

1 **Multivariate genome-wide association analysis of a cytokine** 2 **network reveals variants with widespread immune,** 3 **haematological and cardiometabolic pleiotropy**

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53

54 **Abstract**

55

56 Cytokines are essential regulatory components of the immune system and their aberrant levels
57 have been linked to many disease states. Despite increasing evidence that cytokines operate in
58 concert, many of the physiological interactions between cytokines, and the shared genetic
59 architecture that underlie them, remain unknown. Here we aimed to identify and characterise
60 genetic variants with pleiotropic effects on cytokines – to do this we performed a multivariate
61 genome-wide association study on a correlation network of 11 circulating cytokines measured
62 in 9,263 individuals. Meta-analysis identified a total of 8 loci significantly associated with the
63 cytokine network, of which two (*PDGFRB* and *ABO*) had not been detected previously.
64 Bayesian colocalisation analysis revealed shared causal variants between the eight cytokine
65 loci and other traits; in particular, cytokine network variants at the *ABO*, *SERPINE2*, and
66 *ZFPM2* loci showed pleiotropic effects on the production of immune-related proteins; on
67 metabolic traits such as lipoprotein and lipid levels; on blood-cell related traits such as platelet
68 count; and on disease traits such as coronary artery disease and type 2 diabetes.

69 Introduction

70 Cytokines are signalling molecules secreted by cells that are central to multiple physiological
71 functions, especially immune regulation (1). Broadly-speaking, cytokines include chemokines
72 that drive movement of cells, and growth factors that drive cell growth and proliferation.
73 Changes in circulating cytokine levels have been associated with infection (2), autoimmune
74 diseases (3), malignancies (4), as well as atherosclerosis and cardiovascular disease (5,6). The
75 expression of cytokines can be strongly regulated by genetic variation (7), and several studies
76 have identified cis-acting genetic variants associated with circulating levels of certain
77 cytokines and their receptors under various conditions (8–10). These initial studies laid the
78 foundation for genetic investigation of circulating cytokine levels at a scale and breadth that
79 may improve our understanding of individual differences in immune response, inflammation,
80 infection and common disease susceptibility.

81
82 Despite cytokines operating in concert to facilitate immune regulation, genome-wide
83 association studies (GWAS) have typically focused on individual cytokines (11–18). The most
84 extensive cytokine GWAS to date separately analysed individual levels of 41 circulating
85 cytokines in approximately 8,000 individuals, identifying 27 distinct loci each associated with
86 at least one cytokine (19). Others have identified loci influencing cytokine production in
87 response to pathogens (20,21). While these previous GWAS utilised a univariate framework,
88 analysing each cytokine separately, studies of related traits indicate a multivariate framework
89 can confer greater statistical power, for example by taking advantage of the tightly co-regulated
90 nature of both pro and anti-inflammatory cytokines.

91
92 Several methods for multivariate GWAS of correlated phenotypes have been developed (22–
93 27). Simulations have shown that multivariate analysis can result in increased power to detect
94 genetic associations with small or pleiotropic effects across phenotypes (22,28–30). These have
95 largely been conducted on metabolic traits where they have demonstrated a boost in statistical
96 power. For example, multivariate analysis of four lipid traits led to a 21% increase in
97 independent genome-wide significant variants compared to univariate analysis (23). Similar
98 findings were shown for other metabolic traits (24,31). Moreover, complex genotype-
99 phenotype dependencies have been revealed when jointly testing rare variants with lipoprotein
100 traits (32). Notably, a multivariate GWAS of networks of highly correlated serum metabolites
101 was able to detect nearly twice the number of loci compared to univariate testing, with

102 downstream tissue-specific transcriptional analyses showing that the top candidate genes from
103 multivariate analysis were upregulated in atherosclerotic plaques (31).

104

105 In this study, we focus on correlated immune traits by leveraging the correlation structure
106 within a network of 11 cytokines to perform a multivariate genome-wide scan in 9,263
107 individuals from three population-based cohorts. We then investigate the colocalisation of
108 cytokine-associated variants with those regulating gene expression in numerous tissues and
109 cell types, circulating protein and metabolite levels, haematological traits, and disease states.
110 Finally, we highlight and characterise variants as potential master regulator of the cytokine
111 network, with pleiotropic effects on production of inflammatory proteins, immune cell
112 function, lipoprotein and lipid levels, and cardiometabolic diseases.

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118 **Methods**

119 **Study populations**

120 Approval for the study protocols for each cohort was obtained from their respective ethics
121 committees, and all subjects enrolled in the study gave written informed consent.

122

123 The Cardiovascular Risk in Young Finns Study (YFS) is a longitudinal prospective cohort
124 study commenced in 1980, with follow-up studies carried out every 3 years. The purpose of
125 this study was to monitor the risk factors of cardiovascular disease in children and adolescents
126 from different regions of Finland. In the baseline study, conducted in five Finnish metropolitan
127 areas (Turku, Helsinki, Kuopio, Tampere and Oulu), a total of 3,596 children and adolescents
128 were randomly selected from the national public register, the details of which were described
129 in (33). A total of 2,204 participants responded to the 2007 follow-up study (YFS07), for which
130 the age range was 30-45 years. Ethics were approved by the Joint Commission on Ethics of the
131 Turku University and the Turku University Central Hospital.

132

133 The FINRISK cohorts were part of a cross-sectional population-based survey, which are
134 carried out every five years since 1972 to evaluate the risk factors of chronic diseases in the
135 Finnish population (34). Each survey has recruited a representative random sample of 6,000-

136 8,800 individuals, within the age group of 25-74 years, chosen from the national population
137 information system. This study utilised samples from the 1997 (FINRISK97) and 2002
138 (FINRISK02) collections, which recruited individuals from five or six (for FINRISK02) major
139 regional and metropolitan areas of Finland: the provinces of North Karelia, Northern Savo,
140 Northern Ostrobothnia, Kainuu, and Lapland; the Turku and Loimaa region of south-western
141 Finland; and the Helsinki and Vantaa metropolitan area. In total, 8,444 (aged 24-74 years) and
142 8,798 (aged 51-74 years) individuals participated in the FINRISK97 and FINRISK02 studies,
143 respectively. Importantly, each FINRISK survey is an independent cohort, each comprising a
144 different set of participants. Ethics were approved by the coordinating ethical committee of the
145 Helsinki and Uusimaa hospital district, Finland.

146

147 **Blood sample collection**

148 Blood samples and detailed information on various physical and clinical variables for the YFS
149 and FINRISK cohorts were collected using similar protocols as described previously (33,34).
150 Venous blood was collected following an overnight fast for the YFS cohorts, while non-fasting
151 blood was collected for FINRISK. Samples were centrifuged, and the resulting plasma and
152 serum samples were aliquoted into separate tubes and stored at -70°C for later analyses.

153

154 **Genotype processing and quality control**

155 Genotyping in YFS and FINRISK cohorts was performed on whole blood genomic DNA. For
156 YFS07 (N=2,442), a custom 670K Illumina BeadChip array was used for genotyping. For
157 FINRISK97 (N=5,798), the Human670-QuadCustom Illumina BeadChip platform was used
158 for genotyping. For FINRISK02 (N=5,988), the Human670-QuadCustom Illumina BeadChip
159 (N=2,447) and the Illumina Human CoreExome BeadChip (N=3,541) was used for genotyping.
160 The Illuminus clustering algorithm was used for genotype calling (35) and quality control (QC)
161 was performed using the Sanger genotyping QC pipeline. This included removing SNPs and
162 samples with > 5% genotype missingness followed by removal of samples with gender
163 discrepancies. Genotypes were then imputed with IMPUTE2 (36) using the 1000 Genomes
164 Phase 1 version 3 as the reference panel followed by removal of SNPs with call rate < 95%,
165 imputation “info” score < 0.4, minor allele frequency < 1%, and Hardy-Weinberg equilibrium
166 P -value < 5×10^{-6} . Instances where data was generated using different genotyping platforms,
167 overlapping SNPs were merged using PLINK version 1.90 software ([https://www.cog-
168 genomics.org/plink2](https://www.cog-genomics.org/plink2)) (37). A total of 6,664,959, 7,370,592 and 6,639,681 genotyped and
169 imputed SNPs passed quality control in YFS, FINRISK97 and FINRISK02, respectively.

