Identifying Novel Gene Variants in Coronary Artery Disease and Shared Genes With Several Cardiovascular Risk Factors

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Rationale: **Coronary artery disease (CAD) is a critical determinant of morbidity and mortality. Previous studies have identified several cardiovascular disease risk factors, which may partly arise from a shared genetic basis with CAD, and thus be useful for discovery of CAD genes.**

Objective: **We aimed to improve discovery of CAD genes and inform the pathogenic relationship between CAD and several cardiovascular disease risk factors using a shared polygenic signal–informed statistical framework.**

Methods and Results: **Using genome-wide association studies summary statistics and shared polygenic pleiotropy-informed conditional and conjunctional false discovery rate methodology, we systematically investigated genetic overlap between CAD and 8 traits related to cardiovascular disease risk factors: low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, type 2 diabetes mellitus, C-reactive protein, body mass index, systolic blood pressure, and type 1 diabetes mellitus. We found significant enrichment of single-nucleotide polymorphisms associated with CAD as a function of their association with low-density lipoprotein, high-density lipoprotein, triglycerides, type 2 diabetes mellitus, C-reactive protein, body mass index, systolic blood pressure, and type 1 diabetes mellitus. Applying the conditional false discovery rate method to the enriched phenotypes, we identified 67 novel loci associated with CAD (overall conditional false discovery rate <0.01). Furthermore, we identified 53 loci with significant effects in both CAD and at least 1 of low-density lipoprotein, high-density lipoprotein, triglycerides, type 2 diabetes mellitus, C-reactive protein, systolic blood pressure, and type 1 diabetes mellitus.**

Conclusions: **The observed polygenic overlap between CAD and cardiometabolic risk factors indicates a pathogenic relation that warrants further investigation. The new gene loci identified implicate novel genetic mechanisms related to CAD. (*Circ Res*. 2016;118:83-94. DOI: 10.1161/CIRCRESAHA.115.306629.)**

Key Words: coronary artery disease ■ coronary heart disease ■ genome-wide association study ■ genetic pleiotropy ■ lipids ■ molecular epidemiology ■ myocardial infarction ■ Women's Genome Health Study

Original received April 7, 2014; revision received October 15, 2015; accepted October 20, 2015. In September 2015, the average time from submission to first decision for all original research papers submitted to *Circulation Research* was 12.75 days.

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This manuscript was sent to Elizabeth M. McNally, Consulting Editor, for review by expert referees, editorial decision, and final disposition.

The online-only Data Supplement is available with this article at http://circres.ahajournals.org/lookup/suppl/doi:10.1161/CIRCRESAHA.115.306629/-/DC1.

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Nonstandard Abbreviations and Acronyms

BMI	body mass index
CAD	coronary artery disease
CRP	C-reactive protein
CVD	cardiovascular disease
eQTL	expression quantitative trait locus
FDR	false discovery rate
GWAS	genome-wide association study
HDL	high-density lipoprotein
LCL	lymphoblastoid cells
LDL	low-density lipoprotein
SBP	systolic blood pressure
SNP	single-nucleotide polymorphism
T1D	type 1 diabetes mellitus
T2D	type 2 diabetes mellitus

Coronary artery disease (CAD) is a leading cause of death worldwide. The development of CAD is influenced by both genetic and environmental factors, as evident by its high heritability (40% to 50%), shown in twin and family studies.[1] Genome-wide association studies (GWAS) in CAD have identified a total of 46 genetic variants reaching genome-wide significance for CAD.[2] However, the identified genetic variants explain only a small proportion of estimated heritability,[2] that is, only a small amount of the familial clustering of CAD. This apparent paradox is widely seen across GWAS for complex traits and is termed the missing heritability problem.[3,4] However, recent discoveries suggest that existing GWAS can capture more of the heritability because of common variants if proper statistical tools are used.[5–7]

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Hypertension,[8] obesity,[9] abdominal fat,[10] diabetes mellitus,[11] dyslipidemia,[12–14] and inflammation as reflected by high levels of C-reactive protein (CRP) [15] are associated with CAD. Several studies have found overlapping pathophysiology,[16] but the underlying shared genetic factors and the extent of the polygenic overlap across these phenotypes are mainly unknown. We have developed an analytic framework for complex traits building on the polygenic overlap[17] between two or more phenotypes.[6] This method has the potential to capture more of the polygenic effects in complex traits[18] and has successfully been applied to psychiatric,[6] cardiovascular,[19] neurological diseases,[20] and cancer.[21] This shared polygenic signal method could be particularly informative in CAD, a disease with known comorbidities and overlapping pathophysiology with related cardiovascular and metabolic disorders.[2,22–25]

We used this approach to leverage the power of multiple large genomic studies to describe the extent of the polygenic overlap and identify overlapping single-nucleotide polymorphisms (SNPs) between CAD and 8 associated traits and cardiovascular disease (CVD) risk factors, where recent GWAS results are available: low-density lipoprotein (LDL) cholesterol,[26] high-density lipoprotein (HDL) cholesterol,[26] triglycerides,[26] type 2 diabetes mellitus (T2D),[27] CRP,[28] body mass index (BMI),[29] systolic blood pressure (SBP),[30,31] and type 1 diabetes mellitus (T1D).[32] By combining data from these different GWAS, we hypothesized that the shared polygenic signal approach can improve discovery of CAD genes and inform the pathogenic relationship between CAD and CVD risk factors.

Methods

Participant Samples

We obtained summary statistics from large-scale genomic studies (*P* values and risk allele when available) from public access websites or through collaboration with investigators. The summary statistics are based on the Metabochip[33] for CAD[2] (n=194 427, including 63 746 cases) and T2D[27] (n=149 830), and standard GWAS for LDL[26] (n=95 454), HDL[26] (n=99 900), triglycerides[26] (n=96 568), BMI[29] (n=123 865), SBP[31] (n=203 056), T1D[32] (n=16 559), and CRP (n=66 185).[28] Details on the inclusion criteria and phenotype characteristics of the different GWAS are described in the original publications.

There were some overlapping controls between CAD and T2D and also between CAD and T1D. In both instances, this was mainly because of the inclusion of ≥1 substudies using a shared control design (eg, used by the Wellcome Trust Case Control Consortium and deCODE Genetics[34]; Online Table I). There was also some sample overlap between CAD and LDL, HDL, triglycerides, BMI, and SBP (Online Table I). Note that even without raw data, an upper bound for the amount of sample overlap is obtainable from the original publications by comparing the substudy definitions and samples sizes for CAD and each secondary trait (correlation of uncorrected test statistics because of sample overlap is given in Online Table I; M. LeBlanc et al, unpublished data, 2015).

The Women's Genome Health Study (WGHS), initiated in 1992, is an ongoing prospective cohort including 23 294 initially healthy North American women of European ancestry with whole-genome genotype data and follow-up for major incident health events, including myocardial infarction (MI) and coronary heart disease (CHD; composed of MI, CHD death, and coronary revascularization) are recorded.[35] Over the ≈20 years of follow-up, there were 387 and 1007 cases, respectively, of incident MI and CHD among the 23 294 women.

The relevant institutional review boards or ethics committees approved the research protocol of the individual GWAS, and all participants provided written informed consent.

Statistical Analyses

We use Matlab (version R2013a) for all statistical analysis unless otherwise indicated. First, we looked for evidence of overlapping polygenic signal for CAD and each secondary trait. In the absence of an overlapping polygenic signal, the expectation is that the *P* value distribution for CAD is independent from the *P* values in the secondary trait. The dependency of the *P* value distribution for CAD on each secondary trait can be visually explored using conditional quantile–quantile plots to evaluate genetic 'enrichment' in CAD as a function of a secondary phenotype. Quantile–quantile plots are a descriptive tool for visualizing the difference between an observed distribution and a theoretical distribution. With GWAS, quantiles of the observed (nominal) *P* values, denoted by p, are plotted on the y axis, and the quantiles of the theoretical null distribution (ie, the uniform distribution), denoted by q, are plotted on the x axis. Conventionally, the −log10 transform is used to emphasize tail areas. If there is no deviation from the null distribution and thus no true genetic association present, a quantile–quantile plot falls on the 1:1 line. Leftward deflections of the observed distribution from the null line reflect increased tail probabilities in the distribution of the test statistics, and consequently an overabundance of low *P* values compared with that expected by chance, termed enrichment. Here, we constructed conditional quantile–quantile plots to investigate whether enrichment in the primary phenotype (CAD) is related to significance in a given secondary phenotype, as visualized by a leftward deflection from the null line on the conditional quantile–quantile plot. A conditional quantile–quantile plot was separately constructed for CAD and each of the 8 secondary traits. To test for statistical significance

associated with these conditional quantile–quantile plots, we used the Anderson–Darling test.[21] In brief, this is a statistical test of whether a given sample of data is drawn from a given probability distribution and allows us to determine if an observed leftward deflection is statistically significant.[21] In this case, we used set of SNPs (GWAS $P>0.1$ in the secondary trait), that is, SNPs that are signal depleted in the secondary trait, as the comparison set.

Second, once statistically significant enrichment was confirmed, we computed conditional false discovery rate (FDR), a statistical framework that leverages shared polygenic signal,[6,18] to improve the discovery of SNPs for the primary trait of interest, CAD. The standard FDR is designed to control the expected proportion of incorrectly rejected null hypotheses and is used to correct for multiple comparisons. An extension of the standard FDR is the conditional FDR,[6] which in our application, is used to incorporate information from GWAS summary statistics of a second phenotype. The conditional FDR is defined as the probability of a SNP being null in the first phenotype given that the *P* values in the first and second phenotype are as small as or smaller than the observed ones (Methods in the Online Data Supplement). Importantly, ranking SNPs according to conditional FDR reorders SNPs compared with their raw CAD *P* values, and this new ranking favors SNPs showing signal in both CAD and the given secondary trait. In contrast, the standard FDR does not rerank the SNPs compared with their raw CAD *P* values, but instead suggests a different significance cutoff compared with the Bonferroni correction.

In additional analysis, we computed the conjunctional FDR[18] to detect loci showing strong evidence of association with both CAD and the given secondary trait. Low values in conditional FDR can be driven by association with both phenotypes or with the primary phenotype alone, whereas low values in conjunctional FDR are driven by association with both phenotypes.

The application and interpretation of FDR-based methodology are more challenging for post-GWAS specialized SNP panels, such as the Metabochip.[33] The standard FDR is widely applied in GWAS, where any given SNP is assumed to have the same prior probability of association as all other SNPs. The Metabochip (≈200 000 SNPs) is designed to follow up SNPs of interest relating to metabolic and cardiovascular traits, including fine mapping around genome-wide significant SNPs. As such, the true positives (and the false positives) come in large-dependent clumps. Large-scale dependence in the signal can lead to biased FDR.[36] To correct for this bias, we used a linkage disequilibrium-pruned set of SNPs to estimate the conditional FDR distribution, which was then used for estimating the conditional FDR for the full SNP set (Detailed Methods of this estimation procedure are available in the Online Data Supplement). To visualize the conditional and conjunctional FDR, we constructed Manhattan plots. Detailed information on conditional quantile–quantile plots, Manhattan plots, and conditional and conjunctional FDR can be found in earlier reports[6,18] and in the Online Data Supplement.

The conditional FDR assumes independent samples for CAD and each of the secondary traits. However, several of the participants were included in both a secondary trait GWAS and in the CAD study. Partially overlapping subjects between studies leads to dependencies between the test statistics for different traits for a given SNP under the null hypothesis.[37] We estimated the expected correlation of the cross-trait GWAS test statistics under the null hypothesis of no genetic associations using a similar method to the one described for GWAS meta-analysis[37,38] and corrected for the estimated correlation because of shared subjects using the Mahalanobis transformation (LeBlanc et al in preparation). These corrected test statistics were used in all further analysis.

