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Quantitative and Systems Pharmacology 4. Network-based Analysis of Drug Pleiotropy on Coronary Artery Disease

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Abstract

Despite recent advance of therapeutic development, coronary artery disease (CAD) remains one of the major issues to public health. The use of genomics and systems biology approaches to inform drug discovery and development have offered the possibilities for new target identification and *in silico* drug repurposing. In this study, we propose a network-based, systems pharmacology framework for target identification and drug repurposing in pharmacologic treatment and chemoprevention of CAD. Specifically, we build *in silico* models by integrating known drug-target interactions, CAD genes derived from the genetic and genomic studies, and the human protein-protein interactome. We demonstrate that the proposed *in silico* models can successfully uncover approved drugs and novel natural products in potentially treating and preventing CAD. In case studies, we highlight several approved drugs (e.g., fasudil, parecoxib, and dexamethasone) or natural products (e.g., resveratrol, luteolin, daidzein and caffeic acid) with new mechanism-of-action in chemical intervention of CAD by network analysis. In summary, this study offers a powerful systems pharmacology approach for target identification and *in silico* drug repurposing on CAD.

Graphical Abstract

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Keywords

Drug repurposing; drug-target network; systems pharmacology; genomics; protein-protein interaction; coronary artery disease; natural product

1 INTRODUCTION

Coronary artery disease (CAD) is the leading cause of morbidity and mortality worldwide among multiple types of cardiovascular diseases [1, 2]. Although technological advances have been successfully used to develop new treatment, the mortality rate of CAD has remained unimpressive owing to the heterogeneous factors of causing disease, including genetic factors, environmental factors, and life styles [3]. According to the data from National Health and Nutrition Examination Survey (NHANES) from 2011 to 2014, an estimated 16.5 million adults (age ≥ 20) in America suffer from CAD with the prevalence of 7.4% for males and 5.3% for females [4]. Meanwhile, the estimated direct and indirect cost of myocardial disease in United States (U.S.) is approximate 200 billion annually, placing a heavy burden for economic society [4, 5]. Currently, antihypertensive drugs, lipid reducing drugs, and antiplatelet drugs are the most common medications for treatment or prevention of myocardial diseases (e.g., CAD). However, due to heterogeneous population on CAD, current approved agents are not efficient enough to reduce the disease burden as well as mortality. Thus, it is a pressing need to develop novel treatment and chemoprevention strategies for CAD.

Drug repurposing or repositioning has been recognized as powerful approaches [6, 7] toward treatment of various complex diseases, including CAD [8]. Drug repurposing focuses on the detection and development of new clinical indication from approved drugs, which significantly shortens the time and reduces the cost from random clinical trials and drug development. In addition to approved drugs with ideal pharmacokinetics and well-known pharmacodynamics profile, natural products have been demonstrated another abundant medical resources for developing new treatment strategies [9, 10]. Chinese medicine, including various medicinal natural products, has achieved great success to the health and well-being of the people in Asia [11]. Natural products derived from Chinese medicine have been used to treat or prevent a group of diseases including cardiovascular disease [12]. Accumulating evidence has suggested that Chinese medicine is effective for stable CAD patients, which might serve as a complementary and alternative strategy to the primary prevention of CAD [13, 14]. Altogether, identification of effective treatment and chemoprevention strategies from approved drugs and natural products offer possible strategies to reduce morbidity and mortality of CAD. However, effective identification of approved drugs or natural products with novel mechanism-of-action for treatment of CAD is

very challenging for traditional experimental approaches owing to lack of ideal *in vitro* and *in vivo* models.

The traditional drug discovery approach focusing on “one drug, one target, one disease” is suboptimal for leading to off-target toxicity or unintended beneficial effects. Quantitative and systems pharmacology combines computational and experimental tools toward discovering novel therapeutic agents and understanding of the therapeutic mechanisms of complex diseases [10, 15]. Recent development of systems pharmacology approaches have offered new insights into drug discovery and development, especially focusing on approved drugs (e.g., drug repurposing) and natural products [9, 16–18], offering the possibilities for development of new therapeutic agents and prevention strategies in CAD. For example, recent studies have applied systems pharmacology approaches for discovering new indications for natural products (e.g., resveratrol and quercetin) via unique integration of drug-target interaction (DTI) network and disease-associated genes, in potentially treating cancer and aging disorders [16, 17].

