

A Bivariate Genome-Wide Approach to Metabolic Syndrome

STAMPEED Consortium

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OBJECTIVE—The metabolic syndrome (MetS) is defined as concomitant disorders of lipid and glucose metabolism, central obesity, and high blood pressure, with an increased risk of type 2

diabetes and cardiovascular disease. This study tests whether common genetic variants with pleiotropic effects account for some of the correlated architecture among five metabolic phenotypes that define MetS.

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RESEARCH DESIGN AND METHODS—Seven studies of the STAMPEED consortium, comprising 22,161 participants of European ancestry, underwent genome-wide association analyses of metabolic traits using a panel of ~2.5 million imputed single nucleotide polymorphisms (SNPs). Phenotypes were defined by the National Cholesterol Education Program (NCEP) criteria for MetS in pairwise combinations. Individuals exceeding the NCEP thresholds for both traits of a pair were considered affected.

RESULTS—Twenty-nine common variants were associated with MetS or a pair of traits. Variants in the genes *LPL*, *CETP*, *APOA5* (and its cluster), *GCKR* (and its cluster), *LIPC*, *TRIB1*, *LOC100128354/MTNR1B*, *ABCB11*, and *LOC100129150* were further tested for their association with individual qualitative and quantitative traits. None of the 16 top SNPs (one per gene) associated simultaneously with more than two individual traits. Of them 11 variants showed nominal associations with MetS per se. The effects of 16 top SNPs on the quantitative traits were relatively small, together explaining from ~9% of the variance in triglycerides, 5.8% of high-density lipoprotein cholesterol, 3.6% of fasting glucose, and 1.4% of systolic blood pressure.

CONCLUSIONS—Qualitative and quantitative pleiotropic tests on pairs of traits indicate that a small portion of the covariation in these traits can be explained by the reported common genetic variants. *Diabetes* 60:1329–1339, 2011

Metabolic syndrome (MetS) is defined as a combination of any three metabolic abnormalities, including central obesity, dyslipidemia, insulin resistance and/or glucose intolerance, and elevated blood pressure. These abnormalities tend to cluster in an individual and within families. Using the National Cholesterol Education Program (NCEP) MetS definition, the prevalence among adults in the U.S. was reported to be 23.8% in Caucasians, 21.6% in African Americans, and 31.9% in Mexican Americans (1,2) and 30.2% in adult Finns 40–65 years of age (3). MetS is associated with a higher risk of type 2 diabetes and cardiovascular disease and death (4–6). Because MetS risk factors have been reported

to be heritable, we hypothesized that genes with pleiotropic effects may be responsible for some of the clustering of metabolic abnormalities observed in MetS.

MetS has been previously investigated using multivariate analysis (7–9) and pairwise combinations of its components (10,11). In an investigation of lipid traits, Kullo et al. (12) concluded that pleiotropy (when a gene influences multiple traits) contributes to the covariation among three lipid traits, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and low-density lipoprotein particle size, supporting the hypothesis of genetic pleiotropy as a source of correlation among metabolic traits (13). Although several recent genome-wide association studies (GWAS) have been carried out on MetS components individually (obesity/dyslipidemia/blood pressure [BP] or type 2 diabetes) in several populations (14–21), it is unknown whether the identified variants influence combinations of MetS components.

The current study leverages GWAS results from seven studies participating in the single nucleotide polymorphisms (SNP) Typing for Association with Multiple Phenotypes from Existing Epidemiologic Data (STAMPEED) Consortium. Using the NCEP thresholds for a series of five traits including waist circumference (WC), fasting glucose (GLUC), HDL-C, TG, and BP, we created an affected status for all pairwise combinations of traits in which individuals exceeding the threshold for both traits were considered affected and all others were considered unaffected. By performing association tests with these 10 binary traits, as well as MetS per se, we sought to identify common genetic variants that affect the correlated architecture of these metabolic traits.

RESEARCH DESIGN AND METHODS

The STAMPEED Consortium, established in 2007, was sponsored by the National Heart, Lung, and Blood Institute (NHLBI) and includes 13 independent studies. In this report, we analyze a total of 22,161 participants of European ancestry, originating from seven studies, in alphabetic order: the Atherosclerotic Disease, Vascular function, and Genetic Epidemiology (ADVANCE) study (cases and controls separately), the Atherosclerosis Risk in Communities Study (ARIC) study, the Cardiovascular Health Study (CHS), the NHLBI Family Heart Study (FHS), the Genetic Epidemiology Network of Arteriopathy (GENOA), the Genetic Study of Aspirin Responsiveness (GeneSTAR), and the Northern Finland Birth Cohort 1966 (NFBC). Additional information regarding the characteristics of the original studies (regardless of any subsetting for GWAS genotyping) is available in the Supplementary Data.

The NCEP defines thresholds for five metabolic traits: WC ≥ 102 cm for men or WC ≥ 88 cm for women, HDL-C < 40 mg/dL for men or HDL-C < 50 mg/dL for women, TG ≥ 150 mg/dL, GLUC ≥ 100 mg/dL, and BP threshold as one of the three, systolic BP (SBP) or diastolic BP (DBP) $\geq 130/85$ mmHg or antihypertensive medication use. We defined 10 bivariate traits, HDL-C-WC, HDL-C-TG, HDL-C-GLUC, BP-HDL-C, WC-TG, TG-GLUC, TG-BP, WC-GLUC, BP-GLUC, and WC-BP, in which an individual was considered affected only if s/he exceeded these thresholds for both traits in a pair, otherwise unaffected. For comparison, we also analyzed a MetS per se trait where affected subjects exceed the specified threshold for three or more traits, of the five traits according to NCEP criteria.

