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Network-Based Identification and Prioritization of Key Regulators of Coronary Artery Disease Loci

Yuqi Zhao, Jing Chen, Johannes M. Freudenberg, Qingying Meng, CARDIoGRAM Consortium, Deepak K. Rajpal, and Xia Yang

Department of Integrative Biology and Physiology, University of California, Los Angeles (Y.Z., Q.M., X.Y.); and Target Sciences Computational Biology (US), GSK, King of Prussia, PA (J.C., J.M.F., D.K.R.)

Abstract

Objective—Recent genome-wide association studies of coronary artery disease (CAD) have revealed 58 genome-wide significant and 148 suggestive genetic loci. However, the molecular mechanisms through which they contribute to CAD and the clinical implications of these findings remain largely unknown. We aim to retrieve gene subnetworks of the 206 CAD loci and identify and prioritize candidate regulators to better understand the biological mechanisms underlying the genetic associations.

Approach and Results—We devised a new integrative genomics approach that incorporated (1) candidate genes from the top CAD loci, (2) the complete genetic association results from the 1000 genomes-based CAD genome-wide association studies from the Coronary Artery Disease Genome Wide Replication and Meta-Analysis Plus the Coronary Artery Disease consortium, (3) tissue-specific gene regulatory networks that depict the potential relationship and interactions between genes, and (4) tissue-specific gene expression patterns between CAD patients and controls. The networks and top-ranked regulators according to these data-driven criteria were further queried against literature, experimental evidence, and drug information to evaluate their disease relevance and potential as drug targets. Our analysis uncovered several potential novel regulators of CAD such as *LUM* and *STAT3*, which possess properties suitable as drug targets. We also revealed molecular relations and potential mechanisms through which the top CAD loci operate. Furthermore, we found that multiple CAD-relevant biological processes such as extracellular matrix, inflammatory and immune pathways, complement and coagulation cascades, and lipid metabolism interact in the CAD networks.

Conclusions—Our data-driven integrative genomics framework unraveled tissue-specific relations among the candidate genes of the CAD genome-wide association studies loci and prioritized novel network regulatory genes orchestrating biological processes relevant to CAD.

Correspondence to Xia Yang, PhD, Department of Integrative Biology and Physiology, University of California, Los Angeles, 610 Charles E. Young Dr E, Los Angeles, CA 90095. xyang123@ucla.edu; or Deepak Rajpal, PhD, Target Sciences Computational Biology (US), GSK, 709 Swedeland Rd, King of Prussia, PA 19406. deepak.k.rajpal@gsk.com.

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Disclosures
None.

Keywords

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Coronary artery disease (CAD) is a major cause of morbidity and mortality for both men and women in the Western world. Several clinical risk factors have long been associated with CAD, including high blood pressure, cigarette smoking, elevated low-density lipoprotein cholesterol, and diabetes mellitus, and randomized clinical studies have consistently shown a 30% to 40% reduction in CAD-related deaths by modifying these factors.^{1–3} However, our ability to fully mitigate CAD remains limited because of the incomplete understanding of the molecular basis of CAD. CAD is highly heritable with genetic risk accounting for 40% to 60% of the susceptibility to CAD.⁴ Empowered by the genomic technologies, a total of ≈206 CAD susceptibility loci (58 genome-wide significant and 148 suggestive) have been identified by human genome-wide association studies (GWAS).⁵ The mechanisms of these loci/genes seem to be diverse. For example, genes involved in both lipid metabolism (*APOB*, *ABCG5-ABCG8*, *PCSK9*, *SORT1*, *ABO*, *LDLR*, *APOE*, and *LPA*) and blood pressure regulation (*CYP17A1-NT5C2*, *SH2B3*, *GUCY1A3*, *FES*, and *ZC3HC1*) are among the GWAS loci, providing molecular basis for the long-observed clinical importance of these intermediate clinical traits in CAD development.⁵ However, for the majority of the remaining significant loci, there is a lack of understanding of their functional relevance and molecular mechanisms involved. In addition, together the 206 genetic loci explain ≈25% of the heritability, leaving a large proportion of the genetic heritability to be further explored.^{6,7} These challenges make it difficult to translate the GWAS findings into clinical applications.

To address these challenges, various efforts have been made recently to decode CAD GWAS signals into pathways and gene networks through integrative systems biology approaches.^{8–11} The underlying concept emphasizes that instead of acting alone, disease genes interact through complex regulatory machinery that governs the overall genetic (and epigenetic) control of disease susceptibility.^{12,13} Genetic variants of both strong and subtle effects (ie, loci reaching genome-wide significance and those that are well below the significance cutoff) can perturb parts of the regulatory network, termed subnetworks. Moreover, disease genes do not play equal roles in disease development, that is, some important genes tend to regulate more CAD genes than others do in the regulatory networks, as demonstrated in recent studies of CAD⁸ and late-onset Alzheimer diseases.¹⁴ These studies have unraveled many novel molecular pathways and gene subnetworks in addition to confirming previously known mechanisms, testifying to the power of utilizing context-specific (eg, tissues, drug treatments, and diet changes) regulatory networks in conjunction with genetic signals to retrieve the perturbed subnetworks. Subsequently, the key regulators in the networks can be captured based on the network topology.^{8,9}

Despite the progress, none of these previous studies has focused on the top 206 CAD loci to more closely investigate their tissue-specific network properties and whether they coordinate their actions to affect specific biological functions. Furthermore, the fact that a majority of these top CAD loci only exhibit moderate effects on CAD points to a role of these loci as

disease-modifying genes, rather than strong disease-determining genes.⁶ From the point of view of clinical and therapeutic applications, it is perhaps more productive to identify potential central regulators that coordinate the actions of multiple genes affected by the top CAD loci. These central regulators, when perturbed, may elicit stronger effects on CAD, but can be missed by GWAS because of evolutionary constraints that eliminate common variants affecting the functions of the key regulators in the population.^{8,9,15}

In this study, we devise an integrative network approach that combines multiple types of CAD-related genomic data sets to examine the potential relationship and interactions between the candidate genes at these top CAD loci to better understand the molecular pathways involved in CAD pathogenesis, as well as to identify and prioritize key regulators in tissue-specific networks for CAD. By focusing on the top CAD candidate genes, we aim to (1) delineate the tissue-specific characteristics of CAD gene regulatory networks affected by the top loci, (2) highlight candidate key regulators for CAD, and (3) provide insights into the causal mechanisms of CAD loci/genes.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Overview of the Analytic Pipeline

We designed and implemented an integrative genomics pipeline to pinpoint the gene networks of the top GWAS loci and potential regulators by integrating diverse genomic data sets and tools. The framework can be divided into 4 steps (Figure 1).

First, we retrieve 7 CAD candidate gene lists from GWAS based on different curation and gene mapping criteria (Figure 1A; full list in Table I in the online-only Data Supplement). Comprehensive curation of CAD candidate genes is important because mapping disease-associated loci to candidate genes is not straightforward.¹⁶ The 7 gene lists include.

CAD GWAS Gene List (n=145)—We downloaded the NHGRI GWAS catalog (<https://www.genome.gov/26525384>) and extracted candidate GWAS genes for phenotypes relevant to CAD including coronary heart disease, myocardial infarction, and coronary artery calcification with association $P < 1e-5$.

CAD+C4D Gene List (n=239)—The above CAD GWAS list based on GWAS catalog was supplemented with additional candidate genes identified through the Coronary Artery Disease Genome Wide Replication and Meta-Analysis Plus the Coronary Artery Disease (CARDIoGRAM-C4D) study,⁵ which was based on MetaboChip data and therefore was not included in the GWAS catalog.

CAD GWAS No-Lipid Gene List (n=124)—Lipid metabolism genes were removed from the CAD GWAS gene list to prevent the lipid genes from overshadowing and diluting other novel mechanisms.¹⁷

CAD+C4D No-Lipid Gene List (n=149)—Similarly, lipid metabolism genes were removed from the CAD+C4D gene list to enable better exploration of novel mechanisms.

CAD+C4D Extend Gene List (n=559)—The above 4 CAD candidate lists were primarily based on chromosomal distance-based locus to gene mapping. As suggested by previous studies, disease loci may not affect their most adjacent genes but may function through long-range regulation of distant genes.^{16,18} To address this concern, we used an alternative locus-gene mapping that is based on empirical evidence provided by expression quantitative trait loci from CAD-relevant tissues, as described by Braenne et al.¹⁶ The expression quantitative trait loci studies included were retrieved from Genome-Wide Repository of Associations between single nucleotide polymorphisms and Phenotypes database,¹⁹ Stockholm Atherosclerosis Gene Expression (STAGE)²⁰ study, a liver/adipose study,²¹ Cardiogenics consortium monocyte/macrophages study,²² RNA-sequencing study in blood,^{23,24} and aortic endothelial cells study.²⁵

CAD 1000G Gene List (n=257)—The CAD+C4D list was supplemented with additional candidate genes from the recent 1000 genomes study.⁷

CAD 1000G Extend Gene List (n=880)—We applied the same expression quantitative trait loci-based mapping to the CAD 1000G supplemented loci to map candidate genes as described for the CAD+C4D extended list.