In this study, we presented an integrated systems pharmacology framework (Fig. 1) for development of new therapeutic and prevention strategies in CAD by focusing on approved drugs and natural products. Specifically, we built two drug-target networks for both approved drugs and natural products respectively via assembling experimentally validated drug-target interactions from 11 chemoinformatics and bioinformatics sources (see Methods). We then built *in silico* models to predict new potential associations of approved drugs or natural products on CAD by incorporating drug-target networks and known CAD gene products (proteins) from genetic and genomic studies into the human protein-protein interaction (PPI) network. We demonstrated that our systems pharmacology approaches can identify new therapeutic indications of approved drugs or uncover natural products in treatment and chemoprevention of CAD potentially. If broadly applied, the systems pharmacology-based framework presented here offers useful *in silico* tools for development of new treatment and chemoprevention strategies for CAD and other complex diseases.

2 RESULTS

2.1 Drug–target network analysis in CAD

We constructed the drug-target network for approved drugs (DTnet) and the compound-protein interaction network for natural products (NPnet) respectively by uniquely integrating multiple types of experimental data (see Methods). The DTnet contains 11,333 drug-target interactions connecting 1,812 approved drugs and 1,335 human targets (proteins), and NPnet contains 6,229 compound-protein interactions connecting 500 unique natural products and 2,020 human targets (Table 1). Interestingly, the average degree (connectivity) of natural products (12.46 ± 1.45 , $P = 3.069 \times 10^{-6}$, Fig. S1) in NPnet is significantly stronger than that of approved drugs (6.25 ± 0.33) based on data from DrugCentral database [19], suggesting a significant ‘promiscuity’ of natural products. The detailed information of two drug-target networks are provided in Table S1.

We further reconstructed two disease-centered drug-target networks (Table S1) by focusing on approved drugs or natural products that specifically target proteins encoded by CAD

genes: CADDTnet and CADNPnet (see Methods). Figure 2 displays a bipartite drug-target network for CADDTnet that contains 931 drug-target interactions connecting 634 approved drugs and 58 known CAD proteins (e.g., angiotensin-converting enzyme [ACE] and HMG-CoA Reductase [HMGCR]). Figure 3 displays a bipartite drug-target network for CADNPnet that contains 395 compound-protein interactions connecting 170 unique natural products and 71 CAD proteins. We examined the protein overlap between CADDTnet and CADNPnet. In total, 37 CAD proteins targeted by both approved drugs and natural products, including ACE, nitric oxide synthase (NOS3), phosphodiesterase 5A (PDE5A), and HMGCR. Interestingly, we found that 24.1% (14/58) of CAD proteins in CADDTnet and 38.0% (27/71) of that in CADNPnet are derived from genome-wide association studies (GWAS). This implies that GWAS may contribute to the identification of CAD targets for approved drugs and natural products, consistent with a previous study [20].

We found that the average degree (2.32 ± 0.20 , $P = 0.0027$ [one-side Wilcoxon test], Fig. S2) of natural products in CADNPnet is slightly stronger than that of approved drugs (1.47 ± 0.03) in CADDTnet, further suggesting the ‘promiscuity’ of natural products (Fig. S1). Among 170 natural products (Fig. 3), 9 natural products targeting CAD proteins have degree (K) greater than 8: quercetin ($K = 14$), resveratrol ($K = 14$); hexopyranose ($K = 14$), genistein ($K = 11$), beta-D-Mannose ($K = 11$), arachidonic acid ($K = 9$), daidzein ($K = 9$), acetaldehyde ($K = 9$), and alpha-D-Mannose ($K = 9$). Of note, several natural products (e.g., quercetin and genistein) have been reported to exert cardio-protective effects [21, 22]. For example, quercetin supplementation can reduce the risk of cardiovascular disease in an epidemiologic study [21]. Figure 3 shows that quercetin binds with 14 known CAD proteins, including 3 CAD proteins (NOS3, TGFB1 and VEGFA) identified by GWAS. Quercetin was reported to attenuate endothelial oxidative damage by modulating the endothelial NO synthase (NOS)-related signaling pathway in human umbilical vein endothelial cells (HUVECs) [22]. Genistein interacts with 11 CAD proteins, including 5 CAD proteins (FN1, LDLR, NOS3, TGFB1 and VEGFA) derived from GWAS (Fig. 3). Genistein was reported to regulate low-density lipoprotein receptor (LDLR) expression *in vitro* [23].

Altogether, drug-target network analysis suggests that genetic studies (e.g., GWAS) may contribute to the target identification in CAD. In addition to approved drugs, natural products further offer novel candidates in development of new chemoprevention strategies on CAD. These network analyses promote us develop *in silico* models to further uncover potential therapeutic strategies for CAD by exploiting both approved drugs and natural products.