In each study, ~ 2.5 million SNPs were imputed based on the HapMap (of European origin) CEU panel. Each study provided GWA results for 11 traits to the Data Coordinating Center (Division of Statistical Genomics, Washington University in Saint Louis, MO) for the meta-analysis. SNPs with R^2 -hat < 0.3 (for studies that imputed using MACH), proper info < 0.4 (IMPUTE), or variance ratio < 0.3 (BIMBAM), as well as those with study-specific minor allele frequency $< 5\%$ were filtered out from meta-analysis. As a result, these percentages of imputed markers were used in the meta-analyses: ADVANCE case, 81.4%; ADVANCE control, 81.2%; ARIC, 85.2%; CHS, 69.6%; FHS, 85.5%; GeneSTAR, 79.4%; GENOA, 84.1%; and NFBC, 86.1%; representing ~ 2 million SNPs for the combined analyses. Based on the Q-Q plots for GWAS results, all studies demonstrated a reasonable compliance to the null expectation. A homogeneity test for each SNP across studies is reported in Supplementary Tables 1–6; the tests were not significant, indicating the data could be combined for the meta-analysis.

Statistical methods. The 11 traits were tested for association with each SNP assuming additive SNP effects: $y_{ij} = \beta_0 + \beta_k X_{ijk} + \sum_{c=1}^s \beta_c X_{ijc} + \varepsilon_{ij}$, where the trait y_{ij} is a function of β_0 , the intercept β_k represents the k -th β coefficient from the regression analysis on imputed dosage, evaluated for each SNP ($k = 1, 2, \dots, m$), β_c is the coefficient for covariate effects ($c = 1, 2, \dots, s$); and ε_{ij} represents the residual for subject j ($j = 1, 2, \dots, nPID$) in pedigree i ($i = 1, \dots, nPID$). Individual studies used various software packages to carry out the GWAS (specified in Supplementary Data). Model covariates included sex, age, age², and up to 10 genotypic principal components describing population substructure in the model.

In preparation for meta-analyses, marker panels were aligned with dbSNP, build 36.3 for allele orientation. A meta-analysis of the GWAS results was conducted using a random coefficients model as proposed by DerSimonian and Laird (Supplementary Ref. 18) implemented through the MIXED procedure of SAS. This analysis returns combined estimates of the SNP β coefficients and standard errors, as well as the overall significance level accounting for the variance among estimates across studies. The homogeneity test is conducted to assess whether the β coefficients are combinable across studies ($i = 1$ to k). This test statistic is $Q = \sum_i w_i (\beta_i - \hat{\beta}_w)^2$, where β_i are β coefficients across studies, and the $\hat{\beta}_w = \sum_i w_i \beta_i / \sum_i w_i$, where w_i is inverse of the i -th sampling variance. The Q statistic has an approximate χ^2 with $k-1$ degrees of freedom.

We adopted a genome-wide significance criterion of $P \leq 9.7 \times 10^{-8}$, corresponding to a negative log₁₀ P value of 7.01. This threshold corresponds to the Bonferroni corrected level, by calculating the effective number of independent comparisons after accounting for linkage disequilibrium (LD) among SNPs (Supplementary Ref. 19). Post hoc analyses included investigation of the LD structure in gene regions of interest using HaploView v. 4.2, with HapMap data (v3 release 2/ v2 release 22) on subjects of European descent (CEU).

The top SNPs (that is, those meeting genome-wide significance, choosing one SNP per gene with the smallest P value) identified by the meta-analyses were tested in each study population for their association with each of the five dichotomized traits: WC, HDL-C, TG, GLUC, and BP. In addition, we carried out a specific test of pleiotropy using the full range of variation in the relevant quantitative traits. For subjects using lipid-lowering and/or antihypertensive medications, we imputed untreated traits values based on the estimated average effect of medications from clinical trials, as follows: TG/(1 – 15.2/100); HDL-C/(1 + 6.1/100); and SBP + 14.8 mmHg (Supplementary Ref. 17). An appropriate transformation of the quantitative traits to obtain a good approximation to normality was applied, as needed (e.g., all studies used \ln TG). The pleiotropy test on quantitative traits is a test of the simultaneous effect of the SNP on both quantitative traits, while allowing for a residual correlation: $y_{12ij} = \beta_1 (SNP_{jk} * y_{1ij}) + \beta_2 (SNP_{jk} * y_{2ij}) + \varepsilon_{12ij}$, where y_{12ij} represents a combined vector of traits 1 and 2 for each observation of subject j in family i (if families are present in the data); y_{1ij} and y_{2ij} each represent indicators of 1 s and 0 s if y_{12ij} belong to trait 1 or 2, respectively. The β_1 and β_2 estimates represent the additive contributions of k -th SNP on traits 1 and 2, respectively. We tested the null hypothesis $H_0: \beta_1 = 0$ and $\beta_2 = 0$ versus its alternative $H_1: \beta_1 \neq 0$ and $\beta_2 \neq 0$ with degrees of freedom equal to the number of subjects minus 2. In the family-based studies, subjects were nested within their corresponding pedigree and were considered statistically as repeated units. The tests were implemented with the MIXED procedure of SAS, v. 9.2. All the P values from each study were meta-analyzed.

For all studies, informed consent was obtained from all subjects and approval was granted by participating institutional review boards.

RESULTS

The sample characteristics for the subjects with both valid phenotype and genotype information in each respective study are shown in Table 1. The study populations showed large variation in the prevalence of MetS from 9% in the NFBC sample to 55% in GENOA, in part, reflecting different ascertainment strategies and age ranges represented in each study; the average age ranged from 31 years in the NFBC 1966 study to 73 years in the CHS study (Table 1). Of the five MetS components, TG and HDL-C had the strongest average correlation of any combination of traits (Supplementary Fig. 1).