Stratified Replication Rate

As an internal validation of stratified enrichment, we performed a stratified replication rate analysis using methods described previously,[18] where the contributing studies of the CARDIoGRAMplusC4D Consortium were repeatedly divided into independent discovery and validation sets. The purpose of this analysis is to show that an observed pattern of stratified enrichment is not because of spurious effects. In brief, we randomly selected half of the studies (24) for the discovery set and used the remaining studies for replication and repeated this procedure 200 times. For each SNP in the replication set and the discovery set, we computed a meta-analysis test statistic (Liptak method). For the discovery set, we calculated the associated 2-tailed *P* values, whereas for the replication samples they were converted to 1-tailed *P* values to preserve the direction of effect in the discovery sample. We then created a vector of −log10 (*P* value) cutoffs and binned SNPs according to their *P* values in the discovery set SNPs. For each bin, we kept track of their respective *P* values in the replication set. We can then calculate the replication rate for each bin as defined by the proportion of SNPs in that bin, which has a replication *P* value <0.05. We checked for stratified replication rates by plotting the replication rate curves for 4 strata based on significance in each secondary trait, using the same strata definitions as for the conditional quantile–quantile plots.

Independent Validation

For all novel CAD SNPs identified in the conditional FDR analysis, we checked for nominal replication ($P<0.05$) in the WGHS. Because the WGHS data are collected prospectively, we conducted age-adjusted Cox regression over ≈20 years of follow-up, ending in 2013 for both MI and CHD.

Expression Quantitative Trait Loci Annotation

We tested whether the novel CAD SNPs discovered in the current study are associated with genotype-dependent gene expression in various tissue types. Such SNPs are known as expression quantitative trait loci (eQTLs). To this end, we cross-referenced our novel findings from the conditional FDR analysis with 3 *cis*-eQTL databases: in whole blood[39] (the most powerful eQTL database available), adipose tissue[40] (relevant for metabolic disease), and lymphoblastoid cells (LCL).[40] The whole-blood eQTL data have been collected in a large collaborative effort (n=5311 samples), while the adipose and LCL eQTLs are from a sample size of approximately n=850. We considered a SNP to be an eQTL using an FDR *q* value cutoff of 0.05. The FDR *q* values were already available for whole blood, whereas for adipose tissue and LCL we downloaded the publically available eQTL data and calculated *q* values using the qvalue() package available from Bioconductor (version 2.14) in R (version 3.1.1).

Biological Pathway Analysis

To better understand the biological context of our results, we conducted an Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City, www.qiagen.com/ingenuity) including all previously reported CAD genes and the nearest annotated gene for each novel SNP reported in our study. The available molecules and relationships in the IPA Knowledge Base for mammal (humans, mouse or rat) were considered. We set the confidence filter to relationships where the confidence is experimentally observed. We allowed a maximum size of 35 genes for generating networks, and we allowed ≤25 networks in the overall analysis. IPA computes a score for each network according to the fit of that network to a set of focus gene, and *P* values are calculated using the right-tailed Fisher exact test.

Results

We used a 2-step analysis strategy. First, we assessed overlapping polygenic enrichment for CAD and each of the other traits via conditional quantile–quantile plots and applied the Anderson–Darling test to define which of the 8 secondary traits show significant polygenic overlap. This test requires the direction of the association and as this information was unavailable for SBP, we relied on a visual inspection of the conditional quantile–quantile plot for SBP. As illustrated in Online Table II, all testable traits showed significant enrichment after Bonferroni correction for 21 tests, and SBP showed strong visual evidence for enrichment. Therefore, all 8 secondary traits were retained for the second step of the analysis. Second, we applied conditional and conjunctional FDR

methods to identify new CAD risk loci and to identify overlapping loci between CAD and each of the 8 associated traits. Overall, FDR thresholds of 0.01 and 0.05 were chosen for conditional and conjunctional FDR, respectively. Conservatively adjusting for the 8 secondary traits being considered,[21] this translated to thresholds of 0.01/8 and 0.05/8 for conditional and conjunctional FDR.

Conditional quantile–quantile plots for CAD conditioned on nominal *P* values of association with LDL, CRP, T1D, and T2D showed significant enrichment across different levels of significance (Figure 1). Similar significant enrichment patterns were seen for HDL, triglycerides, SBP, and BMI (Online Figure I). The increasing leftward shift with more strictly defined strata based on nominal *P* values of associated phenotypes suggests a greater proportion of true associations for a given nominal CAD *P* value. This is indicative of cross-trait polygenic enrichment. As illustrated in Figure 1A, LDL, the proportion of SNPs in the $-\log_{10}(p_{LDL}) \geq 3$ category reaching a given significance level (eg, $-\log_{10}(p_{CAD}) > 6$) is much greater than that of all SNP category, indicating a high level of enrichment (Figure 1).

Stratified replication rates were observed for all secondary traits with the exception of BMI (Online Figure II), indicating that the observed enrichment in the conditional quantile–quantile plots is also associated with increased replication rates. The observed pattern of stratified enrichment does not result from spurious effects, and replication rate is increased by conditioning on significance in each of the secondary traits, with the possible exception of BMI.

Conditional and conjunctional FDR were calculated for CAD paired with each of the 8 secondary phenotypes showing enrichment. The results of each analysis were filtered as follows. First, we filtered the lists of significant SNPs by their linkage disequilibrium patterns as observed in the 1000 Genomes[41] data set and report only the most significant result per annotated gene. We considered a SNP to be an independent

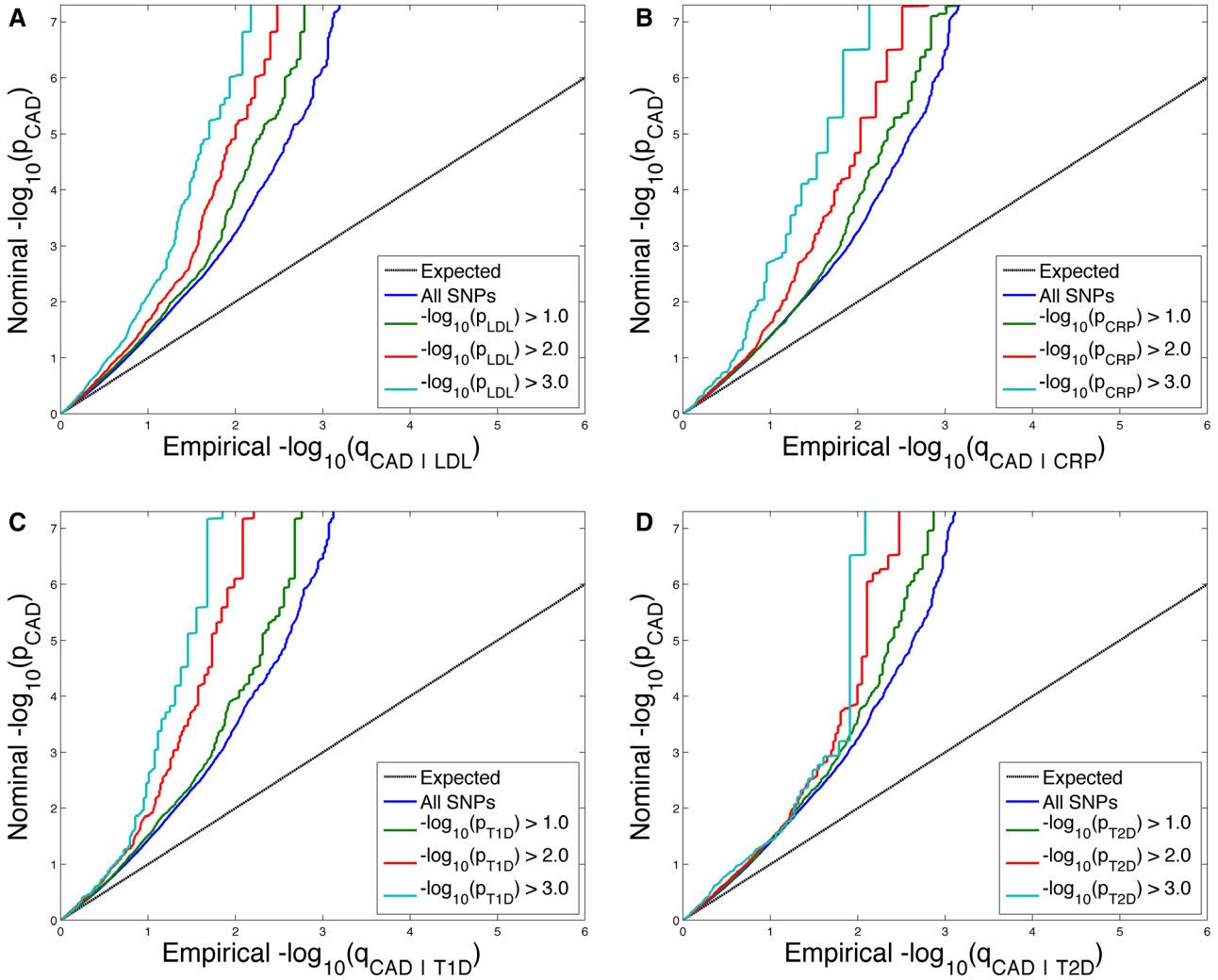

Figure 1. Shared polygenic enrichment. Conditional quantile–quantile plot of nominal vs empirical $-\log_{10}$ *P* values in coronary artery disease (CAD) as a function of significance of association with (**A**) low-density lipoprotein (LDL) cholesterol, (**B**) C-reactive protein (CRP), (**C**) type 1 diabetes mellitus (T1D), and (**D**) type 2 diabetes mellitus (T2D) at the level of $-\log_{10}(P) > 0$, $-\log_{10}(P) > 1$, $-\log_{10}(P) > 2$, and $-\log_{10}(P) > 3$ corresponding to $P<1$, $P<0.1$, $P<0.01$, and $P<0.001$, respectively. Because of the linkage disequilibrium structure on the Metabochip, a linkage disequilibrium–pruned set of single-nucleotide polymorphisms (SNPs) was used for the quantile–quantile plots. Input *P* values were adjusted for shared subjects, if present. Dotted lines indicate the null-hypothesis.

Table 1. Conditional FDR (<0.01), After Controlling for Multiple Testing Across Phenotypes

