleted in tumours and hematopoietic malignancies (74, 75).
355 *DLEU1* is a part of a transcriptionally coregulated gene cluster that modulates the activity of the NF-kB
356 pathway (76) which is also modulated by $\text{TNF}\alpha$ (77). It is largely unknown how the *DLEU1* and related *DLEU2*
357 regulate NF-kB activity (78); our result suggests that $\text{TNF}\alpha$ signalling might be involved in this mechanism.

358 At last, we identified a small effect locus in chr6 harbouring two independent association signals on IL-1 β and
359 showing suggestive association also on IL4 level. This association signal is in the region coding the human
360 leukocyte antigen proteins, and further experimental evidence would be needed to identify the exact
361 mechanism how the locus contributes to interleukin levels.

362 The strengths and limitations of our study should be considered. The sample size of the present study should
363 provide adequate power for detecting genetic associations with circulating markers of systemic inflammation
364 (9). The use of genetically isolated populations, such as inhabitants of Northern Finland, should further
365 enhance the power for locus identification in GWAS settings (79). We were able to perform meta-analyses
366 only for 10 out of the 16 inflammatory phenotypes analysed in the NFBC1966 population and, thus, a
367 replication of the present findings in other populations would be helpful. The inter-assay coefficient of
368 variability measures for sE-selectin and VEGF in particular are notably larger than 15% that is considered to
369 be the limit for acceptable values (Table S1). However, to our consideration, all the findings identified in the
370 present study locate on genome regions with biologically relevant genes. Furthermore, the extremely small
371 p-values ($p=4.48 \times 10^{-305}$ for sE-selectin at the *ABO* locus and $p=4.95 \times 10^{-96}$ for VEGF at the *VEGFA* locus) and the
372 replications of the previously reported loci speak for the data adequacy and adds confidence to the novel
373 associations. Due to mismatches in genotype availability between the present results and open-access data
374 sets or low number of SNPs obtained after exclusions, it was not possible to carry out meaningful
375 comparisons of the genetic effects at all the significant loci.

376 The present results provide novel information on genetic mechanisms influencing levels of inflammatory
377 phenotypes in circulation. The evident role of the *ABO* locus in the regulation of the soluble adhesion
378 molecule levels in circulation likely encompasses at least two distinct mechanisms influencing sE-selectin,
379 sICAM-1 and sVCAM-1 levels. Our findings provide evidence that increased soluble adhesion molecule
380 concentrations per se may not be a risk factor for cardiovascular outcomes. In particular, genetic variation
381 associating with increased sE-selectin or sICAM-1 levels at the *ABO* locus seem to contribute to lower CAD
382 risk. Furthermore, genetic effects at the *ICAM1* locus providing a direct molecular link to sICAM-1

383 concentration do not correlate with the genetic effects on CAD risk nor stroke risk. Overall, the present study
384 extends the knowledge about the precise molecular pathways involved in inflammatory load.

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638 **Conflict of Interest**

639 VS has participated in a conference trip sponsored by Novo Nordisk and received a honorarium from the
640 same source for participating in and advisory board meeting. He also has ongoing research collaboration with
641 Bayer Ltd.

642 Tables

643 Table 1. Basic characteristics of the NFBC1966 study population.

Characteristics	
Total number of individuals	5284
Number of males (%)	2543 (48.1)
Age, years	31.1 ± 0.4
BMI, kg/m ²	24.4 ± 4.0
Glucose, mmol/l	5.1 ± 0.7
LDL-cholesterol, mmol/l	3.0 ± 0.9
HDL-cholesterol, mmol/l	1.6 ± 0.4
Systolic blood pressure, mmHg	124.2 ± 13.6
Diastolic blood pressure, mmHg	76.8 ± 11.7
Values are mean ± standard deviation.	