SNPs in or near 15 genes were significantly associated with at least one of the 11 traits studied (Fig. 1 and Table 2 and details in Supplementary Tables 1–6). MetS per se was associated with several variants in genes including *BUD13* (*BUD13* homolog [*S. cerevisiae*]), *rs10790162*, $P = 5.4E-09$,

TABLE 1

Characteristics of the participants included in genome-wide association analyses (with both nonmissing genotype and phenotype data)

Sample characteristic	ADVANCE control (up to 311)	ADVANCE cases (up to 275)	ARIC (up to 8,127)	CHS (up to 3,262)	FHS (up to 2,432)	GeneSTAR (up to 1,789)	GENOA (up to 1,208)	NFBC (up to 4,757)
% Females	59	59	53	61	55	52	55	52
% Diabetic*	2.5	27	8	12	5	9	10	1
% Smoking†	32.8	63.3	25	11	29	23	14	30
Age (years)								
Men	40 ± 3	42 ± 4	55 ± 6	73 ± 6	50 ± 13	47 ± 13	56 ± 11	31 ± 1
Women	48 ± 5	52 ± 4	54 ± 6	72 ± 5	51 ± 13	47 ± 13	55 ± 11	31 ± 1
BMI (kg/m ²)								
Men	27 ± 5	31 ± 6	27 ± 4	26 ± 4	28 ± 4	29 ± 5	30 ± 5	25 ± 4
Women	26 ± 6	31 ± 8	27 ± 6	26 ± 5	27 ± 6	29 ± 7	31 ± 7	24 ± 5
Waist circumference (cm)								
Men	93 ± 12	102 ± 14	100 ± 10	98 ± 10	100 ± 12	101 ± 14	104 ± 12	89 ± 10
Women	79 ± 13	94 ± 19	93 ± 15	90 ± 14	94 ± 16	94 ± 17	96 ± 17	79 ± 12
HDL cholesterol (mg/dL)								
Men	44 ± 11	40 ± 10	43 ± 12	48 ± 13	43 ± 11	45 ± 13	45 ± 13	55 ± 13
Women	62 ± 14	51 ± 16	57 ± 17	60 ± 16	56 ± 15	57 ± 15	57 ± 17	66 ± 15
Triglycerides (mg/dL)								
Men	151 ± 224	184 ± 165	147 ± 100	138 ± 78	163 ± 113	160 ± 97	194 ± 107	119 ± 75
Women	97 ± 69	162 ± 145	128 ± 81	141 ± 74	138 ± 87	133 ± 79	188 ± 102	94 ± 52
Fasting glucose (mg/dL)								
Men	86 ± 11	104 ± 43	107 ± 27	111 ± 31	102 ± 29	99 ± 27	103 ± 32	93 ± 11
Women	88 ± 11	95 ± 18	102 ± 28	105 ± 27	95 ± 22	92 ± 21	96 ± 27	88 ± 11
SBP (mmHg)								
Men	117 ± 11	114 ± 12	120 ± 16	135 ± 21	119 ± 15	126 ± 16	134 ± 17	130 ± 13
Women	109 ± 13	118 ± 20	117 ± 18	135 ± 21	114 ± 18	121 ± 17	132 ± 17	120 ± 12
DBP (mmHg)								
Men	78 ± 10	73 ± 10	74 ± 10	72 ± 11	72 ± 9	80 ± 11	81 ± 10	80 ± 11
Women	70 ± 9	70 ± 10	70 ± 10	69 ± 11	67 ± 10	76 ± 10	77 ± 9	75 ± 11
% Antihyperlipidemic meds. use	4	81	3	4	10	15	17	0
% Antihypertensive meds. use	7	84	20	35	3	22	65	0.6
% Antiglycemic meds. use‡	1.3	24	3.2	5	0.2	4.3	6.9	0.6
MetS prevalence (%)	13.7	55.2	37.3	41.7	29.7	34.3	55.4	9.1

Values are means ± SD for each quantitative variable and where available split by male/female grouping. Meds., medication. *Definition of type 2 diabetes: fasting blood glucose >126 mg/dL or antiglycemic medication use. †Smoking: current smoking. ‡Antiglycemic meds. use: oral hypoglycemic agents or insulin.

ZNF259 (zinc finger protein 259, *rs2075290*, $P = 2.1E-09$), and *APOA5* (apolipoprotein A-V, *rs2266788*, $P = 1.9E-09$), all of which map within the *APOA*-cluster on chromosome 11 (11q23.3-q23, Supplementary Fig. 2). In addition, variants in *LPL* (lipoprotein lipase, 8p22, *rs295*, $P = 1.7E-09$) and *CETP* (cholesteryl ester transfer protein, plasma, 16q21, *rs173539*, $P = 9.1E-09$) were found associated with MetS. Specifically, variants in *LPL* were associated with BP-HDLc (*rs1441756*, $P = 2.7E-08$), TG-BP (*rs15285*, $P = 1.3E-10$), TG-GLUC (*rs2197089*, $P = 1.6E-09$), HDLC-TG (*rs13702*, $P = 1.0E-16$), and HDLC-WC (*rs301*, $P = 3.2E-11$). *CETP* variants were also significantly associated with BP-HDLc (*rs3764261*, $P = 3.3E-13$), HDLC-GLUC (*rs9939224*, $P = 6.9E-12$), HDLC-TG (*rs173539*, $P = 4.5E-16$), and HDLC-WC (*rs173539*, $P = 1.0E-16$), and variants in the gene *APOA*-cluster (*APOA5*, *BUD13*, and *ZNF259*) were significantly associated with TG-BP, TG-GLUC, HDLC-TG, and WC-TG (Table 2).

The *GCKR*-cluster of genes on chromosome 2p23–2p23.3 (Supplementary Fig. 3), including *GCKR* (glucokinase [hexokinase 4] regulator, 2p23), *ZNF512* (zinc finger protein 512, 2p23), *CCDC121* (coiled-coil domain containing

121, 2p23.3), and *C2orf16* (chromosome 2 open reading frame 16, 2p23.3), were, respectively, significantly associated with WC-TG (*rs780093*, $P = 1.9E-12$; *rs13022873*, $P = 5.0E-09$; *rs3749147*, $P = 1.4E-09$; and *rs1919128*, $P = 2.0E-09$) and also with TG-BP (*rs780093*, $P = 3.0E-10$) for *GCKR* gene. For a specific genotype in the *GCKR* variant studied, the genetic additive effects were inversely associated between TG and GLUC.