SNP	Gene	Chr	CAD T2D	CAD T1D	CAD LDL	CAD HDL	CAD TG	CAD BMI	CAD CRP	CAD SBP	Minimum condFDR	CAD *P* Value
rs10747342*	*HS2ST1*	1	2.59E–03	1.16E–03†	3.11E–03	1.67E–03	1.27E–03	2.45E–03	2.94E–03	3.00E–03	T1D	6.57E–06
rs1418458*	*BC067883*	1	6.55E–03	1.21E–03†	4.43E–03	6.45E–03	6.63E–03	5.72E–03	6.97E–03	6.19E–03	T1D	1.81E–05
rs4268379	*SARS*	1	6.76E–05†	3.20E–04†	8.30E–04†	5.69E–04†	7.20E–04†	2.95E–04†	2.63E–04†	5.81E–04†	T2D	7.46E–07
rs11806316	*NGF*	1	9.55E–04†	2.11E–04†	9.30E–04†	9.70E–04†	5.07E–04†	6.88E–04†	9.78E–04†	1.02E–03†	T1D	1.54E–06
rs10788792*	*GOLPH3L*	1	8.12E–03	NA	1.01E–03†	4.44E–03	4.99E–03	8.66E–03	8.30E–03	7.38E–03	LDL	3.35E–05
rs10800418	*NME7*	1	1.11E–03†	1.06E–04†	1.77E–04†	9.12E–04†	5.83E–04†	6.23E–04†	8.58E–04†	5.96E–04†	T1D	1.19E–06
rs6700559	*DDX59*	1	8.19E–04†	5.55E–05†	6.76E–04†	6.78E–04†	7.20E–04†	4.39E–04†	6.60E–04†	6.00E–04†	T1D	7.79E–07
rs2820315	*LMOD1*	1	1.15E–03†	NA	4.83E–04†	1.31E–03	1.28E–03	3.81E–04†	8.68E–04†	9.08E–04†	BMI	1.70E–06
rs6663784*	*CAPN9*	1	4.03E–03	3.50E–03	3.11E–03	1.26E–03	1.20E–03†	2.51E–03	3.62E–03	2.55E–03	TG	7.35E–06
rs16986953	*AK097927*	2	8.33E–04†	NA	3.67E–04†	8.64E–04†	6.80E–04†	5.19E–04†	7.53E–04†	7.44E–04†	LDL	9.12E–07
rs10186133	*IL1F10*	2	7.23E–03	5.93E–03	4.81E–03	6.45E–03	7.16E–03	6.75E–03	7.28E–04†	6.19E–03	CRP	2.21E–05
rs2322864*	*CXCR4*	2	8.60E–03	NA	6.07E–04†	5.84E–03	5.19E–03	6.21E–03	6.41E–03	7.48E–03	LDL	2.26E–05
rs6435757*	*IKZF2*	2	2.25E–03	1.19E–03†	1.67E–03	2.19E–03	2.01E–03	1.50E–03	1.31E–03	1.71E–03	T1D	3.13E–06
rs13423088*	*BC017935*	2	NA	NA	NA	NA	NA	NA	1.25E–03	6.97E–04†	SBP	2.48E–06
rs7419961*	*AX748264*	2	4.15E–03	NA	8.96E–04†	2.24E–03	2.40E–03	4.09E–03	3.61E–03	3.13E–03	LDL	9.36E–06
rs748431*	*FGD5*	3	2.88E–03	2.79E–03	1.66E–03	3.01E–03	4.43E–03	3.13E–03	4.68E–03	4.95E–04†	SBP	1.18E–05
rs11715915*	*AMT*	3	3.76E–03	NA	2.68E–03	3.35E–03	1.83E–03	2.41E–03	1.12E–03†	2.79E–04†	SBP	6.29E–06
rs1512301*	*GNPDA2*	4	5.05E–03	3.79E–03	1.15E–03†	4.24E–03	4.44E–03	3.55E–03	4.21E–03	4.04E–03	LDL	1.09E–05
rs4690974*	*MAP9*	4	8.50E–03	NA	6.62E–03	8.56E–03	8.54E–03	7.94E–03	7.46E–03	8.62E–04†	SBP	2.52E–05
rs2736100*	*TERT*	5	2.47E–03	5.57E–04†	1.17E–03†	7.17E–04†	9.36E–04†	2.11E–03	1.00E–03†	1.86E–03	T1D	4.33E–06
rs12916*	*HMGCR*	5	3.54E–03	NA	1.40E–02	3.01E–03	5.18E–03	1.13E–03†	4.21E–03	4.04E–03	BMI	1.10E–05
rs246600	*ARHGAP26*	5	9.47E–05†	1.07E–04†	8.11E–05†	1.06E–04†	1.36E–04†	4.80E–05†	5.72E–05†	8.63E–05†	BMI	7.84E–08
rs2814982*	*C6orf106*	6	3.47E–03	3.35E–03	5.20E–04†	3.03E–03	4.21E–03	3.35E–03	2.54E–03	3.46E–03	LDL	7.83E–06
rs1321309*	*CDKN1A*	6	2.25E–03	NA	1.63E–03	2.46E–03	1.86E–03	2.09E–03	1.08E–03†	2.07E–03	CRP	4.18E–06
rs6905288*	*VEGFA*	6	1.85E–03	2.95E–03	1.46E–03	2.56E–03	3.46E–03	1.66E–03	2.94E–03	2.79E–04†	SBP	6.21E–06
rs9367716*	*PRIM2*	6	1.98E–03	2.74E–04†	2.44E–03	2.06E–03	2.91E–03	2.04E–03	1.93E–03	3.42E–03	T1D	7.48E–06
rs4613862	*BC038576*	6	1.49E–03	8.76E–04†	1.30E–03	4.99E–04†	6.22E–04†	5.35E–04†	1.04E–03†	1.09E–03†	HDL	1.73E–06
rs12663498*	*PLEKHG1*	6	5.67E–03	4.25E–03	4.44E–03	2.63E–03	1.86E–03	3.03E–03	1.90E–03	6.73E–04†	SBP	1.29E–05
rs1247351	*MAP3K4*	6	9.58E–04†	7.75E–04†	2.16E–04†	8.64E–04†	7.20E–04†	2.96E–04†	8.00E–04†	5.92E–04†	LDL	9.01E–07
rs10278591*	*MAD1*	7	2.25E–03	1.67E–03	1.82E–03	1.35E–03	1.21E–03†	1.65E–03	2.00E–03	1.49E–03	TG	3.78E–06
rs11204085	*SLC18A1*	8	2.32E–04†	NA	1.79E–04†	9.24E–04†	1.50E–03	1.58E–04†	1.38E–04†	1.63E–04†	CRP	1.69E–07
rs6984210	*BMP1*	8	NA	NA	NA	NA	NA	NA	9.67E–04†	8.66E–04†	SBP	1.22E–06
rs12343412*	*SLC44A1*	9	2.55E–03	3.33E–04†	1.34E–03	3.02E–03	2.63E–03	1.47E–03	2.65E–03	2.37E–03	T1D	4.78E–06
rs867764*	*CAMK1D*	10	2.32E–03	1.67E–03	1.97E–03	1.41E–03	1.61E–03	9.86E–04†	1.79E–03	1.44E–03	BMI	3.22E–06
rs3748242	*ANXA11*	10	5.54E–04†	4.40E–04†	4.98E–04†	4.65E–04†	4.88E–04†	4.32E–04†	2.83E–04†	3.69E–04†	CRP	5.29E–07
rs1980653	*OBFC1*	10	1.43E–03	1.08E–03†	4.54E–04†	9.12E–04†	8.15E–04†	8.21E–04†	1.10E–03†	1.06E–03†	LDL	1.59E–06
rs425325*	*PLEKHA7*	11	5.77E–03	NA	3.86E–03	3.64E–03	4.69E–03	4.53E–03	4.46E–03	6.73E–04†	SBP	1.29E–05
rs12801636	*PCNXL3*	11	5.49E–04†	9.15E–04†	2.63E–04†	6.50E–04†	9.87E–04†	6.02E–04†	9.33E–05†	1.77E–04†	CRP	1.15E–06
rs590121	*SERPINH1*	11	6.67E–04†	NA	5.97E–04†	7.20E–04†	6.80E–04†	5.66E–04†	5.52E–04†	6.42E–04†	CRP	7.76E–07
rs7306455*	*NDUFA12*	12	2.64E–03	9.15E–04†	1.95E–03	2.44E–03	2.49E–03	1.23E–03†	2.00E–03	2.07E–03	T1D	3.70E–06
rs10774613	*CUX2*	12	3.63E–04†	1.97E–05†	5.87E–05†	3.96E–04†	5.12E–04†	1.91E–04†	2.93E–04†	2.10E–04†	T1D	3.96E–07
rs7296651*	*ALDH2*	12	NA	1.25E–04†	NA	NA	NA	NA	1.83E–04†	1.54E–04†	T1D	3.15E–06
rs11066320	*PTPN11*	12	1.23E–04†	4.05E–06†	1.28E–05†	8.40E–05†	1.10E–04†	1.14E–05†	6.92E–05†	5.55E–06†	T1D	6.70E–08
rs1015249*	*RPH3A*	12	5.01E–03	3.32E–04†	4.25E–04†	4.09E–03	2.15E–03	2.29E–03	4.41E–03	1.58E–03	T1D	9.64E–06
rs2708081	*OASL*	12	6.75E–05†	6.86E–05†	1.22E–04†	1.14E–04†	3.56E–04†	2.34E–04†	3.41E–05†	2.70E–04†	CRP	3.08E–07
rs825483*	*ZNF664*	12	8.27E–04†	3.08E–03	3.11E–03	2.56E–03	2.48E–03	1.49E–03	2.94E–03	2.15E–03	T2D	5.81E–06
rs11057830	*SCARB1*	12	1.73E–04†	NA	7.08E–05†	9.07E–05†	3.17E–04†	8.59E–05†	1.51E–04†	1.04E–04†	LDL	1.48E–07
rs9603710*	*TTL/TEL*	13	1.98E–03	NA	1.22E–03†	2.01E–03	2.01E–03	1.62E–03	1.90E–03	1.80E–03	LDL	3.21E–06
rs9316753	*BC044614*	13	1.01E–03†	7.33E–04†	3.92E–04†	4.08E–04†	7.33E–04†	5.35E–04†	7.53E–04†	6.80E–04†	LDL	9.12E–07
rs2273996*	*LMO7*	13	1.40E–02	1.22E–03†	1.20E–02	9.47E–03	1.36E–02	1.16E–02	1.26E–02	1.13E–02	T1D	5.13E–05

(*Continued*)

Table 1. Continued

SNP	Gene	Chr	CAD T2D	CAD T1D	CAD LDL	CAD HDL	CAD TG	CAD BMI	CAD CRP	CAD SBP	Minimum condFDR	CAD *P* Value
rs2146238	*CYP46A1*	14	8.55E–04†	NA	1.00E–04†	8.60E–04†	7.20E–04†	5.19E–04†	8.00E–04†	7.22E–04†	LDL	9.54E–07
rs6494488*	*RBPMS2*	15	1.58E–03	1.17E–03†	1.42E–03	1.56E–03	1.30E–03	6.60E–04†	1.25E–03†	1.22E–03†	BMI	1.99E–06
rs7202877*	*CTRB1*	16	5.30E–03	7.07E–04†	6.99E–03	7.59E–03	8.89E–03	5.07E–03	7.12E–03	6.82E–03	T1D	2.65E–05
rs4888378*	*CFDP1*	16	7.52E–03	1.99E–03	2.72E–03	3.62E–03	3.65E–03	4.41E–03	6.09E–03	7.17E–04†	SBP	1.80E–05
rs4299203	*LRRC48*	17	2.14E–04†	NA	1.90E–04†	1.94E–04†	1.67E–04†	7.01E–05†	1.67E–04†	1.51E–04†	BMI	1.59E–07
rs17608766*	*GOSR2*	17	1.74E–03	9.00E–04†	1.30E–03	2.72E–04†	1.50E–03	6.52E–04†	1.50E–03	1.26E–04†	SBP	2.37E–06
rs3179840*	*ZNF652*	17	8.77E–03	7.73E–03	5.47E–03	4.25E–03	4.61E–03	7.15E–03	7.46E–03	9.99E–04†	SBP	2.43E–05
rs1867624*	*AX746971*	17	1.66E–03	1.40E–03	4.46E–04†	8.74E–04†	1.58E–03	6.70E–04†	1.68E–03	1.26E–04†	SBP	2.25E–06
rs13465*	*ILF3*	19	1.56E–03	4.71E–04†	1.62E–03	1.55E–03	1.61E–03	1.06E–03†	1.33E–03	1.13E–03†	T1D	2.12E–06
rs17616661*	*KANK2*	19	3.08E–03	1.38E–03	7.09E–04†	1.55E–03	2.35E–03	1.97E–03	1.19E–03†	2.48E–03	LDL	5.03E–06
rs12459996*	*CYP2F1*	19	1.67E–03	1.82E–03	5.87E–04†	6.31E–04†	2.23E–03	9.48E–04†	1.69E–03	7.70E–04†	LDL	3.35E–06
rs12460848	*MARK4*	19	1.61E–03	NA	1.04E–03†	1.13E–03†	1.42E–03	8.05E–04†	5.76E–04†	1.02E–03†	CRP	1.68E–06
rs4802322*	*STRN4*	19	1.01E–02	8.50E–04†	5.33E–03	8.59E–03	9.51E–03	9.74E–03	7.49E–03	8.05E–03	T1D	3.00E–05
rs867186	*EDEM2*	20	4.54E–05†	1.46E–04†	1.56E–04†	1.15E–04†	1.05E–04†	1.21E–04†	1.21E–04†	1.21E–04†	T2D	1.36E–07
rs3827066*	*ZNF335*	20	5.77E–03	NA	1.46E–03	1.55E–03	1.22E–03†	5.07E–03	2.52E–03	4.72E–03	TG	1.35E–05
rs1882961*	*NRIP1*	21	7.63E–03	9.09E–03	4.64E–03	9.43E–03	1.10E–02	1.04E–02	3.48E–03	1.04E–03†	SBP	3.39E–05
rs9608859*	*OSM*	22	1.76E–03	NA	1.81E–03	8.06E–04†	1.79E–03	1.48E–03	1.41E–03	1.43E–03	HDL	2.68E–06

Independent (r^2<0.2) SNP(s) with a condFDR <0.01 (after Bonferroni correction for 8 traits) in CAD given the significance level in the associated phenotype. We defined the most significant CAD SNP in each linkage disequilibrium (LD) block based on the minimum condFDR for each associated phenotype. The most significant SNPs in each gene of the LD block are listed along with the associated phenotype that provided the signal. BMI indicates body mass index; CAD, coronary artery disease; Chr, chromosome number; condFDR, conditional FDR; CRP, C-reactive protein; FDR, false discovery rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; SNPs, single-nucleotide polymorphisms; T1D, type 1 diabetes; T2D, type 2 diabetes; and TG, triglycerides. The most significant phenotype association per gene is shown (min condFDR). NA indicates that a given SNP was not available for a given trait.

