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## Association of Novel Genetic Loci with Circulating Fibrinogen Levels: A Genome-Wide Association Study in Six Population-Based Cohorts:

### Dehghan Genome-wide Association Study on Fibrinogen

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## Abstract

**Background**—Fibrinogen is both central to blood coagulation and an acute phase reactant. We aimed to identify common variants influencing circulation fibrinogen levels.

**Methods and Results**—We conducted a genome-wide association analysis on six population-based studies, the Rotterdam Study, the Framingham Heart Study, the Cardiovascular Health Study, the Atherosclerosis Risk in Communities Study, the MONICA/KORA Augsburg Study, and the British 1958 Birth Cohort Study, including 22,096 participants of European ancestry. Four loci were marked by one or more single nucleotide polymorphisms (SNPs) that demonstrated genome-wide significance ( $p < 5.0 \times 10^{-8}$ ). These included a SNP located in the fibrinogen  $\beta$  chain (*FGB*) gene and three SNPs representing newly identified loci. The high-signal SNPs were rs1800789 in exon 7 of *FGB* ( $p = 1.8 \times 10^{-30}$ ), rs2522056 downstream from the interferon regulatory factor 1 (*IRF1*) gene ( $p = 1.3 \times 10^{-15}$ ), rs511154 within intron 1 of the propionyl coenzyme A carboxylase (*PCCB*) gene ( $p = 5.9 \times 10^{-10}$ ), and rs1539019 on the NLR family, pyrin domain containing 3 isoforms (*NLRP3*) gene ( $p = 1.04 \times 10^{-8}$ ).

**Conclusions**—Our findings highlight biological pathways that may be important in regulation of inflammation underlying cardiovascular disease.

## Keywords

genome-wide association; fibrinogen; gene; FGB; IRF1; PCCB; NLRP3

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Elevated levels of fibrinogen within or above the normal range are consistently associated with an increased risk of cardiovascular disease.<sup>1</sup> Fibrinogen has a key role in blood coagulation but is also known as a marker of inflammation. Studies in persons of European ancestry have estimated the heritability of multivariable-adjusted fibrinogen levels from 24% in multiplex families<sup>2</sup> to more than 50% in twins.<sup>3</sup> The three genes encoding the three fibrinogen protein chains explain only a small part of the total estimated genetic variance of circulating levels of fibrinogen.<sup>4</sup>

The objective of this study was to identify novel genetic loci related to plasma fibrinogen levels. A meta-analysis of genome-wide association (GWA) findings was conducted on six population-based studies. We analyzed GWA data of 2,661,766 SNPs from one or more studies from a total of 22,096 participants of European descent.

## Methods

The setting for this meta-analysis is primarily the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.<sup>5</sup> CHARGE includes the Rotterdam Study (RS), the Framingham Heart Study (FHS), the Cardiovascular Health Study (CHS), and the

Atherosclerosis Risk in Communities (ARIC) Study. In addition, data from the British 1958 Birth Cohort (B58C) and the MONICA/KORA Augsburg Study (KORA) has been included.

### Rotterdam Study (RS)

The RS is a prospective, population-based cohort study of determinants of several chronic diseases in older adults.<sup>6</sup> In brief, the study comprised 7,983 inhabitants of Ommoord, a district of Rotterdam in the Netherlands, who were 55 years or over. The baseline examination took place between 1990-1993.

Genotyping was conducted using the Illumina 550K array. SNPs were excluded for minor allele frequency  $\leq 1\%$ , Hardy-Weinberg equilibrium (HWE)  $p < 10^{-5}$ , or SNP call rate  $\leq 90\%$  resulting in data on 530,683 SNPs. Imputation was done with reference to HapMap release 22 CEU using the maximum likelihood method implemented in MACH. The final population for this fibrinogen analysis comprised 2,068 individuals.

### Framingham Heart Study (FHS)

The FHS started in 1948 with 5,209 randomly ascertained participants from Framingham, Massachusetts, US, who had undergone biannual examinations to investigate cardiovascular disease and its risk factors.<sup>7</sup> In 1971, the Offspring cohort<sup>8,9</sup> (comprised of 5,124 children of the original cohort, and the children's spouses) and in 2002, the Third Generation (consisting of 4,095 children of the Offspring cohort), were recruited<sup>10</sup>. FHS participants in this study are of European ancestry.

Genotyping was carried-out as a part of the SHARe project using the Affymetrix 500K mapping array (250K Nsp and 250K Sty arrays) and the Affymetrix 50K supplemental gene focused array on 9,274 individuals. Genotyping resulted in 503,551 SNPs with successful call rate  $> 95\%$  and HWE  $p > 10^{-6}$  on 8481 individuals with call rate  $> 97\%$ . Imputation of  $\sim 2.5$  million autosomal SNPs in HapMap with reference to release 22 CEU sample was conducted using the algorithm implemented in MACH. The final population for fibrinogen analysis included 7,022 individuals (Original Cohort  $n=383$ , Offspring  $n=2,806$ , Third Generation  $n=3,833$ ).

### Cardiovascular Health Study (CHS)

The CHS is a population-based, observational study of risk factors for clinical and subclinical cardiovascular diseases.<sup>11</sup> The study recruited participants 65 years of age and older from four US communities in two phases: 5,201 participants in 1989-1990, and 687 (primarily African American participants) in 1992-1993. A GWA study was conducted in a subset of CHS participants ( $n=3,980$ ), all of whom were without clinical cardiovascular disease at their baseline clinical visit and provided consent to use their DNA for research. The study sample used in the fibrinogen analysis represented the first two of three rounds of genotyping, which was a stratified probability sample. Weights were assigned to each observation to reflect the likelihood of sampling from the 3,980 participants. The analysis was restricted to participants of European decent.