Additional variants were significantly associated with several binary traits, even if not with MetS. A total of 27 unique variants in 16 genes associated to some bivariate combinations of the five metabolic traits. Variants within *LIPC* (lipase hepatic, 15q21-q23) associated with HDLC-GLUC, (*rs2043085*, $P = 1.3E-08$) and with WC-HDLc (*rs10468017*, $P = 5.5E-08$); *ABCB11* (ATP-binding cassette, subfamily B [MDR/TAP], member 11, 2q24) associated with HDLC-GLUC (*rs569805*, $P = 8.5E-08$); *TRIB1* (tribbles homolog 1 [*Drosophila*], 8q24.13) variants were associated, respectively, with HDLC-TG (*rs2954026*, $P = 7.9E-09$) and TG-BP (*rs2954033*, $P = 8.5E-09$); and *TFAP2B* (transcription factor AP-2 β [activating enhancer binding protein 2 β], 6p12) was associated at a borderline

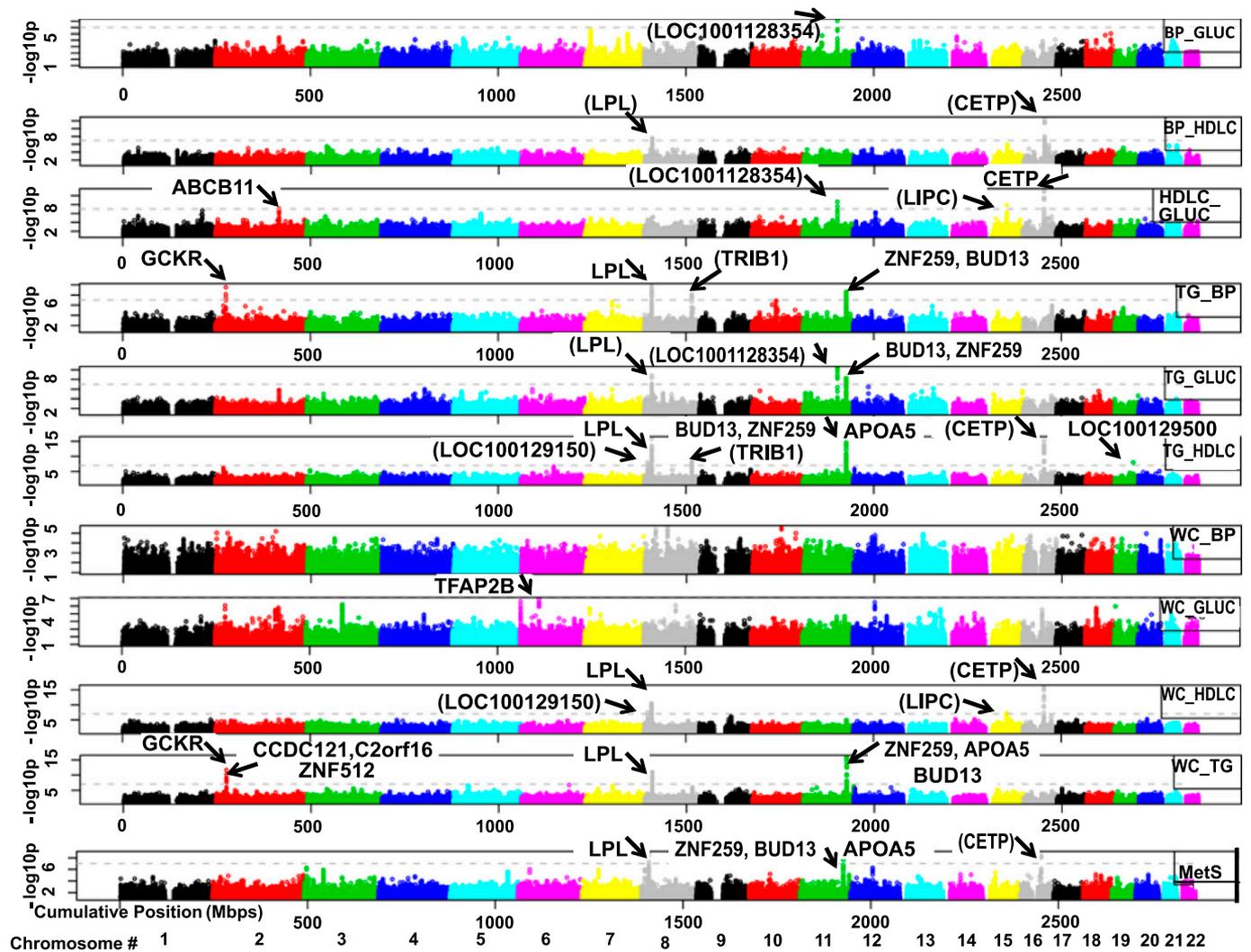


FIG. 1. Genome-wide meta-analyses results. Arrow annotated loci represent genes that show significant associations with MetS and/or individual binary bivariate traits. Each subgraph exemplifies results from a binary bivariate meta-analysis results or MetS meta-analysis. A dashed line in each subgraph represents a significance threshold of negative log₁₀*P* value of 7.01 corresponding to a *P* value of 9.7*10⁻⁸ (see Supplementary Data). A gene name in parentheses annotates a variant close to that particular gene. (A high-quality color representation of this figure is available in the online issue.)

significance level with WC-GLUC (*rs2206277*, *P* = 1.3E-07). Two SNPs located between *LOC100128354* (similar to small nuclear ribonucleoprotein polypeptide G, 11q21) and *MTNR1B* (melatonin receptor 1B) were significantly associated with BP-GLUC (*rs1387153*, *P* = 8.1E-09), with HDLC-GLUC (*rs1387153*, *P* = 2.4E-09), and with TG-GLUC (*rs10830956*, *P* = 4.8E-11) (Supplementary Fig. 4). In addition, *rs439401* of *LOC100129500* (protein coding hypothetical *LOC100129500*, 19q13.2) was associated with HDLC-TG (*P* = 1.0E-08), and *LOC100129150* (protein coding LP5624) variants with HDLC-TG (*rs9987289*, *P* = 1.1E-08) and HDLC-WC (*rs9987289*, *P* = 3.7E-08) (Fig. 1, Table 2, and details in Supplementary Tables 1–6).