Second, we perform the key driver analysis (KDA)^{26–28} to identify potential network regulators, termed KDs of the CAD gene lists using 13 tissue-specific regulatory networks (adipose, aorta, artery, blood, brain, cardiac muscle, heart, islet, kidney, liver, skeletal muscle, smooth muscle, and vascular endothelium) and a protein–protein interaction network (Figure 1B; details of network construction in Supplemental Text in the online-only Data Supplement). We chose these tissues or network types because they are involved in CAD and related risk phenotypes. This step is essential for detecting the interactions and central orchestrators of the CAD genes.

Third, we evaluate the association strength between CAD and the predicted KDs as well as their direct partners in the gene subnetworks to confirm their relationship with CAD, based on 3 criteria: (1) the consistency of KDA performance for each KD across the 7 CAD gene lists and the 14 networks, (2) the CAD association strength of the KD subnetworks in CAD GWAS, and (3) tissue-specific gene expression pattern changes of the KD subnetworks between myocardial infarction and control subjects (Figure 1C). The KDs are scored and ranked based on these 3 data-driven criteria.

Finally, the associations between KDs and CAD are further validated using diverse bioinformatics databases and tools such as phenotypes from knockout mouse models, drug target databases, and literature mining (Figure 1D).

Identification of KD Genes in Tissue-Specific Networks

From the 98 sets of KDA (7 CAD lists × 14 networks), we detected a total of 287 KD genes at false discovery rate <0.05 (detailed KDs for each CAD gene set are listed in separate

spreadsheets in Table II in the online-only Data Supplement), with 156 appearing in at least 2 KDA sets and 44 in at least 2 tissue-specific networks. Then, a meta-analysis, which considers both the consistency across the 98 sets of KDA analysis and the KDA statistics, was performed to derive meta P values for the KDs. This yielded 139 KDs with meta $P < 0.01$ (listed in the last spreadsheet in Table II in the online-only Data Supplement), among which 49 were captured by at least 3 networks and CAD gene lists and 22 were detected as multiple-tissue KDs (Table 1). Therefore, many of the KDs are less sensitive to the curation methods used to retrieve the top CAD GWAS candidate genes (ie, the 7 CAD gene lists) or the networks used in KDA.

We retrieved literature-based information about the KDs to assess their CAD relevance. Among the 139 KDs at meta $P < 0.01$, 29 are within the 7 CAD candidate gene lists. These include *APOA1*, *GBPI*, *GBP2*, *PLG*, *VEGFA*, *COL4A2*, *KCNK13*, *CXCL12*, *ABCG5*, *APOC2*, *APOB*, *ADM*, *SLIT3*, *PHACTR1*, *OSM*, *LARGE*, *IRF1*, *APOA5*, *LDLR*, *KLF6*, *APOC4*, *FN1*, *HEMGN*, *APOC3*, *ABCG8*, *NME6*, *SHC1*, *THADA*, and *PTPN11*. There are 12 KDs satisfying GWAS significance of $P < 5.0 \times 10^{-8}$, including *PHACTR1*, *APOA1*, *APOA5*, *APOC3*, *LDLR*, *COL4A2*, *PTPN11*, *PLG*, *APOC2*, *APOC4*, *APOB*, and *CXCL12* in CARDIoGRAM or CAD 1000 genomes GWAS. Notably, many of these KDs are lipid genes, confirming the importance of lipid metabolism in CAD. We found that 61 of the 139 KDs, when genetically perturbed in knockout mouse models, showed phenotypes directly related to CAD or CAD risk phenotypes such as lipid level changes, hypertension, and obesity based on the Mouse Genome Informatics database (<http://www.informatics.jax.org/>), representing a significant enrichment for genes linked to CAD phenotypes (Fisher exact test $P = 6.31 \times 10^{-16}$, fold change = 5.09). In addition, 23 of the 139 KDs were included in the CADgene V2.0 database,²⁹ which contains 583 CAD-related genes and detailed CAD association information from ≈ 5000 publications (Fisher exact test, $P = 44.36 \times 10^{-13}$; fold enrichment, 7.67). These lines of literature-based evidence support the relevance of the KDs to CAD.

Data-Driven Assessment of the Relevance of KDs and KD Subnetworks to CAD

To more objectively evaluate the CAD relevance of the KDs and their subnetworks, we used multiple data-driven approaches. The KD subnetworks are relatively independent (Figure I in the online-only Data Supplement). First, we utilized the 1000 Genomes-based CAD GWAS to assess the overall enrichment of the KD subnetwork genes for stronger CAD association, as compared to subnetworks derived from random genes or those derived from the CAD top genes in our initial CAD lists (detailed in Materials and Methods in the online-only Data Supplement). Our results indicate that KD subnetworks tend to include stronger CAD GWAS signals than the subnetworks of random genes (Table III in the online-only Data Supplement) or those of the CAD top genes (Table 2; Table III in the online-only Data Supplement), supporting that KDs and their subnetworks may play more important roles in CAD development than the candidate genes of the top CAD loci. For example, 26.7% (12 of 45) of the member genes in the brain subnetwork of *LUM* had CAD GWAS $P < 0.001$ in 1000 genome-based CAD GWAS. In contrast, the average percentages of genes showing CAD association at the same GWAS P value cutoff were much lower in the subnetworks of randomly selected genes (1.9%). The GWAS P value distribution of the *LUM* subnetwork

genes was significantly different from random networks ($P < 1.0e-16$ in 1-sided 2-sample Kolmogorov–Smirnov test) and those of the CAD top GWAS genes ($P = 1.14e-3$). Similarly, for another KD example *KNG1*, 44.8% of the members in the *KNG1* subnetwork in muscle had CAD GWAS $P < 0.001$, in contrast to the much lower percentages for random genes (1.9%; $P < 1.0e-16$ by Kolmogorov–Smirnov test) and CAD top GWAS genes (23.9%; $P = 3.4e-3$). These results support that our data-driven systems biology approach that focuses on the aggregate behavior and properties of CAD genes in gene networks derives potentially stronger candidate genes than standard, individual locus-based methods.

Second, we hypothesize that if the KD subnetworks are important for CAD, the expression levels of the genes in the subnetworks are more likely to be perturbed in patients with CAD. To this end, we analyzed the expression profile alterations of the KD subnetworks between non-CAD subjects and patients with acute myocardial infarction, in 10 CAD-relevant cell types or tissues, namely, endothelial cells, CD34⁺ stem cells, CD4⁺ T cells, resting CD14⁺ monocytes, stimulated monocytes, peripheral blood mononuclear cells, whole blood, epicardial adipose tissue and subcutaneous adipose tissue, and macrophages. Among the 170 KD subnetworks of the 139 KDs (some KDs have subnetworks from multiple tissues), 58 and 81 showed significant up- or downregulation patterns in CAD versus controls in at least 1 cell type at false discovery rate $< 1\%$ and false discovery rate $< 5\%$, respectively (Table IV in the online-only Data Supplement). Furthermore, 12 KD subnetworks were detected to be significantly perturbed in > 2 cell types at false discovery rate $< 5\%$. For example, the *LUM* subnetwork was upregulated in CAD endothelial cells ($P < 1.0e-16$; normalized enrichment score = 1.95), whereas down-regulated in resting CD14⁺ monocytes ($P < 1.0e-16$; normalized enrichment score = -1.68) and T cells ($P = 1.92e-3$; normalized enrichment score = -1.18), with a meta P value of $1.38e-13$ (Figure 2).

Scoring and Ranking of the KDs

We assigned a summary score to each KD according to the scores from measurements of KDA strength and consistency, subnetwork enrichment for 1000 genome-based CAD GWAS signals, and gene expression alterations between CAD and controls (top 20 genes are shown in Table 3). Based on the summary scores, the 10 top-ranked KDs are *LUM*, *COL1A1*, *DUSP6*, *HGD*, *MSN*, *CXCL12*, *DUSP1*, *GBP2*, *MTMR11*, and *CCL18*. It is reassuring that the ranking of most of these genes is consistent across the 3 scoring methods. For example, *LUM*, *COL1A1*, *HGD*, *MSN*, *CXCL12*, and *GBP2* are all within the top 40% in each of the 3 scoring schemes. Notably, none of the top 20 KDs was lipid-related genes although many lipid genes were among the 139 KDs.