2.2 Chemical diversity of approved drugs and natural products on CAD

To investigate the chemical feature of known therapeutic agents on CAD, we performed clustering analysis (Fig. 4) for 634 approved drugs and 170 natural products that target at least one CAD protein. The chemical clustering analysis was performed by computing the root-mean-square-difference (RMSD) of the Tanimoto distance of pairwise compounds using FCFP_4 fingerprint implemented in Discovery Studio 4.0 (version 4.0, Accelrys Inc.). In total, 170 natural products are clustered into 10 chemical groups with cluster centers: hexadecane, cysteine, toluene, spermine, luteolin, acetaldehyde, cetyl alcohol, oleic acid,

acetic acid, and ligustrazine, respectively (Fig. 4A). Among them, cluster 5 (Cluster center: luteolin) are represented as flavonoids, with the largest number (N=77) of natural products. For example, kaempferol [24] and quercetin [25] belonging to flavonoids, have been reported to reduce the risk of CAD [26]. In addition, 634 approved drugs in CADDtNet are clustered into 10 groups with cluster centers: diprophylline, isoflurane, dilevalol, dodecanoic acid, toliprolol, betamethasone, esflurbiprofen, encainide, clomipramine, and diclofenac. Compared with natural products, the second largest cluster (cluster 6) of approved drugs is steroids, including 96 approved drugs (Fig. 4B). For example, digoxin, a glycoside with steroid scaffold, is an FDA-approved drug for congestive cardiac insufficiency, arrhythmias and heart failure [27]. However, a recent study has revealed that long-term anabolic-androgenic steroid use may cause adverse cardiovascular complications, such as myocardial dysfunction and accelerated coronary atherosclerosis [28]. Taken together, natural products (i.e., flavonoid) and approved drugs (i.e., steroids) show unique chemical features (Fig. 4), offering diverse pharmacologic mechanisms-of-action for deep understanding of cardiovascular therapeutics *versus* side effects of approved drugs and natural products.

2.3 Uncovering new association of CAD with approved drugs or natural products

We next turn to build *in silico* models for predicting new associations of CAD with approved drugs or natural products through integrating experimentally validated drug-target networks, known CAD proteins, and the human protein-protein interactions. By applying the threshold of adjusted p-value (q) < 0.05, we computationally identified potential associations for 61 approved drugs (Fig. 5A) and 46 natural products (Fig. 6A) on CAD. In total, 51 approved drugs as well as 27 natural products are predicted to have significant associations on CAD ($q < 10^{-5}$), including several well-known anti-CAD natural products (e.g., quercetin and luteolin) and known cardiovascular drugs (e.g., eplerenone and metoprolol).

Based on 19 approved CAD drugs defined by first-level Anatomical Therapeutic Chemical (ATC) classification codes [29], we found that the area under the ROC curve (AUC) was 72 % (Fig. S3), which is comparable to a previous network proximity approach [30]. We further retrieved previously reported literature data of potential associations for the 61 significantly predicted, approved drugs and 46 natural products on CAD (Table S2). In total, we found 24 approved drugs (a success rate of 39.3 % [24/61]) and 19 natural products (a success rate of 41.3 % [19/46]) that have the reported experimental data to support the predicted anti-CAD effects, suggesting a high hit rate compared to traditional virtual screening approaches [31, 32]. After excluding the compounds with reported experimental evidences, the remaining 37 approved drugs and 27 natural products offer novel, potential anti-CAD candidates.

2.4 Mechanism-of-action to approved drugs on CAD: a network-based analysis

We next selected three typical categories of approved drugs (Those three drugs have the most reported experimental evidences), including Rho-kinase (ROCK) inhibitors, nonsteroidal anti-inflammatory drugs (NSAIDs), and glucocorticoids, to illustrate the mechanism-of-action on CAD.