Genotyping was performed using the Illumina 370 CNV BeadChip system. Samples were excluded for sex mismatch, discordance with prior genotyping, or call rate  $< 95\%$ . SNPs were excluded from analysis when monomorphic, when HWE  $p < 10^{-5}$ , and when call rates were  $< 95\%$ . Imputation was performed using BIMBAM v0.95 with reference to HapMap CEU using release 21A build. The population available for the fibrinogen analysis included 1,993 individuals.

### The Atherosclerosis Risk in Communities (ARIC)

The ARIC study is a longitudinal cohort study of atherosclerosis and its clinical sequelae. It recruited a population-based sample of 15,792 men and women aged 45-64 years from four US communities in 1987-89.<sup>12</sup> The analysis was restricted to subjects of European descent.

Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0. SNPs were excluded for not being autosomal SNPs, not passing laboratory QC, no chromosome location, minor allele frequency  $\leq 1\%$ , SNP call rate  $< 90\%$ , or HWE  $p < 10^{-6}$ . This resulted in data on 716,442 SNPs. Imputation to HapMap SNPs was performed using MACH. After excluding subjects who disallowed DNA use, subjects with a mismatch between called and phenotypic sex, with a mismatch on  $> 10$  of 47 previously analyzed SNPs in ARIC, all but one in sets of first degree relatives, and other individuals who were genetic outliers, the final population for fibrinogen analysis comprised 8,051 individuals.

### The MONICA/KORA Augsburg Study (KORA)

The presented data were derived from the third population-based Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Cooperative Health Research in the Region of Augsburg (KORA) survey S3.<sup>13</sup> This cross-sectional survey covering the city of Augsburg (Germany) and two adjacent counties was conducted in 1994/95 to estimate the prevalence and distribution of cardiovascular risk factors among individuals aged 25 to 74 years as part of the WHO MONICA study. The MONICA/KORA S3 study comprises 4,856 subjects. Among them, 3,006 subjects participated in a follow-up examination of S3 in 2004/05 (MONICA/KORA F3). All participants underwent standardized examinations including blood withdrawals for plasma and DNA. For the KORA genome-wide association study, 1,644 subjects, aged 45 to 69 years were selected from the KORA S3/F3 samples.

Genotyping was performed using the Affymetrix 500K Array Set. Samples were excluded for sex mismatch, discordance with prior genotyping, or call rate  $< 95\%$ . SNPs were excluded from analysis when monomorphic ( $MAF < 0.01$ ), when call rates per SNP were  $< 0.1$  and per individual were  $< 0.1$ . Imputation was done using maximum likelihood method implemented in MACH 1.0. The final population available for the fibrinogen analysis included 1,523 individuals.

### British 1958 birth cohort (B58C)

The B58C is a national population sample followed periodically from birth. At age 44-45 years, 9,377 cohort members were examined by a research nurse in the home as described previously.<sup>14</sup> For this study we used a total of 1,480 cell-line-derived DNA samples from unrelated subjects of European ancestry, with nationwide geographic coverage, which were used as controls by the Wellcome Trust Case Control Consortium (WTCCC).<sup>15</sup>

Genotyping was performed using the Affymetrix 500K Mapping Array Set using the call algorithm CHIAMO as implemented by the WTCCC.<sup>15</sup> Genotypes at other loci were imputed by the program IMPUTE version 0.1.2, using 490,032 autosomal SNPs with CHIAMO calls and the linkage disequilibrium patterns in the HapMap CEU panel. Analysis of imputed genotypes used Marchini's SNPTEST version 1.1.3 and supplementary regression modeling used STATA version 10.0. A final sample size of 1,459 individuals was included in the fibrinogen analysis.

### Measurement of fibrinogen

In the KORA study, fibrinogen was determined by an immunonephelometric method (Dade Behring Marburg GmbH, Marburg, Germany) on a Behring Nephelometer II analyzer. FHS study used the Clauss method<sup>16</sup> in the offspring and the third generation subjects, and a

modified method of Ratnoff and Menzie<sup>17</sup> in the original cohort subjects. In the RS, fibrinogen levels were derived from the clotting curve of the prothrombin time assay using Thromborel S as a reagent on an automated coagulation laboratory 300 (ACL 300, Instrumentation Laboratory, Zaventem, Belgium). The other studies used the Clauss method for measuring plasma fibrinogen.<sup>16</sup>

### Statistical analysis

Each study independently analyzed their genotype-phenotype data. Except for FHS, which has a family structure, all studies conducted analyses of all directly genotyped and imputed SNPs using linear regression on untransformed fibrinogen measures using an additive genetic model adjusted for age, sex, and site of recruitment (if necessary). In FHS, a linear mixed effects model was employed with a fixed additive effect for the SNP genotype, fixed covariate effects, random family specific additive residual polygenic effects to account for within family correlations<sup>18</sup>, and a random environment effect. In addition, FHS adjusted for population stratification using principal components of the directly measured SNPs which were computed using the Eigenstrat software.

To account for residual stratification, p-values were adjusted for genomic inflation. The inflation of the association test statistic, stated as inflation factor lambda ( $\lambda_{gc}$ ), was small for all studies: 0.995 for RS, 1.016 for FHS, 1.031 for CHS, 1.024 for ARIC, 1.012 for KORR, and 1.008 for B58C. Using the study-specific results, we conducted a fixed effect model meta-analysis based on inverse-variance weighting. MetABEL, a package running under R was used to perform the meta-analysis. We used Bonferroni correction to deal with the problem of multiple testing. Simulation studies show that the effective number of independent tests in a GWA analysis is nearly one million.<sup>19</sup> Based on one million tests, we selected a p-value threshold of  $5 \times 10^{-8}$  as the level of genome-wide significance.