Twenty-nine unique SNPs in association with binary traits and MetS per se were further tested with each of the individual dichotomized traits WC, HDLC, TG, GLUC, and BP (Fig. 2). None of the SNPs demonstrated a significant (*P* < 0.05) association with three or more traits and none of them with BP, even though some of the SNPs were in fact identified based on their association with MetS. Thus individual dichotomized trait associations do not necessarily reflect what one can discover with the combination

of traits. Several variants were associated with two individual dichotomized traits: *rs2266788* of *APOA5*, *rs2075290* of *ZNF259*, *rs11820589* of *BUD13*, and *rs13702* of *LPL* associated with TG and HDLC and *rs780093* of *GCKR* with TG and GLUC. We chose a single SNP with the strongest significance per gene to follow-up with a formal test of pleiotropy using the respective quantitative traits. Figure 3 shows a depiction of the pleiotropic relationships among traits based on the meta-analysis results for the pleiotropy test.

Finally, 11 of the top SNPs in 16 genes exhibited association *P* values with MetS meeting nominal significance levels (meta *P* < 0.05) (Table 3). Somewhat surprisingly, none of these was associated with fasting insulin levels (results not shown). On average across studies, the top 16 SNPs together accounted for the following proportions of the total variance for each trait: TG, 9%; HDLC, 5.8%; GLUC, 3.6%; WC, 2.3%; and SBP, 1.4%. To gain some insight as to the possible metabolic relationships among these genes, we used the *KEGG* database (<http://www.kegg.jp/>) to determine the pathways in which these genes participate. *LPL* and *APOA5* are classified as part of *hsa03320*

TABLE 2
A summary of STAMPEED β -meta-analyses of the most significant results

Gene	Variant	Trait	Ch	Position	Meta- β	Meta-SE	Meta <i>P</i>	Homog. <i>P</i> value	Coded	Genotype
<i>GCKR</i>	rs780093	TG-BP	2	27596107	0.18	0.03	3.0E-10	0.70	A	A/G
<i>GCKR</i>	rs780093	WC-TG	2	27596107	0.19	0.03	1.9E-12	0.64	A	A/G
<i>C2orf16</i>	rs1919128	WC-TG	2	27655263	-0.18	0.03	2.0E-09	0.82	A	A/G
<i>ZNF512</i>	rs13022873	WC-TG	2	27669014	-0.17	0.03	5.0E-09	0.47	A	A/C
<i>CCDC121</i>	rs3749147	WC-TG	2	27705422	-0.18	0.03	1.4E-09	0.79	C	C/T
<i>ABCB11</i>	rs569805	HDLC-GLUC	2	169491126	0.16	0.03	8.5E-08	0.46	A	A/T
<i>TFAP2B</i>	rs2206277	WC-GLUC	6	50906485	0.17	0.03	1.3E-07	0.75	A	A/G
<i>(LOC100129150)</i>	rs9987289	HDLC-TG	8	9220768	0.25	0.04	1.1E-08	0.54	A	A/G
<i>(LOC100129150)</i>	rs9987289	HDLC-WC	8	9220768	0.24	0.04	3.7E-08	0.58	A	A/G
<i>LPL</i>	rs295	MetS	8	19860518	0.17	0.03	1.7E-09	0.47	A	A/C
<i>LPL</i>	rs301	HDLC-WC	8	19861214	-0.22	0.03	3.2E-11	0.58	C	C/T
<i>LPL</i>	rs13702	HDLC-TG	8	19868772	0.29	0.03	1.0E-16	0.67	A	A/G
<i>LPL</i>	rs15285	TG-BP	8	19868947	-0.27	0.04	1.3E-10	0.65	A	A/G
<i>(LPL)</i>	rs2197089	TG-GLUC	8	19870653	0.18	0.03	1.6E-09	1.00	C	C/T
<i>(LPL)</i>	rs1441756	BP-HDLC	8	19912666	-0.18	0.03	2.7E-08	0.43	G	G/T
<i>(TRIB1)</i>	rs2954026	HDLC-TG	8	126553708	-0.16	0.03	7.9E-09	0.46	G	G/T
<i>(TRIB1)</i>	rs2954033	TG-BP	8	126562928	0.17	0.03	8.5E-09	0.55	A	A/G
<i>(LOC100128354)</i>	rs1387153	BP-GLUC	11	92313476	-0.19	0.03	8.1E-09	0.48	C	C/T
<i>(LOC100128354)</i>	rs1387153	HDLC-GLUC	11	92313476	-0.21	0.03	2.4E-09	0.49	C	C/T
<i>(LOC100128354)</i>	rs10830956	TG-GLUC	11	92320661	-0.20	0.03	4.8E-11	0.67	C	C/T
<i>BUD13</i>	rs11825181	TG-BP	11	116131468	0.32	0.05	3.0E-09	0.98	A	A/G
<i>BUD13</i>	rs11820589	TG-GLUC	11	116139072	0.32	0.06	5.5E-09	0.83	A	A/G
<i>BUD13</i>	rs10790162	HDLC-TG	11	116144314	0.38	0.05	2.8E-15	0.44	A	A/G
<i>BUD13</i>	rs10790162	MetS	11	116144314	0.25	0.04	5.4E-09	0.44	A	A/G
<i>BUD13</i>	rs10790162	WC-TG	11	116144314	0.39	0.05	6.6E-16	0.79	A	A/G
<i>ZNF259</i>	rs11823543	TG-BP	11	116154345	0.35	0.06	2.5E-09	1.00	A	A/G
<i>ZNF259</i>	rs12286037	TG-GLUC	11	116157417	-0.32	0.06	1.1E-08	0.86	C	C/T
<i>ZNF259</i>	rs2075290	HDLC-TG	11	116158506	0.39	0.05	1.5E-14	0.39	C	C/T
<i>ZNF259</i>	rs2075290	MetS	11	116158506	0.26	0.04	2.1E-09	0.64	C	C/T
<i>ZNF259</i>	rs2075290	WC-TG	11	116158506	0.41	0.05	1.1E-16	0.94	C	C/T
<i>APOA5</i>	rs2266788	HDLC-TG	11	116165896	0.39	0.05	4.6E-13	0.36	C	C/T
<i>APOA5</i>	rs2266788	MetS	11	116165896	0.26	0.04	1.9E-09	0.66	C	C/T
<i>APOA5</i>	rs2266788	TG-BP	11	116165896	0.37	0.07	3.5E-08	0.18	C	C/T
<i>APOA5</i>	rs2266788	WC-TG	11	116165896	0.41	0.05	2.2E-16	0.92	A	A/G
<i>(LIPC)</i>	rs10468017	HDLC-WC	15	56465804	0.16	0.03	5.5E-08	0.47	C	C/T
<i>(LIPC)</i>	rs2043085	HDLC-GLUC	15	56468246	-0.17	0.03	1.3E-08	0.83	A	A/G
<i>(CETP)</i>	rs173539	HDLC-TG	16	55545545	0.26	0.03	4.5E-16	0.61	C	C/T
<i>(CETP)</i>	rs173539	HDLC-WC	16	55545545	0.29	0.03	1.0E-16	0.65	C	C/T
<i>(CETP)</i>	rs173539	MetS	16	55545545	0.16	0.03	9.1E-09	0.41	C	C/T
<i>(CETP)</i>	rs3764261	BP-HDLC	16	55550825	0.29	0.04	3.3E-13	0.43	G	G/T
<i>CETP</i>	rs9939224	HDLC-GLUC	16	55560233	-0.31	0.05	6.9E-12	0.46	G	G/T
<i>LOC100129500</i>	rs439401	HDLC-TG	19	50106291	0.24	0.04	1.0E-08	0.44	C	C/T