Rho-kinases has been identified as potentially therapeutic targets for developing anti-cardiovascular agents [33]. Fasudil, known as ROCK inhibitors, is under investigating to treat cardiovascular disease in several clinical trials, including atherosclerosis (NCT00120718) and carotid stenosis (NCT00670202). As shown in Fig. 5B, fasudil interacts with only one CAD protein (encoded by CCL2) as well as 4 PPI partners (encoded by PRKACA, MYLK, ROCK1 and ROCK2). Obviously, fasudil cannot be predicted to have significant indication on CAD based on the curated CAD protein (CCL2) only. After adding 4 PPI partners, fasudil is predicted to have a significant association on CAD ($Z=4.90$, $q=0.04$). Inhibition of ROCK was reported to offer an alternative strategy to treat CAD in a preclinical study [34]. Fasudil has been reported to be a strong inhibitor on both ROCK1 ($IC_{50}=0.26 \mu M$) and ROCK2 ($IC_{50}=0.32 \mu M$) [35]. Interestingly, fasudil has been reported to ameliorate myocardial ischemia in patients with coronary microvascular spasm [36].

Cardiovascular complications account for the withdrawal of multitudinous post-marketed drugs [37]. NSAIDs have been widely reported to induce various adverse effects, especially in patients with cardiovascular complications [38]. For example, parecoxib, a cyclooxygenase-2 (COX2) selective inhibitor, is approved for short term perioperative pain control in the European Union [39]. Parecoxib has been reported to increase the risk of cardiovascular events [40]. Fig. 5B shows that parecoxib binds with both COX1 and COX2 (encoding by *PTGS1* and *PTGS2*) involving CAD. Herein parecoxib was predicted to have a significant association with CAD ($Z=4.75$, $q<10^{-5}$, Fig. 5B), consistent with previous experimental reports [40]. Parecoxib has been reported to be a strong inhibitor of COX2 ($IC_{50}=5 \text{ nM}$) [41], while inhibition of COX2 contributes to several cardiovascular complications, such as coronary atherothrombosis and heart injury due to the decrease of prostaglandin E2 (PGE2) and prostacyclin (PGI2) [42].

Cardiovascular adverse effects are essential concerns for glucocorticoids as well [43, 44]. The understanding of the molecular mechanism of cardiotoxicities of glucocorticoids might offer potential prevention strategies for reducing cardiovascular events, including CAD. Dexamethasone, known as a glucocorticoid agonist, was approved for treatment of multiple types of human diseases [45]. As displayed in Fig. 5B, dexamethasone binds with 4 CAD proteins (NR3C1, NR3C2, IL5, and PTGS2) and two PPI partners (ANXA1 and PGR).

Here dexamethasone was predicted to have potential association with CAD ($Z=6.51$, $q<10^{-5}$), consistent with the clinically reported cardiotoxicities [46]. Activation of glucocorticoid receptor (NR3C1), has been reported to induce cardiovascular complications such as obesity and hypertension [43]. Dexamethasone is a high effective glucocorticoid receptor agonist with half maximal effective concentration (EC_{50}) of 0.1 nM [47], which may help explain the molecular mechanism of its cardiovascular complications.

2.5 Mechanism-of-action to natural products on CAD: a network-based analysis

Previous studies have reported that several Chinese medicines have potential protective effects on CAD [13, 48]. However, the detailed molecular mechanism of protective effects of Chinese medicine on CAD remain unclear. We next turned to illustrate mechanism-of-action of resveratrol and luteolin on cardiovascular systems via a network-based analysis

Resveratrol, a naturally occurring phenol abundant in the skin of grapes and in red wine, shows cardiovascular protective effects in multiple preclinical studies [49–51]. There are over 12 clinical trials (<http://clinicaltrials.gov/>) being conducted or completed to test resveratrol's therapeutic effects on CAD (e.g., NCT02137421) or other cardiovascular diseases (e.g., NCT01449110 and NCT01564381). Fig. 6B shows that resveratrol binds with 15 CAD proteins and 35 PPI partners (e.g., SIRT1). Herein resveratrol was predicted to show a significant association on CAD ($Z = 9.64$, $q < 10^{-5}$). Activation of sirtuins 1 (SIRT1) has been reported to offer therapeutic effect on CAD [52]. Fig. 6B shows that resveratrol not only targets two CAD proteins (IRS1 and NOS3) but also a PPI partner (SIRT1, $EC_{50} = 23.6 \mu\text{M}$ [53]), suggesting a protective mechanism of resveratrol on CAD.