170 Cryptic relatedness was assessed using identity by descent (IBD) estimates and in cases where
171 the pi-hat relatedness was greater than 0.1, one of the two individuals was randomly removed
172 (N=44 for YFS, N=291 for FINRISK97, and N=39 for FINRISK02). Genetic PCs were
173 obtained through principal component analysis (PCA) using FlashPCA (38) on ~60,000 LD
174 pruned SNPs.

175

176 **Measurement of cytokines**

177 Concentrations of cytokines, chemokines, and growth factors (hereafter referred to as
178 cytokines) were measured in serum (YFS07), EDTA plasma (FINRISK97), and heparin plasma
179 (FINRISK02) using multiplex fluorescent bead-based immunoassays (Bio-Rad). A total of 48
180 cytokines were measured in YFS07 (N=2,200) and FINRSK02 (N=2,775) using two
181 complementary array systems: the Bio-Plex Pro™ Human Cytokine 27-plex assay and Bio-
182 Plex Pro™ Human Cytokine 21-plex assay. For FINRISK97, 19 cytokines were assayed on
183 the Human Cytokine 21-plex assay system. All assays were performed in accordance with the
184 manufacturer's instructions, except that the amount of beads, detection antibodies, and
185 streptavidin-phycoerythrin conjugate were used at half their recommended concentration.
186 Fluorescence intensity values determined using the Bio-Rad's Bio-Plex 200 array reader were
187 converted to concentrations from the standard curve generated by the Bio-Plex™ Manager 6.0
188 software. For each cytokine, a standard curve was derived by fitting a five-parameter logistic
189 regression model to the curve obtained from standards provided by the manufacturer.
190 Cytokines with concentrations at the lower and upper asymptotes of the sigmoidal standard
191 curve were set to the concentration corresponding to the fluorescent intensity 2% above or
192 below the respective asymptotes.

193

194 **Cytokine data filtering, normalisation and clustering**

195 The analysis was limited to 18 cytokines (**Table S1**) assayed in all three cohorts. Although
196 Interleukin 1 receptor, type I (IL-1Ra) was assayed in all three cohorts, it was excluded from
197 the analyses due to its inconsistent Pearson correlation pattern with other 18 cytokines across
198 the three datasets.

199

200 Before normalisation, cytokine data was subset to individuals with matched genotype data in
201 YFS07 (N=2,018), FINRISK97 (N=5,728), and FINRISK02 (N=2,775). We excluded
202 individuals in YFS07 reporting febrile infection in the two weeks prior to blood sampling
203 (N=92). To identify extreme outlier samples, PCA was performed on the log₂ transformed

204 cytokine values using the missMDA R package (39). This method first imputed the missing
205 cytokine values using a regularised iterative PCA algorithm implemented in the imputePCA
206 function, before performing PCA. Three and two outlier samples were removed from
207 FINRISK97 and FINRISK02 respectively. Based on IBD analysis described above, 44
208 (YFS07), 291 (FINRISK97), and 39 (FINRISK02) individuals were also removed. After
209 filtering, a total of 1,843, 5,434 and 1,986 individuals passed quality control in YFS07,
210 FINRISK97 and FINRISK02, respectively, and these were used for downstream analysis.

211
212 Since all 18 cytokines displayed non-Gaussian distributions, we performed normalisation of
213 cytokine levels. For YFS07, the lower limit of detection (LOD) was available for each
214 cytokine. Reported values that were below the LOD were indistinguishable from background
215 noise signals or instrument error (40), and were excluded and treated as missing. For
216 FINRISK97 and FINRISK02, the detection limits were not available; however, it was observed
217 that these two datasets exhibited a bimodal distribution, with the leftmost peak below the
218 expected LOD when compared to the YFS dataset. Individuals in the leftmost peak were
219 therefore set to missing. The log₂-transformed cytokine values were then normalised to follow
220 standard Gaussian distributions (with mean of 0 and sd of 1) using rank-based inverse normal
221 transformation (rntransform) as implemented in the GenABEL R package (41). For each study
222 group, residuals for all cytokines were calculated by regressing the normalised cytokine values
223 on age, sex, BMI, lipid and blood pressure medication, pregnancy status (FINRISK97), and
224 the first 10 genetic PCs using a multiple linear regression model.

225
226 Detection of groups of correlated cytokines was done in FINRISK97, the cohort with the
227 largest sample size. Pairwise Pearson correlation was performed amongst residuals of 18
228 cytokines. These cytokines were then subjected to hierarchical clustering, with one minus the
229 absolute correlation coefficient used as the dissimilarity metric. We then defined a cytokine
230 network – a group of 11 cytokines that were moderate- to highly-correlated ($r > 0.57$) – for
231 subsequent use in the multivariate analysis.

232

233 **Statistical Analysis**

234 Univariate association analysis was carried out with linear regression in PLINK (37), where
235 the residuals of each cytokine were regressed on each SNP genotypes. Summary statistics at
236 each marker across three datasets were then combined in a meta-analysis using the METAL
237 software program (42), which implemented a weighted Z-score method.

238

239 Multivariate testing (MV) was performed under the canonical correlation framework
240 implemented in PLINK (MV-PLINK) (22), which extracted the linear combination of traits
241 most highly-correlated with genotypes at a particular SNP. The test is based on Wilks' Lambda
242 ($\lambda = 1 - \rho^2$), where ρ is the canonical correlation coefficient between the SNP and the cytokine
243 network. Corresponding *P*-values were computed by transforming Wilks' Lambda to a statistic
244 that approximates an *F* distribution and the loadings for each cytokine represented their
245 individual contributions toward the multivariate association result (22). Since the multivariate
246 beta-coefficients and standard errors were not calculated by MV-PLINK, the cohort-level
247 multivariate *P*-values were combined in a meta-analysis using the weighted Z-score method
248 (43,44) implemented in the metap R package. Briefly, the *P*-values for each dataset were
249 transformed into Z-scores, weighted by their respective sample sizes and the sum of these
250 weighted Z-scores were then divided by the square root of the sum of squares of the sample
251 size for each study. The combined weighted Z-score obtained was back-transformed into a one-
252 tailed *P*-value.

253

254 To assess the inflation of the test statistics as a result of population structure, quantile-quantile
255 (Q-Q) plots of observed vs. expected- \log_{10} *P*-values were generated from the multivariate
256 analysis of the three datasets, both individually and meta-analysed. Corresponding genomic
257 inflation factor (λ) was calculated by taking the ratio of the median observed distribution of *P*-
258 values to the expected median.

259

260 To investigate the existence of additional independent signals within the significant
261 multivariate loci, a conditional stepwise multivariate meta-analysis was performed within each
262 locus. For each study cohort, the lead SNP at each locus (*P*-value $< 5 \times 10^{-8}$) together with
263 other covariates were fitted in a linear regression model for each cytokine in the network. The
264 resulting residuals were provided as an input for the multivariate test of the locus being
265 assessed. The cohort-level conditional *P*-values were then combined in a meta-analysis. The
266 stepwise conditional analysis was repeated in the univariate model with the lead multivariate
267 SNPs until no additional significant signal was identified.