In Silico Validation of the Top KD Genes

We conducted comprehensive bioinformatics mining of the 20 top-ranked KDs using various databases and tools. As summarized in Table 4, 8 of the top 20 genes have been linked to CAD or risk factors (eg, hypertension and diabetes) using at least one of the literature mining tools including PolySearch (<http://www.wishart.biology.ualberta.ca/polysearch/>), COREMINE (<http://www.coremine.com/medical/>), and Linguamatics (<http://www.linguamatics.com/>). *F2*, *SHC1*, and *CXCL12* have also been considered as CAD targets in drug development. The other genes including *LUM*, *LOXL2*, *ANXA3*, *SLC2A3*,

MSN, *GBP2*, *CCL18*, *SLIT3*, *HGD*, and *STAT3* represent novel CAD genes uncovered from a data-driven approach, with *LUM*, *LOXL2*, *ANXA3*, *SLC2A3*, and *STAT3* suggested as potentially promising druggable targets based on drug databases DrugBank and Pharmaprojects as well as literature^{30,31} (detailed in Materials and Methods in the online-only Data Supplement).

Biological Pathways and Networks of the Top KDs

We conducted pathway-enrichment analysis for the CAD subnetworks of the top 20 KDs using pathways curated in MSigDB to explore the potential biological mechanisms (details in Materials and Methods in the online-only Data Supplement). The top over-represented pathways for the sub-networks of the top KDs are shown in Table 5 and those for all the KD subnetworks are in Table V in the online-only Data Supplement. We found that the subnetworks of the top KDs are enriched in cardiovascular processes, including immune and inflammatory responses, coagulation, cell activation, and lipid metabolism (all Bonferroni-corrected $P < 0.01$ in Fisher exact test), consistent with findings from another recent integrative genomic study that focused on the overall CAD GWAS instead of the top loci.⁸ Notably, the subnetworks of 8 KDs, including *LUM*, *COL1A1*, *DUSP1*, *SLIT3*, *COL4A2*, *ANXA3*, and *SLC2A3*, were enriched for extracellular matrix (ECM) genes. ECM has been highlighted in recent integrative studies of both CVD and type 2 diabetes mellitus.^{9,32}

We further explored the potential relationships between the top KDs and the CAD GWAS genes in gene regulatory networks. As shown in Figure 3, the top KDs and their sub-networks are closely connected, forming a large network linking multiple processes including complement and coagulation cascades, lipid metabolism, ECM, interferon signaling, focal adhesion, and JAK–STAT signaling. Importantly, the ECM genes constitute the center of the network and connect with the other biological processes through tissue-specific interactions. In addition, although some KDs such as *COL4A2*, *CXCL12*, *SHC1*, *GBP2*, *GBP1*, and *SLIT3* are also top CAD GWAS genes, the majority of the CAD GWAS genes are not KDs but peripheral nodes interacting with one another through KDs in the network. This finding is consistent with our previous observations on the relationship between KDs and GWAS genes.^{8,9} Furthermore, the KDs also bring together many additional CAD candidate genes curated in the CADgene database, which are not necessarily among the top CAD GWAS genes we have focused on but nevertheless provide additional support for the relevance of the KD subnetworks to CAD.

The convergence of our data-driven ranking and literature-based evidence supports the robustness of the top KDs, pathways, and networks revealed from our study. The data-driven summary scores provide an objective means to prioritize CAD genes for future studies, whereas the literature information can be used in flexible ways to further prioritize genes for different purposes. For instance, the data-driven score highlights *LUM* as the top-ranked CAD KD. In conjunction with information that it (1) has not been previously reported for CAD, (2) is druggable, (3) is a part of the ECM process that is supported by multiple KDs and also has known connection to CAD, and (4) is a part of a CAD subnetwork connecting with other CAD-relevant pathways, the potential novel role of Lum in CAD warrants further examination. If literature support for CAD involvement is considered important in

prioritization, KDs *CXCL12* and *F2* can be ranked at the top because (1) extensive literature supports their connection to CAD and (2) they are known CAD drug target.

Discussion

The recent explosion in GWAS studies has contributed significantly to the identification of disease-associated genetic loci. However, the improved efficiency and accuracy in GWAS have not been translated into better biological insights and more effective therapeutic targets. As a result, incorporating other sources of evidence (such as gene regulation information and literature mining) is necessary to narrow down the candidate search space.^{16,33} As summarized recently,³³ candidate disease genes can be prioritized through various computational methods, many of which carry the bias toward better characterized known genes for a given disease. In this study, we applied a data-driven approach by utilizing diverse tissue-specific regulatory networks and protein–protein interaction networks, which do not solely rely on previous knowledge about gene and protein functions. We found gene subnetworks that are concentrated with top CAD GWAS genes and identified potential key regulators or KDs of the CAD networks. We further ranked the KDs based on additional layers of data-rich analyses to provide high-confidence candidate genes for CAD. Our analyses not only detected well-implicated CAD disease genes such as lipid genes, *COL4A2*, and *CXCL12*^{34–36} but also unraveled novel key regulators for CAD such as *LUM*, *HGD*, *F2*, *ANXA3*, and *STAT3*.

Among the novel KDs, *LUM* is ranked on the top. This gene has been previously associated with posterior amorphous corneal dystrophy and high myopia,³⁷ and there is little prior evidence for the link between CAD and *LUM*. *LUM* encodes lumican and belongs to a small leucine-rich proteoglycan protein family. SLRPs include lumican, decorin, biglycan, fibromodulin, keratocan, epiphycan, and osteoglycin. These proteins bind collagen fibrils and the highly charged hydrophilic glycosaminoglycans to regulate interfibrillar spacing.³⁸ We speculate that *LUM* might regulate the downstream collagen genes that are important for CAD development. This notion is supported by the literature evidence on the role of *LUM* in collagen fibrillogenesis in CAD^{39,40} and the enrichment of collagen genes such as *COL3A1* and *COL8A1* in the *LUM* subnetworks found in our analysis. Interestingly, the *LUM* subnetworks are also enriched for genes important for complement and coagulation cascades, such as *SERPING1*, *CFH*, and *TFPI*, which have been implicated in CAD development.^{41,42} We also found that the *LUM* subnetwork from the brain tissue contains estrogen signaling-related genes such as *EMPI*, *ANXA1*, *DCN*, *TGFBI*, and *PCOLCE*. Within the brain, estrogen signaling pathway regulates glucose transport, aerobic glycolysis, and mitochondrial function to generate ATP; estrogen signaling also coordinates the brain and peripheral on metabolic homeostasis.⁴³ The role of estrogen in angiogenesis, cellular regrowth, and downstream collagen formation has been well documented for cardiovascular diseases,^{44,45} and the connection between the small leucine-rich proteoglycan *LUM* with estrogen signaling revealed by our network analysis is intriguing and warrants further investigation. Notably, although our network selection included mostly peripheral and vascular tissues and cell types, *LUM* was identified as the no. 1 KD and the *LUM* brain subnetwork stood out as a key component of the CAD network. Based on the data, we feel that *LUM* and brain ECM perhaps play a previously underappreciated role in CAD.

Encouragingly, a recent independent data-driven study provides strong evidence that brain may be one of the key tissues for CAD.⁴⁶ However, it is possible that the brain ECM network derived from our analysis reflects the role of ECM in atherosclerotic plaque caused by homology in network organization between tissues. Without plaque data to assess this possibility in the current study, caution is required in the interpretation of the role of brain and brain ECM in CAD pathogenesis.

In addition to *LUM*, several other ECM genes including *COL1A1* and *COL4A2* were also ranked as top KDs. Even KDs that are not direct ECM components, such as *DUSP1*, *SLIT3*, *ANXA3*, and *SLC2A3*, are highly connected to ECM genes in their respective gene subnetworks, highlighting the importance of ECM. In the tissue-specific regulatory networks, the key regulators tend to bridge ECM with many other molecular mechanisms involved in CAD development,^{47,48} such as inflammatory responses, complement and coagulation, adhesion, and lipid metabolism (Table 5; Figure 3). These results suggest a central role of ECM in CAD, in agreement with the conclusion from a recent multiethnic genetic study,⁹ where the top KDs for the shared genetic signals between CAD and type 2 diabetes mellitus either encode ECM or are involved in cell–matrix interactions. It is postulated that the critical roles of ECM in cell integrity, cell adhesion, and cellular communication/signaling may underlie its connection with multiple CAD processes. Indeed, the importance of ECM in an array of complex diseases has been increasingly recognized.⁴⁹