644

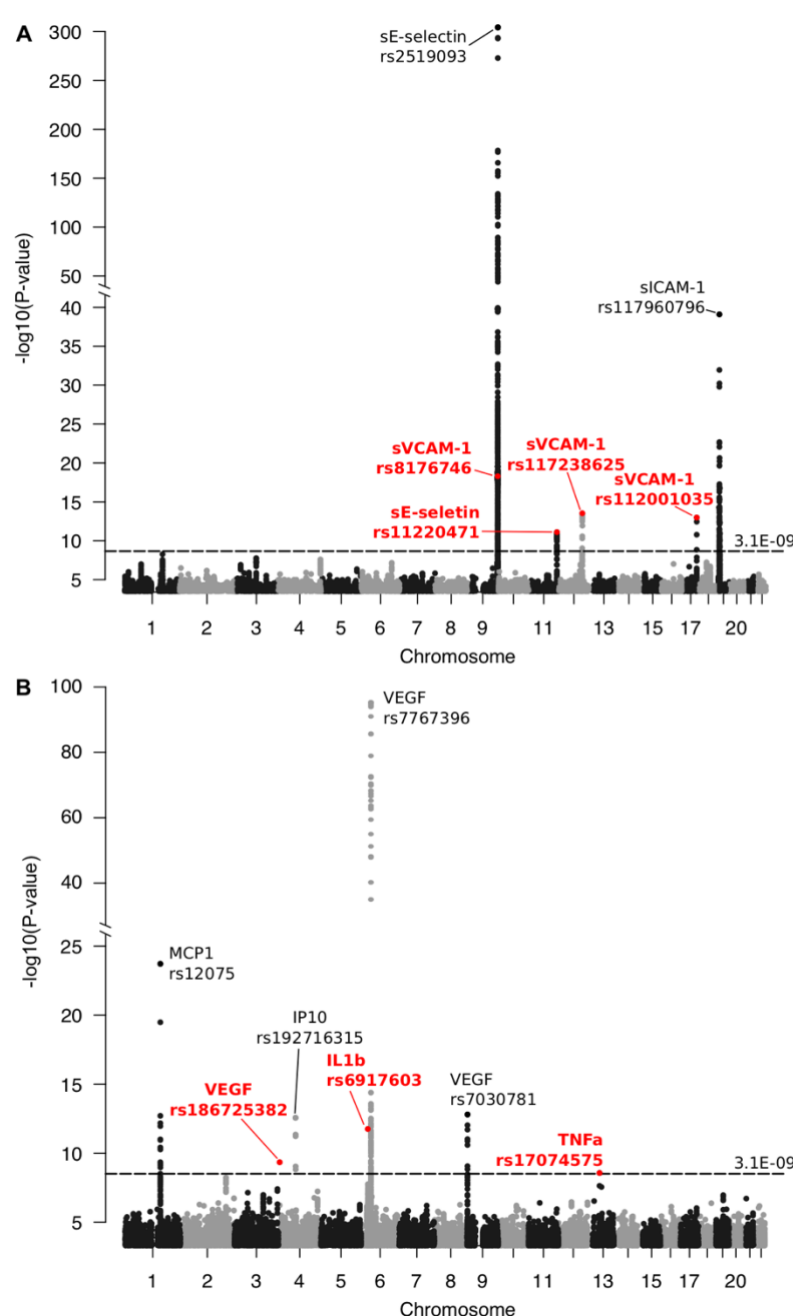
645 Table 2. Significant loci associating with the circulating inflammatory phenotypes.

Study	Marker	Locus	Chr:Position	Candidate gene	Nearest gene(s)	Annotation	dbSNP reference	EA	EAF	Beta	P-value	Variance explained	Total variance explained
NFBC1966	sE-Selectin	9q34.2	9:136141870	<i>ABO</i>	<i>ABO</i>	Intronic	rs2519093	T	0.188	-0.903	4.48E-305	0.249	0.258
		11q24.2	11:126266665	<i>ST3GAL4</i>	<i>ST3GAL4</i>	Intronic	rs11220471	G	0.212	-0.162	7.72E-12	0.009	
	sICAM-1	9q34.2	9:136141870	<i>ABO</i>	<i>ABO</i>	Intronic	rs2519093	T	0.188	-0.352	7.43E-48	0.038	0.118
		19p13.2	19:10383403	<i>ICAM1</i>	<i>ICAM1</i>	Intronic	rs117960796	A	0.012	-1.669	8.03E-40	0.066	
		19p13.2	19:10497360	<i>ICAM1</i>	<i>CDC37</i>	Intergenic	rs74428614	A	0.163	0.226	1.14E-16 *	0.014	
	sVCAM-1	9q34.2	9:136131322	<i>ABO</i>	<i>ABO</i>	Missense	rs8176746	T	0.129	0.256	5.06E-19	0.015	0.038
		12q23.3	12:104448391	<i>HSP90B1</i>	<i>GLT8D2</i>	Intronic	rs117238625	A	0.023	0.510	2.90E-14	0.012	
meta-analyses		17q24.2	17:66823805	<i>ABCA8</i>	<i>ABCA8</i>	Intergenic	rs112001035	A	0.060	-0.324	1.04E-13	0.012	
	IL1 β	6p22.1	6:30017071		<i>HLA locus</i>	Intronic	rs6917603	C	0.251	-0.163	1.76E-12	0.010	0.015
		6p22.1	6:30013887		<i>HLA locus</i>	Intronic	rs9261224	T	0.035	0.261	1.31E-09 *	0.005	
	IP10	4q21.1	4:76899176	<i>CXCL10</i>	<i>SAD1</i>	Intronic	rs192716315	C	0.003	1.513	2.71E-13	0.014	0.014
	MCP1	1q23.2	1:159175354	<i>ACKR1</i>	<i>ACKR1</i>	Missense	rs12075	G	0.561	0.125	1.90E-24	0.008	0.008
	TNF α	13q14.3	13:51141997	<i>DLEU1</i>	<i>DLEU1</i>	Intronic	rs17074575	G	0.002	2.131	2.71E-09	0.018	0.018
	VEGF	4p16.2	4:5636073	<i>STK32B</i>	<i>EVC2</i>	Intronic	rs186725382	A	0.001	-2.380	4.53E-10	0.011	0.052
		6p21.1	6:43927050	<i>VEGFA</i>	<i>C6orf223</i>	Intergenic	rs7767396	G	0.422	0.272	4.95E-96	0.036	
		9p24.2	9:2686273	<i>VLDLR</i>	<i>VLDLR</i> , <i>KCNV2</i>	Intergenic	rs7030781	T	0.373	0.099	1.57E-13	0.005	

Statistical significance is considered at $p < 3.1 \times 10^{-9}$. Novel findings are highlighted with bold font. All positions correspond to human genome build 37. Asterisk (*) indicates associations that are significant after conditioning the analyses on the locus specific lead variant on the preceding row. EA, effect allele; EAF, effect allele frequency.