268

269 **Colocalisation analysis**

270 Bayesian colocalisation tests between cytokine network-associated signals and the following
271 trait- and disease-associated signals were performed using the COLOC R package (45). For

272 whole blood *cis*-eQTLs, we downloaded publicly-available summary data from the eQTLGen
273 Consortium portal (<http://www.eqtlgen.org/>). The eQTLGen Consortium analysis is the largest
274 meta-analysis of blood eQTLs to date and comprises of 31,684 blood and PBMC samples from
275 a total of 37 datasets (46). For immune cell *cis*-eQTLs, we either generated *cis*-eQTL summary
276 data in resting B-cells (47), resting monocytes (48), and stimulated monocytes with interferon-
277 γ or lipopolysaccharide (48), or obtained publicly-available *cis*-eQTL summary data generated
278 by the BLUEPRINT consortium in neutrophils and CD4⁺ T-cells (49). For *cis*-eQTL mapping
279 in B-cells and monocytes (resting and stimulated), information on accessing the raw gene
280 expression and genotype data, data pre-processing, and *cis*-eQTL analysis has been described
281 in a previous study (50). The BLUEPRINT immune cell summary statistics was downloaded
282 from: ftp://ftp.ebi.ac.uk/pub/databases/blueprint/blueprint_Epivar/. For protein QTLs, we used
283 publicly-available SomaLogic plasma protein GWAS summary statistics from the INTERVAL
284 study (17). For disease or complex trait associations, we compiled summary statistics of 185
285 diseases and quantitative traits from GWAS studies conducted in
286 European ancestry individuals, which were accessed from the UK biobank (**Table S10**), or
287 downloaded from either ImmunoBase (<https://www.immunobase.org/>), the NHGRI-EBI
288 GWAS Catalog (<https://www.ebi.ac.uk/gwas/>), or LD Hub (<http://ldsc.broadinstitute.org/>).
289 Here, we only considered immune-related and cardiometabolic diseases. For each cytokine
290 network locus, we only tested traits or diseases with the minimum association *P*-value $< 1 \times$
291 10^{-6} at this locus. COLOC requires either beta-coefficients and its variance, or *P*-values, for
292 each SNP, in addition to MAF and sample size. Since PLINK multivariate did not produce beta
293 values and standard errors, we instead used meta-analysed *P*-values for the multivariate
294 cytokine GWAS summary data. For each association pair assessed for colocalisation, SNPs
295 within 200kb of the lead multivariate cytokine GWAS SNP were considered. COLOC
296 (*coloc.abf*) was run with default parameters and priors. COLOC computed posterior
297 probabilities for the following five hypotheses: PP0, no association with trait 1 (cytokine
298 GWAS signal) or trait 2 (e.g. eQTL signal); PP1, association with trait 1 only (i.e. no
299 association with trait 2); PP2, association with trait 2 only (i.e. no association with trait 1); PP3,
300 association with trait 1 and trait 2 by two independent signals; PP4, association with trait 1 and
301 trait 2 by shared variants. In practice, evidence of colocalisation were defined by
302 $PP3 + PP4 \geq 0.99$ and $PP4/PP3 \geq 5$, a cut off previously suggested (50).

303
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307

308 **Results**

309 **Summary of cohorts and data**

310 Our final dataset comprised a total of 9,267 individuals enrolled in three population-based
311 studies, YFS07 (N=1,843), FINRISK97 (N=5,438), and FINRISK02 (N=1,986), all of whom
312 had genome-wide genotype data and quantitative measurements of 18 cytokines (**Table S1**).
313 Characteristics of the study cohorts are summarised in **Table 1**. Genotypes for the three
314 datasets were imputed with IMPUTE2 (36) using the 1000 Genomes Phase 1 version 3 of the
315 reference panel. After quality control, a total of 6,022,229 imputed and genotyped SNPs were
316 available across all cohorts. Cytokine levels were measured in serum and plasma using Bio-
317 Plex Pro™ Human Cytokine 27-plex and 21-plex assays, then subsequently normalised and
318 adjusted for covariates including age, sex, BMI, pregnancy status, blood pressure lowering
319 medication, lipid lowering medication, and population structure (**Methods**). An overview of
320 the study is shown in **Figure 1**.

321

322 **A correlation network of circulating cytokines**

323 To characterise the correlation structure of circulating cytokines, we utilised the largest dataset
324 available (FINRISK97) and the set of 18 cytokines overlapping all three cohorts. IL-18 was
325 very weakly correlated with other cytokines (**Figure 2A**), while TRAIL, SCF, HGF, MCP-1,
326 EOTAXIN and MIP-1b showed moderate correlation with the others. A distinct set of 11
327 cytokines showed high correlation amongst themselves (median $r=0.75$). In the smaller cohorts
328 (YFS07 and FINRISK02), the cytokine correlation structure was similar but weaker (**Figure**
329 **S1**), with the set of 11 cytokines also showing relatively high correlation (YFS07 median
330 $r=0.42$; FINRISK02 median $r=0.46$). We utilised this set of 11 cytokines (denoted below as the
331 cytokine network) for multivariate association analysis.

332

333 The cytokine network included both anti-inflammatory (IL-10, IL-4, IL-6) and pro-
334 inflammatory (IL-12, IFN- γ , IL-17) cytokines as well as growth factors (FGF-basic, PDGF-
335 BB, VEGF-A, G-CSF) and a chemokine (SDF-1a) involved in promoting leukocyte
336 extravasation and wound healing (51–53). These cytokines were all positively correlated,
337 which is likely indicative of counter-regulatory (negative-feedback) mechanisms amongst pro-
338 inflammatory and anti-inflammatory pathways, such as that of IFN- γ and IL-10 (54).

339

340 **Multivariate genome-wide association analysis for cytokine loci**

341 We performed a multivariate GWAS on the cytokine network in each cohort separately, then
342 cohort-level results were combined using meta-analysis (**Methods**). Since one hypothesis test
343 (corresponding to the cytokine network) was performed for each SNP, a genome-wide
344 significance threshold of $P < 5 \times 10^{-8}$ was used. Minimal inflation was observed for the cohort-
345 level and meta-analysis test statistics with lambda (λ) inflation ranging between 1.00-1.02
346 (**Figure S2A – D**).

347

348 We identified 8 loci reaching genome-wide significance for the cytokine network (**Figure 2B**;
349 **Table 2**). The strongest association was rs7767396 (meta- P -value = 6.93×10^{-306}), a SNP
350 located 172kb downstream of vascular endothelial growth factor A (*VEGFA*) (**Figure S3A**).
351 The *VEGFA* locus was previously identified in GWAS for individual cytokine levels including
352 VEGF-A, IL-7, IL-10, IL-12, and IL-13 (14,19). Consistent with these earlier results, we found
353 that VEGF-A, IL-10, and IL-12 were the top three cytokines based on their trait loadings
354 (relative contribution of each cytokine to the multivariate association result) in each cohort and
355 also significantly associated with this locus in the univariate scans (**Figure S4A**). Multivariate
356 analysis also confirmed four other previously known associations (14,16,19), including loci
357 harbouring *SERPINE2* (rs6722871; meta- P -value = 1.19×10^{-59}), *ZFPM2* (rs6993770; meta- P -
358 value = 4.73×10^{-8}), *VLDLR* (rs7030781; meta- P -value = 3.78×10^{-13}), and *PCSK6*
359 (rs11639051; meta- P -value = 1.93×10^{-58}) (**Figure 2B**; **Table 2**; **Figure S3B – E**). The
360 cytokine with the highest loading at each of these loci was consistent with those previously
361 identified in univariate analysis (**Figure S4B – E**).