Luteolin, a flavonoid rich in fruits and herbs, has been reported to show cardio-protective effects *in vitro* and *in vivo*, including CAD, heart failure, and atherosclerosis [54, 55]. The exact molecular mechanism of cardio-protective effects (e.g., CAD) by luteolin remains unclear. In Fig. 6B, luteolin interacts with 7 CAD proteins and 16 PPI partners (e.g. AKT1 and MAPK1), and is predicted to have potential association on CAD ($Z = 5.83$, $q < 10^{-5}$). AKT regulates the apoptotic pathological process of myocardial ischemia/reperfusion (I/R) injury, while mitogen-activated protein kinases (MAPKs) has been reported to regulate cardiomyocyte function after I/R injury [56, 57]. Recent studies have demonstrated that luteolin can suppress apoptosis by activation of AKT in a simulated I/R model and inhibit MAPK pathway in myocardial I/R injury [58, 59], suggesting a protective mechanism by luteolin on CAD. In summary, quantitative network analysis offers potential mechanism for deep understanding of cardio-protective effects by natural products on CAD and other cardiovascular systems if broadly applied (Fig. 6B).

2.6 Elucidating protective effects of natural products on coronary artery disease via targeting inflammatory-associated proteins.

Recent experimental and clinical studies have suggested that reducing inflammation reduce the risk of cardiovascular disease, including CAD [60]. We next inspected whether natural products target inflammatory response pathways on CAD, by showcasing two typical natural compounds: daidzein and caffeic acid. Daidzein, a soybean isoflavone, has been reported to show cardiovascular protective and anti-inflammatory effects in several preclinical studies [61, 62]. Here daidzein was computationally predicted to have significant association on CAD ($Z = 7.37$, $q < 10^{-5}$). Figure 7 reveals that daidzein binds with 9 inflammatory-associated proteins (e.g., NF- κ B1 and PPARG) and 9 CAD proteins. Daidzein was reported to attenuate I/R-induced myocardial damage via inhibiting NF- κ B activation [61]. In addition, daidzein regulated pro-inflammatory adipokines by activation of PPARG [62]. Caffeic acid, a phenolic acid, is effectively used as a natural antioxidant. Figure 7 shows that caffeic acid interacts with 6 inflammation-associated proteins (e.g., NF- κ B1 and TNF) and 5 CAD proteins (e.g., MMP9 and LMNA). We found that caffeic acid was predicted to have a significant association on CAD ($Z = 5.51$, $q < 10^{-5}$). Specifically, caffeic acid has been reported to improve cardiac mitochondrial dysfunction in Wistar rats [63] and to inhibit tumor necrosis factor alpha (TNF- α)-induced vascular inflammation in HUVECs by inhibiting NF- κ B activation [64]. Collectively, *in silico* prediction and network analysis

reveal that daidzein and caffeic acid have potential protective effects on CAD by targeting inflammatory response pathways. However, further pre-clinical and clinical studies are warranted to inspect these network-predicted anti-inflammatory pathways on CAD further.

3 DISCUSSION AND CONCLUSION

Network-based systems pharmacology approaches offer potential tools for developing chemical intervention strategies on CAD and help use better characterization of pharmacological mechanisms of approved drugs and natural products on cardiovascular systems. In this study, we computationally investigated approved drugs and natural products on CAD using an integrated systems pharmacology framework: (i) constructing two global drug-target networks for both approved drugs and natural products, (ii) building *in silico* models for predicting potential associations of approved drugs and natural products on CAD through integrating drug-target networks, known CAD proteins, and the protein-protein interactions, (iii) identifying potential mechanism-of-action of the computationally predicted therapeutic profiles or adverse effects of approved drugs and natural products on CAD by network analysis. In summary, we demonstrated a powerful systems pharmacology framework for the development of potential therapeutic and prevention strategies for CAD by exploiting the wealth of approved drugs and natural products.

We highlighted several significant improvements compared to previous studies [9, 16, 17]. First, we integrated the human protein-protein interaction of known CAD proteins, which can complement the incompleteness of known disease genes of CAD and increase the statistical power of *in silico* models [65]. We thereby predicted potential associations on CAD for those approved drugs or natural products targeting only one or none of known CAD proteins, such as fasudil. Second, we extended the systems pharmacology framework by searching cardio-protective natural products, offering potential natural products in treating and chemoprevention of CAD comparing to our recent network proximity-based approach that focused on the approved drugs only [30].