*SNP would not have been detected using standard (unconditioned) FDR methodology at the same threshold.

†CondFDR values <0.01, after adjusting for multiple testing across phenotypes.

finding if the linkage disequilibrium, defined using r^2, was <0.2 with all other SNPs in the filtered list. Second, we further filtered the list of significant SNPs for novelty with respect to previously published CAD SNPs. We filtered out any previously reported genes and SNPs, including SNPs in linkage disequilibrium (r^2>0.2) with those previously reported SNPs. Thus, the list of significant SNPs presented in Table 1 represent, to the best of our knowledge, independent novel SNPs for CAD. The corresponding conditional Manhattan plot is given in Figure 2.

Over all 8 secondary traits, we identify 101 SNPs associated with CAD, 67 of which have not previously associated with CAD (previously reported SNPs not shown). Many of these new loci are located in regions with borderline significant association with CAD in previous studies,[42] as is evident by the CAD association *P* value column given in Table 1. Of interest, several of the identified loci are found across the conditional analysis from several risk factors. These loci are not found using standard methods applying a genome-wide Bonferroni correction.

We looked to the WGHS for independent validation of these 67 new CAD SNPs and 12 of these show nominal replication for at least 1 end point (CHD or MI); Online Table III.

Of the 67 novel CAD loci, 32 show genotype-dependent gene expression in whole blood regulating the expression of 57 unique genes, and 42 of these 67 SNPs would not have been detected using the standard (unconditioned) FDR. We found evidence for 16 and 18 loci having an eQTL effect in adipose tissue and LCL, respectively (Table 2). For 6 of these loci, we observed an eQTL effect on the same gene in both whole blood and adipose tissue. Interestingly, 18 loci show an effect on the gene expression of >1 gene.

To further evaluate genetic overlap, we used the conjunctional FDR to identify SNPs with significant effects in both CAD and its associated risk factors. The conjunctional Manhattan plot for CAD is shown in Online Figure II. We identified 53 loci achieving conjunctional FDR<0.05, after adjustment for using multiple risk factors and pruning the results in the same manner as for the conditional FDR (Online Table IV; corresponding *z* scores in Online Table V).

Follow-up IPA identified highly significantly associated top canonical pathways relevant to CAD (eg, liver X receptor [LXR]/retinoid X receptor [RXR]), as well as farnesoid X receptor/RXR Activation and Atherosclerosis Signaling; Online Table V). Additionally, in "Top Diseases and Bio Function," CAD relevant diseases and functions are on top (Cardiovascular Disease and Lipid Metabolism) in the subgroups "Diseases and Disorders" and "Molecular and Cellular Functions."

Discussion

Combining data from large-scale genomic studies from different phenotypes in a conditional FDR framework, we show polygenic overlap between CAD and several CVD risk factor phenotypes and identify 67 novel CAD susceptibility loci. Furthermore, conjunctional FDR analysis identified 53 novel loci associated with both CAD and the CVD risk

Table 2. Novel Coronary Artery Disease SNPs That Are Also eQTLs in Blood and Adipose Tissue and LCL

SNP	Chr	Nearest Gene	Blood eQTL for	Blood eQTL *P* Value	Adipose eQTL for	Adipose eQTL *P* Value	LCL eQTL for	LCL eQTL *P* Value
rs10747342	1	*HS2ST1*	*LMO4*	2.63E–05	*LMO4*	6.08E–05		
rs10788792	1	*GOLPPH3L*	*ARNT*	2.38E–05	*CTSS*	3.01E–04	*CTSS*	6.35E–36
			CTSK	4.98E–32			*LASS2*	4.72E–06
			CTSS	3.86E–150			*CTSK*	2.19E–16
rs2820315	1	*LMOD1*	*IPO9*	4.10E–06	*LMOD1* *RNPEP*	1.43E–11 7.19E–05		
rs6700559	1	*DDX59*	*DDX59*	4.10E–106			*DDX59*	6.27E–08
rs10800418	1	*NME7*			*NME7*	2.58E–12	*NME7*	5.12E–18
rs6663784	1	*CAPN9*			*AGT*	6.65E–06		
rs10186133	2	*IL1F10*	*PSD4*	9.43E–07				
rs6435757	*2*	*IKZF2*					*IKZF2*	4.96E–06
rs748431	3	*FGD5*			*FGD5*	2.31E–04		
rs11715915	3	*AMT*	*USP4*	1.86E–24	*RBM6*	1.24E–04	*KLHDC8B*	1.08E–04
			NICN1	9.50E–29			*APEH*	2.51E–05
rs1321309	6	*CDKN1A*	*CDKN1A*	4.12E–23			*CDKN1A*	6.74E–19
			Z85996.1-1	1.04E–42				
rs2814982	6	*C6orf106*	*c6orf106*	1.56E–11				
rs9367716	6	*PRIM2*	*RAB23*	6.47E–15				
rs10278591	7	*MAD1*	*MAD1L1*	1.05E–06			*MAD1L1*	2.46E–04
rs11204085	8	*SLC18A1*	*LPL*	1.00E–04				
rs3748242	10	*ANXA11*	*AL512662.8-2,SFTPD*	7.62E–05			*c10orf58*	3.45E–05
			ANXA11	2.24E–19			*ANXA11*	1.93E–05
rs12801636	11	*PCNXL3*	*SIPA1*	1.19E–06				
rs590121	11	*SERPINH1*	*GDPD5*	8.69E–10				
rs1015249	12	*RPH3A*	*OAS1*	2.77E–13				
rs2708081	12	*OASL*	*CAMKK2*	1.83E–10	*c12orf43*	1.15E–05	*CAMKK2*	2.86E–04
			c12orf43	5.55E–20				
			P2RX4	1.95E–10				
			OASL	7.32E–10				
rs10774613	12	*CUX2*					*IFT81*	2.92E–04
rs7296651	12	*ALDH2*	*ERP29*	1.96E–21	*c12orf30*	3.97E–05	*c12orf30*	3.84E–04
			TMEM116	1.49E–67	*TMEM116*	1.12E–08	*TMEM116*	7.17E–12
							ACAD10	4.64E–04
							FLJ30092	4.62E–08
rs7306455	12	*NDUFA12*	*NDUFA12*	8.57E–06				
rs825483	12	*ZNF664*	*CCDC92*	9.29E–06	*CCDC92*	2.48E–16		
rs6494488	15	*RBPMS2*	*ANKDD1A*	2.21E–18	*TRIP4*	1.55E–04	*TRIP4*	1.53E–09
			RBPMS2	1.29E–72				
rs4888378	16	*CFDP1*	*CFDP1*	1.63E–10			*CHST6*	1.50E–04
rs7202877	16	*CTRB1*	*CFDP1*	6.33E–12				
rs17608766	17	*GOSR2*	*GOSR2*	6.42E–06				
rs3179840	17	*ZNF652*	*GNGT2*	1.23E–36			*GNGT2*	8.18E–10
			PHOSPHO1	2.62E–08				
rs4299203	17	*LRRC48*	*ATPAF2*	3.97E–09			*DRG2*	3.11E–07
			c17orf39	1.73E–19				
			DRG2	1.73E–09				
			TOM1L2	7.10E–08				
			SREBF1	6.45E–51				

(*Continued*)

Table 2. Continued

SNP	Chr	Nearest Gene	Blood eQTL for	Blood eQTL *P* Value	Adipose eQTL for	Adipose eQTL *P* Value	LCL eQTL for	LCL eQTL *P* Value
rs1867624	17	*AX746971*			*PECAM1*	5.97E–10		
rs12459996	19	*CYP2F1*	*HNRNPUL1*	3.86E–16			*BLVRB*	5.69E–04
							SNRPA	4.01E–04
							B9D2	2.97E–04
rs12460848	19	*MARK4*	*CKM*	7.91E–08				
			KLC3	4.92E–12				
			VASP	5.67E–14				
rs17616661	19	*KANK2*	*KANK2*	5.60E–17			*KANK2*	5.26E–06
rs4802322	19	*STRN4*	*CALM3*	7.14E–06	*FKRP*	2.53E–07		
			PRKD2	1.10E–23				
			FKRP	3.11E–31				
			SLC1A5	6.12E–10				
rs3827066	20	*ZNF335*	*AL162458.10–3,MMP9*	1.13E–04	*WFDC3*	1.78E–04	*PLTP*	1.20E–06
			CD40	1.27E–05	*NEURL2*	6.50E–09		
			DNTTIP1	2.52E–14	*PLTP*	1.60E–06		
			TNNC2	3.98E–19				
rs867186	20	*EDEM2*	*ACSS2*	2.99E–07	*PROCR*	3.24E–15		
			EIF6	2.25E–101				
			TRPC4AP	8.04E–108				
rs9608859	22	*OSM*	*SF3A1*	4.29E–94	*THOC5*	6.97E–05		
			MTP18	1.63E–08				

A SNP was considered to be an eQTL using an false discovery rate threshold of 0.05. Chr indicates chromosome number; eQTL, expression quantitative trait locus; LCL, lymphoblastoid cell; and SNPs, single-nucleotide polymorphisms.

factors, LDL, HDL, triglycerides, T1D, T2D, CRP, and SBP. Importantly, we validated the conditional FDR approach by showing that replication rates in independent CAD substudies increase as a function of *P* value in each secondary trait, with the possible exception of BMI. Furthermore, we see nominal replication for 12/67 SNPs in the WGHS. Overall, these results suggest that a proportion of the clinically and epidemiologically observed association between these phenotypes can be explained by overlapping genetic loci (pleiotropy) and not simply shared environmental risk factors. Furthermore, the findings provide further evidence that CAD is a highly polygenic disease.

Our findings of polygenic overlap provide novel insights into the relationship between CAD and major CVD risk factors. We demonstrate an interesting genetic dissociation among these risk factors and CAD, with strong enrichment for lipids, inflammation, and metabolic disorders. The combination of dyslipidemia (ie, high triglycerides and LDL cholesterol and low HDL cholesterol), T2D, and high blood pressure forms the metabolic syndrome,[12–14,43,44] and all of these factors (particularly LDL) showed strong genetic overlap with CAD. This is in agreement with a recent reports suggesting a common genetic basis for regulation of lipid and glucose homeostasis,[45] whereas previous studies did not show common genes for the different components of the metabolic syndrome,[46] but revealed strong lipid gene contribution. It is further supported by the pathway analysis that identified atherosclerosis signaling and farnesoid X receptor /RXR activation among the 3 most relevant pathways. Genes activated by the farnesoid X receptor has been shown to influence vascular tension and regulate the unloading of cholesterol from foam cells.[47] Another important finding is the overlap between CAD and T2D. Based on conditional analysis of these 2 phenotypes, 21 novel loci were identified. This is in line with previous single gene studies suggesting a genetic link between T2D and CAD.[48]

The strong shared polygenic signal between LDL and CAD emphasizes the important role of LDL in CAD development and supports the notion that risk genes for atherosclerosis, such as LDL genes, are causal for CAD as recently suggested.[49] Finally, 2 of the phenotypes most strongly overlapping with CAD were CRP and T1D, 2 immune-related phenotypes. CRP is regarded as a reliable marker of systemic inflammation, and its role as a biomarker in CAD has been attributed to its ability to reflect upstream inflammatory pathways. However, the finding in this study suggests that the link between CRP and CAD may also reflect overlapping genetic loci. T1D is related to autoimmune mechanisms, and its genetic overlap with CAD underlines the important role of the immune system in CAD and could be because of a large number of overlapping genes between immune and lipid phenotypes.[50] In fact, the bidirectional interaction between inflammation and lipids is regarded as a phenotypic hallmark of atherosclerosis, and our findings suggest that this