In addition, we estimated the effect of the top SNPs in strata of sex and smoking status. Gene-by-sex and gene-by-smoking interaction was tested in each study by introducing an interaction term into the linear model. We used a sample size weighted meta-analysis to combine the reported interaction p-values across studies for each of the top SNPs.

### Replication in Women's Health Genome Study (WHGS)

We used the WHGS to replicate our genome wide significant findings and other loci for which our meta-analysis generated more modest evidence of an association (p-value of  $5 \times 10^{-7}$ ). Participants in WHGS are derived from the genetic arm of the Women's Health Study and include American women with no prior history of cardiovascular disease, cancer, or other major chronic illness who provided a baseline blood sample during the enrollment phase of the Women's Health Study between 1992 and 1995.<sup>20</sup> Fibrinogen levels were measured using an immunoturbidimetric assay (Kamiya Biomedical, Seattle, Wash), which was standardized to a calibrator from the World Health Organization. Genotyping was done using the Illumina Infinium II assay to query a genome-wide set of 315,176 haplotype-tagging SNP markers (Human HAP300 panel) as well as a focused panel of 45,882 missense and haplotype tagging SNPs. For this analysis, the evaluation was performed on 17,686 non-diabetic individuals who were of Caucasian ancestry and were not taking lipid-lowering agents. The GWA results of the WHGS are reported in a companion manuscript.

## Results

The sample size and participant characteristics from each study are shown in Table 1. A quantile-quantile plot (Q-Q plot) of the observed against expected p-value distribution is shown in Figure 1. Figure 2 illustrates the primary findings from the meta-analysis and presents p-

values for each of the interrogated SNPs across the 22 autosomal chromosomes. A total of 73 SNPs (supplemental Table 1) exceeded the threshold of genome-wide significance and clustered around four loci on chromosomes 1 (2 SNPs), 3 (12 SNPs), 4 (23 SNPs), and 5 (36 SNPs) (Figure 3).

The strongest statistical evidence for an association was for rs1800789 which is located at 4q31.3 in exon 7 of the fibrinogen  $\beta$  (*FGB*) gene (minor allele frequency [MAF]: 0.20-0.24, meta-analysis p-value =  $1.75 \times 10^{-30}$ , fibrinogen level change per minor allele [ $\Delta$ ]: 0.087 g/L). The other significant loci were marked by rs2522056, which is located at 5q23.3, 25 kb downstream of the interferon regulatory factor 1 (*IRF1*) gene (MAF: 0.17-0.21, p =  $1.3 \times 10^{-15}$ ,  $\Delta$ : -0.063 g/L), rs511154, which is located at 3q22.3, in intron 1 of the propionyl coenzyme A carboxylase, beta polypeptide (*PCCB*) gene (MAF: 0.21-0.24, p =  $5.94 \times 10^{-10}$ ,  $\Delta$ : 0.045 g/L) and rs1539019 which is located at 1q44, on the NLR family, pyrin domain containing 3 isoforms (*NLRP3*) gene (MAF: 0.37-0.42, p =  $1.04 \times 10^{-8}$ ,  $\Delta$ : -0.038 g/L). Cohort-specific findings are presented for the top SNP within each locus in Table 2. Results did not change materially when we adjusted the model for other covariates (smoking, alcohol consumption, body mass index, systolic blood pressure, triglyceride, total- and HDL-cholesterol, diabetes, and cardiovascular disease) (data not shown). Table 3 shows the mean and standard deviations for fibrinogen levels by genotype for each of the four SNPs.

We estimated the association of the four SNPs by sex and smoking status separately but none of the SNPs showed a significantly different association between subgroups (Supplementary Table 2 and 3).

A combined risk alleles score summarizing the number of risk alleles was associated with a 15% increase in overall mean fibrinogen level comparing subjects with no risk allele (mean fibrinogen level 2.81 g/L) to subjects with six or more risk alleles (mean fibrinogen level 3.24 g/L). The genetic variants identified in our study explained less than 2% of the overall variance in plasma fibrinogen in all studies except one.

To investigate the validity of our findings, we sought replication of the four loci using WHGS data. Since WHGS did not genotype the identical SNPs as our six cohorts, the best proxy SNP was used for replication. For rs1800789, rs2522056, rs511154, and rs1539019, we used WHGS SNPs rs6056 ( $r^2=0.95$ ; p= $8.04 \times 10^{-39}$ ), rs1016988 ( $r^2=0.80$ ; p= $1.24 \times 10^{-12}$ ), rs684773 ( $r^2=1.0$ ; p= $1.92 \times 10^{-5}$ ), and rs1539019 (p= $2.89 \times 10^{-4}$ ), respectively, as the proxy SNP. The direction of each association in WHGS was consistent with our findings.

In addition to our four genome-wide significant loci, two other loci demonstrated multiple-SNP hits with p-values  $< 5 \times 10^{-7}$ : one on chromosome 2 (rs4251961, p= $3.5 \times 10^{-7}$ ) and one on chromosome 14 (rs8017049, p= $5.6 \times 10^{-7}$ ). When we examined the results for these two loci in the WHGS data, we found evidence for replication on chromosome 2 (rs4251961 in WHGS, p= $8.5 \times 10^{-3}$ ).

## Discussion

We identified four loci associated with circulating fibrinogen level through a meta-analysis of GWA data from six cohort studies comprising 22,096 subjects. We provide strong information of the previously reported associations with the *FGB* locus. Three of our findings are newly identified associations.