However, several potential limitations should be acknowledged. First, although we assembled large-scale, experimentally reported drug-target interactions for both approved drugs and natural products from publicly available databases based on our sizeable efforts, the incompleteness of drug-target networks may exist as well. In addition, some drug-target or compound-protein interactions on natural products come from functional assays, not physical binding studies, which could cause the risk of false positive rate. An integration of the computational predicted drug-target network may help overcome the incompleteness of the known drug-target networks based on our recent studies [66, 67]. In addition, adding large-scale drug-induced transcriptome [68] or proteome [69] data may help improve the accuracy of *in silico* models further, such as Connectivity Map. Second, our current *in silico* models cannot separate the therapeutic effects against side effects owing to lack of the detailed functional effects of drug targets and disease proteins. Drug targets representing nodes within cellular networks are often intrinsically coupled in both therapeutic and adverse profiles [70]. Drugs can inhibit or activate protein functions (including antagonists vs. agonists), while disease alleles from genetic or genomic studies contain loss-of-function or gain-of-function. For example, an inhibitor that targets loss-of-function disease proteins

often causes adverse effects. Hence, integration of functional genomic assays or large-scale disease gene expression profiles (upregulation or downregulation), along with patient data (e.g., health insurance claims data) validation and *in vitro* or *in vivo* mechanistic studies will improve *in silico* drug repurposing models further [30, 71].

In summary, we suggested that a network-based, systems pharmacology framework offered potential strategies for *in silico* drug repurposing in treating and chemoprevention of CAD by exploiting promiscuity of approved drugs and natural products. If broadly applied, the systems pharmacology approaches presented here can be applied for development of new chemical intervention strategies in other diseases as well.

4 EXPERIMENTAL SECTION

4.1 Construction of drug–target networks for approved drugs

To construct a drug–target network for approved drugs, we downloaded the latest data from DrugCentral database (accessed in Dec 2017) [19], and only kept data items by the following criteria: (i) the target organism is homo sapiens; (ii) the target can be transformed into a symbol gene; (iii) the drug can be transformed to canonical SMILES format.

4.2 Construction of compound–protein networks for natural products

We manually collected 6 traditional Chinese medicine (TCM) formulae with clinically-proven efficacy on CAD, including ShengMaiSan (SMS) [72], ZhiGanCao Decoction (ZGC), SuHeXiang Pill (SHX), BuYangHuanWu Decoction (BYHW) [73], ShiXiaoSan (SXS), and XiaoXianXiong Decoction (XXD), from references [72, 73] and website [<http://cnda.cfda.gov.cn>] of China Food and Drug Administration (CFDA).

We next collected the constituting herbs for each TCM formula, and extracted related herb ingredients for 6 TCM formulae from six publicly available TCM data sources: Traditional Chinese Medicine database (TCMDb) [74], Traditional Chinese Medicine integrated database (TCMID) [75], Traditional Chinese Medicine Systems Pharmacology (TCMSP) [76], Traditional Chinese Medicine database@Taiwan (TCM@Taiwan) [77], TCM-MESH [78] and TM-MC [79]. In total, we obtained 6,077 herb-ingredient pairs connecting 30 herbs to 4,297 unique natural products (Table S3).

We further integrated a compound–protein interaction network of natural products from two types of data sources: 1) direct DTI databases (including ChEMBL [80] and BindingDB [81]), and 2) indirect DTI databases (including STITCH 5 [82], Herbal Ingredients' Targets Database (HIT) [83], and TCMID [75]). We downloaded the latest data as below: ChEMBL (v21), and BindingDB (accessed in September 2017). We filtered data items that met the following criteria: (i) inhibitory constant (K_i), dissociation constant (K_d), half maximal inhibitory concentration (IC_{50}) or half maximal effective concentration (EC_{50}) $> 10 \mu M$; (ii) the target is a human protein; (iii) the target has a unique UniProt accession number; (iv) each drug can be transformed to canonical SMILES format. Subsequently all drug structures were carefully standardized by removing salt ions and standardizing dative bonds using Open Babel toolkit (v2.3.2) [84].

We collected indirect DTIs by following the below steps. For STITCH source (accessed in Sep 2017), the thickness of each interaction pair represents the confidence score of the association. Only DTIs from *homo sapiens* were retained, and compound-protein interactions with experimental evidence score over 0.7 were used in this work. We further extracted compound-protein interactions from HIT and TCMID using a web crawler approach, and deleted the duplicated DTIs. Finally, we constructed a global compound-protein network for natural products after integrating direct and indirect interactions (edges) via mapping 4,297 unique natural products into the five databases using the “InChIKey” derived from chemical structures (SMILES format).