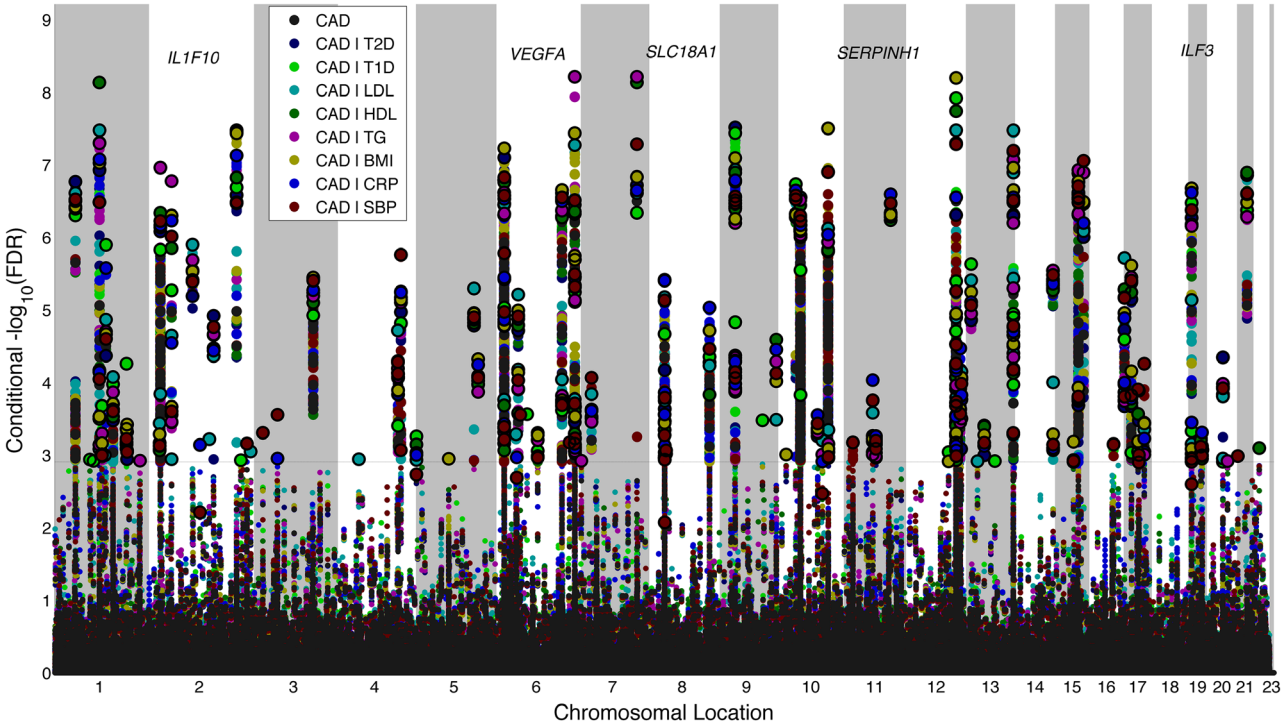

Figure 2. Conditional false discovery rate (FDR) Manhattan plot of –log$_{10}$ (FDR)* values for coronary artery disease (CAD) alone (black), and –log$_{10}$ (conditional FDR) for CAD given type 2 diabetes mellitus (T2D; CAD T2D; navy blue), CAD given type 1 diabetes mellitus (T1D; CAD T1D; light green), and CAD given low-density lipoprotein (LDL; CAD LDL; aqua), CAD given high-density lipoprotein (HDL; CAD HDL; dark green), triglycerides (TG; CAD TG; fuchsia), and body mass index (BMI; CAD BMI; mustard yellow), CAD given C-reactive protein (CRP; CAD CRP; royal blue) and CAD given systolic blood pressure (SBP; CAD SBP; maroon). Single-nucleotide polymorphisms (SNPs) with –log$_{10}$ (conditional FDR) >2.9 (ie, overall FDR<0.01 after Bonferroni correction for 8 traits) are shown with large points. A black circle around the large points indicates the most significant SNP in each linkage disequilibrium block, and this SNP was annotated with the closest gene, which is listed above the symbols in each locus, except for the HLA (human leukocyte antigen) region on chromosome 6, which was excluded from the analysis. Details for the novel loci with –log$_{10}$ (conditional FDR) >2.9 are given in Table 1. *For the –log10 (FDR) for CAD alone, the maximum value displayed in this figure is 6.5. This is done purely for display purposes and as such should be interpreted as >6.5.

phenotype could reflect overlapping genes between these 2 interacting pathophysiological arms of atherogenesis. The pathway analysis revealed LXR/RXR activation as the top-ranked canonical pathway. LXR/RXR is heterodimer nuclear receptors/transcription factors. LXR acts as a cholesterol sensor, and LXR pathway activation has been shown to stimulate lipogenesis and hypertriglyceridemia.[51] LXR/RXR can also modulate inflammatory responses to cholesterol exposure and could represent a regulator of the interaction between lipids and inflammation, being the most important pathway in the pathogenesis of CAD.

In the original CAD GWAS and follow-up Metabochip study, 46 loci were identified.[2] By combining the original CAD results with the CVD risk factor phenotypes GWAS, we identified 101 significant loci associated with CAD, of which 67 are novel, using the conditional FDR approach. Even though the original CAD study was large,[2] the increased power provided by additional GWAS of associated phenotypes together with the conditional FDR method more than doubled gene discovery. The novel SNPs discovered here contribute to explaining more of the missing heritability for CAD, but we cannot quantify how much more is explained because we are working at the summary statistic level. These findings underline the cost-effectiveness of the current statistical methods and highlight several interesting genes in CAD pathology. *IL1F10* (interleukin 1 family, member 10 [theta]) was identified in the pathway analysis of the CAD GWAS, it is known to bind *IL1R* and stimulate nuclear factor-κB pathway. *VEGFA* is well known in the CVD field, but to the best of our knowledge, this has never been shown in genetic studies. *SLC18A1* (solute carrier family 18 [vesicular monoamine transporter], member 1) has been implicated in neuropsychiatric disorders, but not previously in CVD. *SERPINH1* (serpin peptidase inhibitor, clade H [heat shock protein 47], member 1 [collagen-binding protein 1]) is a heat shock protein, known to be involved in atherosclerosis. *ILF3* (interleukin enhancer–binding factor 3, 90 kDa) is a matrix metalloproteinase, well studied in the CVD research field, and the findings of *ILF10* and *ILF3* underscore the role of the *IL-1* cytokine family in CAD.

Although nearest gene annotation can be informative, the vast majority of discovered SNPs are located outside coded DNA regions.[52] Therefore, annotating the identified genetic variants to the correct causal genes for the phenotype of interest often remains challenging.[52] One of the potential mechanisms whereby SNPs may affect phenotype variations is through altered gene expression. We successfully identified eQTL effects in whole blood, LCL, and adipose tissue, suggesting these genes as potential causal

candidates. Of interest, some of the genetic variants showed an effect on the gene expression of >1 gene. We speculate that the shared effect of the genetic variants on the phenotypes under study might be explained by the regulation of several different genes, but further studies would be necessary to connect the genes with altered gene expression seen in Table 2 to the clinical phenotypes. Moreover, the majority of the genes regulated by the genetic variants were different from the nearest annotated gene. Given that the original whole blood has markedly different power and used different statistical eQTL definitions than the LCL and adipose tissue eQTL studies, a detailed cross-tissue comparison is not possible. Further studies are needed to determine the functional mechanisms involved in the novel CAD loci identified here.

There are certain limitations associated with the present results. Because of the overlap in some of the GWAS samples examined, we cannot completely exclude the contribution from environmental or behavioral factors. The shared participants between genomic studies could also affect the findings. However, we did adjust for overlapping subjects and used strict FDR thresholds to account for the 8 secondary traits. Although clinical comorbidity and shared pathophysiology between these phenotypes pose a challenge for the interpretation of the basis of the shared polygenetic signals, their utility for increasing the power to detect new loci for CAD is not affected. The question remains whether the identified shared genes are independent of other phenotypes (biological pleiotropy) or the current findings are results of overlapping phenotypes (mediated by other phenotypes), as several of these risk factors can be co-occurring (mediated pleiotropy).[53] However, it seems reasonable to interpret our findings as reflecting the existence of shared genetically determined pathophysiological processes across CAD and the associated phenotypes. In general, FDR methodology is a less conservative approach to multiple testing than Bonferroni correction. However, using the conditional FDR, we are not simply relaxing the significance threshold, but are increasing power and incorporating useful information from a second trait into the analysis, allowing us to identify the SNPs more likely to replicate. We have not strictly replicated all of these findings in independent samples, but we have shown that replication rates increase by conditioning on significance in the secondary traits and have shown that 12 SNPs nominally replicate in the WGS. Although the prospective design of the WGHS makes it suitable for validation of the candidate CAD associations, the numbers of incident events of MI and CAD were much smaller than in the discovery sample, which was composed of a preponderance of men compared with the all-female composition of the WGHS. However, in spite of much lower power and possibility of differences according to sex, the WGHS is the largest and most relevant independent data set we were able to access, and we found nominal association for novel CAD 12 loci.

In conclusion, we found substantial polygenic overlap between CAD and several-related conditions, importantly LDL, T2D, and CRP, providing more evidence for fundamental pathogenic relationship between these phenotypes that cannot be explained by lifestyle factors. The 67 novel CAD loci identified here provide new insight into genetic mechanisms of CAD and may form the basis for earlier diagnosis and new prevention and treatment strategies.

Acknowledgments

We thank the DIAGRAM Consortium, the CARDIoGRAMplusC4D Consortium, the Global Lipids Genetics Consortium, The International Consortium for Blood Pressure GWAS, the GEFOS Consortium, the Giant Consortium, the Type 1 Diabetes Consortium, and the CHARGE Consortium Inflammation Working Group for the summary statistics GWAS data.

Sources of Funding

The present project was supported by the Research Council of Norway (213837, 223273); the South East Norway Health Authority (2013–123); the Kristian Gerhard Jebsen Foundation; in addition to the National Institutes of Health grants R01AG031224, R01EB000790, and RC2DA29475, and the European Union project CVgenes@target; a grant from the Leducq Foundation, CADgenomics; and BMBF, Forschungskonsortien zur Systemmedizin, e:AtheroSysMED; DFG, SFB 1123. The Women's Genome Health Study is supported by HL043851, HL080467, and HL099355 from the National Heart, Lung, and Blood Institute and CA047988 from the National Cancer Institute with collaborative scientific support and funding for genotyping provided by Amgen. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article.

Disclosures

None.

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Novelty and Significance

What Is Known?

- Previous work has identified 46 genetic risk variants associated with coronary artery disease (CAD).
- Genetic data for traits with overlapping pathophysiology can be combined to improve power for identifying novel genetic risk variants.

What New Information Does This Article Contribute?

- We identified 67 new genetic risk variants for CAD.
- CAD and several cardiometabolic traits share a large number of genetic risk factors.

Clinical and epidemiological evidence suggests a relationship between CAD and cardiometabolic traits. In the presence of a shared polygenic signal (ie, a large number of shared risk variants each with a small effect), traits with overlapping pathophysiology with CAD can be used in combination with novel statistical methodology to improve discovery of variants associated with CAD. Using large-scale genetic data from CAD and genetic data from hypertension, obesity, abdominal fat, diabetes mellitus, dyslipidemia, and inflammation (C-reactive protein), we found a polygenic overlap between CAD and each of these related traits. We identified 67 novel CAD risk variants and 53 risk variants jointly associated with CAD and at least 1 other related trait. These results highlight the importance of shared polygenic risk factors between coronary artery disease and cardiovascular risk factors. Our findings provide important insights into molecular mechanisms underlying coronary artery disease and have potential implications for prevention and treatment strategies.

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Identifying Novel Gene Variants in Coronary Artery Disease and Shared Genes With Several Cardiovascular Risk Factors

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Circ Res. 2016;118:83-94; originally published online October 20, 2015;
doi: 10.1161/CIRCRESAHA.115.306629
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571

LeBlanc et al. Supplemental Material

Supplemental Methods

False Discovery Rate (FDR)

The tail-area based FDR for a given p-value, p, is defined as

$$\text{(standard unconditioned) } FDR(p) = \pi_0 F_0(p) / F(p). \qquad [1]$$

where $F_0(p)$ is the cumulative distribution function (cdf) for null SNPs, $F(p)$ is the cdf for all SNPs and π_0 is the *a priori* proportion of null SNPs. In practice the FDR needs to be estimated from the data and a conservative estimate can be obtained by setting π_0 to 1. For p-values, $F_0(p)$ is the cdf for the uniform distribution and $F(p)$ is estimated by the empirical cdf, $q = N_p / N$, where N_p is the number of SNPs with p-values less than or equal to p, and N is the total number of SNPs.