The most significant SNP in our study was rs1800789 which is located on the *FGB* gene. The *FGB* gene encodes the fibrinogen  $\beta$  chain. A well-characterized SNP at this locus is rs1800787 (-148C/T) which resides 965 base pairs away from our top SNP (rs1800789) and is in high LD with it ( $D' \sim 1.0$ ,  $r^2=0.91$ ). It is known that rs1800787 directly affects gene transcription in

basal and IL6-stimulated conditions in luciferase expression studies.<sup>21</sup> Another well-characterized SNP in this region is rs1800790 (455G/A), which is also in strong LD with rs1800787, is known to be related to plasma fibrinogen<sup>22</sup> and showed a strong association with fibrinogen levels in our study as well ( $p=5.04\times 10^{-27}$ , Supplementary Table 1).

The second locus is located 25 kb downstream from the *IRF1* gene on chromosome 5. *IRF1* is a member of the interferon regulatory transcription factor family and activates transcription of interferon  $\alpha$  and  $\beta$ . *IRF1* also functions as a transcription activator of genes induced by interferon  $\alpha$ ,  $\beta$  and  $\gamma$ . Direct effects of interferons on fibrinogen have not previously been described, but it is known that they play a role in the regulation of acute phase proteins. Notably, the SNP is only 31 kb from a SNP strongly associated with Crohn's disease in a recent meta-analysis (rs2188962,  $p<2.32\times 10^{-18}$ ).<sup>23</sup> Individuals with inflammatory bowel disease (IBD), including Crohn's disease, are at a threefold higher risk of venous thrombosis<sup>24</sup>, accounting for substantial morbidity and mortality in this group.<sup>25</sup> Furthermore, multiple studies have indicated significantly elevated levels of fibrinogen in IBD patients.<sup>26</sup> This suggests that *IRF1* or nearby genes may contribute to Crohn's disease via a mechanism mediated through an increase in acute phase responsiveness and fibrinogen levels.

The third locus on chromosome 3 is located in intron 1 of the *PCCB* gene. The *PCCB* gene is responsible for a particular step in the breakdown of the amino acids isoleucine, methionine, threonine, and valine. However, the available information about *PCCB* does not provide a strong hypothesis about the putative function of the gene in regulation of fibrinogen levels.

The fourth locus on chromosome 1 is located on the *NLRP3* gene. The *NLRP3* gene encodes a pyrin-like protein, which interacts with the apoptosis-associated speck-like protein PYCARD/ASC and is a member of the NALP3 inflammasome complex.<sup>27</sup> Activated NALP3 inflammasome drives processing of the pro-inflammatory cytokine pro-*IL1 $\beta$*  to *IL1 $\beta$* . Recent data indicate that the NALP3 inflammasome can be activated by endogenous 'danger signals' as well as compounds associated with pathogens and triggers an innate immune response.<sup>28</sup>

The finding on chromosome 2 is located in the promoter region (1 kb upstream from the transcription start site) of the interleukin-1 receptor antagonist (*IL1RN*) gene. Fibrinogen is an acute phase protein that is regulated by cytokines, mainly *IL1* and *IL6*, while the *IL6*-mediated transcription of the fibrinogen gene is inhibited by *IL1 $\beta$* .<sup>29</sup> This region has formerly reported to be associated with fibrinogen levels; rs2232354, which is in high LD with our top SNP, rs4251961, was associated with fibrinogen levels in an asymptomatic population.<sup>30</sup>

Our findings were replicated in WGHS. Two of our four SNPs are reported by WGHS as genome-wide significant findings (rs6056 and rs1016988) and the other two have p-values which suggest non-chance findings in a replication (rs684773 and rs1539019). These results provide further credibility that our newly identified loci are valid.

We examined evidence for the top four fibrinogen loci among gene expression QTLs from recent GWA studies in human liver tissues<sup>31</sup> and lymphoblastoid cell lines.<sup>32</sup> In liver tissues, SNPs at the *FGB* locus were strongly associated with the expression of *FGB* (e.g., rs4508864,  $p<1.20\times 10^{-8}$ ) as well as with other *trans*-located mRNAs. Likewise, we observed that several SNPs in the region of the *IRF1* locus were strongly associated with the expression of nearby genes (including *IRF1*, *LOC441108*, and *SLC22A5*) in both liver tissues and lymphoblastoid cell lines (e.g., rs2070729,  $p=4.9\times 10^{-10}$  for expression of the *IRF1* gene). These results from independent genome-wide association studies strongly suggest a functional basis for the observed associations in the *FGB* and *IRF1* loci.

Although heritability estimates for circulating fibrinogen are substantial, the genetic variants identified in our study explain only a small part of the overall variance. Therefore, our SNPs

probably have limited value in prediction of cardiovascular disease. Rare variants, common variants with smaller effects, or variants which interact with other genetic and environmental factors may explain the remaining variation in plasma fibrinogen levels.

Fibrinogen was measured independently in the six cohorts. Though methods for measuring fibrinogen concentration were not standardized, they were all based on the Clauss method or another clotting assay, except for KORA which used nephelometry. Nonetheless, the effect estimates for the top SNPs were comparable between KORA and other studies.

Contributing studies used different genotyping platforms with different groups of SNPs. To enable the meta-analysis, each study imputed ~2.5 million SNPs in HapMap CEU samples. Imputation has previously been shown to be accurate and to increase the power. The power, of course, would have been higher if all SNPs were genotyped in all studies.