Besides the ECM genes, we detected other novel or less well-studied key regulators for CAD, such as *STAT3*, *HGD*, and *GBP2*. Although their direct roles in CAD development are poorly implicated, the biological processes involved in their subnetworks are biologically plausible. For example, *STAT3* is a member of JAK–STAT signaling pathway, the principal signaling mechanism for a wide array of cytokines and growth factors and a critical pathway for many physiological activities such as immune development, hematopoiesis, and adipogenesis.⁵⁰ The activation of JAK–STAT signaling has been implicated as a significant contributing factor to the pathogenesis of CAD,⁵¹ and the interactions between *STAT3* and interleukin-6 signaling may play a role.⁵² In support of the interleukin-6 path, *STAT3* is connected to the CAD GWAS gene *IL6R* in the CAD network derived through our analysis (Figure 3). Moreover, *STAT3* is a hub linking multiple additional CAD GWAS genes such as *PTPN11*, *HNF1A*, and *PDGFRA* in the network. Another KD gene *HGD* encodes the enzyme homogentisate 1 to 2 dioxygenase, which is involved in the catabolism of the amino acids tyrosine and phenylalanine. Defects in *HGD* are responsible for alkaptonuria, a genetic disease with Mendelian autosomal recessive inheritance, by causing accumulation of a proinflammatory and pro-oxidative stress intermediate, 2,5-dihydroxyphenylacetic acid in connective tissues. Patients with alkaptonuria often have cardiovascular disease, kidney disease, and damages to other organs. In our data-driven networks (Figure 3), *HGD* is a KD connected with multiple apolipoproteins (eg, *APOA1*, *APOA5*, *APOC3*, and *APOC4*) and coagulation factors (eg, *PLG*, *SERPINC1*, *F13B*, and *F9*), supporting an intimate interaction between this enzyme and known CAD processes and implying an underappreciated role of this gene in CAD. Another novel KD, *GBP2*, has been identified as a possible control factor for tumor development and a marker of efficient T-cell response.⁵³ In our analysis, *GBP2* subnetworks in both blood and kidney were enriched with interferon signaling genes and may contribute to CAD by modulating inflammatory responses.

In addition to identifying both well-known CAD driver genes such as lipid metabolism genes and novel KDs for the top CAD GWAS genes, our network-based method also unravels intimate connections among the top CAD GWAS genes and pathways (Figure 3), which has not been achieved by previous efforts. For example, many CAD GWAS genes involved in lipid metabolism and transport (eg, *APOA1*, *APOA4*, *APOA5*, *APOB*, and *APOC1-C4*) are joined with coagulation and complement genes (eg, *PLG*, *SERPINC1*) via KD genes *HGD* and *F2*. This lipid metabolism-coagulation subnetwork is further connected to several immune and inflammatory subnetworks, which are in turn connected to ECM subnetworks. For other well-known lipid metabolism genes, such as *PCSK9*, *LDLR*, *LPL*, and *LIPA*, they tend to be spread around different subnetworks rather than strongly clustered (Figure 3), probably because of the limitations of gene regulatory networks in capturing biochemical and enzymatic reactions. These findings shed light on the complex interactions among CAD GWAS genes and between individual CAD processes via KDs, thus unraveling novel insights into the complex mechanisms of CAD development.

Recently, several pathway and network analysis of CAD utilizing CARDIoGRAM CAD GWAS have been conducted.^{8,32,54} These studies use the full set of GWAS, which includes both strong and subtle genetic signals to identify CAD pathways and networks. In contrast to these existing studies, our current analysis specifically focuses on the smaller number of strong and significant CAD GWAS loci. Encouragingly, our current analysis based on the top CAD loci revealed highly consistent pathways and networks when compared with the previous studies utilizing full GWAS data set. Lipid metabolism, complement and coagulation, ECM, inflammatory pathways, focal adhesion, peroxisome proliferator-activated receptors signaling, and cell cycle are all replicated in our current analysis. In addition, the current study involves more comprehensive coverage of networks, a novel, vigorous, and streamlined gene ranking and prioritization scheme, and incorporation of multidimensional data and resources for validation. As a result, the current study provides a detailed illustration of the network architecture connecting the top CAD candidate genes and pinpoint potential regulators of these top loci. The prioritized KDs with full annotation of their tissue-specific subnetworks, biological pathways, literature evidence, and druggability information represent a step further toward a better understanding of the top GWAS loci as well as clinical and therapeutic application of the recent powerful genetic discoveries.

Similar to several previous studies, here we again demonstrate that the KDs identified are not necessarily significant GWAS signals although their neighbors in gene networks tend to be GWAS signals.^{8,9,15} This phenomenon likely explains why GWAS hits have low selection pressure and commonality in the general population, and why each locus only explains a small fraction of heritability. These observations may argue against the importance of a majority of individual GWAS candidate genes as effective therapeutic targets because their subtle effects in disease modification. KDs, on the contrary, may serve as better drug targets because of their importance in regulating other GWAS genes. These hypotheses, however, need to be treated with caution. In fact, some of the GWAS genes such as *HMGCR* and *PCSK9* are proven, effective drug targets in CAD therapeutics, yet they were not detected as KDs in our study. Instead, genes *PMVK* and *LDLR* that functionally relate to the known targets were KDs in our analysis. One potential explanation is that the mostly transcription-based gene regulatory networks used in our analysis cannot capture the various types of

regulation important for CAD and thus may miss additional KDs. Although it is appealing to suggest that KDs are more potent drug targets based on network properties, this hypothesis awaits future validation. The importance of KDs in regulating large numbers of genes may also imply more severe side effects accompanying stronger therapeutic impact. Therefore, selection of KDs with partners and functions specific to CAD is necessary to avoid potential adverse effects.

The lack of signals of KDs in GWAS may be a result of negative selection pressure because of their critical roles in biological networks, as hypothesized and discussed by multiple previous studies.^{8,9,15} This negative pressure hypothesis is supported by the recent discovery of 3230 essential genes out of 31 345 human genes based on exome sequencing of 60 706 humans⁵⁵ (Exome Aggregation Consortium, <http://www.exac.broadinstitute.org/>). These essential genes demonstrate near-complete depletion of truncating variants and have no currently established human disease phenotypes because of the lack of functional variants. Among the top 20 KDs identified in our CAD study, 6 were among the essential genes, including *COL1A1*, *DUSP6*, *F2*, *MSN*, *SLIT3*, and *STAT3*, representing a significant enrichment of essential genes among the top KDs (fold change, 2.91; Fisher exact test, $P=0.0028$). Another recent study revealed that *BRD4* is essential for luminal cancer. However, there is lack of cancer GWAS signals in this gene.⁵⁶ Furthermore, in our previous comparison of KDs versus non-KDs for a set of inflammatory genes shared across >10 diseases,²⁶ we found 63.6% of KDs and 39.2% of non-KDs demonstrated observable phenotypes based on the Mouse Genome Informatics mouse phenome database. This represents a significantly higher percentage of genes with mutant phenotypes among KDs when targeted in mutant animals ($P=0.001$ by proportion test). Therefore, evidence from both humans and mice supports the essential role and stronger negative pressure of KDs. By focusing on gene networks connecting the top CAD GWAS genes and their KDs, our study offers a distinct angle to understand the complex CAD biology and detect more plausible therapeutic targets. In support of the therapeutic potential of the KDs, our study also provides evidence that many of the top KDs uncovered have been subject to drug development as druggable targets (Table 4) for multiple diseases, such as thrombosis, diabetes mellitus, and cancers. Based on their central locations in the network models, enrichment of CAD GWAS signals in their local subnetworks, and gene expression perturbations in myocardial infarction patients, these KDs are plausible potential targets for CAD.

Despite the advantages and novel discoveries discussed above, there are limitations in our study. First, the determination of candidate genes based on GWAS loci is not straightforward. Chromosomal location-based mapping lacks functional support, whereas functional data-supported mapping has incomplete coverage of tissue and lack of power in identifying weak *cis*-association and *trans*-regulation. Therefore, candidate gene mapping that was based on existing data can be far from comprehensive and may carry biases. To address these issues, we incorporated different CAD candidate gene lists according to different single nucleotide polymorphism-gene mapping approaches (ie, chromosomal location versus expression quantitative trait loci) and inclusion/exclusion criteria (eg, whether to consider or exclude genes in well-known lipid loci to avoid overshadowing novel genes and mechanisms by lipid genes). Interestingly, many top KDs do not seem to be

sensitive to the CAD gene curation parameters, supporting the robustness of the network structures and KDs. This carries important implications that the main biological pathways and networks of CAD are relatively stable despite that new genes are continuously discovered and inaccurate mapping of certain candidate genes by any particular mapping method may exist. Second, although we aim to conduct a comprehensive data-driven analysis, our investigation is limited by the availability of tissue-specific regulatory networks, which may introduce bias because networks from other critical cell or tissue types such as vascular smooth muscle cells are missed. Third, the gene networks used in our current analysis do not include other regulatory molecules or elements such as noncoding RNAs and enhances, which will miss essential key regulators that are not protein-coding genes, such as the noncoding ANRIL transcript for the 9p21 locus. Fourth, for the gene expression pattern analysis of the KD subnetworks between CAD and controls, we were not able to identify CAD transcriptomic data sets involving the tissue types perfectly matching those used for the gene networks. Refinement of our analysis is necessary when additional data sets become available in the future. Finally, our study represents a bioinformatics data mining effort and is exploratory in nature. Although we provide multiple lines of in silico evidence to support the importance of the KDs, experimental validation of the novel KDs for their roles in regulating the CAD GWAS genes, networks, and disease development is beyond the scope of the current study but warrant further investigation in future studies.