4.3 Manual curation of disease genes for coronary artery disease and inflammatory response pathways.

In this study, CAD disease-associated genes (CAD genes) were collected from Online Mendelian Inheritance in Man (OMIM) [85], The Human Gene Mutation Database (HGMD) [86], and GWAS Catalog [87], while inflammation-associated genes were integrated from Comparative Toxicogenomics Database (CTD) [88], DisGeNet [89], HGMD [86] and GWAS Catalog [87]. OMIM is a comprehensive bioinformatics resource of curated descriptions of human genes and phenotypes as well as the relationships between them [85], while HGMD is a repository of inherited mutation data manually curated from published references [86]. Only single-nucleotide polymorphism (SNPs) with Genome-wide significance ($P < 5.0 \times 10^{-8}$) on CAD were extracted from GWAS Catalog. Finally, 243 CAD disease-associated genes and 112 inflammation-associated genes were obtained. In this study, we grouped 243 CAD genes into three source types: GWAS, non-GWAS, and their overlapped gene sets. The details are provided in Table S4.

4.4 Construction of a specific PPI network of CAD genes

We integrated a large-scale, high-quality human protein interactome from 5 types of PPIs: high-throughput Y2H binary, kinase-substrate interactions, signaling interactions, protein three-dimensional (3D) interactome, protein complexes (Bioplex 1&2), and literatures, as described in our recent studies [30]. In addition, we mapped 243 CAD genes to the interactome to extract a specific PPI network of CAD genes.

We further filtered PPI interactor genes (neighbors of CAD proteins) that are not significantly expressed in CAD tissue (blood vessel). Firstly, the RNA-seq data (RPKM value) of 32 tissues were downloaded from GTEx V6 release (accessed on April 01, 2016, <https://gtexportal.org/home/>). Those genes with RPKM ≥ 1 in over 80% of samples were defined as tissue-expressed genes and the remaining genes as tissue-unexpressed. Secondly, to quantify the expression significance of tissue-expressed gene *i* in tissue *t*, we computed the average expression $E(i)$ and the standard deviation $\delta_E(i)$ of a gene's expression across all considered tissues. The significance of gene expression in tissue *t* is defined as below:

$$Z_{E(i),t} = (E(i,t) - E(i)) / \delta_E(i) \quad (1)$$

In this study, only the PPI interactor genes with Z-score higher than 0 were kept. Finally, we compiled a specific PPI network of CAD proteins, which consisted of 3,176 PPIs connecting 243 CAD proteins and 1,433 PPI partners (Table S5).

4.5 *In silico* prediction

Here, we built *in silico* models to predict new associations of approved drugs (or natural products) on CAD by incorporating drug-target (compound-protein) interactions, the known CAD genes, and the human PPI network. We first excluded the approved drugs or natural products that had less than two targets as well as those without any known proteins encoded by CAD genes or the first neighbors (direct interacting proteins) of CAD proteins in the human PPI network.

The hypothesis of our framework asserts that an approved drug (or a natural product) with high promiscuity shows a higher possibility to treat CAD if its targets are more likely to be CAD proteins or partners of CAD PPIs. A permutation testing was used to calculate the statistical significance of an approved drug (or a natural product) to be prioritized for potential association on CAD. The null hypothesis supposes that the targets of an approved drug (or a natural product) randomly locate at CAD proteins or their neighbors of CAD PPIs across the human proteome. We performed the permutation testing as below:

$$P = \frac{\# \{S_m(p) > S_m\}}{\# \{ \text{total permutations} \}} \quad (2)$$

A nominal P was computed for each drug (or natural product) by counting the number of observed CAD or PPI interactor genes greater ($S_m(p)$) than the permutations (S_m).

Here we repeated 100,000 permutations by randomly selecting 1,638 proteins (the same number of CAD proteins and their neighbors of CAD PPIs) from the genome-wide simulation (20,462 human protein-coding genes from the NCBI database [<https://www.ncbi.nlm.nih.gov/gene>], Table S6). Then, the nominal P -values from the permutation tests were corrected as adjusted P -values (q) using R based on Benjamini-Hochberg approach [90].

Subsequently, a Z-score was calculated for each drug (or natural product) to be prioritized for potential association on CAD during permutation testing as previously described [9, 16], where “ x ” is the real number of CAD or PPI interactor genes targeted by a given natural product (or drug), μ is the mean number of CAD or their PPI partners targeted by a given natural product (or drug) during 100,000 permutations, and σ is the standard deviation.

$$Z = \frac{x - \mu}{\sigma} \quad (3)$$

4.6 Network Visualization and Statistical Analysis

The statistical analysis in this study was carried out using the Python (v3.2, <http://www.python.org/>) and R platforms (v3.01, <http://www.r-project.org/>). Networks were visualized by Cytoscape (v3.2.0, <http://www.cytoscape.org/>) and Gephi (v0.9.2, <https://gephi.org/>).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Schematic diagram illustrating an integrated systems pharmacology framework for predicting new associations of approved drugs and natural products on coronary artery disease (CAD).