Ch, chromosome number; position, position in base pairs; gene-hugo name, gene name is shown in parentheses when a SNP is near a location of the gene; meta- β , meta- β coefficient; meta-SE, meta-analysis β coefficients SE; homog. *P* value, a *P* value from a test of homogeneity of β coefficients, which has a high bound to one when all β coefficients included in the β -meta-analysis are very similar and is significant (less than 0.05) when coefficients among studies differ drastically; coded, the allele that was considered as coded allele for combining data of different studies accounting for β coefficient direction.

(PPAR signaling) pathway, and *LPL* and *LIPC* are part of *hsa00561* (Glycerolipid metabolism). In addition, *LPL* was classified in *hsa05010* (Alzheimer's disease) and *LIPC* is a member of *hsa01100* (Metabolic pathways); *MTNR1B* is a member of *hsa04080* (Neuroactive ligand receptor interaction), and *ABCB11* is classified as a member of *hsa02010* (ABC transporters) pathway. The rest of reported genes are not classified in pathways at present.

DISCUSSION

We used GWAS of pairs of metabolic traits to discover genetic determinants contributing to the correlated architecture of several metabolic traits that define MetS. Angers

and Biswas (22), who studied mathematical and statistical aspects of bivariate trait combination versus univariate ordinal categorical data, have shown that univariate analysis fails to detect features of the data found by the bivariate analysis. Therefore, this valid approach has the potential to uncover novel determinants not detectable with usual single phenotype-based analyses. Conversely, it is possible that by bivariate subsampling this study identified top SNPs that may further reduce the association power for any other subsample of three- or four- or five-trait combinations.

A meta-analysis approach was used to augment the power to detect such determinants. DerSimonian and Laird's method (Supplementary Ref. 18) has the considerable