In conclusion, we have identified four loci associated with fibrinogen levels through meta-analysis of GWA data from six cohort studies comprising 22,096 subjects. All four loci replicated in a seventh study. In addition, we replicated one of the two other loci which showed a close to significant association in our meta-analysis and is biologically plausible. Three of our findings (*IRF1*, *PCCB*, and *NLRP3*) represent newly identified associations. Among the genes in the novel loci implicated in our study are those that encode proteins playing a role in inflammation representing interesting targets for further research into biological pathways involved in cardiovascular disease and other chronic inflammatory conditions.

#### Clinical summary

Fibrinogen is a major player in the coagulation system, is a determinant of platelet aggregation, and affects blood viscosity. Circulating fibrinogen levels have been consistently associated with risk of coronary heart disease. Although blood fibrinogen levels are influenced by many environmental factors, genes either independently or in combination with environmental factors play an important role in determining circulating fibrinogen levels. The advent of genome-wide association studies provides an opportunity to identify previously unsuspected genetic loci that influence complex traits. In this study, we combined genome-wide association data from six large prospective cohort studies, and identified four genetic loci that are associated with circulating fibrinogen levels. These genetic loci provide valuable insights into the pathways that determine circulating fibrinogen levels. Although additional investigations are needed to understand the exact mechanisms, our findings do highlight the key contribution of inflammatory genes in influencing inter-individual variation in fibrinogen levels. A better understanding of the molecular mechanisms that control circulating fibrinogen levels may spur the development of novel therapeutic strategies that might reduce fibrinogen levels. Such pharmacological agents may be potentially useful for reducing the risk of coronary heart disease.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A full list of principal CHS investigators and institutions can be found at <http://www.chs-nhlbi.org/pi.htm>.

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## Web Resources

1. MACH. <http://www.sph.umich.edu/csg/abecasis/MACH/index.html><http://www.sph.umich.edu/csg/abecasis/MACH/index.html>
2. SHARE. [http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study\\_id=phs000007.v3.p2](http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v3.p2)[http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study\\_id=phs000007.v3.p2](http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v3.p2)
3. SNPTTEST. <http://www.stats.ox.ac.uk/marchini/software/gwas/snptest.html><http://www.stats.ox.ac.uk/marchini/software/gwas/snptest.html>
4. R-project. <http://www.r-project.org><http://www.r-project.org>
5. MetABEL. <http://mga.bionet.nsc.ru/~yurii/ABEL><http://mga.bionet.nsc.ru/~yurii/ABEL>
6. Eigenstrat software. <http://genepath.med.harvard.edu/~reich/EIGENSTRAT.htm><http://genepath.med.harvard.edu/~reich/EIGENSTRAT.htm>
7. IMPUTE. <http://www.stats.ox.ac.uk/~marchini/software/gwas/impute.html><http://www.stats.ox.ac.uk/~marchini/software/gwas/impute.html>