Leveraging pleiotropy by conditional FDR

Conditional FDR exploits the shared polygenic signal between coronary artery disease (CAD) and each secondary trait to increase power for detection of CAD SNPs. Conditional FDR is defined as the posterior probability that a given SNP is null for the first phenotype given that the p-values for both phenotypes are as small or smaller as their observed p-values.

$$\text{Conditional FDR: } FDR(p_1 \mid p_2) = \pi_0(p_2)\, p_1 / F(p_1 \mid p_2), \qquad [2]$$

where p_1 is the p-value for the first phenotype, p_2 is the p-value for the second, $F(p_1 \mid p_2)$ is the conditional cdf and $\pi_0(p_2)$ the conditional proportion of null SNPs for the first phenotype given that p-value for the second phenotype is p_2 or smaller. As with FDR, we obtain a conservative estimate of the conditional FDR by setting π_0 to 1. $F(p_1 \mid p_2)$ is estimated from the data by constructing a two-dimensional grid, with CAD p-value category for columns, and the secondary trait p-value category for rows, and then counting how many SNPs fall into each bin on the grid. The empirical conditional cdf was estimated using the binomial regression model that estimates the probability for falling into a bin conditional on the sum over all bins in one row.

Conjunctional FDR

The conjuctional FDR is defined as follows:

$$FDR_{CAD\ \&\ trait2} = max(FDR_{CAD\ |trait2}, FDR_{trait2\ |\ CAD}).\ [3]$$

FDR calculations for the Metabochip

To estimate the conditional and conjunctional FDR, the joint distribution for p-values for the primary trait (CAD) and the secondary trait (e.g. T2D), *F(p1,p2),* needs to be estimated from the observed data. For GWAS data, this is a straightforward process since SNPs on standard GWAS chips can be treated as a random sample of common variants from the human genome and the size of the LD blocks will be unrelated to the effect of the SNPs on the traits of interest. Therefore there is no a priori reason to believe that this estimate of *F(p1,p2)* is biased. Here we use the CAD metabochip data rather than standard GWAS chip data. Here the estimation of *F(p1,p2)* will be incorrect if we use the whole dataset. The reason for this is that by design since the metabochip follows up previously described cardiometabolic SNPs with fine mapping. This means that there is non-independence between the size of the LD block and statistical significance. Larger blocks of SNPs will be found for non-null SNPs. As such, an unbiased estimate of *F(p1,p2)* was obtained from an LD-pruned set of SNPs.

Online Figure Legends

Online Figure I. Pleiotropic Enrichment. Conditional quantile-quantile plot of nominal versus empirical -$\log_{10}$ p-values in Coronary Artery Disease (CAD) as a function of significance of association with A) body mass index (BMI) B) high density lipoprotein (HDL) C) systolic blood pressure (SBP) and D) triglycerides (TG), at the level of $-\log_{10}(p) > 0$, $-\log_{10}(p) > 1$, $-\log_{10}(p) > 2$, $-\log_{10}(p) > 3$ corresponding to $p < 1$, $p < 0.1$, $p < 0.01$, $p < 0.001$, respectively. Due to the linkage disequilibrium structure on the metabochip, a linkage disequilibrium-pruned set of SNPs was used for the quantile-quantile plots. Input p-values were adjusted for shared subjects, if present. Dotted lines indicate the null-hypothesis.

Online Figure II. Stratified replication rates plots showing the average rate of replication ($p < 0.05$) within the CARDIoGRAMplusC4D contributing studies as a function of significance in a secondary trait: (A) C-reactive protein (CRP), (B) type 1 diabetes (T1D), (C) type 2 diabetes (T2D), (D) low density lipoprotein (LDL), (E) high density lipoprotein (HDL), (F) systolic blood pressure (SBP), (G) body mass index (BMI) and (H) triglycerides (TG), at the level of $-\log_{10}(p) > 0$, $-\log_{10}(p) > 1$, $-\log_{10}(p) > 2$, $-\log_{10}(p) > 3$ corresponding to $p < 1$, $p < 0.1$, $p < 0.01$, $p < 0.001$, respectively.

Online Figure 3 III. Conjunctional FDR Manhattan plot of $-\log_{10}$(conjunctional FDR) for coronary artery disease (CAD) and type 2 diabetes (T2D; CAD&T2D; navy blue), CAD and type 1 diabetes (T1D; CAD&T1D; light green), CAD and low density lipoprotein (LDL; CAD&LDL; aqua). CAD and high density lipoprotein (HDL; CAD&HDL; dark green), CAD and triglycerides (TG; CAD&TG; fuchsia), CAD and body mass index (BMI; CAD&BMI; mustard yellow). CAD and C-reactive protein (CRP; CAD&CRP; royal blue) and CAD and systolic blood pressure (SBP; CAD&SBP; maroon). SNPs with $-\log_{10}$ (conjunctional FDR) > 2.2 (i.e. overall FDR < 0.05 after Bonferroni correction for eight traits) are shown with large points. A black circle around the large points indicates the most significant SNP in each linkage disequilibrium block and this SNP was

annotated with the closest gene which is listed above the symbols in each locus, except for the HLA region on chromosome 6, which was excluded from the analysis. Details for the novel loci with $-\log_{10}$ (conjunctional FDR) > 2.2 are given in Supplemental Table 3.

Online Tables

Online Table I. Correlation due to cross-trait sample overlap and corresponding overlap numbers.

Trait 1	Trait 2	Correlation	Description of overlap
CAD	T2D	0.09 (0.14)	Cases: 26,874 cases unique to T2D 55,780 cases unique to CAD; 7966 shared cases Controls: 75,282 controls unique to T2D 90,982 controls unique to CAD 39,699 shared controls
CAD	T1D	0.03 (NA)	Cases: 7514 cases unique to T1D; 63,746 cases unique to CAD; no shared cases Controls: 5703 controls unique to T1D; 127,339 controls unique to CAD, 3342 shared controls
CAD	LDL	<0.19 (0.10)	188,577 total subjects LDL; 194,427 total subjects CAD, up to which 36,432 are shared subjects
CAD	HDL	<-0.19 (-0.10)	188,577 total subjects HDL; 194,427 total subjects CAD, up to which 36,432 are shared subjects
CAD	TG	<0.19 (0.11)	188,577 total subjects HDL; 194,427 total subjects CAD, up to which 36,432 are shared subjects
CAD	BMI	<0.23 (0.05)	123,865 total subjects BMI; 194,427 total subjects CAD, up to which 37,131 are shared subjects
CAD	SBP	<0.31 (NA)	69,395 total subjects SBP; 194,427 total subjects CAD, up to which 36,545 are shared subjects

Cross-trait correlation of the GWAS test statistics for CAD and each secondary trait calculated using the methods presents in LeBlanc et al. (in prep). Correlation is presented as estimated (observed in data). Where a less than sign, <, is shown, the phenotypic correlation of trait 1 and trait 2 needs to be estimated from epidemiological studies.

Online Table II. Anderson Darling test for enrichment.

	Logp threshold		
	>3	>2	>1
BMD	2.64E-01	2.36E-01	1.20E-01
T2D	**1.70E-03**	5.69E-02	3.47E-01
T1D	**3.58E-04**	**9.56E-04**	3.81E-01
LDL	**1.87E-29**	**4.90E-09**	**7.80E-05**
HDL	**7.65E-06**	8.60E-03	7.47E-02
TG	**1.68E-04**	5.44E-02	5.82E-01
BMI	1.13E-01	**3.48E-06**	**2.10E-03**
CRP	**3.69E-04**	1.60E-02	2.23E-01
SBP*	NA	NA	NA

The bold strata are significant for an overall level of α=0.05 after correction for multiple testing. The set of SNPs (GWAS p>0.1 in the *secondary* trait), i.e., SNPs that are signal depleted in the secondary trait, was used as the comparison set.

*Note that SBP was excluded from the Anderson Darling test since the effect direction was not available and for technical reasons this made the Anderson Darling test unreliable after correction for sample overlap.

Online Table III SNPs that replicate in the WGHS at a nominal p-value of less than 0.05.

SNP	Event	P
rs4888378	CHD	0.0043
rs6905288	MI	0.0048
rs6905288	CHD	0.0066
rs12801636	CHD	0.0088
rs7296651	CHD	0.014
rs7296651	MI	0.017
rs11066320	MI	0.022
rs867764	MI	0.023
rs12801636	MI	0.030
rs1882961	CHD	0.031
rs10747342	MI	0.032
rs10774613	MI	0.034
rs10774613	CHD	0.036
rs2146238	MI	0.037
rs3179840	MI	0.043

MI, myocardial infarction; CHD, coronary heart disease (composed of MI, CHD death, and coronary revascularization)

Online Table IV. Conjunctional FDR (<0.05), after controlling for multiple testing across phenotypes

snp	gene	chr	CAD&T2D	CAD&T1D	CAD&LDL	CAD&HDL	CAD&TG	CAD&BMI	CAD&CRP	CAD&SBP	Min ConjFDR
rs4268379	*SARS*	1	5.07E-02	6.98E-01	**8.30E-04**	4.16E-01	1.00E+00	6.94E-01	4.61E-01	8.89E-01	**LDL**
rs12740374	*CELSR2*	1	3.82E-01	NA	**3.37E-08**	**7.40E-09**	2.22E-01	5.68E-01	3.58E-01	1.00E+00	**HDL**
rs7515901	*MYBPHL*	1	1.00E+00	NA	**2.31E-03**	3.70E-01	1.00E+00	9.68E-01	9.97E-01	1.00E+00	**LDL**
rs10495907	*DYNC2LI1*	2	8.91E-01	1.00E+00	**3.97E-03**	6.77E-01	8.46E-01	9.50E-01	7.41E-01	9.60E-01	**LDL**
rs10186133	*IL1F10*	2	9.63E-01	9.24E-01	4.69E-01	8.11E-01	5.75E-01	1.00E+00	**7.28E-04**	8.89E-01	**CRP**
rs934287	*ICA1L*	2	1.00E+00	3.63E-01	**1.57E-06**	3.28E-01	1.41E-01	2.61E-01	5.17E-01	1.00E+00	**LDL**
rs1250255	*FN1*	2	1.00E+00	1.00E+00	**5.66E-03**	9.04E-01	5.75E-01	9.00E-01	9.97E-01	4.61E-01	**LDL**
rs2176042	*BC017935*	2	**3.36E-03**	NA	4.69E-01	7.07E-03	**5.37E-03**	1.26E-01	7.90E-01	1.99E-01	**T2D**
rs7642590	*MAP4*	3	1.00E+00	NA	6.95E-01	2.89E-01	3.73E-01	4.47E-01	9.17E-01	**2.26E-03**	**SBP**
rs695238	*TRAIP*	3	9.15E-01	NA	9.29E-01	8.35E-03	1.00E+00	3.43E-01	**5.13E-03**	7.44E-01	**CRP**
rs7638389	*BC040632*	3	**5.30E-03**	1.00E+00	1.04E-01	2.89E-01	4.70E-01	2.61E-01	7.90E-01	6.91E-01	**T2D**
rs10512987	*PPP2R3A*	3	1.00E+00	9.58E-01	**3.73E-03**	1.68E-01	7.37E-03	3.43E-01	3.13E-01	8.89E-01	**LDL**
rs7356185	*USP53*	4	1.00E+00	8.93E-01	7.46E-01	9.04E-01	9.27E-01	9.68E-01	1.00E+00	**4.18E-03**	**SBP**
rs1508798	*SNORD123*	5	1.00E+00	9.58E-01	5.85E-01	2.38E-02	**2.34E-03**	1.00E+00	9.17E-01	1.00E+00	**TG**
rs10477741	*C5orf56*	5	6.96E-01	NA	**1.03E-03**	5.68E-01	1.00E+00	1.00E+00	9.47E-01	8.36E-01	**LDL**
rs2814982	*C6orf106*	6	9.63E-01	1.00E+00	**5.20E-04**	**3.03E-03**	8.46E-01	5.06E-01	8.32E-01	1.00E+00	**LDL**
rs1321309	*CDKN1A*	6	8.11E-01	NA	4.69E-01	7.38E-01	**1.86E-03**	1.00E+00	5.74E-01	8.89E-01	**TG**
rs6905288	*VEGFA*	6	3.44E-01	1.00E+00	6.41E-01	**2.56E-03**	**3.46E-03**	8.61E-01	9.97E-01	1.51E-02	**HDL**
rs1564348	*SLC22A1*	6	8.56E-01	1.00E+00	**5.66E-03**	5.15E-01	1.26E-02	9.68E-01	6.32E-01	7.44E-01	**LDL**
rs9365233	*MAP3K4*	6	1.00E+00	1.00E+00	**5.78E-03**	1.00E+00	7.33E-01	5.06E-01	1.00E+00	6.34E-01	**LDL**
rs2237659	*COG5*	7	6.96E-01	1.00E+00	2.60E-02	7.38E-01	**2.66E-03**	8.61E-01	5.17E-01	8.89E-01	**TG**
rs6997340	*NAT2*	8	6.35E-01	9.58E-01	**3.97E-03**	6.22E-01	1.20E-02	9.68E-01	7.41E-01	1.00E+00	**LDL**
rs11204085	*SLC18A1*	8	7.56E-01	NA	9.29E-01	**9.24E-04**	**1.50E-03**	1.00E+00	9.17E-01	1.00E+00	**HDL**
rs343494	*RANBP6*	9	1.00E+00	**5.94E-03**	1.00E+00	6.22E-01	1.00E+00	1.00E+00	9.97E-01	7.44E-01	**T1D**
rs7902355	*TSPAN14*	10	9.63E-01	1.00E+00	3.17E-01	**5.80E-03**	8.46E-01	1.00E+00	9.97E-01	5.75E-01	**HDL**
rs7926335	*PLEKHA7*	11	9.63E-01	NA	9.29E-01	3.70E-01	1.00E+00	9.39E-01	9.97E-01	**7.17E-04**	**SBP**