362

363 The multivariate GWAS also detected novel cytokine associations not identified in any
364 previous univariate tests of these cytokines. These were three loci with genic lead SNPs in the
365 candidate genes *F5*, *PDGFRB*, and *ABO*. The lead variant at the *F5* locus (rs9332599; meta-
366 P -value = 7.17×10^{-12}) is located in intron 12 of *F5* (**Figure S3F**). At the platelet-derived
367 growth factor receptor-beta (*PDGFRB*) locus, the lead variant rs2304058 (meta- P -value = 4.06
368 $\times 10^{-9}$) is within intron 10 of *PDGFRB* (**Figure S3G**). At the *ABO* locus, the lead variant
369 rs550057 (meta- P -value = 2.75×10^{-8}) is within the first intron of *ABO* (**Figure S3H**);
370 furthermore, rs550057 is located ~1.6 kb upstream of the erythroid cell specific enhancer,
371 which contains a GATA-1 transcription factor binding site and has been shown to enhance the
372 transcription of the *ABO* gene (55).

373

374 To investigate the presence of multiple independently associated variants at each of the eight
375 loci, we performed stepwise conditional multivariate meta-analysis. Three loci (*SERPINE2*,
376 *VEGFA*, and *PCSK6*) exhibited evidence of multiple independent signals (**Table S2**). In
377 addition to the lead variants (rs6722871, rs7767396, rs11639051) at each of these three loci,
378 we identified additional association signals (rs55864163; *SERPINE2*, meta- $P_{cond} = 9.03 \times 10^{-29}$;
379 rs112215592, *SERPINE2*, meta- $P_{cond} = 2.10 \times 10^{-12}$; rs4714729; *VEGFA*, meta- $P_{cond} = 7.49$
380 $\times 10^{-10}$; rs6598475, *PCSK6*, meta- $P_{cond} = 2.63 \times 10^{-17}$), which were independently associated
381 with the cytokine network. We also performed conditional univariate analysis that adjusted for
382 the lead multivariate SNPs, which were either the same lead univariate SNPs or in high LD (r^2
383 = 0.99). This univariate analysis also uncovered the same secondary signal at the *VEGFA* locus
384 in association with *VEGFA* cytokine levels (rs4714729; meta- $P_{cond} = 8.8 \times 10^{-13}$) (**Table S2**).
385

386 **Colocalisation of cytokine variants with *cis*-eQTLs in whole blood**

387 To characterise the regulatory effects of the multivariate cytokine-associated loci, we queried
388 the largest publicly-available set of results for whole blood *cis*-eQTLs from a meta-analysis of
389 31,684 individuals, which was obtained from the eQTLGen Consortium database (46). We
390 found SNPs, lead or LD-proxy ($r^2 > 0.5$), at seven of the eight cytokine loci (*ABO*, *F5*, *PCSK6*,
391 *PDGFRB*, *SERPINE2*, *VEGFA*, *VLDLR*) with *cis*-regulatory effects (P -value $< 1 \times 10^{-6}$) on
392 gene expression (a total of 17 unique genes) in blood (**Table S3**). Using Bayesian
393 colocalisation analysis, we further demonstrated that associations at three of these loci
394 colocalised with *cis*-eQTLs for *ABO*, *PCSK6*, and *SERPINE2* expression (**Figure 3A – C**;
395 **Table S4**).

396

397 **Colocalisation of cytokine variants with immune cell-specific *cis*-eQTLs**

398 Next, we investigated the cell type- or context-dependent regulatory effects of genetic variants
399 associated with the cytokine network by interrogating previously published *cis*-eQTLs specific
400 to resting B-cells (47), resting monocytes (48), stimulated monocytes with interferon- γ or
401 lipopolysaccharide (48), resting neutrophils (56), naive CD4⁺ T-cells (49,56) and CD8⁺ T-cells
402 (49), all isolated from healthy donors of European ancestry (**Table S5**). Three out of the eight
403 cytokine network loci harboured *cis*-eQTLs (P -value $< 1 \times 10^{-6}$) in at least one immune cell
404 type, in either stimulated or non-stimulated state (**Table S6**). For example, SNPs at the

405 *SERPINE2* locus were reported to have *cis*-eQTL effects across multiple immune cell types,
406 including B-cells, CD4⁺ and CD8⁺ T-cells (**Table S6**).

407

408 Further, colocalisation analysis showed that the cytokine network variants at *SERPINE2* had
409 strong evidence of sharing a causal variant with *SERPINE2 cis*-eQTLs in CD4⁺ T-cells and B-
410 cells, similar to the colocalisation we observe in whole blood (**Figure 3B**; **Table S7**).

411

412 **Colocalisation of cytokine variants with plasma protein QTLs**

413 To investigate protein-level effects of cytokine network variants, we utilised plasma protein
414 QTLs (pQTLs) from the INTERVAL study (17). Colocalisation analysis, considering only
415 pQTLs with association *P*-value $< 1 \times 10^{-6}$, showed all the eight cytokine network loci had
416 strong evidence of shared causal variants with plasma levels of a total of 146 proteins (out of
417 the 215 tested) (**Table S8**). Of these, the *ABO* and *ZFPM* cytokine network loci strongly
418 colocalised with pQTL signals for 55 (out of 81) and 87 (out of 98) proteins, respectively
419 (**Table 3**; **Table S8**). Of these, 14 and 75 proteins shared the same causal lead pQTLs with the
420 lead cytokine network variants at the *ABO* (rs550057) and *ZFPM2* (rs6993770) loci,
421 respectively, suggesting these variants have widespread effects.

422

423 The *ABO* locus colocalised with pQTLs for several membrane proteins (B3GN2, endoglin,
424 GOLM1, OX2G, TPST2) and cell surface receptors (IL-3RA, LIFR, IGF-I R, HGF receptor).
425 *ABO* colocalisation was also observed with pQTLs for adhesion and immune-related molecules
426 involved in leukocyte recruitment, cell adhesion, and transmigration, including sGP130,
427 sICAM-1, sICAM-2, LIRB4, and P-selectin (**Table 3**; **Table S8**). At the *ZFPM2* locus,
428 colocalisation was seen with pQTLs for proteins generally found in platelet granules (e.g.
429 VEGFA, PDGF-AA, PDGF-BB, PDGF-D, angiopoietin, P-selectin). At the *SERPINE2* locus,
430 we observed that in addition to colocalising with the *cis*-eQTL signal for *SERPINE2*
431 expression, the cytokine network-associated variants colocalised with the *cis*-pQTL variants
432 for *SERPINE2* protein levels (**Table S8**). Likewise, the *VEGFA* locus colocalised with a *cis*-
433 pQTL for VEGFA, and the *PDGFRB* locus with a *cis*-pQTL for PDGFRB.

434

435 **Relationships of cytokine network variants with complex traits and diseases**

436 Using the NHGRI GWAS Catalog (57,58), we found that, across all eight cytokine network
437 loci, 55 SNPs matched SNPs previously associated with quantitative traits and diseases. (**Table**
438 **S9**). The lead cytokine network variant at *ZFPM2* (rs6993770) has previously been associated

439 with various platelet traits, including platelet count, distribution width, plateletcrit (total
440 platelet mass) and mean volume (17,59) (**Table S9**).

441

442 Next, GWAS summary statistics from a broad range of traits and diseases (**Table S10**),
443 including hematopoietic traits, circulating metabolites, immune- and cardiometabolic-related
444 diseases were compiled for colocalisation analysis with the cytokine network loci. The two
445 cytokine network-associated loci, *ABO* and *ZFPM2*, exhibited strong evidence of
446 colocalisation for several traits and diseases. The *ZFPM2* locus not only colocalised with
447 signals for several platelet trait associations, but also with other haematological trait-associated
448 signals including white blood cell counts, and specifically neutrophil and basophil counts
449 (**Table 3; Table S11**). The *ABO* locus showed colocalisation with various QTLs for
450 haematological traits including red blood cell traits (haemoglobin concentration, red blood cell
451 count, and hematocrit) and white blood cell counts, including granulocyte count and
452 specifically eosinophil count (**Table 3; Table S11**). This is consistent with the *ABO* locus being
453 identified as a pQTL for proteins involved in leukocyte activation as identified previously.
454 Cytokine network variants at the *ABO* locus colocalised with those of intermediate density,
455 low density, and very low-density lipoprotein subclasses as well as glycosylated haemoglobin
456 (HbA1c) (**Table 3; Table S11**), suggesting both inflammatory and metabolic effects. Notably,
457 the same cytokine network variants at the *ABO* locus also strongly colocalised with signals
458 associated with coronary artery disease (CAD), pulmonary embolism, ischemic stroke, and
459 type 2 diabetes (T2D) (**Table 3, Table S11**).

