Protein Interaction-Based Genome-Wide Analysis of Incident Coronary Heart Disease

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Background—Network-based approaches may leverage genome-wide association (GWA) analysis by testing for the aggregate association across several pathway members. We aimed to examine if networks of genes that represent experimentally determined protein-protein interactions (PPIs) are enriched in genes associated with risk of coronary heart disease (CHD).

Methods and Results—Genome-wide association analyses of approximately ≈700 000 single-nucleotide polymorphisms in 899 incident CHD cases and 1823 age- and sex-matched controls within the Nurses' Health and the Health Professionals Follow-up Studies were used to assign genewise *P* values. A large database of PPIs was used to assemble 8351 unbiased protein complexes and corresponding gene sets. Superimposed genewise *P* values were used to rank gene sets based on their enrichment in genes associated with CHD. After correcting for the number of complexes tested, 1 gene set was overrepresented in CHD-associated genes ($P=0.002$). Centered on the β1-adrenergic receptor gene (*ADRB1*), this complex included 18 protein interaction partners that have not been identified as candidate loci for CHD. Of the 19 genes in the top complex, 5 are involved in abnormal cardiovascular system physiological features based on knockout mice (4-fold enrichment; Fisher exact test, $P=0.006$). Ingenuity pathway analysis revealed that canonical pathways, especially related to blood pressure regulation, were significantly enriched in the genes from the top complex.

Conclusions—The integration of a GWA study with PPI data successfully identifies a set of candidate susceptibility genes for incident CHD that would have been missed in single-marker GWA analysis. (***Circ Cardiovasc Genet*. 2011;4:549-556.**)

Key Words: genetics of cardiovascular disease ■ acute myocardial infarction ■ epidemiology

Genome-wide association (GWA) studies provide a unique opportunity for the unbiased exploration of novel genetic variation of importance to phenotypic traits. The first series of GWA studies of coronary heart disease (CHD) and more broadly defined cardiovascular disease (CVD) phenotypes elucidated DNA sequence variations at the 9p21.3 locus as a robustly replicated risk-conferring region,[1–3] but through a series of larger GWA study consortia, approximately 10 susceptibility loci have been reported.[4–6] The recent publication of results from the multi-ethnic Coronary Artery Disease Genetics Consortium,[7] the first Han Chinese GWA study,[8] and the CARDIoGRAM consortium with >20 000 coronary artery disease cases,[9] yielded an additional 18 new loci. However, the complexity of the phenotype,[10] small effect sizes, and between-study differences may complicate the identification of many true associations in meta-analyses that necessarily assume homogeneity across the individual studies. Most GWA studies have focused on the identification of the strongest single-locus associations, but the identification of combined effects of many weakly associated variants is especially appealing for complex diseases, such as CHD, that are likely not caused by single variants or by a single biological pathway. Thus, another suggested approach for reducing the noise inherent in moderately powered high-density data collected within internally homogeneous populations is the integration of additional biological data on pathway organization through the use of protein-protein interaction (PPI) databases.[11–16] By enabling tests of sets of single-nucleotide polymorphisms (SNPs) within physically interacting gene products (direct or indirect), PPI data can augment GWA analysis because a set of SNPs, each with a moderate, but genuine, association, in aggregate may have improved statistical significance. Although several databases provide gene sets that resemble well-known canonical pathways, high-confidence PPI data may, to a larger degree, mimic the unbiased nature of GWA studies because of their

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increased coverage and detail of even noncanonical pathways.[11,17] Initial approaches have proved useful for suggesting novel genes and gene networks involved in other complex phenotypes, such as obesity,[18] type 2 diabetes mellitus,[13] breast and pancreatic cancer,[19] multiple sclerosis,[20] and Crohn disease,[21] that were not identified in the traditional GWA analysis. The completeness of such integrative analysis relies strongly on the gene sets tested. We aimed to examine if networks of genes that represent experimentally determined PPIs are enriched in genes associated with the risk of incident CHD. To leverage our GWA analysis of CHD within 2 homogeneous US prospective cohorts, including 899 incident cases collected through >10 years of follow-up, we used our PPI database InWeb,[14] which covers approximately ≈13 000 human proteins and 173 500 high-confidence experimentally derived PPIs based on 11 publicly available PPI databases.

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Methods

Study Population

The Nurses' Health Study (NHS) enrolled 121 701 female nurses, aged 30 to 55 years, who returned a mailed questionnaire in 1976 regarding lifestyle and medical history. The Health Professionals Follow-up Study (HPFS) enrolled 51 529 males, aged 40 to 75 years, who returned a similar questionnaire in 1986. Participants of both cohorts have received follow-up questionnaires biennially to record newly diagnosed illnesses. Detailed descriptions of the study cohorts have been previously published.[22,23]

Blood Collection and DNA Extraction in a Nested Case-Control Study

Between 1989 and 1990, a blood sample was requested from all active participants in the NHS and collected from 32 826 women. Similarly, blood samples were requested between 1993 and 1995 and obtained from 18 225 HPFS participants. For details on storage of blood samples, please see the online supplement.

In both cohorts, nested case-control studies were designed using incident CHD, with nonfatal myocardial infarction and fatal CHD as the outcome. A diagnosis of myocardial infarction was confirmed on the basis of the criteria of the World Health Organization (symptoms plus either diagnostic electrocardiographic changes or elevated levels of cardiac enzymes). Deaths were identified from state vital records and the National Death Index or reported by the participant's next of kin or the postal system. A fatal CHD was confirmed by an examination of hospital or autopsy records, by the listing of CHD as the cause of death on the death certificate, if CHD was the underlying and most plausible cause, and if evidence of previous CHD was available. Among participants who provided blood samples and who were free of diagnosed CVD or cancer at blood draw, we identified 474 women and 454 men with incident CHD between blood draw and June 2004. By using risk-set sampling,[24] controls were selected randomly and matched in a 1:2 ratio on age, smoking, and month of blood return, among participants who were free of CVD when CHD was diagnosed in the case. In this study design, a control for an early case may be included again if the person develops CHD during follow-up; thus, after counting such converters only once (as cases), the total number of samples sent for genotyping were 1354 HPFS samples and 1521 NHS samples.

The present study was approved by the institutional review boards at Brigham and Women's Hospital and Harvard School of Public Health.

Genotyping and Quality Control

Details on the protocol for DNA extraction have been included in the online supplement. Genotyping was performed using the Affymetrix Genome-Wide Human 6.0 array and the Birdseed calling algorithm.[25] Genotypic data for 1330 HPFS samples (98%) passed laboratory technical quality control criteria, and the missing call rate was <0.05. Likewise, 96% of the NHS samples were successfully genotyped. A subset of 312 NHS samples were not genotyped, together with the remaining CHD case-control sets, because they overlapped with previous GWA studies of breast cancer (Illumina 550) and type 2 diabetes (Affymetrix 6.0). These samples were processed and subjected to quality control as part of the earlier GWAS (leaving 272 samples with available data), and SNPs also present on the Affymetrix 6.0 platform were subsequently merged with the cleaned CHD data. Details on methods for data cleaning and assessment of population structure in the data sets are included in the online supplement. Because of few samples with substantial evidence of non-European genetic ancestry, these samples were excluded from subsequent analysis (n=24). SNPs that were monomorphic, had a missing call rate ≥2%, an Hardy-Weinberg Equlibrium (HWE) P value $<1\times10^{-4}$, or a minor allele frequency (MAF) <0.02 were excluded, leaving 724 881 SNPs that passed quality control in HPFS and 721 316 in NHS for analysis of called genotypes. Imputation of approximately ≈2.5 million SNPs was performed using MACH software (version 1.0.16) with HapMap CEU phased II data (Release 22) as the reference panel.