8. BIMBAM. <http://stephenslab.uchicago.edu/software.html>  
<http://stephenslab.uchicago.edu/software.html>

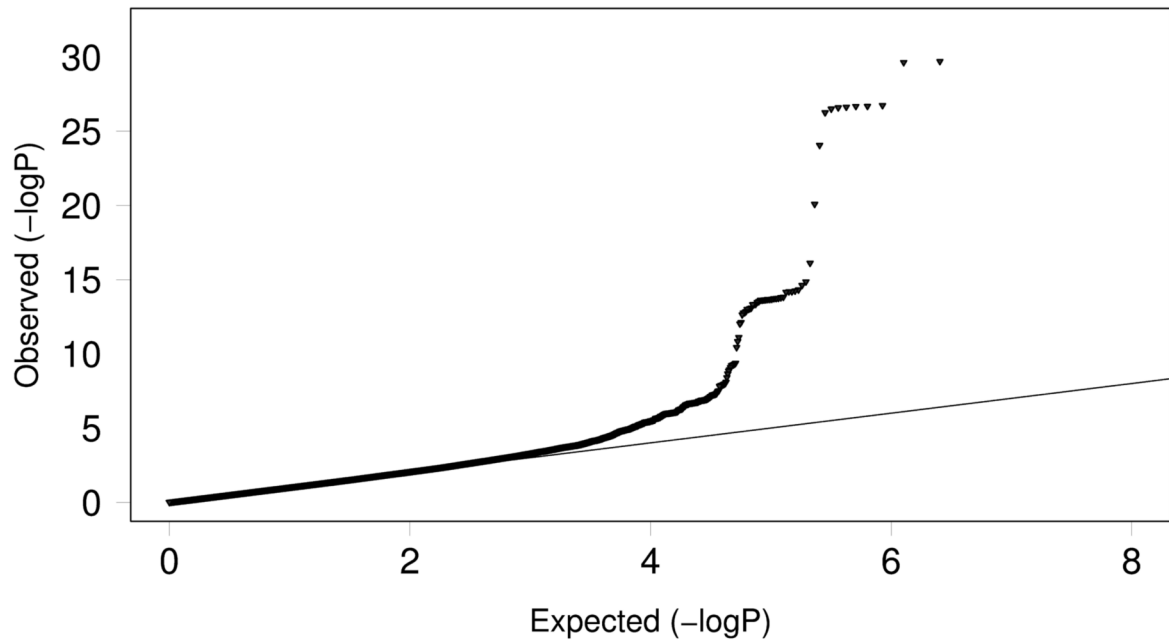
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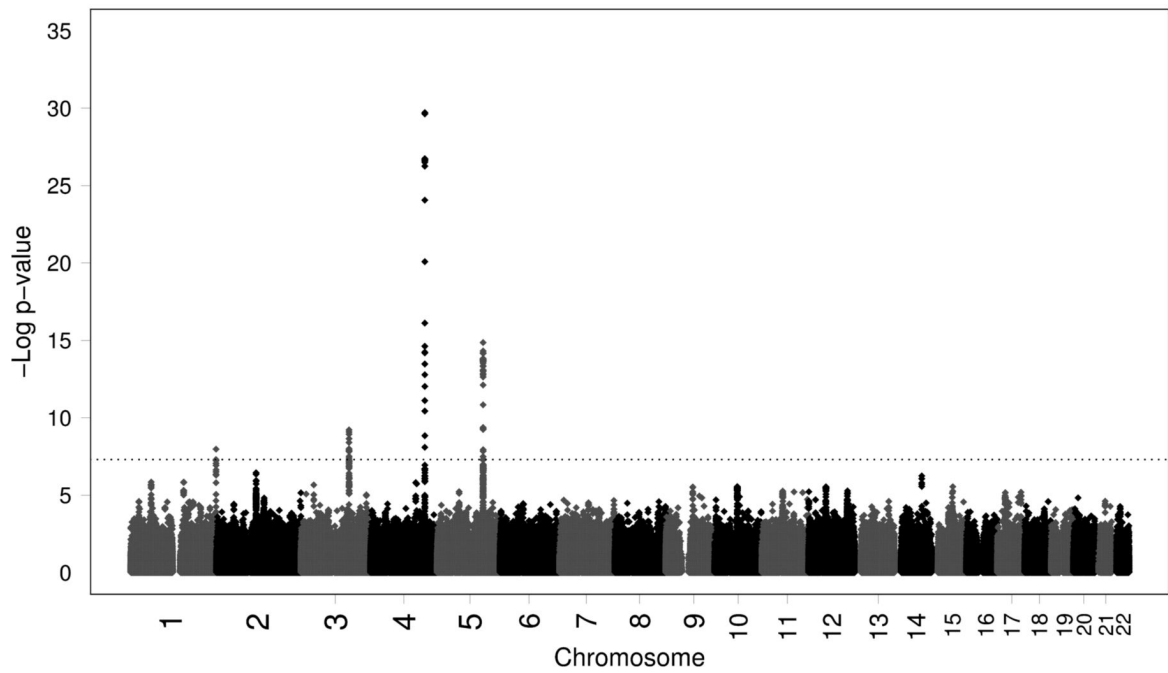
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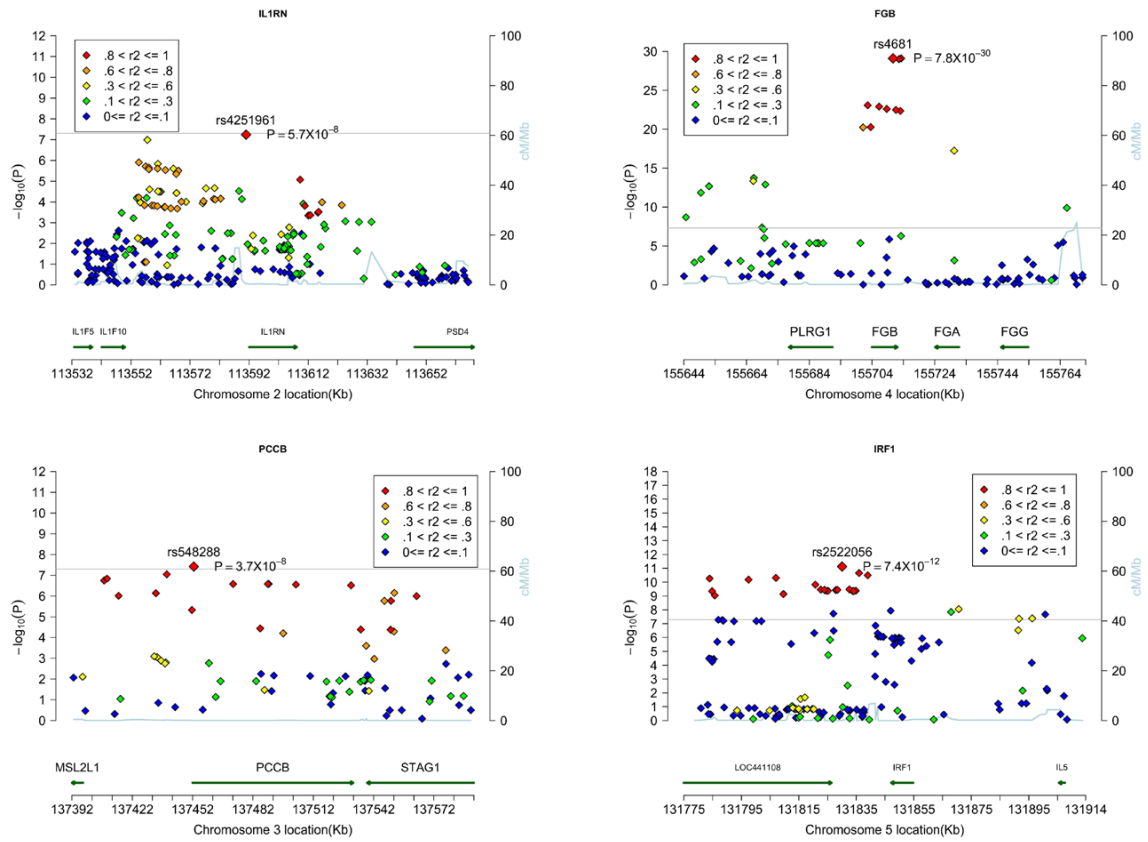


**Figure 1.** QQ-plot for the meta-analysis results. Quantile-quantile plot of the observed and the expected distribution of p-values for all 2,661,766 SNPs and their association with fibrinogen levels based on meta-analyzed data.