460

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465 **Discussion**

466 In this study, we first identified a network of 11 correlated cytokines which are known to
467 participate in a broad array of immune responses in circulation. These cytokines include those
468 involved in the classical TH1 (IL-12, IFN- γ), TH2 (IL-4, IL-6, and IL-10), TH17 (IL-6, IL-17, and
469 G-CSF), and T_{reg} (IL-10) responses (51,52) as well as the promotion of angiogenesis, tissue
470 repair and remodelling typically coinciding with inflammatory and post-inflammatory states
471 (VEGF-A, FGF-basic and PDGF-BB) (53). Although previous *in vitro* challenge studies
472 (20,21) indicate antagonistic relationships amongst selected cytokines in the network, our

473 analyses in >9,000 individuals are consistent with previous study utilising similar data (19),
474 showing that these 11 circulating cytokines are positively correlated in the general population.
475 Therefore, at the population level, it is more likely that an equilibrium in circulating levels of
476 disparate cytokines exists, possibly maintained by counter-regulatory mechanisms.

477

478 Our multivariate GWAS meta-analysis identified eight loci associated with the cytokine
479 network; confirming six previously-reported associations for circulating cytokine levels
480 (14,16,19) as well as uncovering two additional signals (*PDGFRB* and *ABO*), empirically
481 demonstrating the statistical power of multivariate approaches. Further, integrative genetic
482 analyses revealed evidence for shared genetic influences between these loci, molecular QTLs,
483 and complex trait and disease associations. This study identified several regions harbouring
484 cytokine-associated signals that colocalise with whole blood and/or immune cell-specific *cis*-
485 eQTLs for a number of genes, including *SERPINE2*, *ABO*, and *PCSK6*, suggesting these genes
486 are possible candidates underlying the collective expression of cytokines in the cytokine
487 network – or vice versa. Our findings also highlight that the cytokine network associations at
488 the pleiotropic loci, *ABO* and *ZFPM2*, overlap with signals associated with multiple traits,
489 including cardiometabolic diseases, immune-related proteins, and platelet traits.

490

491 *SERPINE2* encodes protease nexin-1, an inhibitor of serine proteases such as thrombin and
492 plasmin, and is therefore implicated in coagulation, fibrinolysis and tissue remodelling (60). It
493 shares similar functions with its better-known homolog *SERPINE1*, or plasminogen activator
494 inhibitor-1 (PAI-1), the elevation of which is associated with thrombosis and cardiovascular
495 risk (60). However, there is also evidence that *SERPINE2* has pleiotropic roles in immune and
496 inflammatory regulation, that could be either dependent or independent of its function as a
497 serine protease. It is expressed in many tissue types, and its expression can be induced by pro-
498 inflammatory cytokines such as IL-1 α (61,62). Conversely, *SERPINE2* can itself influence
499 inflammatory status: *SERPINE2* is a candidate susceptibility gene for chronic obstructive
500 pulmonary disease, and *SERPINE2*-knock-out mice exhibited extensive accumulation of
501 lymphocytes in the lungs, through a mechanism linked to thrombin and NF κ B activation (62).
502 We observed in our data that the cytokine network associations overlapped with the *SERPINE2*
503 pQTL signal. Moreover, using immune cell-specific *cis*-eQTL data, we further demonstrated
504 colocalisation between the cytokine network and *SERPINE2* *cis*-eQTL signals specifically in
505 CD4⁺ T-cells and B-cells. This suggests that the association between *SERPINE2* and the
506 cytokine network at this locus is at least partially-driven by lymphocytic expression –

507 consistent with *SERPINE2* itself influencing chemotaxis and recruitment of lymphocytes (62).
508 Our analyses demonstrate that the importance of *SERPINE2* in regulating immune and
509 inflammatory processes is potentially greater than previously anticipated, and warrants further
510 targeted research.

511

512 Like *SERPINE2*, the *ABO* locus has widespread pleiotropic effects. The most well-known
513 function of *ABO* is its determination of blood group. The human *ABO* gene has three major
514 alleles (A, B, and O) that determine ABO blood type. The A and B alleles encode for distinct
515 “A” versus “B” glycosyltransferases that add specific sugar residues to a precursor molecule
516 (H antigen) to form A versus B antigens, respectively (63). The O allele results in a protein
517 without glycosyltransferase activity (63). The lead cytokine-associated variant rs550057 and
518 its proxies in moderate LD ($r^2 = 0.6$; rs507666, rs687289) have been previously shown to
519 determine the *ABO* allele (64), but they have also been associated with circulating levels of
520 inflammatory proteins such as ICAM-1, P-selectin, and ALP (17,65,66). Our study showed that
521 cytokine network associations at the *ABO* locus share colocalised signals with a host of other
522 proteins and traits, including lipoproteins (IDL, LDL, VLDL), proteins of immune function,
523 immune cell subsets, and cardiometabolic diseases (**Table 3**), highlighting the potential for
524 shared molecular etiology amongst these traits. Our analyses highlight the potential genetic
525 basis for numerous previous observations linking ABO blood group to an array of similar traits
526 and phenotypes (18,67–71).

527

528 It could therefore be speculated that the *ABO* gene influences the risk of cardiometabolic
529 disease due to its involvement in multiple inflammatory, haemostatic and metabolic processes;
530 however, our current understanding of the mechanisms behind this remains unclear. For
531 instance, non-O blood groups have been associated with increased risk of both cardiovascular
532 disease, venous thromboembolism, stroke, and T2D (68,72). However, the O blood group has
533 itself been linked to elevated IL-10 and worse outcomes given existing coronary disease (risk
534 of cardiovascular death, recurrent myocardial infarction and all-cause mortality) (64). Other
535 studies have suggested a role for von Willebrand factor (VWF), a coagulative factor which also
536 expresses ABO antigens – in particular, the O phenotype is associated with lower VWF, which
537 may explain reduced thrombotic and cardiovascular risk (64,73). It has been suggested that the
538 link between ABO blood group type and venous thromboembolism (VTE) is potentially driven
539 by VWF and Factor VIII – non-O blood group individuals presented a higher risk of venous
540 thromboembolism and had elevated levels of both VWF and Factor VIII (74,75). Also relevant

541 is the link between *ABO* and adhesion molecules such as E-selectin and sICAM-1 which are
542 overexpressed in inflammatory states (18,66,70,71). sICAM-1 is a known positive correlate
543 with cardiovascular disease; however, it is the A blood group, not O, that is associated with
544 reduced sICAM-1 levels, again complicating the picture (70). Inferring the exact causal
545 relationships amongst all these entities will require intricate follow-up experimental
546 investigation, involving simultaneous examination of all key players. It is particularly unclear
547 whether the link with cardiometabolic diseases may be due to its direct modification of H
548 antigen, or on the glycosyltransferase activity of the encoded enzyme on other proteins, or
549 some combination of both. In our study, formal causal inference (e.g. with Mendelian
550 Randomisation) was not possible because the corresponding multivariate beta-coefficients and
551 standard errors are not currently calculable and the locus itself has extensive pleiotropy.