GWA Analysis of CHD

To analyze the association between each SNP (coded as counts of minor alleles) and risk of CHD, we ran logistic regression analysis using PLINK software.[26] We adjusted for matching factors used in the design of the nested case-control study (age and smoking) and the top 3 eigenvectors. We also analyzed the MACH dosage files of the imputed SNPs (with MAF ≥0.05) in logistic regression models (adjusting for the same covariates as previously given) using the ProbABEL package from the ABEL set of programs.[27] A fixed-effects meta-analysis was performed to combine the study-specific β estimates using the METAL package.[28]

Systems biology–based approaches that integrate data on protein interactions are necessarily restricted to the protein-coding part of the genome. We mapped all GWA SNPs that passed quality control to 21 800 protein-coding genes (423 450 mapped SNPs, approximately ≈57% of all SNPs on the Affymetrix 6.0 arrays) (Figure 1, step 1). This process is gene centric such that SNPs that are not within genes or their 70-kb upstream and 20-kb downstream flanking regions were discarded. SNPs were allowed to map to >1 gene. Each gene was assigned a P value based on the SNP with the lowest GWA P value within the gene transcript(s) and its flanking regions. Subsequently, the Šidàk correction was applied to adjust the P value for each gene by the number of effective tests (uncorrelated number of SNPs within each gene and its flanking regions, as described by Galwey,[29] and provided as a web tool through MetaRanker).[16]

PPIs and CHD-Specific Protein Complexes

Protein-protein interactions comprise both transient interactions (eg, phosphorylation events) and stable interactions (eg, the cytoskeleton). Our comprehensive, experimentally derived database of protein-protein interactions, InWeb (version 2.9), covers approximately ≈13 000 human proteins and 350 029 PPIs, of which 173 500 can be regarded as high-confidence interactions (as described later).[14] The database is updated on a monthly basis with interactions retrieved from all major experimental PPI databases (details available in the online supplement). The strengths of the InWeb database include the relatively high coverage (4-fold increase in the number of interactions compared with the Human Protein Reference Database)[30] and a quantitative assessment of confidence in the reported interactions. The (continuous) confidence score (ranging from 0 [low support] to 1 [strong support]) is assigned by accounting for the number and quality of the publications reporting each of the interactions and the number of shared interaction partners of 2 interacting proteins.[14] The assembly of 8351 gene sets was accomplished by iteratively assigning each protein in the database and its first-order interaction partners to a protein complex (Figure 1, step 1b). As such a construction of protein complexes results in a relatively large number of overlapping complexes; complexes that were >80% similar (similarity of gene sets assessed by the Jaccard

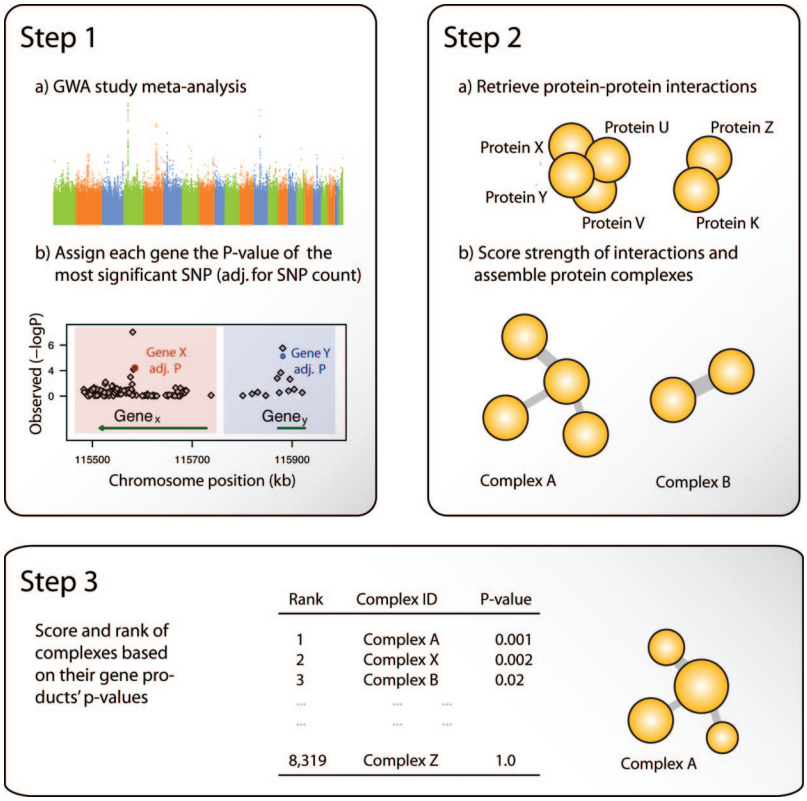

Figure 1. Conceptual framework for the integration of GWA data with PPI data. The approach consists of 3 overall steps: (1) GWA meta-analysis, SNPs are mapped to genes, genes are scored based on the most significant SNP, and the gene scores are adjusted by the number of independent SNPs mapped to the given gene; (2) protein complexes are assembled based on experimentally derived PPIs; and (3) the gene sets underlying the protein complexes are scored based on their genes' *P* values and their PPI confidence scores.

Index) were merged. After superimposing the genewise *P* values from the GWA analysis onto the network, we used a modified version of an approach published by Ideker et al[31] to iteratively assess whether any of the gene sets that were derived from the protein complexes were enriched in CHD-associated genes. Given a gene set of size k, this was accomplished as follows: (1) converting all k gene *P* values to *Z* scores using the inverse normal cumulative distribution function, (2) weighting them with the interaction confidence score of the PPI with the central hub protein (a step that was not part of the original algorithm), (3) calculating a gene-set score by summing the weighted *Z* scores, and then (4) subtracting the sum of an average gene set of size k (calculated based on 100 000 randomized gene-set scores) and dividing by the SD of an average subnetwork of size k. Formally, step 1 can be formulated as $z_i = F^{-1}(1-p_i)$, $i \in \{1,\ldots,k\}$, steps 2 and 3 as $S_{\text{gene-set}} = \frac{1}{k}\left(z_{hub} + \sum_{j=1}^{k-1} cs^j_{\text{hub}} z_j\right)$, and step 4 as $Z_{gene\text{-}set} = \frac{S_{gene\text{-}set} - \mu_k}{\sigma_k}$, where p_i denotes the *P* value of gene i, z_i denotes the *Z* score of gene i, z_j denotes the *Z* score of the interacting gene product j, F^{-1} denotes the inverse normal cumulative distribution function, cs^j_{hub} the confidence score for the PPI between interacting gene product j and the hub gene product, $S_{gene\text{-}set}$ denotes the score of the gene set after steps 1 to 4, μ_k and σ_k denote the mean and SD of 100 000 randomized gene-set scores, and $Z_{gene\text{-}set}$ denotes the final gene-set *Z* score. By using this method, all gene sets were ranked based on their computed *Z* scores (Figure 1, step 3). Because SNPs were allowed to map to several overlapping genes, some gene sets may be assigned artificially inflated scores if they comprise genes that overlap on a given chromosome and are scored based on the same low SNP *P* value. To avoid this potential bias, we discarded 1 of the genes in any overlapping gene pair in a given complex (genes were considered to overlap if their transcripts were closer than 200 kb to each other). This approach is conservative because it avoids inflated complex scores, but in some cases it may reduce the significance of truly associated complexes that comprise colocalizing gene products with independent associations. In our present analysis, the top complex remained the same with or without discarding overlapping genes (and for different exclusion thresholds). We assessed the significance of our observed top-scoring complex by comparing its score with a background distribution of 100 scores generated under the null hypothesis that the complex is not associated with CHD case-control status. The background distribution was estimated on the basis of 100 permutations of our GWA meta-analysis (randomizing the case-control status) and recomputations of the gene scoring and complex scoring step for each permutation. An ideal scenario would include at least 1 million permutations, but the aggregate computing times for the GWA analysis, the gene scoring step, and the complex enrichment analysis did not allow for this.

After identification of the top-ranking complex, we searched the literature to see if the genes were known as human CVD candidate genes. To assess overrepresentation of known CVD susceptibility genes, we used a list of 123 genes reported by Samani et al[3] and updated it with GWA findings in the National Institutes of Health Catalog of Genome-Wide Association Studies (supplemental Table I).[32] We also tested for overrepresentation of a list of 889 genes that affected cardiovascular system physiological features (MP:0001544) in knockout mice (Mouse Genome Informatics database, http://www.informatics.jax.org; Jackson Laboratory, Bar Harbor, ME), of which 837 were among the gene products in our PPI database. To ensure that the observation that genes from our top complex were overrepresented in the mouse cardiovascular physiological gene set was not because of chance, we compared the observed enrichment score with a background distribution of 10 000 scores computed based on randomly sampled protein complexes. Each of the random complexes matched the observed complex in size, and each gene product was sampled with a probability equal to its observed prevalence in the total set of protein complexes. In addition to the enrichment analysis of known human and mouse CHD risk genes, we used the Ingenuity Pathway Analysis (IPA) software tool, version 9.0 (Ingenuity Systems Inc, 2011) to systematically test the complex genes for pathway enrichment.