(A) Construction of drug-target (compound-protein) networks for both approved drugs and natural products. (B) Building a protein-protein interaction (PPI) network for CAD proteins. (C) Predicting new associations of approved drugs and natural products on CAD via *in silico* models (see Methods).

Fig. 2. A bipartite drug–target interaction network (CADDTnet) for coronary artery disease (CAD)-associated drugs from DrugCentral database [19]. CADDTnet contains 931 interactions connecting 634 approved drugs and 58 CAD proteins (including 12 proteins from GWAS, 44 from non-GWAS, and 2 overlapping genes shared by multiple data sources [see Methods]). The CADDTnet was drawn using Gephi (v0.9.2). The label font size and node size are proportional to degree (connectivity). The label name is shown only when its degree is higher than 5. The color of drug node is colored by their first-level Anatomical Therapeutic Chemical Classification (ATC) codes from the DrugBank database [29].

Fig. 3. A bipartite natural product–protein interaction network (CADNPnet) for coronary artery disease (CAD)-associated natural products from 6 traditional Chinese medicine (TCM) formulae.

CADNPnet contains 395 interactions connecting 170 natural products and 71 CAD proteins (including 23 proteins from GWAS, 44 from non-GWAS, and 4 overlapping proteins shared by multiple sources). The CADNPnet was drawn using Gephi (v0.9.2). The label font size and node size are proportional to degree (connectivity). The label name is shown only when its degree is higher than 5. The color of drug nodes is colored by the chemical clustering analysis (see Fig. 4).

Fig. 4. Chemical diversity analysis for natural products (A) from 6 traditional Chinese medicine (TCM) formulae (see Methods) and for approved drugs (B) from DrugCentral database [19]. The structures of 10 cluster centers during chemical structural clustering analysis for 171 natural products (A) and 634 approved drugs (B) were illustrated. N denotes the number of drugs or natural products in each cluster.

Fig. 5. The predicted associations of approved drugs on coronary artery disease (CAD). (A) Circos plot representing the predicted associations connecting 61 approved drugs with CAD. The predicted q values with corresponding Z-score are exhibited as connected lines (edges). The drugs with significant q values ($q < 10^{-5}$) are highlighted in bold font. Drugs are grouped by their first-level Anatomical Therapeutic Chemical Classification (ATC) codes. The circos plot was drawn using Circos (v0.69). (B) A discovered drug-target-disease network connecting three selected drugs (fasudil, parecoxib, and dexamethasone) as well as

their corresponding targets of CAD proteins and their protein-protein interaction neighbors. The thickness of blue line is proportional to the predicted Z-score.

Fig. 6. The predicted associations of natural products on coronary artery disease (CAD). (A) Circos plot representing the predicted associations connecting 46 natural products with CAD. The predicted q values with corresponding Z-score are exhibited as connected lines (edges). Natural products with significant q values ($q < 10^{-5}$) are highlighted in bold font. The circos plot was drawn using Circos (v0.69). (B) A discovered drug-target-disease network connecting three typical natural products (resveratrol, luteolin and myricetin) as well as their corresponding targets of CAD proteins and their protein-protein interaction neighbors. The thickness of blue line is proportional to the predicted Z-score.

Fig. 7. A discovered drug-target-disease network for two typical natural products by targeting inflammatory response pathways.

The network displays the predicted associations of two natural products (daidzein and caffeic acid) on coronary artery disease (CAD) as well as their corresponding CAD proteins (targets) and genes on inflammatory response pathways (see Methods).

Table 1.

The statistics comparison of four drug-target (compound-protein) networks.

Data set	N _D	N _T	N _{DTI}	Sparsity (%)
DTnet	1,812	1,335	11,333	0.468
CADDTnet	634	58	931	2.535
NPnet	500	2,020	6,229	0.617
CADNPnet	170	71	395	3.273

N_D: the number of drugs in network, N_T: the number of targets in network, N_{DTI}: the number of drug-target interactions (DTIs) or compound-protein interactions, Sparsity: the ratio of N_{DTI} to the number of all possible DTIs. CADDTnet represents the specific network connecting approved drugs with coronary artery disease (CAD) proteins derived from DTnet, while CADNPnet denotes to the specific network connecting natural products with CAD proteins extracted from NPnet.