552

553 The *ZFPM2* locus has been associated with platelet traits (59), and our findings highlight its
554 importance as a determinant of platelet and angiogenic cytokine activity. *ZFPM2* encodes a
555 zinc finger cofactor that regulates the activity of *GATA4*, a transcription factor reported to play
556 a critical function not only in heart development (76) but also modulation of angiogenesis. In
557 particular, *GATA4* directly binds to the promoter of angiogenic factor *VEGFA* and regulates
558 its expression (77), and it has been shown that disruption of *ZFPM2*-*GATA4* interaction alters
559 the expression of *VEGFA* and other angiogenesis-related genes (78). *VEGFA* and *PDGFR-BB*,
560 which are part of the cytokine network, have been found to be released via alpha granules of
561 activated platelets, and serum *VEGFA* levels correlate closely with blood platelet counts (79–
562 81). In our study, we show that the cytokine-associated signal at the *ZFPM2* locus colocalised
563 with GWAS signals for platelet traits and platelet proteins. The lead cytokine network SNP
564 rs6993770 has been reported to be a *trans*-eQTL in whole blood for gene products typically
565 found in platelets and their receptors (e.g. *CXCL5*, *GP9*, *MYL9*, *VWF*) (46). Collectively,
566 these findings suggest that this locus regulates the number and/or cytokine activity of
567 circulating platelets, and that this potentially occurs via interaction with *GATA4* and regulation
568 of *VEGFA*.

569

570 In conclusion, our study illustrates the utility of multivariate analysis of correlated immune
571 traits and highlights potentially fruitful avenues of biological investigation for multivariate
572 genetic signals. Our results highlight that certain gene loci drive the expression of a cytokine
573 network with immune, inflammatory and tissue repair functions; and, simultaneously, these
574 loci are implicated in the regulation of other haemostatic and metabolic functions, with

575 relevance to human health and disease. This stresses the fact that the processes of inflammation,
576 haemostasis and repair often run concurrent with each other after injury, and that biological
577 systems often feature ample redundancy and feedback loops within individual effectors.

578

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582

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- 830

831 **Figures**

832 **Figure 1: Overview of the study populations, design, and the analyses conducted.**

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834

835 **Figure 2: Multivariate GWA analysis of a network of 11 correlated cytokines in three**

836 **Finnish cohorts. (A)** Correlation heatmap of the 18 cytokines in the FINRISK97 cohort. Each

837 cell presents the pair-wise Pearson's correlation coefficient between the normalised cytokine

838 residuals. The cytokines are ordered by hierarchical clustering, using 1 minus the absolute

839 value of the correlations as the distance matrix. The colour scale denotes the strength of the

840 correlations, where red is a high positive correlation. The group of 11 tightly correlated

841 cytokines (black box) was used for multivariate analysis. **(B)** Manhattan plot for meta-analysis

842 results from the multivariate GWAS of the cytokine network. The statistical strength of

843 association ($-\log_{10}$ meta- P -value; y -axis) is plotted against all the SNPs ordered by

844 chromosomal position (x -axis). The sky-blue horizontal dashed line represents the genome-

845 wide (meta- P -value $< 5 \times 10^{-8}$) significance threshold. The lead SNP (lowest meta- P -value) at

846 each locus and the nearby genes are shown.

847

848 **Figure 3: Regional plots for the cytokine network association, and whole blood and**

849 **immune cell *cis*-eQTL association signals at the *ABO*, *PCSK6* and *SERPINE2* locus. (A)**

850 The cytokine network GWAS signal (top) colocalises with the whole blood *cis*-eQTLs signal

851 for *ABO* (bottom) at the *ABO* locus on chromosome 9; **(B)** colocalises with whole blood *cis*-

852 eQTLs for *PCSK6* expression (bottom) at the *PCSK6* locus on chromosome 15; **(C)** colocalises

853 with the *cis*-eQTL signals for *SERPINE2* expression in whole blood (middle), B-cells (middle),

854 and CD4⁺ T-cells (bottom) at the *SERPINE2* locus on chromosome 2. For each plot, the circles

855 represent the $-\log_{10}$ association P -values (y -axis) of SNPs plotted against their chromosomal

856 position (x -axis). The eQTL association plots show the lead cytokine network GWAS SNP

857 tested in the colocalisation analysis. The lead cytokine network GWAS SNP rs6722871 was

858 not present in the B-cell and CD4⁺ T cell eQTL dataset, instead, the next top GWAS SNP

859 present in each of the eQTL dataset (rs861442, B-cell; rs1438831, CD4⁺ T-cell) is shown. For

860 all regional plots, pairwise LD (r^2) in the region is coloured with respect to the lead cytokine

861 network GWAS SNP. LD was calculated from the 1000 Genomes European population.

862

863 **Tables**

864

865 **Table 1: Summary of descriptive characteristics of the three study cohorts.**

Characteristics	FINRISK97	FINRISK02	YFS07
Collection year	1997	2002	2007
Number of individuals with matched cytokine & genotype data	5438	1986	1843
Number of males (%)	2637 (48.5)	991(49.9)	841 (45.6)
Mean age in years (and range)	47.6 (24-74)	60.3(51-74)	37.7 (30-45)
BMI (kg/m ²); mean \pm SD	26.6 \pm 4.6	28.1 \pm 4.5	25.9 \pm 4.6
Number of individuals on lipid lowering drugs (%)	174 (3.2)	284 (14.3)	40 (2.2)
Number of individuals on blood pressure treatment drugs (%)	698 (12.8)	512 (25.8)	127 (6.9)

866 Abbreviations: BMI, body mass index; YFS, Young Finns Study. The numbers beside the
867 cohort names refer to the calendar year (collection year) in which the samples and clinical
868 information were obtained from each cohort.

869 **Table 2: Meta-analysed results of multivariate GWAS of cytokine network**

Locus	Locus Region	Top SNP	Average MAF	Top Multivariate Meta- <i>P</i> -value	Univariate Meta- <i>P</i> -value (Top Cytokine)	Detection
<i>F5</i>	1q24.2	rs9332599	0.294	7.17×10^{-12}	9.21×10^{-3} (SDF1a)	Multivariate
<i>SERPINE2</i>	2q36.1	rs6722871	0.311	1.19×10^{-59}	3.55×10^{-18} (PDGF-BB)	Both
<i>PDGFRB</i>	5q32	rs2304058	0.379	4.06×10^{-9}	1.52×10^{-5} (IL4)	Multivariate
<i>VEGFA</i>	6p21.1	rs7767396	0.471	6.93×10^{-306}	3.10×10^{-201} (VEGF-A)	Both
<i>ZFPM2</i>	8q23.1	rs6993770	0.221	4.73×10^{-8}	1.01×10^{-7} (IL12p70)	Multivariate
<i>ABO</i>	9q34.2	rs550057	0.306	2.75×10^{-8}	4.9×10^{-3} (IL4)	Multivariate
<i>VLDLR</i>	9p24.2	rs7030781	0.413	3.78×10^{-13}	6.78×10^{-14} (VEGF-A)	Both
<i>PCSK6</i>	15q26.3	rs11639051	0.255	1.93×10^{-58}	1.19×10^{-26} (PDGF-BB)	Both
<i>JMJD1C</i>	10q21.3	rs9787438	0.374	* 1.30×10^{-7}	* 8.96×10^{-12} (VEGFA)	Univariate

870 The table shows the meta-analysis *P*-values for the top SNP (lowest *P*-value) at each locus
871 associated with the cytokine network in the multivariate analysis at genome-wide significance
872 threshold (5×10^{-8}). The corresponding lowest meta-*P*-value for the same top SNP in the
873 univariate analysis with any single cytokine present in the cytokine network, given in brackets
874 beside the meta-*P*-value, was also reported. *Instance where the top SNP at a locus crossed
875 only the univariate significance threshold ($P < 4.55 \times 10^{-9}$), then the corresponding meta-*P*-
876 value for that SNP in the multivariate was also given. The univariate significance threshold was
877 calculated from a Bonferroni correction for 11 cytokines tested ($5 \times 10^{-8}/11$).
878

879 **Table 3: Colocalisation of cytokine network-associated variants at the *ABO* and *ZFPM2* loci with those of plasma protein levels,**
880 **quantitative traits, and disease risk.** Evidence: evidence of colocalisation; Strong: $PP3+PP4 > 0.99$ and $PP4/PP3 > 5$; Suggestive: $PP3 + PP4 >$
881 0.75 and $PP4/PP3 > 3$; None: association signal for the trait at the locus, but no evidence of colocalisation.