Results

The characteristics of incident cases and matching controls in the 2 cohorts are presented in Table 1. The women in the

Table 1. Baseline Characteristics of Women and Men in Whom CHD Developed During Follow-Up and Matched Controls in the NHS and the HPFS*

	HPFS		NHS	
Characteristic	Cases (n = 425)	Controls (n = 878)	Cases (n = 464)	Controls (n = 945)
Age, y	64.5 (8.6)	64.2 (8.5)	60.2 (6.3)	59.8 (6.3)
Female sex, %	0	0	100	100
Hypertension, %*	37.2	29.0	50.2	27.3
Diabetes, %*	9.0	3.8	14.4	6.24
Current smoker, %	9.7	8.7	27.8	26.1
Cholesterol, mmol/L				
Total	5.5 (1.0)	5.2 (1.0)	6.1 (1.0)	5.9 (1.0)
HDL	1.1 (0.3)	1.2 (0.3)	1.3 (0.4)	1.6 (0.4)
Triglyceride, mmol/L	1.8 (1.5)	1.5 (2.2)	1.6 (1.0)	1.3 (0.7)
BMI, kg/m^2	26.0 (3.2)	25.6 (3.3)	26.0 (6.6)	24.5 (5.8)

Values are means (SDs) of continuous covariates (except triglyceride levels, which are reported as median [interquartile range]) or percentages. Age and smoking were matching factors. Triglyceride levels were log transformed before analysis and only reported in fasting participants (HPFS, 65%; NHS, 79%). BMI, body mass index; HDL, high-density lipoprotein.

*Self-reported diagnosis before blood draw.

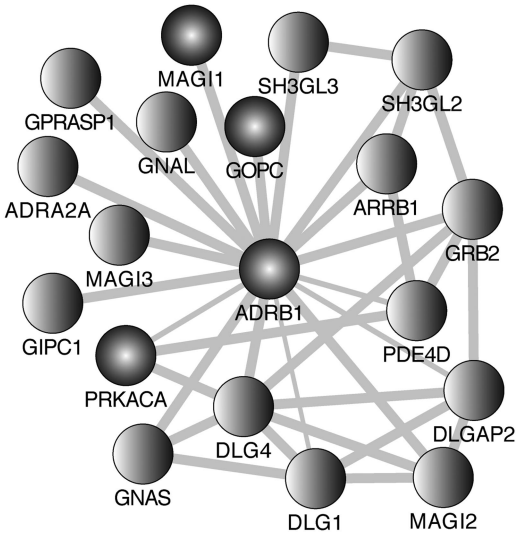

Figure 2. Top-ranking protein complex from the genome-wide analysis of CHD in the NHS and the HPFS. Edges between the nodes denote experimentally-derived protein-protein interactions and are scaled in size according to our confidence measure (larger indicates higher confidence). Nodes with radial gradients denote genes in the complex with corrected gene-wise *P*-values <0.05. Full gene names are available in the online-only Supplement.

NHS were slightly younger, more likely to smoke, and more likely to report a diagnosis of hypertension or diabetes. Genome-wide association analysis of each cohort separately and in a meta-analysis did not reveal any markers that exceeded the genome-wide significance threshold (supplemental Figure I).

Based on the InWeb database, 8351 protein complexes were assembled based on large-scale proteomics data from human and model organisms. We restrained our analyses to high-confidence PPIs only, including a subset that we recently validated experimentally in human heart tissue.[39] The resulting protein complexes were tested for enrichment in CHD-associated genes by using the genewise *P* values from the GWA analysis to create *Z* scores and ranking the complexes (gene sets) by their combined *Z* scores, adjusted for the size of each gene set and weighted by the confidence of the interactions between the peripheral gene products and the central protein of the complex. After correcting for the number of complexes tested, 1 gene set was significantly overrepresented in CHD-associated genes from our GWA meta-analysis ($P=0.002$). The gene complex was centered on the known candidate gene for the β1-adrenergic receptor (*ADRB1*) (Figure 2). To ensure that the top complex was not merely significantly enriched in genes with low *P* values but was significantly associated with CHD case-control status, we permuted the phenotype-genotype association in the GWA analysis 100 times and recomputed the complex score at each iteration. We found that the score for the observed *ADRB1* complex was superior to any of the scores for the randomized complexes. In Figure 2, the additional 18 genes that were part of the complex of interacting proteins are shown. Edges between them are scaled in size according to our confidence measure. As shown in more detail in Table 2, the genes (membrane-associated guanylate kinase inverted 1 [*MAGI1*], the protein kinase cAMP-dependent catalytic α [*PRKACA*], and the Golgi-associated PDZ and coiled-coil motif containing [*GOPC*]) were nominally significant after correcting for the number of independent SNPs in each gene, whereas the remainder showed weaker or no association. In the combined test of a gene set, all known interaction partners are included regardless of their GWA signal, and the strength of the association for the complex relies on the sum of all genewise *P* values of the interacting genes. Our results did not change when we based our analysis on the imputed GWA data rather than on the hard-call genotypes.

Next, we assessed whether the *ADRB1* complex was enriched in known human or mouse CVD risk genes. No significant overlap with the list of 123 susceptibility genes reported by Samani et al[3] and the genetic loci identified in GWA studies of CVD was observed ($P=0.1$).[32] To test for enrichment in CHD-specific evidence from mouse studies, we searched for the genes in the top complex in an a priori–defined set of genes causing abnormal cardiovascular physiological features in knockout mice. Among 889 genes reported for that phenotype, 837 human homologs were among the 12 793 genes included in our analysis, and 5 were part of the 19 genes in the *ADRB1* complex, representing a 4-fold enrichment (Fisher exact test, $P=0.006$). The 5 genes also found in mice knockout gene sets were *ADRB1*, *ADRA2A*, *ARRB1*, *PDE4D*, and *GRB2*, of which all except *PDE4D* played a role in the regulation of blood pressure, cardiac function, and hypertrophy. Because proteins that are known to interact physically are more likely to have similar functional annotation,[33] possible chance correlations resulting in a gene with a low *P* value could potentially result in a falsely associated complex if the falsely associated gene's annotation resembles the phenotype of interest. To test for this possible bias, we subjected the mouse gene set enrichment analysis to 10 000 random complexes sampled from the PPI network and found that only 13 of the 10 000 randomized enrichment scores were lower than our observed score ($P=0.001$).

Table 2. Genes and Primary SNPs in the Top-Ranking Protein Complex Based on the GWA Meta-Analysis of Risk of CHD in the NHS and HPFS

Gene	Gene *P* Value*	Top SNP	MAF	OR	Top SNP, Raw *P* Value	No. of SNPs in the Gene	No. of Independent SNPs in the Gene
MAGI1	7.8E-04	*rs7620106*	0.40	1.30	9.1E-06	251	86
PRKACA	0.004	*rs40282*	0.46	1.20	0.002	2	2
GOPC	0.028	*rs12664183*	0.28	1.23	0.001	100	27
ADRB1	0.041	*rs17653278*	0.06	0.70	0.003	41	12
MAGI3	0.073	*rs4839312*	0.26	1.21	0.005	62	14
MAGI2	0.086	*rs2065198*	0.46	1.22	0.001	579	149
GRB2	0.107	*rs7223674*	0.05	0.72	0.014	32	8
DLGAP2	0.143	*rs7836020*	0.45	1.18	0.005	100	33
ARRB1	0.217	*rs2279129*	0.08	0.75	0.013	34	19
DLG4	0.251	*rs5412*	0.16	1.15	0.069	7	4
GNAL	0.277	*rs2848465*	0.22	0.83	0.009	85	36
GIPC1	0.304	*rs4926215*	0.47	0.89	0.042	15	8
DLG1	0.335	*rs7616531*	0.26	1.17	0.020	56	20
GPRASP1	0.348	*rs17340189*	0.11	1.15	0.090	6	5
ADRA2A	0.355	*rs7908645*	0.34	1.13	0.056	15	8
SH3GL3	0.441	*rs8025427*	0.42	1.15	0.018	68	31
GNAS	0.508	*rs1022697*	0.43	1.13	0.032	50	21
SH3GL2	0.562	*rs10810813*	0.16	0.83	0.019	162	43
PDE4D	0.677	*rs17799450*	0.08	1.34	0.015	312	74

*Adjusted for the number of independent SNPs within loci (ie, independent SNPs in the gene). Full gene names are available in the online supplement.

We used IPA to examine whether the annotations of the genes in the *ADRB1* complex were enriched for any particular phenotype. Between 10 and 12 of the 19 genes were reported in cardiovascular, neurological, endocrine, and immunologic disorders (Table 3). Moreover, several cardiovascular-related pathways were enriched in genes from the complex. The top canonical pathway was cardiac hypertrophy signaling. To better ensure that the observed enrichment was not because of chance, we sampled 100 random gene sets comprising 19 genes each and performed IPA analysis based on each set. Only 1 random gene set exhibited enrichment in CVD genes as strong as the observed enrichment for the *ADRB1* complex gene set. Thus, we conclude that our top complex was significantly enriched in genes associated with CVD ($P<0.05$). None of the random gene sets were significantly enriched in the cardiac hypertrophy canonical pathway, suggesting that the *ADRB1* complex gene set was significantly enriched in genes from this pathway as well. We confined this IPA permutation analysis to 100 iterations because the software does not allow automation and all runs were performed manually.