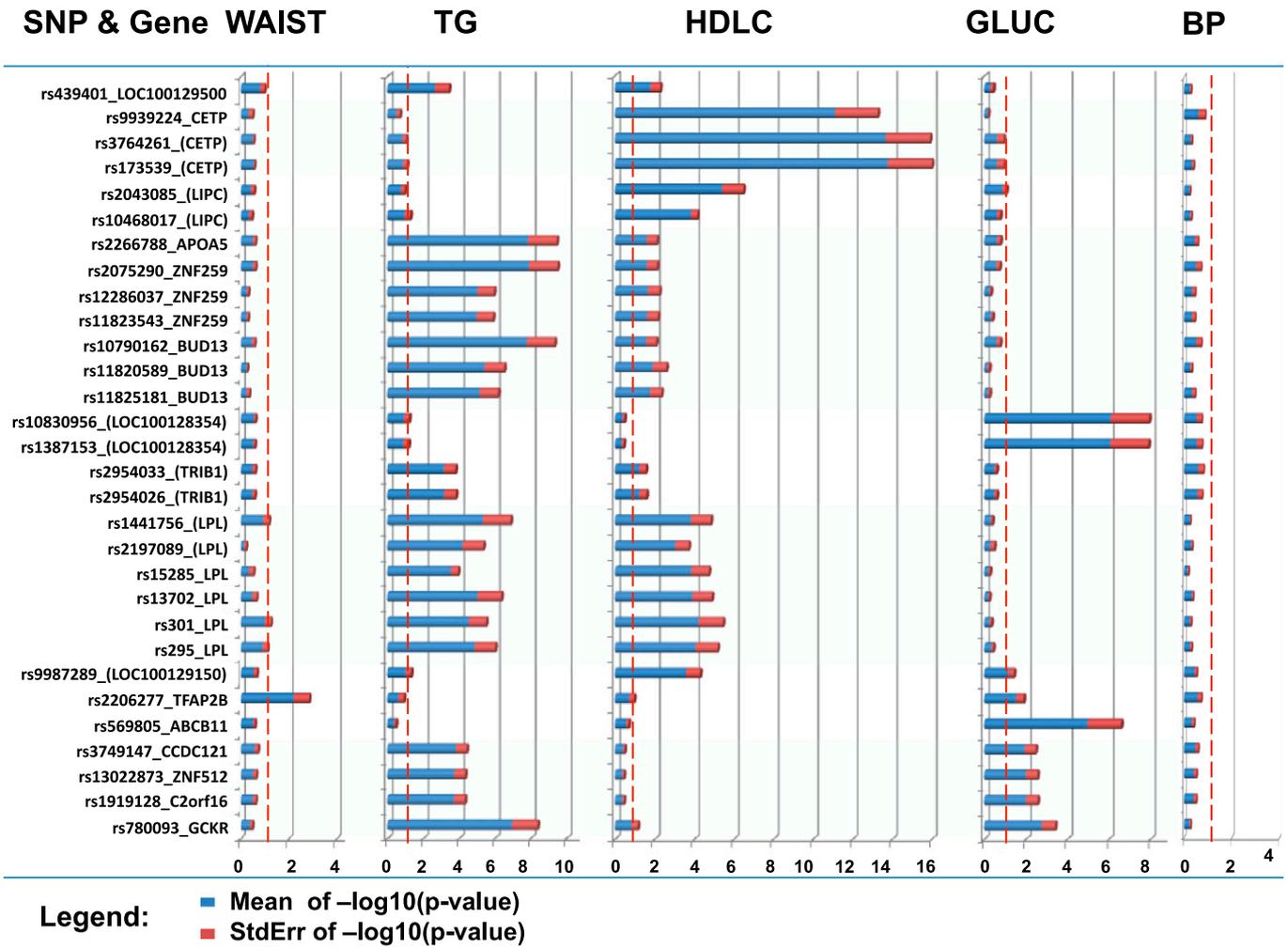


FIG. 2. Top significant SNPs from meta-analyses of MetS and bivariate traits associations, tested now for their association with dichotomized risk traits (WC, HDLC, TG, GLUC, and BP) as defined in the MetS NCEP definition. The results shown in the graph are the sample weighted mean of negative $\log_{10} P$ values (blue bars) per trait association, for all studies combined. On the top of blue bars (mean) added are the corresponding standard errors (red bars) of these negative $\log_{10} P$ values. The minimal threshold of negative $\log_{10} (0.05) P$ value is shown with a vertical red dashed line. A gene name in parentheses means the corresponding SNP is located in a region near the gene. (A high-quality color representation of this figure is available in the online issue.)

advantage of dynamically accounting for study differences by including the variance of the SNP-wise β parameter estimates in the calculation of the meta-analysis P value. This is particularly valuable in the context of the present analysis, with the variety of ascertainment strategies and ages across individual studies.

We identified 29 unique variants in or near 15 genes associated with binary pairwise traits or with MetS per se at the genome-wide significance level ($P < 9.7 \times 10^{-8}$), and an additional one at borderline significance. Interestingly, all but two of these results included a lipid abnormality, suggesting that genetic effects on lipid levels are more pronounced than for other traits. However, this observation is also consistent with the proposal of McGarry (23) that dyslipidemia is a fundamental component in the development of MetS. Moreover, the predominant pleiotropic pattern was for HDLC and TG, consistent with their well-known inverse correlation (Supplementary Fig. 1). The most influential variants in the correlation among traits were in or near *LPL*, *CETP*, *APOA5*, *ZNF259*, *BUD13*, *TRIB1*, *LOC100129500*, and *LOC100128154* (Table 2). Genes with variants influencing MetS per se included *LPL*,

CETP, and the *APOA*-cluster (*APOA5*, *ZNF259*, and *BUD13*), which are known to play an important role in lipid metabolism (24–37).

Variants in *LPL* were significantly associated with MetS and also with binary traits HDLC-BP, TG-BP, TG-GLUC, TG-HDLC, and WC-HDLC. *LPL* encodes lipoprotein lipase, which hydrolyzes TG in circulating very low-density lipoprotein cholesterol and chylomicrons, providing free fatty acids and monoacylglycerol for utilization by the surrounding target tissues, particularly in skeletal and cardiac muscle and adipose tissue. Previously *LPL* variants have been associated with individual components of the MetS (14,24,25), as well as with insulin resistance (26) and CHD (27). Our results indicate that *LPL* has pleiotropic effects on TG and HDLC validated by pleiotropy tests on the quantitative traits (Fig. 3). In Supplementary Fig. 5 we have simulated TG distributions for each study, based on the number of subjects, mean, and standard deviations of TG reported from each study. The *LPL* variant *rs13702* (3' UTR) had the lowest P value in the association tests within *LPL* gene, and the prevalent genotype ('AA') was associated with increased levels of TG and