Figures

Figure 1. The combined Manhattan plots for significant associations with inflammatory markers studied in (A) NFBC1966 and in (B) meta-analyses with three other Finnish population cohorts. Significance threshold $p < 3.1 \times 10^{-9}$ derives from the standard p-value limit for genome-wide significance $p < 5 \times 10^{-8}$ corrected for 16 markers examined in the present study. Novel association signals are highlighted with red font and replicated loci are marked with black font.



654 Figure 2. The effects of the ABO blood types and the A1 subtype on soluble adhesion molecule levels.

655 The effects of the ABO blood types on sE-selectin, sICAM-1 and sVCAM-1 levels were evaluated in linear

656 models, where adjusted (sex, age, BMI, and the ten first genetic principal components) and transformed

657 soluble adhesion molecule concentrations were used as outcomes and the ABO blood type served as

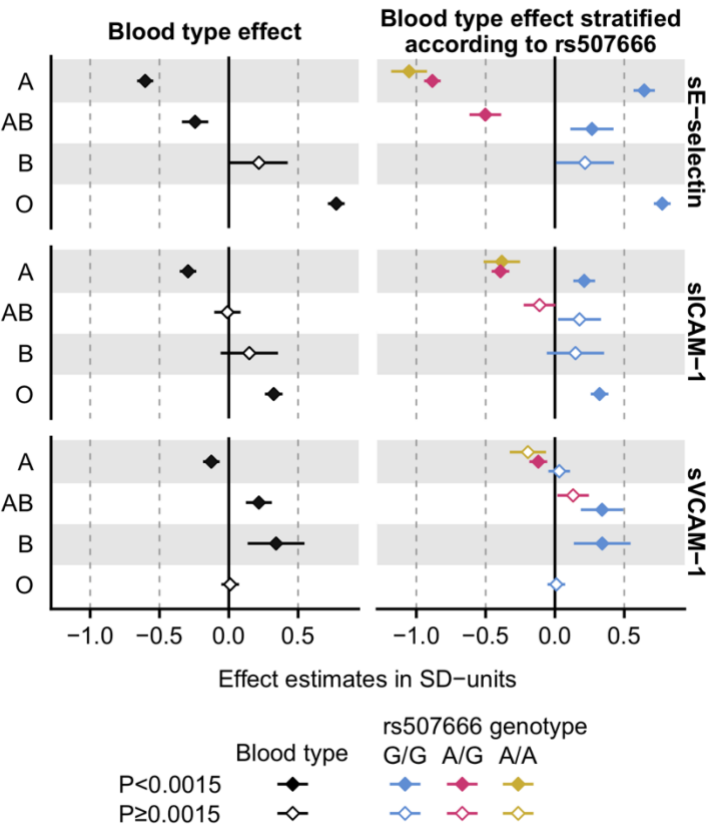
658 categorical variable (A versus non-A, etc.). Corresponding models were fitted for the ABO blood types

659 stratified by the rs507666-A allele count (0, 1 or 2), where the A allele tags the ABO subtype A1 having

660 enhanced glycosyltransferase activity (25). No individuals were found to have B or O blood type and one or

661 more copies of the rs507666-A allele and, thus, it was not possible to perform stratification within these

662 blood types.



664 Figure 3. The correlations of the SNP effects on soluble adhesion molecule levels versus other cardiovascular
665 health-related traits at the *ABO* locus.

666 The correspondence of the SNP effects on inflammatory phenotypes versus cardiovascular health-related
667 traits were determined for the SNPs associating with (A) sE-selectin, (B) sICAM-1, and (C) sVCAM-1 levels at
668 the *ABO* locus. SNP effects on coronary artery disease (CAD), stroke, and LDL-C or HDL-C were extracted from
669 CARDIoGRAM (26), Stroke Consortia (27), and a metabolomics GWAS (28) summary statistics, respectively.
670 The correlations (Pearson's r) of the genetic effects were estimated using subsets of representative SNPs
671 extracted from the summary statistics of the present GWAS using a clumping function in PLINK and r^2
672 threshold of 0.2. Prior to clumping, data were filtered to include only the SNPs available in all the three
673 datasets. Correlations with $p \geq 0.05$ are marked with a cross. The SNPs used for estimating the correlations
674 are listed in Table S5 and scatter plot representations as well as correlations at the other loci and other
675 inflammatory phenotypes are shown in Figure S3.