In summary, our study provides a novel integrative genomics framework to understand the relations among CAD GWAS genes and prioritize the genes impacted by genetic variations in CAD. Our findings offer novel mechanistic hypotheses about CAD pathogenesis and pinpoint potential drug targets to more effectively counteract CAD. Other complex diseases or phenotypes such as cancers, Alzheimer disease, and drug responses also involve multiple genetic determinants. Our analytic framework can be adapted for other disease areas to help generate new hypotheses and benefit drug target discovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

CAD	coronary artery disease
ECM	extracellular matrix
GWAS	genome-wide association studies

KD	key driver
KDA	key driver analysis

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Significance

Genome-wide association studies have successfully identified hundreds of regions in the genome that harbor genetic variants that increase risks for coronary artery disease (CAD). However, it has been challenging to understand the molecular mechanisms through which these loci contribute to CAD, and the clinical implications and utilities of these findings remain largely unknown. In this work, we designed and implemented an integrative method that leverages the genetic association strength, tissue-specific gene regulations, and gene expression perturbations in patients with CAD, to understand the relations among the CAD loci and prioritize causal genes for CAD that are more suitable for drug development. Our approach revealed intimate interactions among the genes underlying the CAD loci and identified potential novel regulators, many of which druggable, that coordinate the actions of CAD genes and pathways to impact CAD pathogenesis.

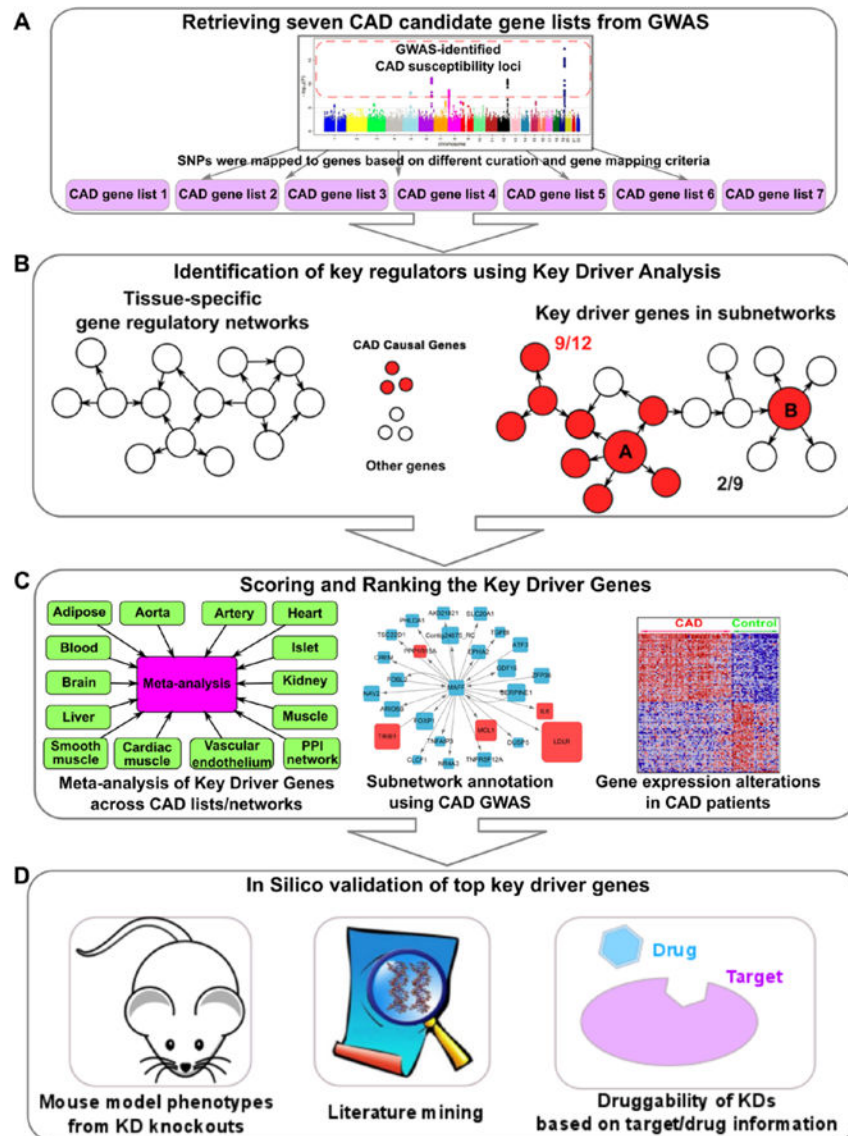


Figure 1.

A framework for prioritizing coronary artery disease (CAD) genes using network-based approaches. The workflow contains 4 steps, including (A) retrieving CAD gene lists from genome-wide association studies (GWAS) and other functional studies, (B) key driver (KD) analysis, (C) scoring and ranking based on network consistency of KD analysis, GWAS gene annotation, and gene expression profiles between myocardial infarction and normal subjects, and (D) in silico validation.

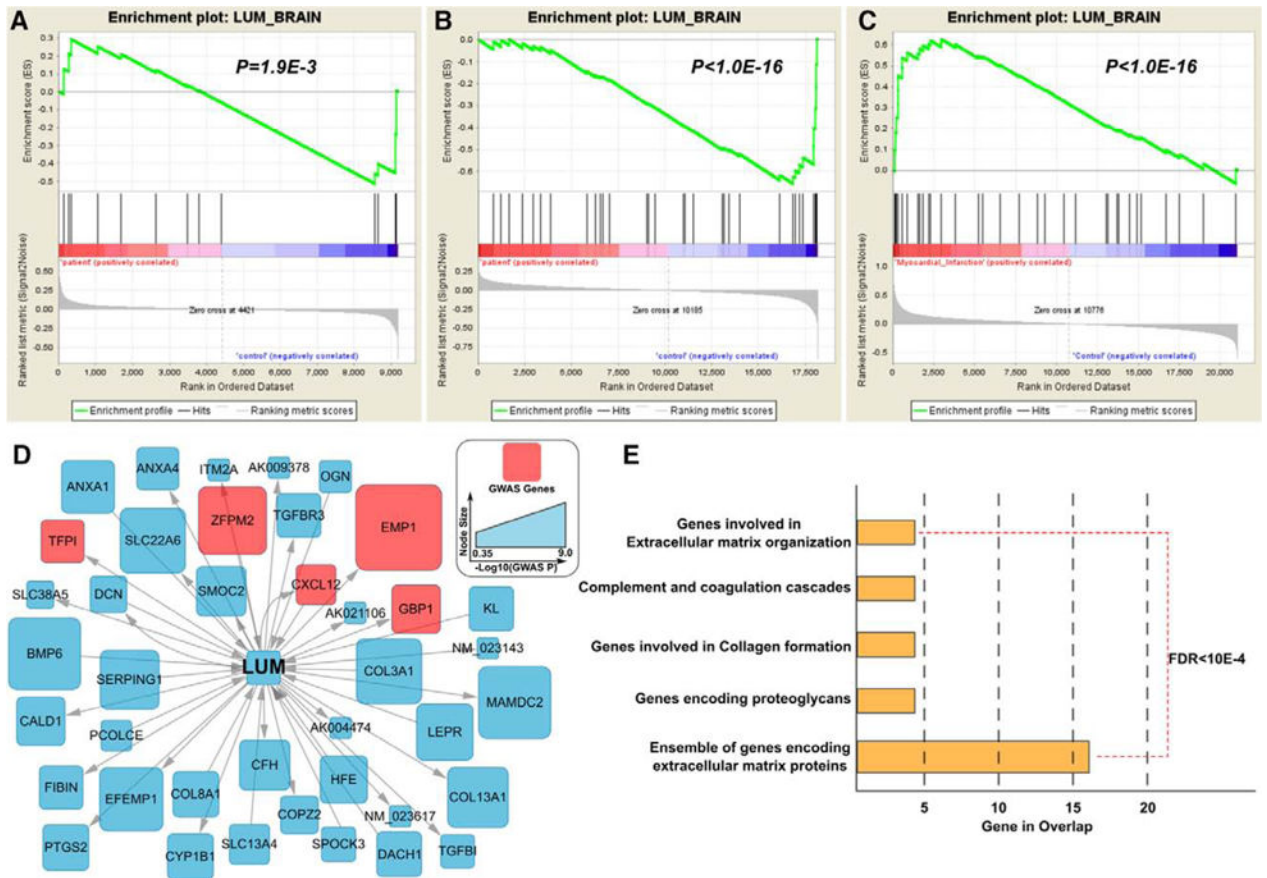


Figure 2. Gene expression perturbations of LUM subnetwork in brain tissue in myocardial infarction (MI) patients. The gene set enrichment analysis revealed significant gene expression perturbation in the LUM subnetwork in T cells (A), resting monocytes (B), and endothelial cells (C) in MI cases. Plots for the 7 nonsignificant cell or tissue types are not shown, but results are available in Table IV in the online-only Data Supplement. The red nodes in the LUM subnetwork (D) are coronary artery disease (CAD) genes from CAD extend list. Pathway analysis for the LUM subnetwork was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID), with all the pathways satisfying false discovery rate (FDR) $< 10e-4$ (E).