Table 3. Diseases, Disorders, and Canonical Pathways Enriched in Genes From the Top Complex, Identified by IPA

IPA Variables	*P* Value for Enrichment	Gene Variables
Disease/disorder		
Respiratory	2.34E–07–5.00E–02	3*
Cardiovascular	1.12E–05–4.31E–02	12*
Neurological	2.81E–05–3.51E–02	12*
Endocrine system	3.63E–05–2.94E–02	10*
Immunologic	3.63E–05–1.56E–02	11*
Canonical pathway		
Cardiac hypertrophy signaling	1.62E–07	0.024 (6/246)†
G $\beta\gamma$ signaling	3.28E–06	0.034 (4/117)†
cAMP–mediated signaling	5.75E–06	0.023 (5/216)†
PTEN signaling	6.83E–06	0.033 (4/123)†
Cardiac β–adrenergic signaling	1.43E–05	0.026 (4/151)†

*Number of genes.
†Ratio (number of genes in the top complex/ total number of genes in the pathway).

Discussion

We conducted a protein network-based GWA analysis to leverage our moderately powered GWA study of CHD. By using GWA data from 2 individually homogeneous studies, we integrated the genewise *P* values with a large database of PPIs. By exploiting the complementary nature of genetic variation and biochemical data, we successfully identified a gene complex of 19 candidate genes that may play a role in the etiology of incident CHD. Subsequent pathway enrichment analysis indicated that the top complex was significantly enriched in (1) genes from the canonical cardiac hypertrophy signaling pathway (the highest-ranking pathway in the IPA analysis), (2) genes annotated with CVD (the second most enriched trait in the IPA analysis), and (3) mouse genes annotated in cardiovascular system physiological features. Our results provide preliminary evidence that known CHD-related genes coalesce onto distinct protein complexes. Most of the genes in the top complex had relatively small effect sizes, making them unlikely findings in traditional single-locus GWA analyses of CHD.

To our knowledge, our study of incident CHD is the first attempt at integration of data on the human interactome with GWA data in relation to incident CHD. As shown in the enrichment analyses, the top complex comprises several genes

Table 4. Overview of Genes in the Identified Top Complex and Their Implication in the IPA CVD Set, the Cardiac Hypertrophy Signaling Pathway (Hypertrophy), and the Mouse Knockout Models of Abnormal Cardiovascular Physiology (MGI)

Gene	SNP	CVD	Hypertrophy	MGI
MAGI1	*rs7620106*	Yes	No	No
PRKACA	*rs40282*	No	Yes	No
GOPC	*rs12664183*	No	No	No
ADRB1	*rs17653278*	Yes	Yes	Yes
MAGI3	*rs4839312*	Yes	No	No
MAGI2	*rs2065198*	Yes	No	No
GRB2	*rs7223674*	Yes	Yes	Yes
DLGAP2	*rs7836020*	Yes	No	No
ARRB1	*rs2279129*	No	No	Yes
DLG4	*rs5412*	No	No	No
GNAL	*rs2848465*	Yes	No	No
GIPC1	*rs4926215*	No	Yes	No
DLG1	*rs7616531*	No	No	No
GPRASP1	*rs17340189*	No	No	No
ADRA2A	*rs7908645*	Yes	Yes	Yes
SH3GL3	*rs8025427*	Yes	No	No
GNAS	*rs1022697*	Yes	Yes	No
SH3GL2	*rs10810813*	Yes	No	No
PDE4D	*rs17799450*	Yes	No	Yes

*Full gene names are available in the online supplement.

that previously have been annotated to CVD and, in particular, the cardiac hypertrophy signaling pathways. Except for *ADRB1*, these known genes were not nominally significant by themselves but were leveraged because of their interaction with genes that comprised SNPs, which exhibited an association with CHD in our GWA study. In addition, the top complex was significantly enriched in genes from the Mouse Genetics Initiative database that were annotated in the "cardiovascular system physiology." The genes *ADRB1*, *GRB2*, and *ADRA2A* overlapped between all 3 a priori–defined gene sets (overview provided in Table 4). It is well-known that the β1-adrenergic receptor plays an important role in the regulation of cardiac contractility. In candidate genetic studies, *ADRB1* SNPs have been associated with blood pressure[34] and risk of future CHD, which might be particularly true for individuals with an elevated blood pressure.[35] Studies on the adrenergic pathway genes, including *ADRA2A*, that encode the α2A-adrenergic receptor, have not shown consistent associations. However, recently, a polymorphism in *ADRA2A* that caused overexpression of the protein strongly reduced insulin secretion from pancreatic cells and was associated with an elevated risk of type 2 diabetes.[36] The *GRB2* gene encodes the growth factor receptor-bound protein 2. So far, information on this genetic locus links it to an important role in lymphocytes and growth cells, but no human genetic epidemiological studies have investigated this locus in relation to cardiometabolic disorders.

Alternative approaches for augmenting GWA data by testing significance beyond single-locus associations include pathway-based approaches, such as methods that search the protein interactome for dense subnetworks enriched in the GWA signal[19,20] and methods that assess predefined gene sets for enrichment in the GWA signal.[16,17,37] The former class of methods is inspired by the early work of Ideker[31] and Chuang[38] and colleagues and uses a heuristic search algorithm to identify subnetworks that are enriched in gene products that, in aggregate, associate with the phenotype. The advantage of these methods is that they do not assume any a priori delineation of pathways. However, the main drawback is that they rely on user-specified parameters that control the size of the subnetworks identified by the algorithm. In addition, none of them incorporate information on the confidence of the experimentally derived PPIs. Although our approach resembles the recently presented dmGWAS approach,[19] only our approach incorporated a score on confidence in the reported interactions. Another strength of our approach is that it is based on a PPI database that, despite its high coverage (our analysis includes twice as many interactions as those used in the dmGWAS method), solely includes high-confidence experimentally derived interactions. Although InWeb does not rely on predicted PPIs, which are more prone to false-positive interactions, it still entails approximately 173 500 interactions from 11 databases. Our integration-based approach has strengths and limitations. One of the inherent limitations is that it only covers approximately 60% of all SNPs present on genotyping platforms. Consequently, SNPs within distal enhancer regions are discarded, as are other long-range regulatory relationships. However, systematic tissue- and condition-specific expression quantitative trait loci analyses are increasingly contributing to the development of more refined SNPs to gene-mapping schemes. Among other limitations, we had a relatively small sample size in our GWA study of incident CHD and we were limited to whites. However, the application of the novel PPI approach still allowed us to uncover gene sets that were not otherwise identified. Replication in another prospective study setting is important to verify and demonstrate the significance of the *ADRB1* complex in incident CVD. Genome-wide data in a cohort with prospectively collected CHD cases would be preferable. It is likely that only larger GWA consortia will have a sufficient number of incident cases to accomplish this. Other approaches to follow up on our proof-of-principle approach and findings include investigations of rare variants through targeted resequencing or expression profiling across CHD-relevant tissues from appropriate cases and controls.

In conclusion, our approach suggests that integration of other layers of biological evidence with a moderately powered GWA study of CHD in 2 homogeneous study populations can yield potentially interesting sets of candidate genes that would be missed in traditional statistical GWA analyses. We identified 1 gene set, centered on *ADRB1*, that was overrepresented in CHD-associated genes in our GWA study and that was also enriched in genes involved in the CVD phenotype and particularly in blood pressure regulation pathways. Our novel approach highlighted 19 genes that warrant further association and functional studies in terms of risk of CHD and blood pressure.