**Figure 2.**

-Log plot for the meta-analysis. -Log p-values for each of the 2,661,766 tests performed as part of the genome-wide association analysis of fibrinogen levels. The grey dashed horizontal lines correspond to the p-value threshold of  $5 \times 10^{-8}$  and the grey solid line corresponds to  $5 \times 10^{-6}$ .



**Figure 3.** Regional plots of loci associated with fibrinogen. (a-d). The association p-values ( $-\log_{10}$  transformed, indicated by the left y-axis) for SNPs in a 60kb region of each of the four loci (*FGB*, *IRF1*, *PCCB*, *NLRP3*) are plotted against their chromosome positions (NCBI build 36) on x-axis. Each diamond represents a SNP with the color indicating the linkage disequilibrium (estimated using HapMap CEU sample) between the SNP and the top associated SNP that is plotted by a red diamond with size larger than all other diamonds, and with the SNP name displayed on the top, and p-value on the right. Shown in light blue are the estimated recombination rates in HapMap with values indicated by the right y-axis. The bottom panel displays the genes in the region based on the UCSC Genome Browser March 2006 assembly, with the arrow to right (left) indicating +(-) strand. The grey horizontal line corresponds to the p-value threshold of genome-wide significance,  $5 \times 10^{-8}$ .

Table 1

Characteristics of the Study Participants

	Study Sample						
	RS	FHS	CHS	ARIC	KORA	B58C	
Number	2,068	7,022	1,993	8,051	1,523	1,459	
Age, Mean (SD) (y)	70.8 (9.0)	46.6 (11.5)	73.2 (5.8)	54.3 (5.6)	52.1 (10.2)	44.9 (0.4)	
Male (%)	36.8	46.1	44.4	47.1	49.3	50.0	
Body mass index, Mean (SD) ((kg/m <sup>2</sup> ))	26.5 (3.9)	27.0 (5.2)	26.4 (4.5)	27.0 (4.9)	27.3 (4.1)	27.4 (4.8)	
Current smoker (%)	22.2	18.9	11.3	25.1	15.4	23.0	
Alcohol drinker (%)	77.1	74.5	52.2	44.8	70	94.6	
Systolic blood pressure, Mean (SD) (mmHg)	138.2 (21.4)	121.1(17.1)	137.7 (21.7)	118.5 (17.0)	133.0 (18.3)	126.7 (15.3)	
Diastolic blood pressure, Mean (SD) (mmHg)	72.2 (11.2)	75.0 (10.0)	70.6 (11.5)	71.7 (10.0)	81.9 (10.9)	79.1 (10.2)	
Total cholesterol, Mean (SD) (mmol/l)	6.7 (1.3)	5.1 (1.0)	5.5 (1.0)	5.5 (1.1)	6.1 (1.1)	5.9 (1.0)	
HDL cholesterol, Mean (SD) (mmol/l)	1.3 (0.4)	1.4 (0.4)	1.4 (0.4)	2.8 (0.9)	1.4 (0.4)	1.5 (0.4)	
Prevalent cardiovascular disease (%)	7.9	12.6	0	6.7	0.7	4.3	
Prevalent diabetes (%)	11.8	4.8	29.7	8.5	3.7	1.9	
Hypertension (%)	34.8	11.5	37.9	19.8	23.5	21.4	
Fibrinogen, Mean (SD) (g/L)	2.7 (0.7)	3.2 (0.7)	3.2 (0.6)	2.97 (0.6)	2.9 (0.7)	3.0 (0.6)	

RS: The Rotterdam Study; FHS: The Framingham Heart Study; CHS: The Cardiovascular Health Study; ARIC: The Atherosclerosis Risk in Communities Study; KORA: The MONICA/KORA Augsburg Study; B58C: British 1958 birth cohort



Table 2

Association of the Top SNPs in Four Loci with Plasma Fibrinogen Levels

	RS	FHS	CHS	ARIC	KORA	B58C	Combined
rs1800789 (A/G)	2,068	7,022	1,993	8,051	1,523	1,458	22,096
Chromosome: 4	2.59×10 <sup>-05</sup>	8.01×10 <sup>-13</sup>	1.50×10 <sup>-02</sup>	2.19×10 <sup>-09</sup>	1.00×10 <sup>-04</sup>	1.22×10 <sup>-01</sup>	1.75×10 <sup>-30</sup>
Location: 155702193	0.108	0.103	0.179	0.068	0.097	0.042	0.087
Gene: FGB	0.026	0.014	0.036	0.011	0.025	0.027	0.008
Modeled Allele: A	0.955	0.929	0.845	0.981	0.874	0.958	-
R square (%)	0.8	0.8	2.5	0.4	0.8	0.2	-
Effective allele frequency	0.20	0.21	0.21	0.22	0.24	0.20	-
rs2522056 (G/A)	2,068	7,022	1,993	7,856	1,523	1,459	21,901
Chromosome: 5	2.40×10 <sup>-01</sup>	2.59×10 <sup>-05</sup>	6.20×10 <sup>-04</sup>	5.59×10 <sup>-07</sup>	4.80×10 <sup>-03</sup>	4.00×10 <sup>-03</sup>	1.31×10 <sup>-15</sup>
Location: 131829625	-0.031	-0.058	-0.168	-0.060	-0.076	-0.082	-0.063
Gene: IRF1	0.026	0.014	0.049	0.012	0.027	0.029	0.008
Modeled Allele: A	0.979	0.979	0.442	1.000	0.940	0.968	-
R square (%)	0.1	0.3	1.6	0.4	0.5	0.6	-
Effective allele frequency	0.19	0.21	0.17	0.209	0.21	0.18	-
rs511154 (A/G)	2,068	7,022	1,993	8,051	1,523	1,459	22,096
Chromosome: 3	1.96×10 <sup>-02</sup>	2.12×10 <sup>-03</sup>	3.20×10 <sup>-03</sup>	1.7×10 <sup>-04</sup>	8.26×10 <sup>-01</sup>	1.30×10 <sup>-02</sup>	5.94×10 <sup>-10</sup>
Location: 137433611	0.057	0.041	0.089	0.043	0.006	0.063	0.045
Gene: PCCB	0.024	0.013	0.030	0.011	0.027	0.026	0.007
Modeled Allele: A	0.996	1.017	0.993	0.989	0.973	0.945	-
R square (%)	0.3	0.1	0.7	0.2	<0.1	0.4	-
Effective allele frequency	0.22	0.24	0.23	0.22	0.21	0.24	-
rs1539019 (A/C)	2,068	7,022	1,993	7,773	1,523	1,434	21,818
Chromosome: 1	4.22×10 <sup>-02</sup>	9.76×10 <sup>-04</sup>	9.94×10 <sup>-01</sup>	3.13×10 <sup>-04</sup>	6.50×10 <sup>-03</sup>	1.64×10 <sup>-01</sup>	1.04×10 <sup>-08</sup>
Location: 245666924	-0.043	-0.041	0.000	-0.037	-0.061	-0.030	-0.038
Gene: NLRP3	0.021	0.013	0.027	0.010	0.022	0.022	0.007