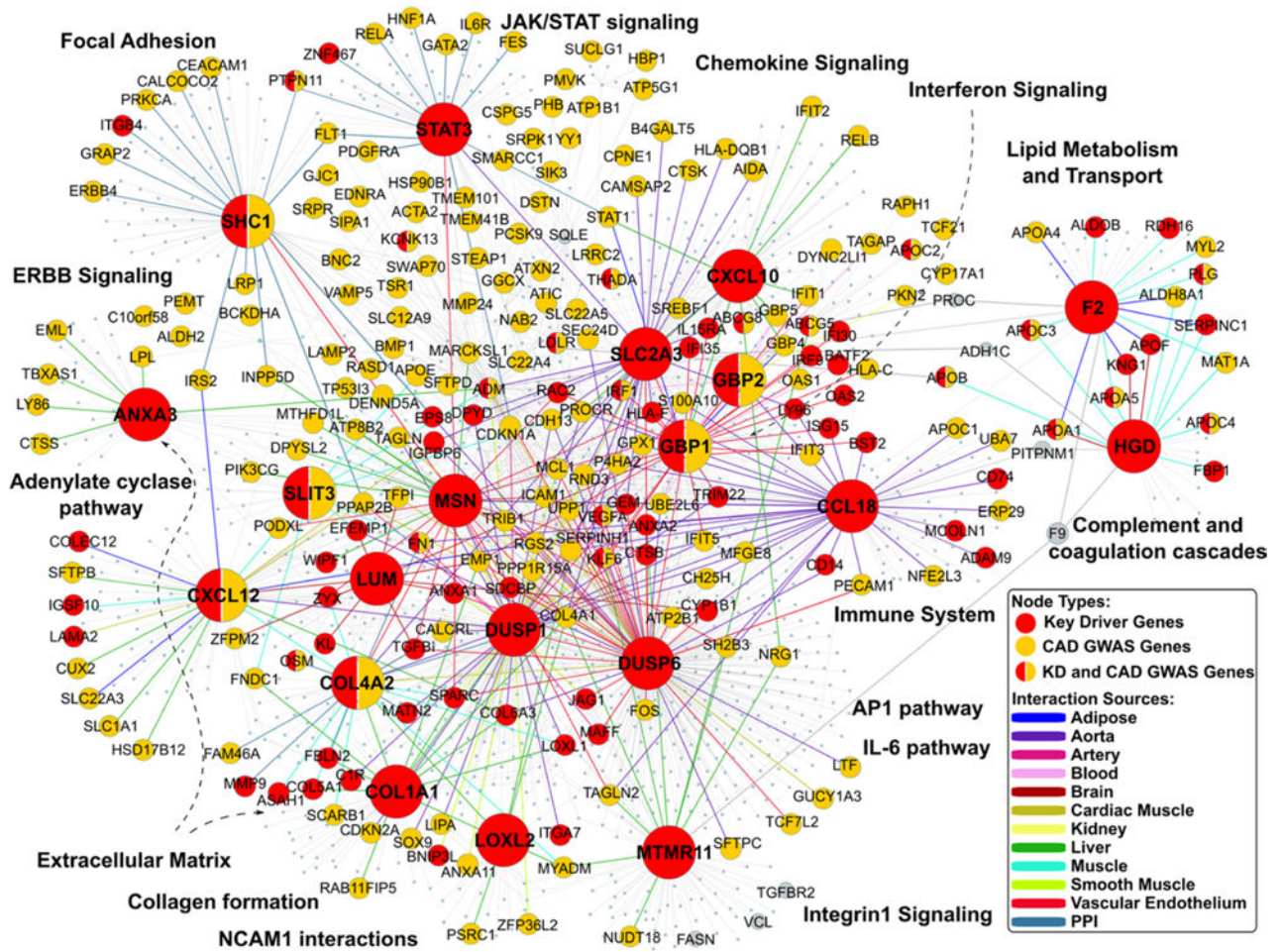


Figure 3. Top key driver (KD) subnetworks in tissue-specific gene regulatory and protein–protein interaction (PPI) networks. The largest nodes are the top 20 KDs. The nodes highlighted in red are KDs, whereas the yellow nodes are the coronary artery disease (CAD) genome-wide association studie (GWAS) genes. KDs that are also CAD GWAS genes are labeled half red and half yellow. The edge colors between genes denote the tissue or network sources of gene–gene interactions. JAK-STAT indicates Janus kinase-signal transducers and activators of transcription.

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Table 1

Top Multitissue KD Genes in Gene Regulatory and Protein-Protein Interaction Networks Ranked by KD Analysis Meta *P* Value Across CAD Gene Lists and Networks

KD	Ad	Ao	Ar	Bl	Br	CM	Ht	Is	Kd	Li	Mu	PP	SM	VE	Tissue Consist	List Consist	Meta <i>P</i> Values	KD <i>P</i> Values in 1000G CAD GWAS
<i>DUSP6</i>	0	2	2	0	0	3	0	0	0	0	0	0	6	2	5	15	1.35e-34	1.47e-03
<i>DUSP1</i>	0	1	6	0	0	0	0	0	0	0	0	0	4	0	3	11	2.77e-26	2.07e-03
<i>KNG1</i>	0	0	0	0	5	0	0	0	0	0	4	0	0	0	2	9	8.60e-15	2.65e-03
<i>F2</i>	5	0	0	0	0	0	0	0	0	0	4	0	0	0	2	9	4.22e-13	2.62e-04
<i>APOA1</i>	4	0	0	0	5	0	0	0	0	0	2	0	0	0	3	11	1.93e-09	5.60e-05
<i>HGD</i>	0	0	0	0	4	0	0	0	0	0	5	0	0	0	2	9	1.05e-08	4.15e-03
<i>GBP1</i>	2	0	0	2	0	0	0	0	0	0	0	0	0	2	3	6	3.41e-08	6.76e-04
<i>GBP2</i>	0	0	0	2	0	0	0	0	2	0	0	0	2	0	3	6	1.60e-07	6.76e-04
<i>ANXA1</i>	0	0	3	0	0	0	0	0	0	0	0	0	1	0	2	4	9.70e-07	9.12e-03
<i>PLG</i>	4	0	0	0	0	0	0	0	0	1	0	0	0	0	2	5	3.13e-06	2.88e-34
<i>COL4A2</i>	0	0	0	0	0	0	0	0	0	0	4	3	0	0	2	7	4.04e-06	1.83e-10
<i>MSN</i>	0	0	0	0	0	0	0	0	0	2	0	0	0	1	2	3	7.30e-06	1.00e+00
<i>ANXA2</i>	0	0	0	0	0	2	0	0	0	0	0	2	0	0	2	4	1.47e-05	3.43e-03
<i>CXCL12</i>	1	0	0	0	0	1	0	0	0	3	1	0	0	0	4	6	2.28e-05	2.95e-10
<i>ABCG5</i>	1	0	0	0	0	0	0	0	0	2	0	0	0	0	2	3	3.70e-05	1.35e-07
<i>APOB</i>	2	0	0	0	0	0	0	0	0	0	0	3	0	0	2	5	6.48e-05	3.09e-08
<i>ADM</i>	0	0	1	0	0	0	0	0	0	0	0	0	1	0	2	2	7.95e-05	2.77e-04
<i>RDH16</i>	0	0	0	0	2	0	0	0	0	0	2	0	0	0	2	4	0.000115	3.17e-03
<i>PAPSS2</i>	0	0	0	0	0	1	0	0	0	0	0	0	2	0	2	3	0.000136	4.68e-03
<i>APOA5</i>	0	0	0	0	1	0	0	0	0	2	0	0	0	0	2	3	0.0005	5.60e-05
<i>FNI</i>	0	0	0	0	0	0	0	0	1	0	0	3	0	0	2	4	0.000759	5.14e-07
<i>IRF7</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0.000912	8.68e-03

The numbers in each cell represent how many of the 7 CAD gene lists yielded the KD using the specific tissue network. Ad indicates adipose; Ao, aorta; Ar, artery; Bl, blood; Br, brain; CAD, coronary artery disease; CM, cardiac muscle; GWAS, genome-wide association studies; Ht, heart; Is, islet; Kd, kidney; KD, key driver; Li, liver; Mu, muscle; PP, protein-protein interaction; SM, smooth muscle; and VE, vascular endothelium networks.