Disclosures

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References

1. Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, Jonasdottir A, Sigurdsson A, Baker A, Palsson A, Masson G, Gudbjartsson DF, Magnusson KP, Andersen K, Levey AI, Backman VM, Matthiasdottir S, Jonsdottir T, Palsson S, Einarsdottir H, Gunnarsdottir S, Gylfason A, Vaccarino V, Hooper WC, Reilly MP, Granger CB, Austin H, Rader DJ, Shah SH, Quyyumi AA, Gulcher JR, Thorgeirsson G, Thorsteinsdottir U, Kong A, Stefansson K. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science*. 2007;316:1491–1493.
2. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, Hinds DA, Pennacchio LA, Tybjaerg-Hansen A, Folsom AR, Boerwinkle E, Hobbs HH, Cohen JC. A common allele on chromosome 9 associated with coronary heart disease. *Science*. 2007;316:1488–1491.
3. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, Dixon RJ, Meitinger T, Braund P, Wichmann HE, Barrett JH, Konig IR, Stevens SE, Szymczak S, Tregouet DA, Iles MM, Pahlke F, Pollard H, Lieb W, Cambien F, Fischer M, Ouwehand W, Blankenberg S, Balmforth AJ, Baessler A, Ball SG, Strom TM, Braenne I, Gieger C, Deloukas P, Tobin MD, Ziegler A, Thompson JR, Schunkert H. Genomewide association analysis of coronary artery disease. *N Engl J Med*. 2007;357:443–453.
4. Kathiresan S, Voight BF, Purcell S, Musunuru K, Ardissino D, Mannucci PM, Anand S, Engert JC, Samani NJ, Schunkert H, Erdmann J, Reilly MP, Rader DJ, Morgan T, Spertus JA, Stoll M, Girelli D, McKeown PP, Patterson CC, Siscovick DS, O'Donnell CJ, Elosua R, Peltonen L, Salomaa V, Schwartz SM, Melander O, Altshuler D, Ardissino D, Merlini PA, Berzuini C, Bernardinelli L, Peyvandi F, Tubaro M, Celli P, Ferrario M, Fetiveau R, Marziliano N, Casari G, Galli M, Ribichini F, Rossi M, Bernardi F, Zonzin P, Piazza A, Mannucci PM, Schwartz SM, Siscovick DS, Yee J, Friedlander Y, Elosua R, Marrugat J, Lucas G, Subirana I, Sala J, Ramos R, Kathiresan S, Meigs JB, Williams G, Nathan DM, MacRae CA, O'Donnell CJ, Salomaa V, Havulinna AS, Peltonen L, Melander O, Berglund G, Voight BF, Kathiresan S, Hirschhorn JN, Asselta R, Duga S, Spreafico M, Musunuru K, Daly MJ, Purcell S, Voight BF, Purcell S, Nemesh J, Korn JM, McCarroll SA, Schwartz SM, Yee J, Kathiresan S, Lucas G, Subirana I, Elosua R, Surti A, Guiducci C, Gianniny L, Mirel D, Parkin M, Burtt N, Gabriel SB, Samani NJ, Thompson JR, Braund PS, Wright BJ, Balmforth AJ, Ball SG, Hall AS, Schunkert H, Erdmann J, Linsel-Nitschke P, Lieb W, Ziegler A, Konig I, Hengstenberg C, Fischer M, Stark K, Grosshennig A, Preuss M, Wichmann HE, Schreiber S, Schunkert H, Samani NJ, Erdmann J, Ouwehand W, Hengstenberg C, Deloukas P, Scholz M, Cambien F, Reilly MP, Li M, Chen Z, Wilensky R, Matthai W, Qasim A, Hakonarson HH, Devaney J, Burnett MS, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Epstein SE, Rader DJ, Scheffold T, Berger K, Stoll M, Huge A, Girelli D, Martinelli N, Olivieri O, Corrocher R, Morgan T, Spertus JA, McKeown P, Patterson CC, Schunkert H, Erdmann E, Linsel-Nitschke P, Lieb W, Ziegler A, Konig IR, Hengstenberg C, Fischer M, Stark K, Grosshennig A, Preuss M, Wichmann HE, Schreiber S, Holm H, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Engert JC, Do R, Xie C, Anand S, Kathiresan S, Ardissino D, Mannucci PM, Siscovick D, O'Donnell CJ, Samani NJ, Melander O, Elosua R, Peltonen L, Salomaa V, Schwartz SM, Altshuler D. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. *Nat Genet*. 2009;41:334–341.
5. Erdmann J, Grosshennig A, Braund PS, Konig IR, Hengstenberg C, Hall AS, Linsel-Nitschke P, Kathiresan S, Wright B, Tregouet DA, Cambien F, Bruse P, Aherrahrou Z, Wagner AK, Stark K, Schwartz SM, Salomaa V, Elosua R, Melander O, Voight BF, O'Donnell CJ, Peltonen L, Siscovick DS, Altshuler D, Merlini PA, Peyvandi F, Bernardinelli L, Ardissino D, Schillert A, Blankenberg S, Zeller T, Wild P, Schwarz DF, Tiret L, Perret C, Schreiber S, El Mokhtari NE, Schafer A, Marz W, Renner W, Bugert P, Kluter H, Schrezenmeir J, Rubin D, Ball SG, Balmforth AJ, Wichmann HE, Meitinger T, Fischer M, Meisinger C, Baumert J, Peters A, Ouwehand WH, Deloukas P, Thompson JR, Ziegler A, Samani NJ, Schunkert H. New susceptibility locus for coronary artery disease on chromosome 3q22.3. *Nat Genet*. 2009;41:280–282.
6. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature.* 2007;447:661–678.
7. Peden JF, Hopewell JC, Saleheen D, Chambers JC, Hager J, Soranzo N, Collins R, Danesh J, Elliott P, Farrall M, Stirrups K, Zhang W, Hamsten A, Parish S, Lathrop M, Watkins HC, Clarke R, Deloukas P, Kooner JS, Goel A, Ongen H, Strawbridge RJ, Heath S, Malarstig A, Helgadottir A, Ohrvik J, Murtaza M, Potter S, Hunt SE, Delepine M, Jalilzadeh S, Axelsson T, Syvanen AC, Gwilliam R, Bumpstead S, Gray E, Edkins S, Folkersen L, Kyriakou T, Franco-Cereceda A, Gabrielsen A, Seedorf U, Eriksson P, Offer A, Bowman L, Sleight P, Armitage J, Peto R, Abecasis G, Ahmed N, Caulfield M, Donnelly P, Froguel P, Kooner AS, McCarthy MI, Samani NJ, Scott J, Sehmi J, Silveira A, Hellenius ML, van't Hooft FM, Olsson G, Rust S, Assmann G, Barlera S, Tognoni G, Franzosi MG, Linksted P, Green FR, Rasheed A, Zaidi M, Shah N, Samuel M, Mallick NH, Azhar M, Zaman KS, Samad A, Ishaq M, Gardezi AR, Memon FU, Frossard PM, Spector T, Peltonen L, Nieminen MS, Sinisalo J, Salomaa V, Ripatti S, Bennett D, Leander K, Gigante B, de Faire U, Pietri S, Gori F, Marchioli R, Sivapalaratnam S, Kastelein JJ, Trip MD, Theodoraki EV, Dedoussis GV, Engert JC, Yusuf S, Anand SS. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. *Nat Genet*. 2011;43:339–344.
8. Wang F, Xu CQ, He Q, Cai JP, Li XC, Wang D, Xiong X, Liao YH, Zeng QT, Yang YZ, Cheng X, Li C, Yang R, Wang CC, Wu G, Lu QL, Bai Y, Huang YF, Yin D, Yang Q, Wang XJ, Dai DP, Zhang RF, Wan J, Ren JH, Li SS, Zhao YY, Fu FF, Huang Y, Li QX, Shi SW, Lin N, Pan ZW, Li Y, Yu B, Wu YX, Ke YH, Lei J, Wang N, Luo CY, Ji LY, Gao LJ, Li L, Liu H, Huang EW, Cui J, Jia N, Ren X, Li H, Ke T, Zhang XQ, Liu JY, Liu MG, Xia H, Yang B, Shi LS, Xia YL, Tu X, Wang QK. Genome-wide association identifies a susceptibility locus for coronary artery disease in the Chinese Han population. *Nat Genet*. 2011;43:345–349.
9. Schunkert H, Konig IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, Preuss M, Stewart AF, Barbalic M, Gieger C, Absher D, Aherrahrou Z, Allayee H, Altshuler D, Anand SS, Andersen K, Anderson JL, Ardissino D, Ball SG, Balmforth AJ, Barnes TA, Becker DM, Becker LC, Berger K, Bis JC, Boekholdt SM, Boerwinkle E, Braund PS, Brown MJ, Burnett MS, Buysschaert I, Carlquist JF, Chen L, Cichon S, Codd V, Davies RW, Dedoussis G, Dehghan A, Demissie S, Devaney JM, Diemert P, Do R, Doering A, Eifert S, Mokhtari NE, Ellis SG, Elosua R, Engert JC, Epstein SE, de Faire U, Fischer M, Folsom AR, Freyer J, Gigante B, Girelli D, Gretarsdottir S, Gudnason V, Gulcher JR, Halperin E, Hammond N, Hazen SL, Hofman A, Horne BD, Illig T, Iribarren C, Jones GT, Jukema JW, Kaiser MA, Kaplan LM, Kastelein JJ, Khaw KT, Knowles JW, Kolovou G, Kong A, Laaksonen R, Lambrechts D, Leander K, Lettre G, Li M, Lieb W, Loley C, Lotery AJ, Mannucci PM, Maouche S, Martinelli N, McKeown PP, Meisinger C, Meitinger T, Melander O, Merlini PA, Mooser V, Morgan T, Muhleisen TW, Muhlestein JB, Munzel T, Musunuru K, Nahrstaedt J, Nelson CP, Nothen MM, Olivieri O, Patel RS, Patterson CC, Peters A, Peyvandi F, Qu L, Quyyumi AA, Rader DJ, Rallidis LS, Rice C, Rosendaal FR, Rubin D, Salomaa V, Sampietro ML, Sandhu MS, Schadt E, Schafer A, Schillert A, Schreiber S, Schrezenmeir J, Schwartz SM, Siscovick DS, Sivananthan M, Sivapalaratnam S, Smith A, Smith TB, Snoep JD, Soranzo N, Spertus JA, Stark K, Stirrups K, Stoll M, Tang WH, Tennstedt S, Thorgeirsson G, Thorleifsson G, Tomaszewski M, Uitterlinden AG, van Rij AM, Voight BF, Wareham NJ, Wells GA, Wichmann HE, Wild PS, Willenborg C, Witteman JC, Wright BJ, Ye S, Zeller T, Ziegler A, Cambien F, Goodall AH, Cupples LA, Quertermous T, Marz W, Hengstenberg C, Blankenberg S, Ouwehand WH, Hall AS, Deloukas P, Thompson JR, Stefansson K, Roberts R, Thorsteinsdottir U, O'Donnell CJ, McPherson R, Erdmann J, Samani NJ. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat Genet*. 2011;43:333–338.
10. Kitsios GD, Dahabreh IJ, Trikalinos TA, Schmid CH, Huggins GS, Kent DM. Heterogeneity of the phenotypic definition of coronary artery disease and its impact on genetic association studies. *Circ Cardiovasc Genet*. 2011;4:58–67.
11. Elbers CC, van Eijk KR, Franke L, Mulder F, van der Schouw YT, Wijmenga C, Onland-Moret NC. Using genome-wide pathway analysis to unravel the etiology of complex diseases. *Genet Epidemiol.* 2009;33:419–431.
12. Cantor RM, Lange K, Sinsheimer JS. Prioritizing GWAS results: A review of statistical methods and recommendations for their application. *Am J Hum Genet*. 2010;86:6–22.
13. Perry JR, McCarthy MI, Hattersley AT, Zeggini E, Weedon MN, Frayling TM. Interrogating type 2 diabetes genome-wide association data using a biological pathway-based approach. *Diabetes*. 2009;58:1463–1467.
14. Lage K, Karlberg EO, Storling ZM, Olason PI, Pedersen AG, Rigina O, Hinsby AM, Tumer Z, Pociot F, Tommerup N, Moreau Y, Brunak S. A human phenome-interactome network of protein complexes implicated in genetic disorders. *Nat Biotechnol*. 2007;25:309–316.
15. Brorsson C, Hansen NT, Lage K, Bergholdt R, Brunak S, Pociot F. Identification of T1D susceptibility genes within the MHC region by combining protein interaction networks and SNP genotyping data. *Diabetes Obes Metab*. 2009;11(suppl 1):60–66.
16. Pers TH, Hansen NT, Lage K, Koefoed P, Dworzynski P, Miller ML, Flint TJ, Mellerup E, Dam H, Andreassen OA, Djurovic S, Melle I, Borglum AD, Werge T, Purcell S, Ferreira MA, Kouskoumvekaki I,