rs12801636	*PCNXL3*	11	4.70E-01	1.00E+00	3.17E-01	**6.50E-04**	**1.02E-03**	9.26E-01	4.40E-02	6.43E-02	**HDL**
rs644740	*OVOL1*	11	6.35E-01	8.93E-01	1.38E-02	1.15E-02	8.46E-02	1.00E+00	7.90E-01	**2.26E-03**	**SBP**
rs7933887	*ST3GAL4*	11	1.00E+00	NA	1.04E-01	**4.69E-03**	3.73E-01	1.00E+00	1.00E+00	7.91E-01	**HDL**
rs4149033	*SLCO1B1*	12	1.00E+00	1.00E+00	6.41E-01	1.00E+00	**3.98E-03**	9.26E-01	1.00E+00	8.89E-01	**TG**
rs2681472	*ATP2B1*	12	8.11E-01	1.00E+00	1.00E+00	1.00E+00	4.70E-01	1.00E+00	7.90E-01	**3.40E-03**	**SBP**
rs1056618	*AJ276555*	12	1.00E+00	NA	**5.78E-03**	7.38E-01	9.27E-01	5.68E-01	8.32E-01	1.00E+00	**LDL**
rs7398833	*CUX2*	12	9.15E-01	**1.85E-04**	**1.94E-03**	3.70E-01	**3.25E-03**	8.11E-01	2.02E-01	**2.29E-04**	**TG**
rs11066320	*PTPN11*	12	9.63E-01	**4.05E-06**	**1.28E-05**	**8.40E-05**	3.73E-01	1.88E-02	9.17E-01	**5.55E-06**	**T1D**
rs7315519	*RPH3A*	12	8.91E-01	**4.86E-04**	**2.99E-03**	**3.59E-03**	9.27E-01	1.26E-01	1.00E+00	1.21E-01	**T1D**
rs692902	*SPPL3*	12	3.19E-02	9.24E-01	2.75E-01	2.89E-02	7.85E-01	1.00E+00	**4.77E-03**	8.89E-01	**CRP**
rs2708081	*OASL*	12	1.02E-01	3.10E-01	**6.70E-04**	1.94E-01	8.46E-01	9.26E-01	**3.41E-05**	8.89E-01	**CRP**
rs825461	*ZNF664*	12	**4.29E-03**	1.00E+00	9.29E-01	**3.59E-03**	1.65E-01	5.68E-01	1.00E+00	3.60E-01	**T2D**
rs11057830	*SCARB1*	12	9.36E-01	NA	**3.49E-04**	1.95E-02	**2.69E-03**	9.26E-01	9.97E-01	4.09E-01	**LDL**
rs4932370	*FURIN*	15	3.82E-01	9.24E-01	4.69E-01	3.28E-01	3.73E-01	9.50E-01	7.41E-01	**7.93E-05**	**SBP**
rs2072142	*DHX38*	16	9.15E-01	NA	**1.93E-03**	6.22E-01	7.85E-01	8.02E-02	1.00E+00	NA	**LDL**
rs9927309	*CTRB2*	16	**5.30E-03**	**8.50E-04**	9.29E-01	4.64E-01	1.00E+00	9.00E-01	1.00E+00	7.91E-01	**T2D**
rs1838105	*GOSR2*	17	1.00E+00	NA	1.48E-01	**5.97E-03**	6.29E-01	8.11E-01	1.00E+00	8.76E-02	**HDL**
rs12940887	*ZNF652*	17	9.36E-01	NA	7.46E-01	8.89E-02	1.92E-02	8.61E-01	9.97E-01	**5.15E-03**	**SBP**
rs2812	*PECAM1*	17	9.36E-01	NA	6.41E-01	1.24E-01	9.27E-01	8.61E-01	7.41E-01	**3.63E-03**	**SBP**
rs13465	*ILF3*	19	9.63E-01	4.89E-01	**1.62E-03**	2.22E-01	8.46E-01	9.68E-01	1.00E+00	7.44E-01	**LDL**
rs12052058	*SMARCA4*	19	8.91E-01	8.14E-01	**7.34E-06**	7.38E-01	1.00E+00	8.11E-01	5.74E-01	1.00E+00	**LDL**
rs892115	*SPC24*	19	9.63E-01	1.19E-01	**4.75E-03**	9.04E-01	1.00E+00	9.00E-01	6.88E-01	7.44E-01	**LDL**
rs17616661	*KANK2*	19	1.00E+00	6.98E-01	**1.57E-03**	**1.55E-03**	3.29E-01	9.50E-01	5.17E-01	9.60E-01	**HDL**
rs2241718	*CYP2F1*	19	9.63E-01	NA	**2.99E-03**	9.04E-01	5.75E-01	1.00E+00	9.97E-01	NA	**LDL**
rs1415771	*EDEM2*	20	1.00E+00	1.02E-01	5.89E-02	**4.25E-03**	1.00E+00	8.61E-01	1.00E+00	9.60E-01	**HDL**
rs3827066	*ZNF335*	20	1.00E+00	NA	2.75E-01	**1.55E-03**	7.00E-03	9.68E-01	5.74E-01	1.00E+00	**HDL**
rs4822458	*DDT*	22	1.00E+00	8.58E-01	1.00E+00	9.04E-01	**5.76E-03**	9.50E-01	8.66E-01	1.00E+00	**TG**

Independent ($r^2 < 0.2$) of SNP(s) with a conjunctional FDR (conjFDR) < 0.05 (after Bonferonni correction for 8 traits) in Coronary Artery Disease (CAD) given the significance level in the associated phenotype. We defined the most significant CAD SNP in each LD block based on the minimum conjunctional FDR for each associated phenotype. The most significant SNPs in each gene of the LD block are listed along with the associated

phenotype that provided the signal. Coronary artery disease (CAD), low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides (TG), type 2 diabetes (T2D), C-reactive protein (CRP), body mass index (BMI), systolic blood pressure (SBP), type 1 diabetes (T1D), chromosome number (Chr). Conjunctional FDR values < 0.05, after adjusting for multiple testing across phenotypes are in bold. The most significant phenotype association per gene is shown (min conjFDR). NA indicates that a given SNP was not available for a given trait.

Online Table V. Signed z-scores for the SNPs presented in Supplemental Table 3.

snp	gene	chr	CAD z-score	BMI z-score	T2D z_score	LDL z-score	HDL z-score	TG z-score	CRP z-score	**Min conjFDR**	**Trait**
rs4268379	*SARS*	1	-4.95	1.64	3.57	-12.00	1.85	-0.42	2.09	**8.30E-04**	**LDL**
rs12740374	*CELSR2*	1	8.70	-1.91	-2.22	36.59	-8.37	2.75	-2.24	**7.40E-09**	**HDL**
rs7515901	*MYBPHL*	1	4.48	-0.57	-0.06	9.40	-1.98	0.15	0.43	**2.31E-03**	**LDL**
rs10495907	*DYNC2LI1*	2	-4.22	-0.98	-1.29	-7.81	1.21	-0.96	-1.51	**3.97E-03**	**LDL**
rs10186133	*IL1F10*	2	4.24	-0.10	-0.46	1.84	0.41	1.59	-5.98	**7.28E-04**	**CRP**
rs934287	*ICA1L*	2	5.84	-2.49	0.08	-5.46	-2.20	-2.48	-1.93	**1.57E-06**	**LDL**
rs1250255	*FN1*	2	3.44	-1.22	0.50	-4.75	0.24	-1.25	0.47	**5.66E-03**	**LDL**
rs2176042	*BC017935*	2	-4.33	2.72	-6.59	-1.84	8.47	-7.43	-1.38	**3.36E-03**	**T2D**
rs7642590	*MAP4*	3	-3.96	2.01	-0.75	-1.36	2.11	-2.11	-0.90	**2.26E-03**	**SBP**
rs695238	*TRAIP*	3	-4.04	-2.43	-1.19	0.32	4.38	-0.12	-4.02	**5.13E-03**	**CRP**
rs7638389	*BC040632*	3	3.95	2.62	-5.33	-2.40	1.78	-1.38	-1.44	**5.30E-03**	**T2D**
rs10512987	*PPP2R3A*	3	-4.07	-2.43	-0.09	-3.85	2.50	-4.20	2.36	**3.73E-03**	**LDL**
rs7356185	*USP53*	4	3.80	-0.49	-0.10	1.19	-0.68	-0.18	0.24	**4.18E-03**	**SBP**
rs1508798	*SNORD123*	5	4.24	0.51	0.01	-1.15	2.95	-4.55	-0.83	**2.34E-03**	**TG**
rs10477741	*C5orf56*	5	-4.75	-0.26	-1.88	-4.25	1.49	-0.25	-0.58	**1.03E-03**	**LDL**
rs2814982	*C6orf106*	6	-4.47	-2.16	0.45	4.44	6.75	-0.94	-1.21	**5.20E-04**	**LDL**
rs1321309	*CDKN1A*	6	-4.60	-0.31	-1.65	-1.84	1.11	-4.56	-1.81	**1.86E-03**	**TG**
rs6905288	*VEGFA*	6	-4.52	1.24	-2.53	1.05	6.61	-7.61	-0.44	**2.56E-03**	**HDL**
rs1564348	*SLC22A1*	6	-4.23	0.56	1.31	-9.62	1.67	-3.64	1.77	**5.66E-03**	**LDL**
rs9365233	*MAP3K4*	6	4.57	-1.97	0.23	3.78	-0.32	1.32	-0.04	**5.78E-03**	**LDL**
rs2237659	*COG5*	7	-4.22	1.26	1.63	-3.28	1.00	3.92	1.99	**2.66E-03**	**TG**
rs6997340	*NAT2*	8	4.02	-0.52	1.92	4.07	0.87	6.24	1.54	**3.97E-03**	**LDL**
rs11204085	*SLC18A1*	8	5.23	0.31	1.80	-0.19	-14.03	14.24	0.78	**9.24E-04**	**HDL**
rs343494	*RANBP6*	9	3.53	-0.05	0.73	-0.05	-1.26	-0.15	-0.38	**5.94E-03**	**T1D**
rs7902355	*TSPAN14*	10	4.24	0.63	-0.41	2.21	3.58	-0.45	0.40	**5.80E-03**	**HDL**
rs7926335	*PLEKHA7*	11	-4.25	0.84	-0.78	-0.76	-1.54	-0.29	0.40	**7.17E-04**	**SBP**