	RS	FHS	CHS	ARIC	KORA	B58C	Combined
Modeled Allele: A	0.990	0.846	0.97	1.000	0.664	0.829	-
Observed/expected variance	0.2	0.2	<0.1	0.3	0.4	0.1	-
R square (%)	0.39	0.40	0.41	0.37	0.42	0.41	-
Effective allele frequency							

RS: The Rotterdam Study; FHS: The Framingham Heart Study; CHS: The Cardiovascular Health Study; ARIC: The Atherosclerosis Risk in Communities Study; KORA: The MONICA/KORA Augsburg Study; B58C: British 1958 birth cohort

\* The beta coefficient is for an age and sex adjusted model

Table 3

## Plasma Fibrinogen Level by Genotype

SNP	Study	Sample size	Fibrinogen level Mean(SD), g/L	Sample size	Fibrinogen level Mean(SD), g/L	Sample size	Fibrinogen level Mean(SD), g/L
rs1800789	RS	1,328	2.8 (0.69)	652	2.9 (0.66)	88	3.0 (0.69)
	FHS	4,489	3.2 (0.69)	2,250	3.3 (0.66)	283	3.4 (0.70)
	CHS	1,290	3.1 (0.62)	624	3.2 (0.63)	79	3.3 (0.61)
	ARIC	4,908	2.9 (0.62)	2,730	3.0 (0.59)	413	3.0 (0.67)
	KORA	884	2.8 (0.64)	533	3.0 (0.70)	106	3.0 (0.66)
	B58C	928	2.9 (0.61)	471	3.0 (0.55)	59	3.0 (0.60)
rs2522056	RS	1,349	2.8 (0.68)	641	2.8 (0.69)	78	2.7 (0.72)
	FHS	4,364	3.3 (0.68)	2353	3.2 (0.66)	305	3.1 (0.74)
	CHS	1,239	3.2 (0.61)	754	3.1 (0.63)	0	NA
	ARIC	4,946	2.9 (0.62)	2,592	2.9 (0.61)	318	2.8 (0.63)
	KORA	951	2.9 (1.01)	493	2.8 (1.01)	79	2.7 (1.02)
	B58C	971	3.0 (0.59)	438	2.9 (0.58)	50	2.9 (0.59)
rs511154	RS	1,253	2.8 (0.67)	706	2.8 (0.70)	109	2.9 (0.74)
	FHS	4,047	3.2 (0.67)	2,536	3.3 (0.70)	439	3.2 (0.66)
	CHS	1,206	3.2 (0.60)	687	3.2 (0.63)	100	3.3 (0.69)
	ARIC	4,925	2.9 (0.61)	2,712	2.9 (0.62)	414	3.0 (0.62)
	KORA	946	2.9 (0.68)	503	2.9 (0.63)	74	3.0 (0.62)
	B58C	834	2.9 (0.60)	541	3.0 (0.57)	84	3.1 (0.59)
rs1539019	RS	762	2.8 (0.69)	987	2.8 (0.69)	319	2.8 (0.65)
	FHS	2,419	3.3 (0.68)	3,428	3.2 (0.69)	1,175	3.2 (0.65)

SNP	Study	Sample size	Fibrinogen level Mean(SD), g/L	Sample size	Fibrinogen level Mean(SD), g/L	Sample size	Fibrinogen level Mean(SD), g/L
	CHS	686	3.2 (0.63)	991	3.2 (0.61)	316	3.2 (0.61)
	ARIC	3,041	2.9 (0.63)	3,721	2.9 (0.60)	1,011	2.9 (0.60)
	KORA	530	3.0 (0.73)	715	2.9 (0.63)	278	2.8(0.60)
	B58C	524	3.0 (0.62)	653	2.9 (0.57)	257	2.9(0.59)

RS: The Rotterdam Study; FHS: The Framingham Heart Study; CHS: The Cardiovascular Health Study; ARIC: The Atherosclerosis Risk in Communities Study; KORA: The MONICA/KORA Augsburg Study; B58C: British 1958 birth cohort