Workman CT, Hansen T, Mors O, Brunak S. Meta-analysis of heterogeneous data sources for genome-scale identification of risk genes in complex phenotypes. *Genet Epidemiol*. 2011;35:318–332.
17. Wang K, Li M, Bucan M. Pathway-based approaches for analysis of genomewide association studies. *Am J Hum Genet*. 2007;81:1278–1283.
18. Liu YJ, Guo YF, Zhang LS, Pei YF, Yu N, Yu P, Papasian CJ, Deng HW. Biological pathway-based genome-wide association analysis identified the vasoactive intestinal peptide (VIP) pathway important for obesity. *Obesity*. 2010;18:2339–2346.
19. Jia P, Zheng S, Long J, Zheng W, Zhao Z. dmGWAS: Dense module searching for genome-wide association studies in protein-protein interaction networks. *Bioinformatics*. 2011;27:95–102.
20. Baranzini SE, Galwey NW, Wang J, Khankhanian P, Lindberg R, Pelletier D, Wu W, Uitdehaag BM, Kappos L, Polman CH, Matthews PM, Hauser SL, Gibson RA, Oksenberg JR, Barnes MR. Pathway and network-based analysis of genome-wide association studies in multiple sclerosis. *Hum Mol Genet*. 2009;18:2078–2090.
21. Wang K, Zhang H, Kugathasan S, Annese V, Bradfield JP, Russell RK, Sleiman PM, Imielinski M, Glessner J, Hou C, Wilson DC, Walters T, Kim C, Frackelton EC, Lionetti P, Barabino A, Van LJ, Guthery S, Denson L, Piccoli D, Li M, Dubinsky M, Silverberg M, Griffiths A, Grant SF, Satsangi J, Baldassano R, Hakonarson H. Diverse genome-wide association studies associate the IL12/IL23 pathway with Crohn disease. *Am J Hum Genet*. 2009;84:399–405.
22. Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. *J Womens Health*. 1997;6:49–62.
23. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of a expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol*. 1992;135:1114–1126.
24. Prentice RL, Breslow NE. Retrospective studies and failure time models. *Biometrika*. 1978;65:153–158.
25. Korn JM, Kuruvilla FG, McCarroll SA, Wysoker A, Nemesh J, Cawley S, Hubbell E, Veitch J, Collins PJ, Darvishi K, Lee C, Nizzari MM, Gabriel SB, Purcell S, Daly MJ, Altshuler D. Integrated genotype calling and association analysis of SNPs, common copy number polymorphisms and rare CNVs. *Nat Genet*. 2008;40:1253–1260.
26. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575.
27. Aulchenko YS, Struchalin MV, Van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics*. 2010;11:134.
28. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, Heath SC, Timpson NJ, Najjar SS, Stringham HM, Strait J, Duren WL, Maschio A, Busonero F, Mulas A, Albai G, Swift AJ, Morken MA, Narisu N, Bennett D, Parish S, Shen H, Galan P, Meneton P, Hercberg S, Zelenika D, Chen WM, Li Y, Scott LJ, Scheet PA, Sundvall J, Watanabe RM, Nagaraja R, Ebrahim S, Lawlor DA, Ben-Shlomo Y, vey-Smith G, Shuldiner AR, Collins R, Bergman RN, Uda M, Tuomilehto J, Cao A, Collins FS, Lakatta E, Lathrop GM, Boehnke M, Schlessinger D, Mohlke KL, Abecasis GR. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet*. 2008;40:161–169.
29. Galwey NW. A new measure of the effective number of tests, a practical tool for comparing families of non-independent significance tests. *Genet Epidemiol*. 2009;33:559–568.
30. Peri S, Navarro JD, Amanchy R, Kristiansen TZ, Jonnalagadda CK, Surendranath V, Niranjan V, Muthusamy B, Gandhi TK, Gronborg M, Ibarrola N, Deshpande N, Shanker K, Shivashankar HN, Rashmi BP, Ramya MA, Zhao Z, Chandrika KN, Padma N, Harsha HC, Yatish AJ, Kavitha MP, Menezes M, Choudhury DR, Suresh S, Ghosh N, Saravana R, Chandran S, Krishna S, Joy M, Anand SK, Madavan V, Joseph A, Wong GW, Schiemann WP, Constantinescu SN, Huang L, Khosravi-Far R, Steen H, Tewari M, Ghaffari S, Blobe GC, Dang CV, Garcia JG, Pevsner J, Jensen ON, Roepstorff P, Deshpande KS, Chinnaiyan AM, Hamosh A, Chakravarti A, Pandey A. Development of human protein reference database as an initial platform for approaching systems biology in humans. *Genome Res*. 2003;13:2363–2371.
31. Ideker T, Ozier O, Schwikowski B, Siegel AF. Discovering regulatory and signalling circuits in molecular interaction networks. *Bioinformatics*. 2002;18(suppl 1):S233–S240.
32. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci U S A*. 2009;106:9362–9367.
33. Hartwell LH, Hopfield JJ, Leibler S, Murray AW. From molecular to modular cell biology. *Nature*. 1999;402:C47–C52.
34. Johnson AD, Newton-Cheh C, Chasman DI, Ehret GB, Johnson T, Rose L, Rice K, Verwoert GC, Launer LJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, Caulfield M, Van Duijn CM, Ridker PM, Munroe PB, Levy D.Association of hypertension drug target genes with blood pressure and hypertension in 86 588 individuals. *Hypertension*. 2011;57:903–910.
35. Leineweber K, Heusch G. Beta 1- and beta 2-adrenoceptor polymorphisms and cardiovascular diseases. *Br J Pharmacol*. 2009;158:61–69.
36. Rosengren AH, Jokubka R, Tojjar D, Granhall C, Hansson O, Li DQ, Nagaraj V, Reinbothe TM, Tuncel J, Eliasson L, Groop L, Rorsman P, Salehi A, Lyssenko V, Luthman H, Renstrom E. Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. *Science*. 2010;327:217–220.
37. Segre AV, Groop L, Mootha VK, Daly MJ, Altshuler D. Common inherited variation in mitochondrial genes is not enriched for associations with type 2 diabetes or related glycemic traits. *PLos Genet*. 2010;6:e1001058.
38. Chuang HY, Lee E, Liu YT, Lee D, Ideker T. Network-based classification of breast cancer metastasis. *Mol Syst Biol*. 2007;3:140.
39. Lage K, Møllgård K, Greenway S, Wakimoto H, Gorham JM, Workman CT, Bendsen E, Hansen NT, Rigina O, Roque FS, Wiese C, Christoffels VM, Roberts AE, Smoot LB, Pu WT, Donahoe PK, Tommerup N, Brunak S, Seidman CE, Seidman JG, Larsen LA. Dissecting spatio-temporal protein networks driving human heart development and related disorders. *Mol Syst Biol*. 2010;6:381.