ABO locus (Chromosome 9)			
Traits/ Diseases	Group/ Functions	Evidence	Names
Diseases	Cardiometabolic diseases	Strong	Pulmonary embolism, ischemic stroke, coronary artery disease, type 2 disease,
		None	Deep vein thrombosis
Blood cell traits	Blood cell counts	Strong	White blood cell, granulocytes, basophils + eosinophils, basophils + neutrophils, eosinophils + neutrophils, eosinophils, neutrophils, haematocrit (%), haemoglobin, myeloid, red blood cells, platelet distribution width
		Suggestive	Basophils, reticulocytes
		None	Monocyte, platelet, plateletcrit (%), red cell distribution width
Metabolites	IDL particle constituents	Strong	Total cholesterol (IDL-C), free cholesterol (IDL-FC), total lipids (IDL-L), total particle concentration (IDL-P), phospholipids (IDL-PL), triglycerides (IDL-TG)
	LDL subclass particle constituents	Strong	For large particles: total cholesterol (L-LDL-C), cholesterol esters (L-LDL-CE), free cholesterol (L-LDL-FC), total lipids (L-LDL-L), total particle concentration (L-LDL-P), phospholipids (L-LDL-PL), For medium particles: total cholesterol (M-LDL-C), cholesterol esters (M-LDL-CE), total lipids (M-LDL-L), total particle concentration (M-LDL-P), phospholipids (M-LDL-PL) For small particles: total cholesterol (S-LDL-C), total lipids (S-LDL-L), total particle concentration (S-LDL-P)
	VLDL subclass particle constituents	Strong	For small particles: total cholesterol (S-VLDL-C), For extra-small particles: total lipids (XS-VLDL-L), phospholipids (XS-VLDL-PL)
	Other	Strong	HbA1c, Apolipoprotein B, total LDL cholesterol, total serum cholesterol
Proteins	Chemokine activity	Strong	FAM3B, FAM3D, MIP-5, TECK,
		Suggestive	CCL28
	Chemokine receptors	Strong	IL-3RA, HGF receptor, sGP130, VEGF-R2, VEGF-R3
		None	TCCR
	Receptor function and/or signalling	Strong	F177A, GP116, IGF-1R, IR, JAG1, MBL, PEAR1, PYY, SECTM1, SEMA6A, TLR4
		Suggestive	PLXB2
		None	CD109, CD209, GFRAL, GPIV, LIF-R, Notch-1, PEAR1, sTIE1, sTIE2
	Cell adhesion	Strong	Cadherin-1, E-selectin, Endoglin, ICAM-4, ISLR2, Laminin, NCAM-L1, OX2G, P-selectin, sICAM-1, sICAM-2, sICAM-5
		None	ADAM23, BCAM, Cadherin-5, Desmoglein-2, ESAM
	Enzyme function	Strong	B3GN2, B4GT1, B4GT2, Cathepsin-S, CLIC5, DPEP2, FA20B, FUT10, GLCE, GNS, IAP, LPH, MA1A2, NDST1, QSOX2, ST4S6, TPST2, XXLT1
None		ATS13, BGAT, CEL, CHSTB, DYR, MINP1, TLL1	
Miscellaneous	Strong	C1GLC, CASC4, GOLM1, KIN17, THSD1, TUFT1,	
	None	Factor VIII, OBP2B	

ZFPM2 locus (Chromosome 8)			
Traits/ Diseases	Group/ Functions	Evidence	Names
Blood cell traits	Blood cell counts	Strong	White blood cells, granulocytes, basophils + neutrophils, neutrophils + eosinophils, basophils, neutrophils, myeloid, platelets, plateletcrit (%), platelet distribution width, mean platelet volume
Proteins	Cytokine/chemokine activity	Strong	EDA, IL-7, PDGF-AA, PDGF-BB, PDGF-D, VEGF-A, NAP-2, RANTES, TARC
	Immune response	Strong	CLM2, COCH, CYTF, DB119
	Receptor function and/or signalling	Strong	ANG-1, APP, BDNF, CD44, CGB2, CRIM1, Dkk-1, Dkk-4, EDAR, EPHB2, EPHB3, GI24, GRP, LIRB4, Mammaglobin-2, OBP2A, P2RX6, PAP1, PTPRD, RGS10, RGS3, RHOG, THA, MESD2
		Suggestive	Ephrin-A3
		None	UNC5H4, sRAGE
	Cell adhesion	Strong	Galectin-7, KIRR2, MAdCAM-1, MFGM, ON, P-Selectin, PCDG8, SCF, SPARCL1, (<i>CDHR3, OBCAM</i>)
	Enzyme activity	Strong	Arylsulfatase A, ASM3A, B4GT7, Cathepsin A, CHSTB, CPXM1, FUT8, GSTM1-1, INP5E, MMEL2, MYSM1, PAI-1, PDIA5, RIFK, SIRT5, SPTC1, UD2A1
		None	PDE3A, ZFP91, LAML2, HECW1
	Enzyme inhibitor	Strong	SERPINE2, SPINK5, TICN3, WFD13
	Transcription/translation	Strong	APBB1, CENPW, HIF-1a, PAIP1
Suggestive		ID2	
Miscellaneous	Strong	4EBP2, APLP2, ARL1, ASIC4, CA063, Coactosin-like protein, CQ089, DJB11, MPP7, NSG2, PROL1, RBM28, SATB1, SYT11, SYT17, TXNDC4	
	None	CNA2	