rs12801636	*PCNXL3*	11	4.86	1.25	2.25	-1.73	-5.60	4.43	3.28	**6.50E-04**	**HDL**
rs644740	*OVOL1*	11	3.95	0.06	1.94	-3.05	-4.41	2.94	1.40	**2.26E-03**	**SBP**
rs7933887	*ST3GAL4*	11	4.47	-0.29	-0.05	2.80	-3.88	2.10	-0.23	**4.69E-03**	**HDL**
rs4149033	*SLCO1B1*	12	-3.82	1.04	-0.43	1.05	0.00	3.72	0.14	**3.98E-03**	**TG**
rs2681472	*ATP2B1*	12	-3.84	-0.12	1.37	-0.35	0.36	1.41	1.45	**3.40E-03**	**SBP**
rs1056618	*AJ276555*	12	3.96	-1.86	0.30	-3.32	-1.03	0.67	1.30	**5.78E-03**	**LDL**
rs7398833	*CUX2*	12	4.56	-1.45	1.17	-3.81	-2.03	4.08	2.58	**3.25E-03**	**TG**
rs11066320	*PTPN11*	12	5.40	-3.80	0.86	-6.24	-5.54	2.18	0.92	**4.05E-06**	**T1D**
rs7315519	*RPH3A*	12	4.34	-2.76	-0.91	-3.71	-4.36	-0.17	0.18	**4.86E-04**	**T1D**
rs692902	*SPPL3*	12	3.65	0.27	4.76	2.24	2.91	1.02	-9.03	**4.77E-03**	**CRP**
rs2708081	*OASL*	12	5.12	1.28	3.35	4.38	1.97	1.03	-12.98	**3.41E-05**	**CRP**
rs825461	*ZNF664*	12	-4.44	1.84	-4.49	-0.71	6.37	-2.68	-0.35	**4.29E-03**	**T2D**
rs11057830	*SCARB1*	12	-5.26	1.02	0.52	-4.52	3.48	-4.15	-0.54	**3.49E-04**	**LDL**
rs4932370	*FURIN*	15	-5.69	0.75	-2.47	1.31	-1.90	1.50	1.53	**7.93E-05**	**SBP**
rs2072142	*DHX38*	16	-4.27	-3.22	-1.17	-4.90	-0.87	-1.14	-0.06	**1.93E-03**	**LDL**
rs9927309	*CTRB2*	16	4.18	-1.17	5.20	0.62	-1.76	0.12	-0.14	**5.30E-03**	**T2D**
rs1838105	*GOSR2*	17	3.96	-1.40	0.48	2.63	4.19	1.56	0.08	**5.97E-03**	**HDL**
rs12940887	*ZNF652*	17	-3.73	-1.55	0.61	-1.16	2.83	-5.09	-0.54	**5.15E-03**	**SBP**
rs2812	*PECAM1*	17	-4.48	1.27	0.57	1.00	-2.24	-0.79	1.54	**3.63E-03**	**SBP**
rs13465	*ILF3*	19	-4.74	0.57	0.44	-12.12	2.41	-0.95	0.34	**1.62E-03**	**LDL**
rs12052058	*SMARCA4*	19	6.42	-1.52	-1.21	17.44	-1.10	0.10	1.85	**7.34E-06**	**LDL**
rs892115	*SPC24*	19	4.11	-1.18	0.91	6.60	-0.77	0.42	1.65	**4.75E-03**	**LDL**
rs17616661	*KANK2*	19	-4.56	-0.95	-0.62	-4.14	4.41	-2.16	-1.96	**1.55E-03**	**HDL**
rs2241718	*CYP2F1*	19	4.15	0.12	0.88	-3.48	0.18	-1.16	0.38	**2.99E-03**	**LDL**
rs1415771	*EDEM2*	20	-4.22	-1.52	-0.62	2.54	4.03	0.12	0.06	**4.25E-03**	**HDL**
rs3827066	*ZNF335*	20	-4.35	-0.74	-0.22	1.77	-4.33	3.32	-1.82	**1.55E-03**	**HDL**
rs4822458	*DDT*	22	4.21	-0.70	-0.30	0.46	0.26	3.85	-1.08	**5.76E-03**	**TG**

Coronary artery disease (CAD), low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides (TG), type 2 diabetes (T2D), C-reactive protein (CRP), body mass index (BMI), systolic blood pressure (SBP), type 1 diabetes (T1D), chromosome number (Chr).

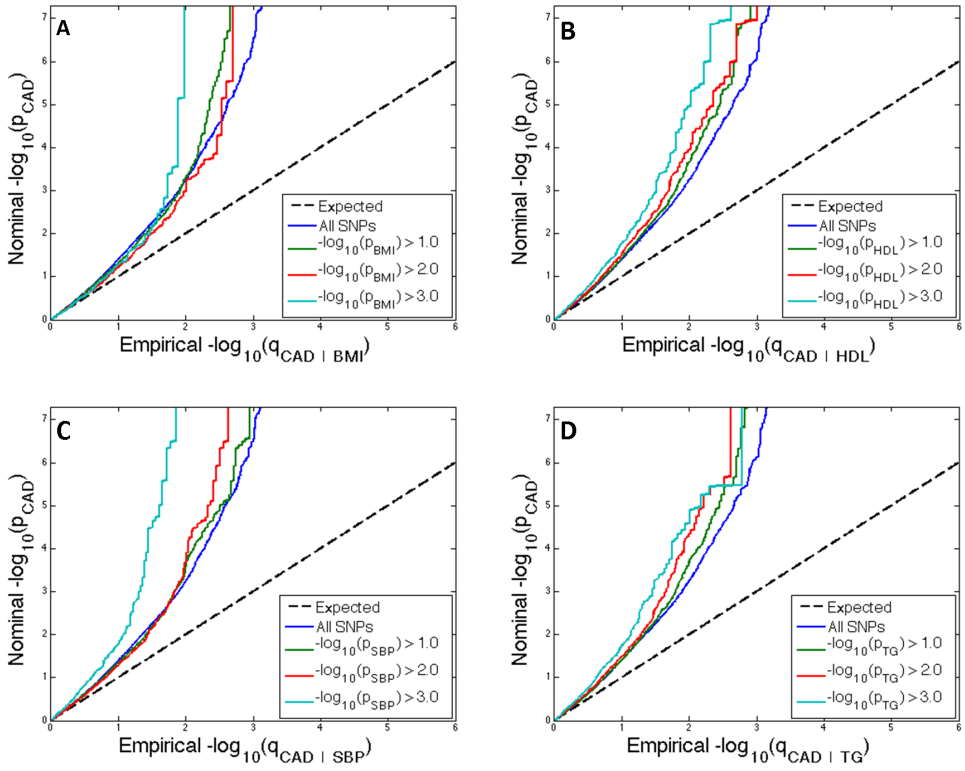
Note that signed z-scores for T1D and SBP are not publically available and are therefore excluded from this table. The most significant phenotype association per gene is shown (min conjFDR).

Online Table VI. Ingenuity Pathway Analysis (IPA) including our novel CAD SNPs and previously published CAD SNPs.

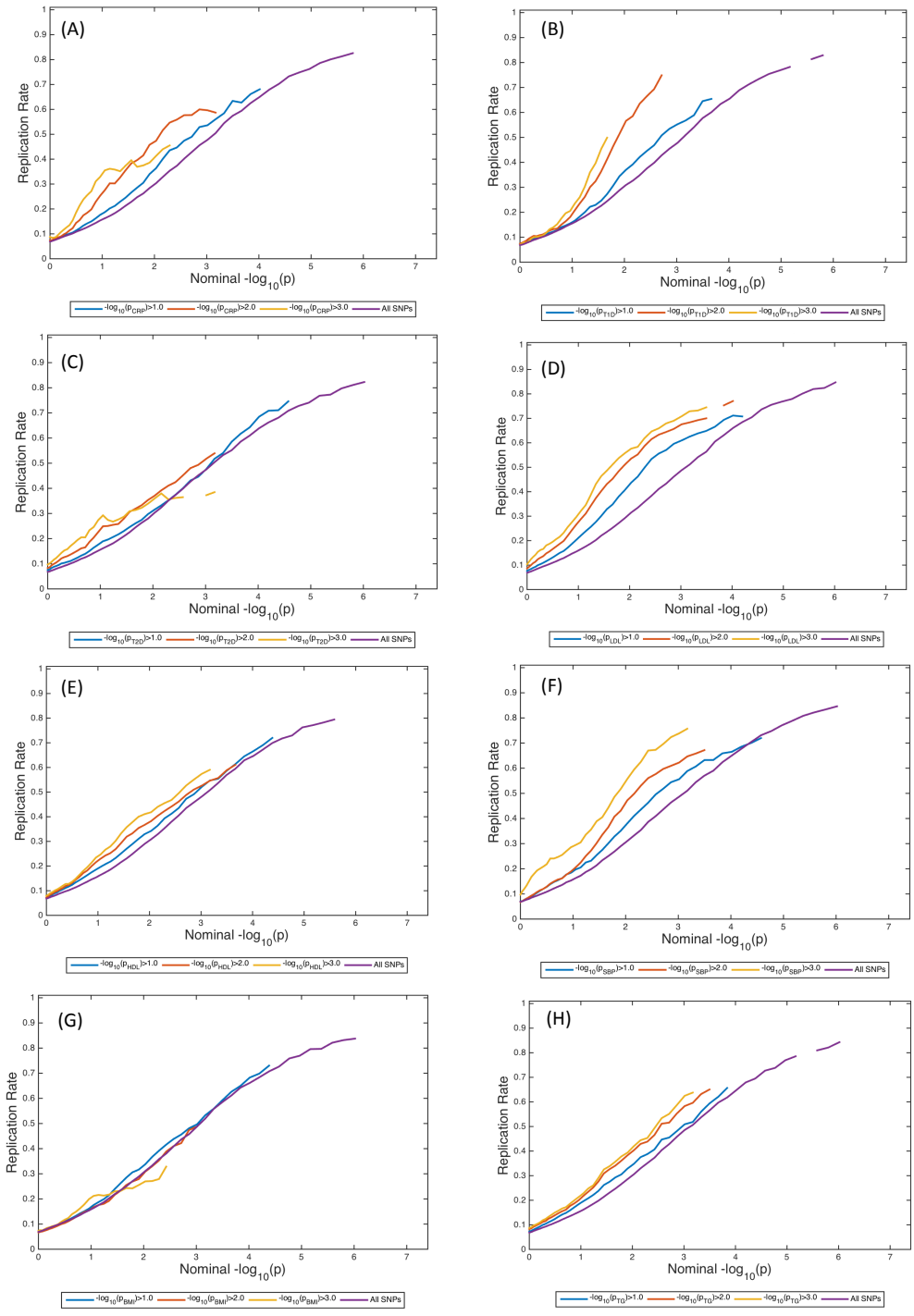
Top Canonical Pathways		
Name	**p-value**	
LXR/RXR Activation	2.22E-14	
Atherosclerosis Signaling	1.36E-11	
FXR/RXR Activation	3.66E-10	
Clathrin-mediated Endocytosis Signaling	2.18E-07	
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	1.57E-05	
Diseases and Disorders		
Name	**p-value**	**#candidate genes**
Cardiovascular Disease	4.24E-14 -5.02E-04	47
Organismal Injury and Abnormalities	9.65E-14 - 5.02E-04	57
Metabolic Disease	1.80E-12 - 3.71E-04	41
Neurological Disease	1.28E-09 - 1.54E-04	29
Psychological Disorders	1.28E-09 - 1.54E-04	26
Molecular and Cellular Functions		
Name	**p-value**	**#candidate genes**
Lipid Metabolism	5.06E-14 - 3.71E-04	40
Molecular Transport	5.06E-14 - 4.12E-04	50
Small Molecule Biochemistry	5.06E-14 - 3.71E-04	51
Vitamin and Mineral Metabolism	1.30E-13 - 3.71E-04	17
Protein Synthesis	1.41E-13 - 4.45E-05	14

*P-values are from a right-tailed Fisher exact test and represent significance of overrepresentation of candidate genes within respective gene groups. P-value ranges indicate values for various disease sub-classifications (not shown).

Online Figure I. Conditional Q-Q plots for BMI, HDL, SBP and TG.



Online Figure II. Stratified replication rates plots



Online Figure III. Conjunctional Manhattan plot