CLINICAL PERSPECTIVE

New approaches for the analysis of genome-wide association (GWA) data that allow for integration with complementary data are needed for phenotypes that do not support large-scale recruitment schemes or meta-analysis. Most GWA studies have focused on the identification of the strongest single-locus associations, but the identification of combined effects of many weakly associated variants is especially appealing for complex diseases, such as coronary heart disease (CHD), that are likely not caused by single variants or by a single biological pathway. We used a network-based analysis based on protein-protein interaction data and GWA results for CHD in 899 CHD cases and 1823 controls from the Nurses' Health and the Health Professionals Follow-up Studies. *P* values for each gene were assigned according to the smallest *P* values for single-nucleotide polymorphisms within 70 kb upstream and 20 kb downstream of the gene and corrected for the effective number of independent single-nucleotide polymorphisms for each gene. Networks of genes that represent direct protein-protein interactions were examined to identify protein complexes with evidence for association in aggregate. After correcting for the number of complexes tested, a significant association was observed between CHD and genes that encode proteins that interact with the β1-adrenergic receptor gene (*ADRB1*). This complex included 18 protein interaction partners that have not been identified as candidate loci for CHD. This comprehensive approach highlights that network-based analyses have the potential to reveal additional novel genes of interest to a phenotype beyond those discovered in GWA studies of common variants.

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Protein Interaction-Based Genome-Wide Analysis of Incident Coronary Heart Disease

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SUPPLEMENTAL MATERIAL

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Protein interaction-based genome-wide analysis of incident coronary heart disease.

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* Dr's Jensen and Pers contributed equally to the manuscript

Methods

Blood collection and DNA extraction in nested case-control study

Blood samples collected from 32,826 NHS participants between 1989 and 1990, and 18,225 HPFS participants between 1993 and 1995, were returned to our laboratory using overnight courier. Upon arrival, whole blood samples were centrifuged and aliquoted into cryotubes as plasma, buffy coat, and red blood cells, which were stored in the vapor phase of liquid nitrogen freezers.

DNA extraction

High-quality DNA was extracted from buffy coats using the QIAmpTM (QIAGEN Inc., Chatsworth, CA) 96-spin blood kit protocol. The average yield from 50μl of buffy coat (based on 1,000 samples) is 5.5μg with a standard deviation of 2.2 (range 2.0-16.4).

GWAS data cleaning

Samples were removed from all datasets based on the following criteria: 1) accidental duplicates as identified by pairwise identity-by descent (n=2); 2) samples identified as siblings (n=0), 3) discordant genotypic and phenotypic sex (n=2); and 4) missing call rate ≥2% (n=0). Population structure was investigated by principal component analysis. We used a set of 12,021 SNPs selected to have very low levels of LD and to have MAF >0.05 in Caucasians.[1] Study subjects passing quality control were analyzed together with a set of 209 HapMap II founders (59 CEU, 60 YRI, 45 JPT and 45 CHB). Subjects within the means of the first and second principal components (± 3 s.d.) among self-described whites were classified as having primarily European ancestry.

Protein-protein interaction database

The InWeb PPI database retrieves information from all major major experimental PPI databases, including the following: BIND,[2] DIP,[3] BioGRID,[4] HPRD,[5] IntAct,[6] MPact,[7] MIPS,[8] DOMINO,[9] Corum,[10] PDZBase,[11] and MINT.[12]

Supplemental Tables and Figures

Supplemental Table 1. List of cardiovascular susceptibility genes identified by Samani et al (NEJM 2007)[13] and the Human Genome Catalogue.

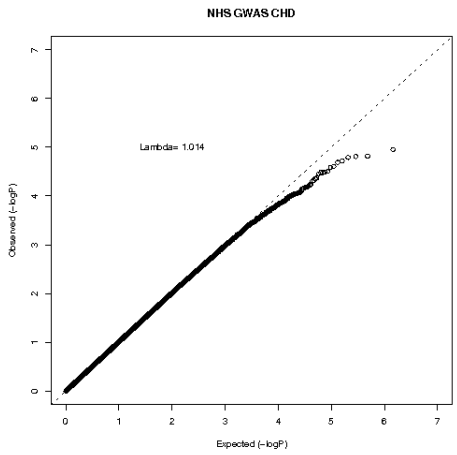
ABCA1
ADD1
ADH1C
ACDC
ADRB1
ADRB2
ADRB3
AGER
AGT
AGTR1
AGTR2
ALOX5AP
APOA1
APOA4
APOE
CCL11
CCL5
CCR2
CD14
CD36
CETP
MHC2TA
COMT
CPB2
CX3CR1
CYBA
CYP11B2
CYP1A1

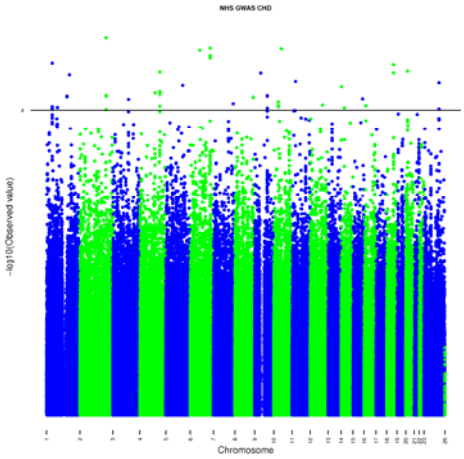
CYP1A2
CYP2C9
ENPP1
ESR1
F12
F13A1
F2
F3
F5
F7
FGB
GCK
GCLM
GJA4
GNB3
GP1BA
GP6
HMOX1
HTR2A
ICAM1
IL1B
IL6
ITGA2
ITGB2
ITGB3
LGALS2
LIPC
LPA
LPL
LRP1
LTA
MGP
MMP3
MMP9

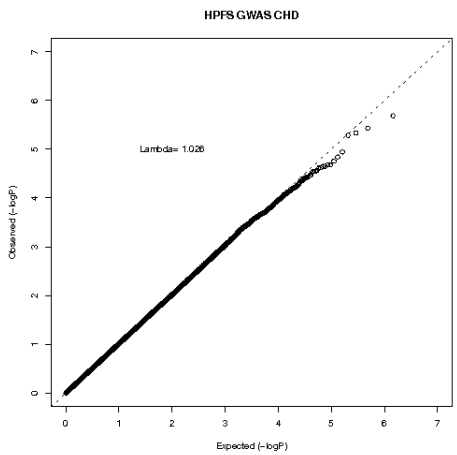
MTCO2
MTHFR
MTR
MTTP
NOS3
NPPA
NR3C1
OLR1
PCSK9
PECAM1
PLA2G7
PON1
PON2
PPARA
PPARG
SELE
SELP
SELPLG
TFPI
TGFB1
THBD
THBS1
THBS4
TLR4
TNF
TNFRSF1A
WRN
MIA3
MRAS
HNF1A-C12orf43
PHACTR1
WDR12
CXCL12
CDKN2A/CDKN2B