Table 2

Top KDs Based on CAD GWAS Enrichment in the KD Subnetworks

KD	Subnetwork Size	No. of Genes With CAD 1000 Genomes GWAS $P < 0.001$ in KD Subnetworks	Genes With CAD 1000 Genomes GWAS $P < 0.001$ in KD Subnetworks	P Values Compared With GWAS Genes
<i>SLIT3</i>	28	11	<i>APOE, SLIT3, EPB41L2, NID1, FNI, TGFBR3, DOCK1, ADM, SCARA5, PROCR, THBS3</i>	<0.00114
<i>AZGP1</i>	23	8	<i>PLG, SLC22A1, UPB1, APOA5, AZGP1, ABCG5, CYP2A13, FBPI</i>	<0.00114
<i>APOF</i>	45	17	<i>PLG, APOC4, ITIH3, GLS2, APOB, APOA5, ARG1, APOF, AGXT, LIPC, ITIH4, MAT1A, PAH, PON1, CFI, APOC2, CYP2A13</i>	<0.00114
<i>LDLR</i>	11	7	<i>APOE, APOB, LRPAP1, LDLR, PCSK9, DAB1, AP1M2</i>	<0.00114
<i>ACCN1</i>	13	4	<i>LARGE, ACCN1, ITGA2, CYP17A1</i>	<0.00114
<i>RIFI</i>	11	2	<i>MALAT1, NBEAL1</i>	<0.00114
<i>STAT3</i>	102	35	<i>CDKN1A, PDGFRA, JUN, FGFR3, FLT1, CXCR4, RPS6KA5, BRCA1, HDAC3, STMN1, NLK, STAT5B, STAT3, ZNF467, MAP3K7, MTOR, NMI, RAC1, PML, KAT5, EIF2AK2, PRKCD, PTPN11, JAK3, FOXM1, PDGFRB, HNF1A, SUMO4, FES, IL2RA, IL6R, IGF1R, RELA, FER, ZNF148</i>	<0.00114
<i>OAS2</i>	12	4	<i>IFIT1, APOA4, OAS1, MMP12</i>	<0.00114
<i>F2</i>	Adipose (49); muscle (47)	Adipose (12); muscle (19)	Adipose (<i>PLG, SERPIND1, SEC14L2, APOC3, APOA5, APOA4, APOF, LIPC, PAH, AKR1C1, APOA1, F2</i>); Muscle (<i>PLG, SERPIND1, HPX, APOC3, ATP2A2, KLKB1, UPB1, TNNT1, APOA5, MYL2, ARG1, ASGR1, GNMT, MAT1A, PAH, AKR1C1, CFI, APOA1, F2</i>)	Adipose (0.0182); muscle (0.00114)
<i>HGD</i>	Brain (40); muscle (48)	Brain (8); muscle (12)	Brain (<i>PLG, APOC4, HPX, APOA5, APOF, PAH, AKR1D1, APOA1</i>); Muscle (<i>PLG, APOC4, CA5A, UROCI, APOC3, UPB1, APOB, APOA5, AGXT, MAT1A, CYP2A13, FBPI</i>)	Brain (0.00114); muscle (0.0136)
<i>LUM</i>	45	12	<i>CXCL12, COL3A1, TGFBR3, BMP6, FIBIN, TFPI, GBPI, ANXA4, SMOC2, SPOCK3, PCOLCE, ZFPM2</i>	0.00114
<i>MTMR11</i>	80	25	<i>DUSP8, ARHGAP22, ARL2BP, VCL, NRG1, SORBS2, TAGLN2, NID1, COL3A1, COL4A1, SMOX, SH2B3, ABCG5, MYO9B, FST, ARF5, HELLS, NUDT18, WFDC3, PDGFRB, SCD, SLC20A1, ST6GALNAC4, SGSM1, OTUD7B</i>	0.00114
<i>COL1A1</i>	73	23	<i>COL4A2, NCAM1, FBLN2, COL6A3, GINS2, TMSB10, ITGBL1, COL3A1, GAS6, COL4A1, LOXL4, WT1, SERPINH1, IGFBP2, CD163, PDGFRB, EFEMP2, MMP12, CCDC3, TREM2, EGR2, PCOLCE, LOXL1</i>	0.00114
<i>CXCL12</i>	Adipose (47); cardiac muscle (170); liver (30); muscle (36)	Adipose (10); cardiac muscle (54); liver (9); muscle (11)	Adipose (<i>PODXL, MMP3, CXCL12, TRERF1, KDR, SLC6A6, MRV1, KLK10, SLC22A3, RAB19</i>); Cardiac_muscle (<i>CAV2, PYGL, HLA-DRA, COL4A2, ZCCHC24, CTGF, CXCL12, PPAP2B, ST5, GRK5, AXL, LRPI, NID1, ZCCHC14, PECAM1, SNED1, FGFR1, EPS8, WIPF1, VPS13D, PTRF, HLA-B, CRISPLD2, TMEM176A, NR2F2, COL4A1, LIPA, ZHX3, NPR1, CXCL1, SLC24A3, SFRP2, RHOQ, LPAR1, MYLK, RAB13, SGK1, CD302, FTO, PRCP, USP25, TRIB2, ZBTB20, PSD3, TIMP3, WIP1, PCOLCE, LTBP1, LAMB2, GUCY1A3, LAMC1, ANGPT1, NFIB, KLC1</i>); Liver (<i>PLEKHA1, CXCL12, COL6A3, SLC2A1, RERG, SLC6A6, CUX2, HSD17B12, SFTPB</i>); Muscle (<i>COL4A2, AHR, PODXL, CXCL12, S100Z, SCUBE3, BRPF1, RNF213, ARHGDIB, CNIH3, OR4X2</i>)	Adipose (0.0534); cardiac muscle (0.0182); liver (0.00341); muscle (0.00114)

KD	Subnetwork Size	No. of Genes With CAD 1000 Genomes GWAS $P < 0.001$ in KD Subnetworks	Genes With CAD 1000 Genomes GWAS $P < 0.001$ in KD Subnetworks	<i>P</i> Values Compared With GWAS Genes
<i>CFB</i>	20	10	<i>PLG, APOC1, C3, APOA5, C4B, APOF, HP, CFI, APOC2, APOA1</i>	0.00114
<i>APOC2</i>	27	10	<i>APOC1, SLC22A1, APOC3, APOA5, AGT, CYP4F12, ARG1, ABCG5, APOF, APOC2</i>	0.00114
<i>RDH16</i>	Brain (43); muscle (36)	Brain (10); muscle (11)	Brain (<i>APOC4, SLC22A1, APOC3, KLKB1, MBL1P, APOA5, APOF, PONI, AKR1C1, CFI</i>); Muscle (<i>PLG, SEC14L2, APOC3, HAAO, APOB, ABCG5, C8G, APOA4, GNMT, CYP2A13, F2</i>)	Brain (0.0273); muscle (0.00114)
<i>NGRN</i>	32	10	<i>HDDC3, TRIM68, FURIN, KLHL25, RCCD1, SPC24, SLC22A3, MESDC1, APOC2, AKAP13</i>	0.00114
<i>FN1</i>	Kidney (16); PPI (75)	Kidney (3); PPI (21)	Kidney (<i>ERBB4, ADAM12, FN1</i>); PPI (<i>COL4A2, FBLN2, COL4A3, ITGB6, CXCL12, FSTL3, MMP9, GALNT6, FN1, COL7A1, COL4A1, SDC2, FST, LPA, FASLG, COL4A4, SMAD9, MEP1B, LTBPI, IGFBP5, FBLN1</i>)	Kidney (0.00114); PPI (0.0477)
<i>NCOR2</i>	12	7	<i>NCOR2, ACAD10, UBC, AACS, CUX2, PTPN11, VPS33A</i>	0.00114

The detailed information for all the KD genes is available in Table III in the online-only Data Supplement. CAD indicates coronary artery disease; GWAS, genome-wide association studies; KD, key driver; and PPI, protein-protein interaction.