883 Refer to Table S8 for full descriptions of the proteins.

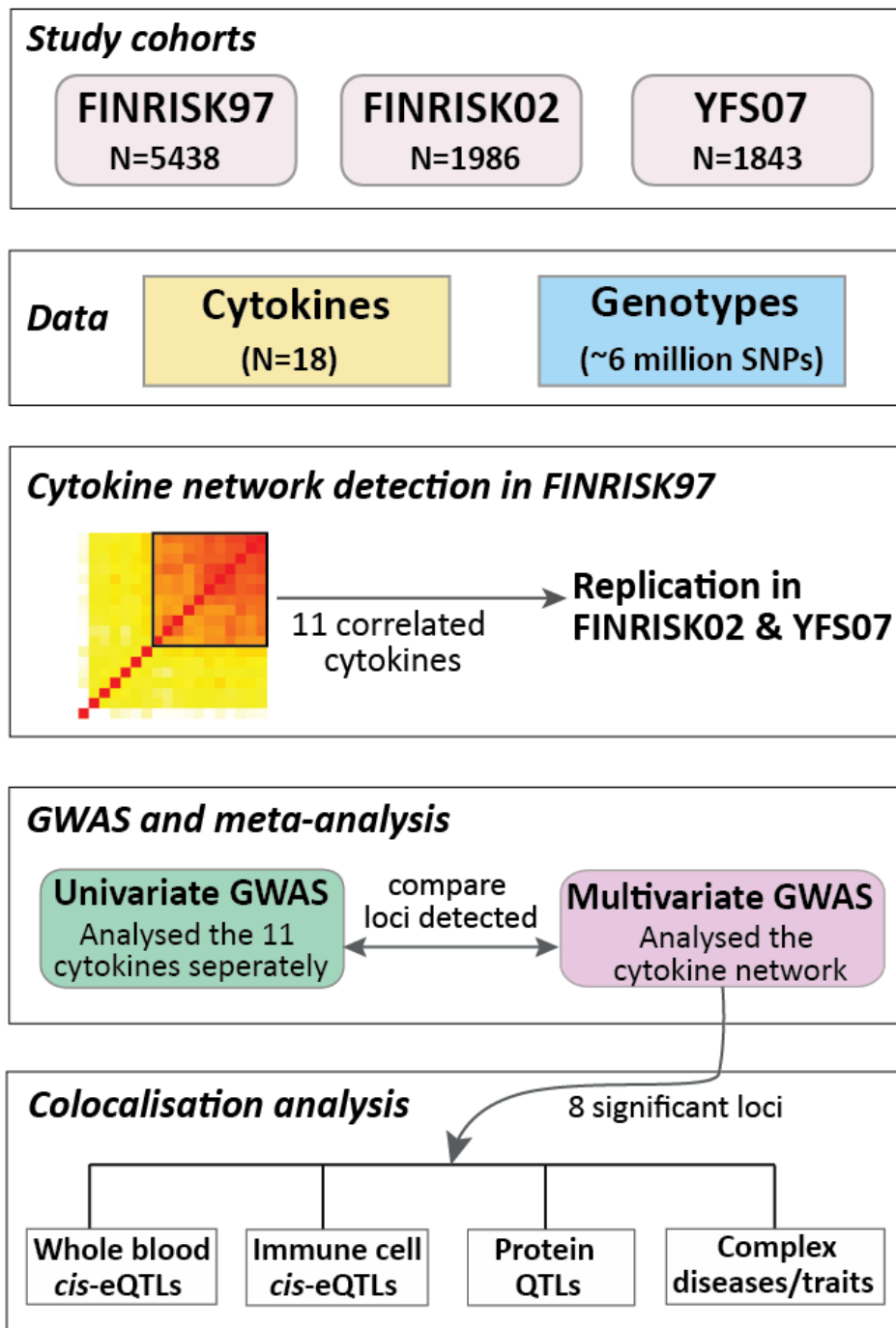
884 **Figures**

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887 **Figure 1: Overview of the study populations, design, and the analyses conducted.**

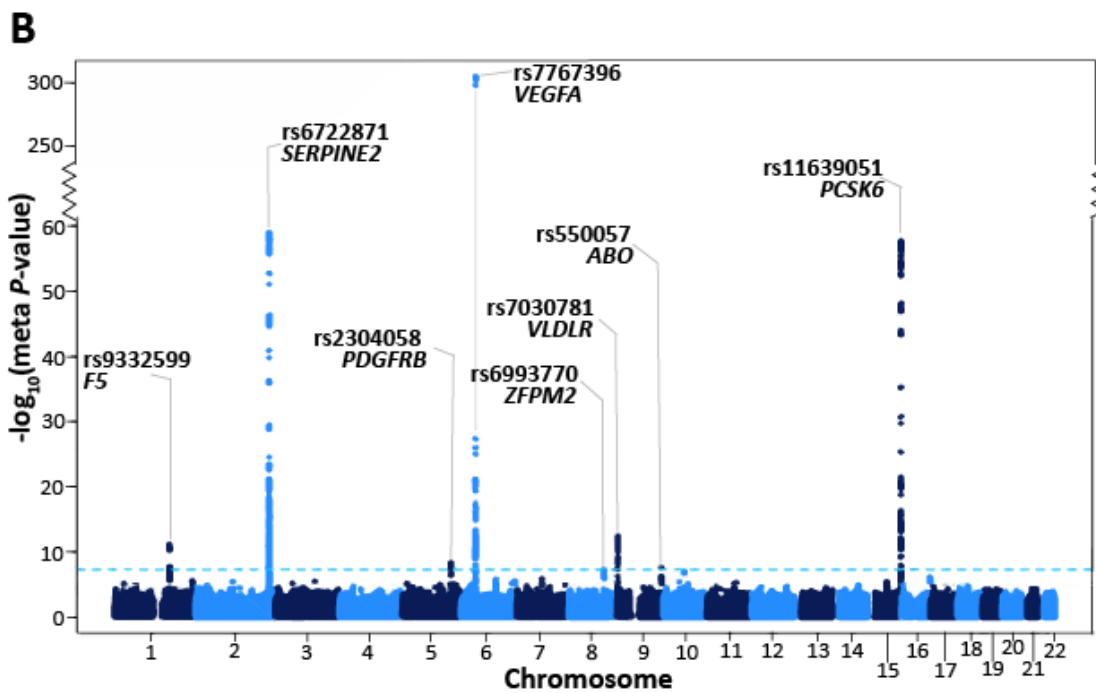
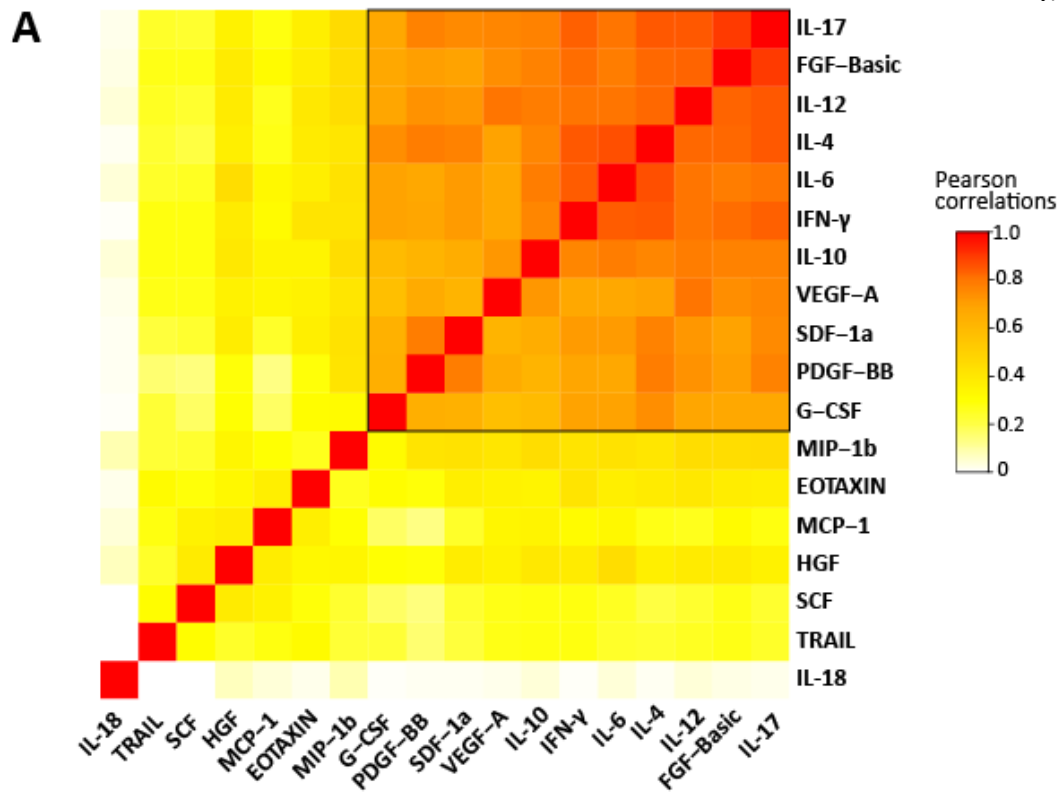
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930 **Figure 2: Multivariate GWA analysis of a network of 11 correlated cytokines in three**
931 **Finnish cohorts**
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976 **Figure 3: Cytokine network-associated loci colocalise with whole blood and immune cell**
 977 ***cis*-eQTLs**
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