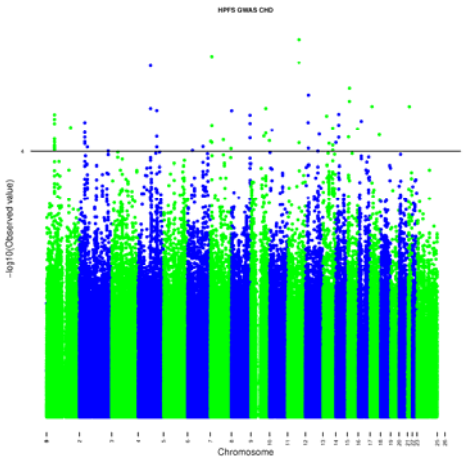
LDLR
PCSK9
MTHFD1L
SMAD3
SLC22A3
ABCG8
CELSR2-PSRC1-SORT1

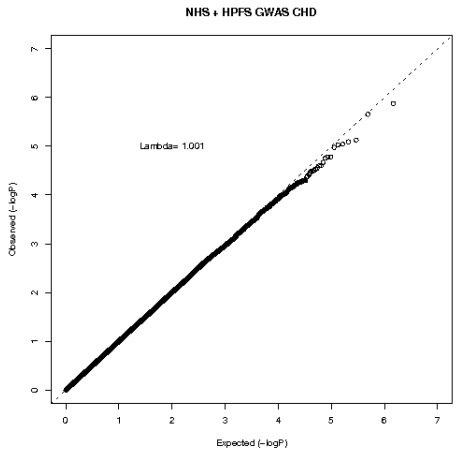
Supplemental Figure 1. Genome-wide association analysis for risk of CHD in the NHS and HPFS and the combined study population (QQ and Manhattan plots).

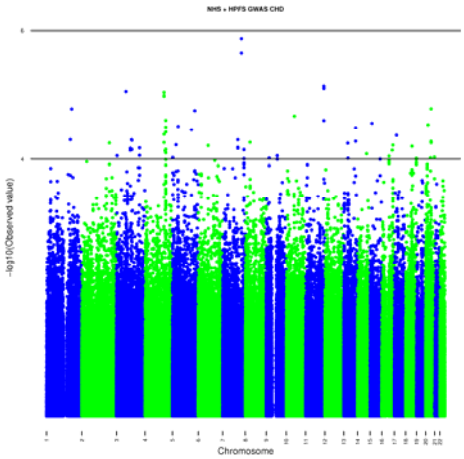












Supplemental Table 2. Full gene names for genes in the top complex

HUGO GENE NOMENCLATURE	***Full name***
MAGI1	membrane-associated guanylate kinase inverted 1
PRKACA	protein kinase cAMP-dependent catalytic alpha
GOPC	Golgi associated PDZ and coiled-coil motif containing
ADRB1	beta-1-adrenergic receptor
MAGI3	Membrane-associated guanylate kinase inverted 3
MAGI2	Membrane-associated guanylate kinase inverted 2
GRB2	growth factor receptor-bound protein 2
DLGAP2	discs, large (Drosophila) homolog-associated protein 2
ARRB1	arrestin, beta 1
DLG4	discs, large homolog 4 (Drosophila)
GNAL	guanine nucleotide binding protein, alpha activating activity polypeptide, olfactory type
GIPC1	GIPC PDZ domain containing family, member 1.
DLG1	discs, large homolog 1 (Drosophila)
GPRASP1	G protein-coupled receptor associated sorting protein 1.
ADRA2A	adrenergic, alpha-2A-, receptor
SH3GL3	SH3-domain GRB2-like 3
GNAS	GNAS complex locus
SH3GL2	SH3-domain GRB2-like 2
PDE4D	phosphodiesterase 4D, cAMP-specific.

References

1. Yu K, Wang Z, Li Q, Wacholder S, Hunter DJ, Hoover RN, Chanock S, Thomas G. Population substructure and control selection in genome-wide association studies. PLoS One. 2008; 3:e2551.

2. Bader GD, Donaldson I, Wolting C, Ouellette BF, Pawson T, Hogue CW. BIND--The Biomolecular Interaction Network Database. Nucleic Acids Res. 2001; 29:242-245.

3. Salwinski L, Miller CS, Smith AJ, Pettit FK, Bowie JU, Eisenberg D. The Database of Interacting Proteins: 2004 update. Nucleic Acids Res. 2004; 32:D449-D451.

4. Stark C, Breitkreutz BJ, Reguly T, Boucher L, Breitkreutz A, Tyers M. BioGRID: a general repository for interaction datasets. Nucleic Acids Res. 2006; 34:D535-D539.

5. Peri S, Navarro JD, Amanchy R, Kristiansen TZ, Jonnalagadda CK, Surendranath V, Niranjan V, Muthusamy B, Gandhi TK, Gronborg M, Ibarrola N, Deshpande N, Shanker K, Shivashankar HN, Rashmi BP, Ramya MA, Zhao Z, Chandrika KN, Padma N, Harsha HC, Yatish AJ, Kavitha MP, Menezes M, Choudhury DR, Suresh S, Ghosh N, Saravana R, Chandran S, Krishna S, Joy M, Anand SK, Madavan V, Joseph A, Wong GW, Schiemann WP, Constantinescu SN, Huang L, Khosravi-Far R, Steen H, Tewari M, Ghaffari S, Blobe GC, Dang CV, Garcia JG, Pevsner J, Jensen ON, Roepstorff P, Deshpande KS, Chinnaiyan AM, Hamosh A, Chakravarti A, Pandey A. Development of human protein reference database as an initial platform for approaching systems biology in humans. Genome Res. 2003; 13:2363-2371.

6. Kerrien S, am-Faruque Y, Aranda B, Bancarz I, Bridge A, Derow C, Dimmer E, Feuermann M, Friedrichsen A, Huntley R, Kohler C, Khadake J, Leroy C, Liban A, Lieftink C, Montecchi-Palazzi L, Orchard S, Risse J, Robbe K, Roechert B, Thorneycroft D, Zhang Y, Apweiler R, Hermjakob H. IntAct--open source resource for molecular interaction data. Nucleic Acids Res. 2007; 35:D561-D565.

7. Guldener U, Munsterkotter M, Oesterheld M, Pagel P, Ruepp A, Mewes HW, Stumpflen V. MPact: the MIPS protein interaction resource on yeast. Nucleic Acids Res. 2006; 34:D436-D441.

8. Mewes HW, Frishman D, Mayer KF, Munsterkotter M, Noubibou O, Pagel P, Rattei T, Oesterheld M, Ruepp A, Stumpflen V. MIPS: analysis and annotation of proteins from whole genomes in 2005. Nucleic Acids Res. 2006; 34:D169-D172.

9. Ceol A, Chatr-aryamontri A, Santonico E, Sacco R, Castagnoli L, Cesareni G. DOMINO: a database of domain-peptide interactions. Nucleic Acids Res. 2007; 35:D557-D560.

10. Ruepp A, Brauner B, Dunger-Kaltenbach I, Frishman G, Montrone C, Stransky M, Waegele B, Schmidt T, Doudieu ON, Stumpflen V, Mewes HW. CORUM: the comprehensive resource of mammalian protein complexes. Nucleic Acids Res. 2008; 36:D646-D650.

11. Beuming T, Skrabanek L, Niv MY, Mukherjee P, Weinstein H. PDZBase: a protein-protein interaction database for PDZ-domains. Bioinformatics. 2005; 21:827-828.

12. Chatr-aryamontri A, Ceol A, Palazzi LM, Nardelli G, Schneider MV, Castagnoli L, Cesareni G. MINT: the Molecular INTeraction database. Nucleic Acids Res. 2007; 35:D572-D574.

13. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, Dixon RJ, Meitinger T, Braund P, Wichmann HE, Barrett JH, Konig IR, Stevens SE, Szymczak S, Tregouet DA, Iles MM, Pahlke F, Pollard H, Lieb W, Cambien F, Fischer M, Ouwehand W, Blankenberg S, Balmforth AJ, Baessler A, Ball SG, Strom TM, Braenne I, Gieger C, Deloukas P, Tobin MD, Ziegler A, Thompson JR, Schunkert H. Genomewide association analysis of coronary artery disease. N Engl J Med. 2007; 357:443-453.