Table 3
Top 20 KDs Based on an Overall Ranking Score Derived From 3 Data-Driven Criteria

KD	Meta <i>P</i> Value for KDA	<i>P</i> Value for GWAS Enrichment	Meta <i>P</i> Value for Differential Expression	Score 1 (KDA Strength and Consistency)	Score 2 (Subnetwork Enrichment for CARDIoGRAM-C4D GWAS Signals)	Score 3 (Gene Expression Alterations Between CAD and Controls)	Summary Score
<i>LUM</i>	5.74e-08	1.14e-03	1.38e-13	0.935	0.923	0.978	2.836
<i>COL1A1</i>	4.13e-06	1.14e-03	1.55e-05	0.848	0.923	0.819	2.590
<i>DUSP6</i>	1.35e-34	6.82e-03	1.00e-16	1.000	0.538	1.000	2.538
<i>HGD</i>	1.05e-08	1.14e-03	3.76e-02	0.957	0.923	0.616	2.496
<i>MSN</i>	7.30e-06	4.55e-03	4.44e-16	0.826	0.619	0.993	2.438
<i>CXCL12</i>	2.28e-05	1.14e-03	1.62e-02	0.797	0.923	0.652	2.372
<i>DUSP1</i>	2.77e-26	1.02e-02	2.46e-08	0.993	0.427	0.928	2.348
<i>GBP2</i>	1.60e-07	3.41e-03	9.42e-04	0.920	0.698	0.725	2.343
<i>MTMR11</i>	8.93e-07	1.14e-03	3.61e-01	0.913	0.923	0.486	2.322
<i>CCL18</i>	1.39e-06	9.09e-03	8.61e-11	0.877	0.474	0.949	2.300
<i>SLIT3</i>	2.04e-04	1.00e-03	2.44e-01	0.717	1.000	0.507	2.225
<i>GBP1</i>	3.41e-08	1.82e-02	1.05e-11	0.942	0.226	0.964	2.132
<i>F2</i>	4.22e-13	1.14e-03	9.53e-01	0.971	0.923	0.232	2.126
<i>COL4A2</i>	4.04e-06	5.68e-03	1.94e-03	0.855	0.582	0.688	2.126
<i>LOXL2</i>	1.68e-04	3.41e-03	2.96e-02	0.725	0.698	0.638	2.061
<i>CXCL10</i>	2.74e-04	1.02e-02	3.37e-12	0.659	0.427	0.971	2.058
<i>ANXA3</i>	3.06e-03	1.14e-03	7.02e-07	0.246	0.923	0.877	2.046
<i>SLC2A3</i>	2.74e-04	7.95e-03	4.76e-07	0.652	0.500	0.884	2.036
<i>SHC1</i>	4.26e-03	1.14e-03	1.32e-09	0.167	0.923	0.942	2.032
<i>STAT3</i>	3.50e-03	1.00e-03	3.27e-05	0.210	1.000	0.812	2.022

CAD indicates coronary artery disease; CARDIoGRAM-C4D, Coronary Artery Disease Genome Wide Replication and Meta-Analysis Plus the Coronary Artery Disease Consortium; KDA, key driver analysis; and GWAS, genome-wide association studies.

Table 4
In Silico Validation of the Top 20 Key Driver Genes Using Text-Mining and Drug Information

KD	PolySearch (Z Score >0.5)	COREMINE (Significance <10 ⁻⁴)	Top Associated Diseases in Linguamatics (PubMed >5)*	Drug Development [†]	Druggability [‡]
<i>LUM</i>	No	No	Melanoma, myopia, breast neoplasms	N/A	Yes
<i>COL1A1</i>	No	Yes	Osteogenesis imperfecta, otosclerosis, osteoporosis	Cancer, solid, unspecified; macular degeneration, age; scleroderma	Yes
<i>DUSP6</i>	No	No	Malignant neoplasm pancreas, carcinogenesis, obesity	N/A	N/A
<i>HGD</i>	No	No	Alkaptouria, metabolic diseases, Barrett esophagus	N/A	N/A
<i>MSN</i>	No	No	Neoplasm metastasis, pseudohermaphroditism female	N/A	N/A
<i>CXCL12</i>	Yes	Yes	Diabetes mellitus, ischemia, myocardial infarction	Infection, HIV/AIDS; cancer, unspecified; infarction, myocardial	Yes
<i>DUSP1</i>	No	No	Malignant neoplasm, diabetes mellitus	N/A	N/A
<i>GBP2</i>	No	No	Breast neoplasms, neoplasm metastasis, vesicular stomatitis	N/A	N/A
<i>MTMR11</i>	No	No	Obesity, prostatic neoplasms, atherosclerosis	N/A	N/A
<i>CCL18</i>	No	No	Breast carcinoma	N/A	N/A
<i>SLIT3</i>	No	No	Breast neoplasms, rheumatoid, brain ischemia	N/A	N/A
<i>GBP1</i>	No	No	Inflammatory bowel diseases, vascular diseases	Anticancer, other; Gene therapy	Yes
<i>F2</i>	Yes	Yes	Thrombosis, myocardial infarction, stroke	Thrombosis; cardiovascular disease, unspecified; atherosclerosis	Yes
<i>COL4A2</i>	No	Yes	Anoxia, cerebral hemorrhage, edema	N/A	N/A
<i>LOXL2</i>	No	No	Tumor progression	Anticancer, other; hepatoprotective; respiratory; vulnherary	Yes
<i>CXCL10</i>	No	Yes	Communicable diseases, infection, inflammation	Traumatic brain injury; Crohn's disease; arthritis, rheumatoid	Yes
<i>ANXA3</i>	No	No	Neoplasm metastasis, ovarian neoplasms, prostatic neoplasms	N/A	Yes
<i>SLC2A3</i>	No	No	Carcinoma, squamous cell; lipidoses	N/A	Yes
<i>SHC1</i>	Yes	No	Breast neoplasms, carcinogenesis, diabetes mellitus	Neurological disease, unspecified; cardiovascular disease, unspecified	N/A
<i>STAT3</i>	No	No	Breast neoplasms, carcinogenesis, inflammation	Inflammatory disease, unspecified; cancer, unspecified; Alzheimer disease	Yes

N/A indicates not applicable.

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* The key driver genes were searched against all the disease PubMed databases using Linguamatics.

† The drug target information was collected from DrugBank and Pharmaprojects databases. The listed diseases were the most targeted ones in the drug development.

‡ The druggability information of the key drivers was retrieved from.^{30,31}

Table 5

Functional Enrichment of Subnetworks of Top 20 KDs

KD	Subnetwork Sources	Subnetwork Size	Over-Represented Pathway Categories
<i>LUM</i>	Brain	45	ECM; proteoglycans
<i>COL1A1</i>	Liver	73	ECM; collagen formation
<i>DUSP6</i>	Aorta	253	FRA pathway
<i>DUSP6</i>	Artery	50	IL-6, IL-7 pathway; syndecan 4 pathway
<i>DUSP6</i>	Cardiac muscle	108	API pathway; IL-6, IL-7 pathway
<i>DUSP6</i>	Smooth muscle	17	MAPK targets nuclear events mediated by map kinases; ERBB1 downstream pathway
<i>DUSP6</i>	Vascular endothelium	122	IL-6, IL-7 pathway
<i>HGD</i>	Brain	40	PPAR signaling pathway; synthesis of bile acids and bile salts via 7alpha hydroxycholesterol
<i>HGD</i>	Muscle	48	Complement and coagulation cascades
<i>MSN</i>	Liver	56	Fcγ R-mediated; CXCR4 pathway
<i>MSN</i>	Vascular endothelium	105	Focal adhesion; integrin1 pathway
<i>CXCL12</i>	Adipose	47	Adenylate cyclase activating and inhibitory pathway
<i>CXCL12</i>	Liver	30	Intestinal immune network for IGA production; amino acid and oligopeptide SLC transporters
<i>DUSP1</i>	Aorta	179	ECM
<i>DUSP1</i>	Artery	71	Integrin1 pathway
<i>DUSP1</i>	Smooth muscle	63	HIF1 pathway; FRA pathway
<i>GBP2</i>	Blood	20	Interferon signaling
<i>GBP2</i>	Kidney	12	Interferon signaling
<i>MTMR11</i>	Liver	80	Integrin3 pathway
<i>CCL18</i>	Aorta	214	Immune system
<i>SLIT3</i>	Muscle	28	ECM
<i>GBP1</i>	Adipose	25	Interferon signaling
<i>GBP1</i>	Blood	18	Interferon signaling
<i>GBP1</i>	Vascular endothelium	92	Interferon signaling
<i>F2</i>	Adipose	49	Complement and coagulation cascades; AMI pathway
<i>F2</i>	Muscle	47	Complement and coagulation cascades; intrinsic pathway
<i>COL4A2</i>	Muscle	28	Collagens
<i>COL4A2</i>	PPI	19	ECM; platelet amyloid precursor protein pathway
<i>LOXL2</i>	Liver	28	Collagen formation; NCAM1 interactions
<i>CXCL10</i>	Liver	46	Chemokine signaling pathway
<i>ANXA3</i>	Liver	49	Matrisome associated; ECM regulators
<i>SLC2A3</i>	Aorta	190	ECM; P53 downstream pathway
<i>SHC1</i>	PPI	118	ERBB signaling pathway; focal adhesion
<i>STAT3</i>	PPI	102	JAK-STAT signaling pathway; pathways in cancer

Fisher exact test was performed for the enrichment of the KD subnetworks in MSigDB canonical pathway database. Only the top 2 functional terms are shown based on the *P* values with Bonferroni corrections. All the pathways shown satisfy Bonferroni-corrected $P < 0.01$. ECM indicates extracellular matrix; KD, key driver; PPAR, peroxisome proliferator-activated receptors; and PPI, protein–protein interaction.

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