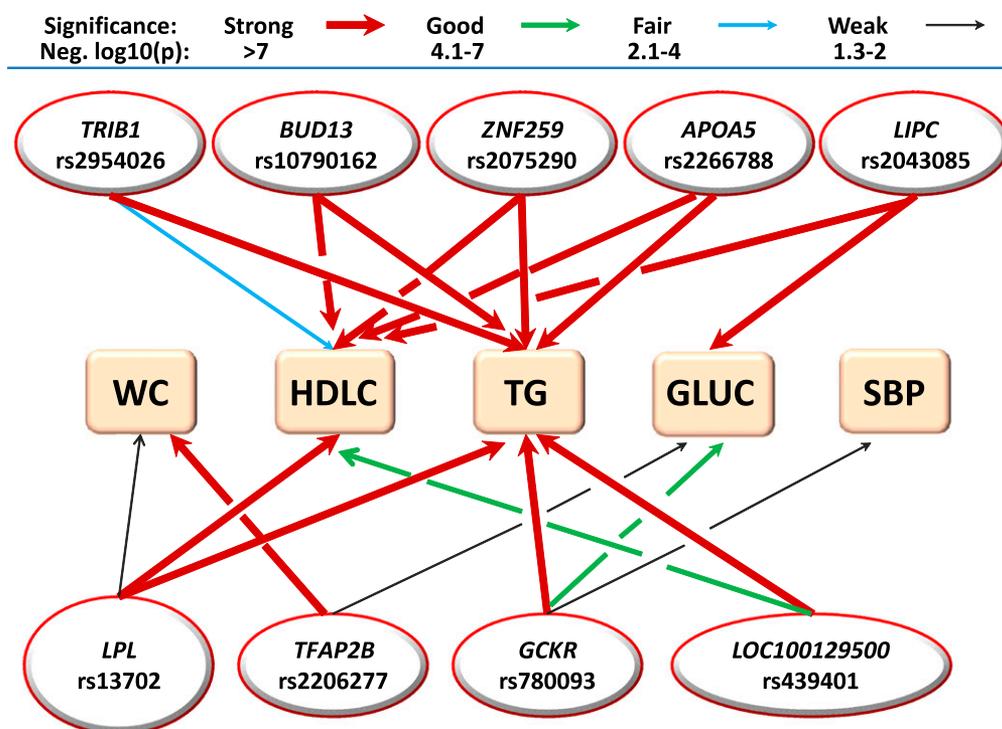


FIG. 3. A summary of meta-analysis on pleiotropy effects for selected SNPs on pair combinations of quantitative traits. Each study performed a pleiotropy test for selected SNPs with corresponding quantitative trait combinations. The identified meta-significant results show that variants associated more with two lipid measures and fasting glucose. (A high-quality color representation of this figure is available in the online issue.)

lower levels of HDLC. We provide detailed directions of association tests for each study in Supplementary Tables 1–6.

CETP is an independent gene that enables the transfer of cholesteryl esters in HDLC toward TG-rich lipoproteins, thereby contributing to lower HDLC. Variants of *CETP* were significantly associated with MetS, TG-HDLC, HDLC-GLUC, HDLC-BP, and WC-HDLC. Variants in *CETP* have been associated with HDLC levels (28,29). In our study *rs9939224* ‘TT’ rare genotype was associated with higher TG and lower HDLC levels. Although *CETP* genotypes have been reported extensively in association with CVD, its exact role in disease pathogenesis is unclear (30).

APOA5 is a member of the cluster of genes *APOA1–C3–A4–A5*, located on 11q23. *APOA5* encodes for apolipoprotein A5, a protein found in chylomicrons, VLDL, and HDL particles (31). *APOA5* has functional role in the endocytosis of TG-rich particles through its binding to the LDL receptor (32). Variants of the *APOA5* gene associate with elevated TG levels and may increase CHD risk (33). *APOA5* variants also have been reported to be associated with MetS (34,35). The ‘CC’ genotype of *rs2266788* (3’ UTR) was observed to have pleiotropic effects on (increasing) TG and (lowering) HDLC levels. By contrast, the functions of nearby genes *ZNF259* and *BUD13* are currently unknown, although they may reflect common haplotype associations (Supplementary Fig. 2).

TG-BP and WC-TG were both associated with an intronic SNP, *rs780093* in *GCKR*. The ‘AA’ genotype for *rs780093* was associated with higher TG levels and, in most of the studies, with lower levels of GLUC. *GCKR* also has been previously implicated as a susceptibility gene for type 2 diabetes (36). Functional studies suggest that *rs1260326*, a nonsynonymous SNP, has an effect on plasma glucose and triglyceride levels through increased glucokinase

activity in liver (37). However, this coding SNP is in a different LD block than is *rs780093* identified in our study (Supplementary Fig. 3). Furthermore, *rs1260326* and the intronic *rs780094* within *GCKR* have been reproducibly associated with multiple quantitative metabolic traits, but in the study of Sparsø et al. (38) with reduced risk for type 2 diabetes. It also is associated with TG (38–40), LDL cholesterol (41), C-reactive protein (42), and WC (43). These reports together with our evidence support the broad pleiotropic effects of *GCKR*.

LIPC variants demonstrated pleiotropic effects, specifically *rs10468017* on WC-HDLC and *rs2043085* on HDLC-GLUC. *LIPC*, which is expressed in liver, has the capacity to catalyze hydrolysis of phospholipids; mono, di, and triglycerides; and acyl-CoA thioesters and is considered an important enzyme in HDLC metabolism (28,44–46). Our results confirm those of Kathiresan et al. (15), who showed that the minor ‘T’ allele at *rs10468017* was associated with lower *LIPC* expression and increased HDLC levels. The ‘AA’ genotype of *rs2043085* also was associated with higher HDLC levels. Variants near *TRIB1* (8q24.13) (*rs2954026* and *rs2954033*) associated with TG-BP and TG-HDLC. The *rs2954026* and *rs2954033* SNPs locate 34 and 43 Kb downstream of *TRIB1*, respectively, a gene that has been associated with hyperlipoproteinemia (47). *TRIB1* and *TRIB2* genes also have been found to be upregulated in regions of human atherosclerotic plaque.

Other interesting variants are *rs10830956* and *rs13887153*, which map 10 and 3 Kb downstream of *LOC100128354*, respectively, and about 22 and 29 Kb upstream of *MTNR1B*. These were associated with TG-GLUC and BP-GLUC, HDLC-GLUC. These variants also showed highly significant associations with fasting glucose levels (Fig. 2). The less common ‘TT’ genotype of *rs10830956* showed higher levels of TG and GLUC. These variants are in

TABLE 3
Meta-analysis among studies of 16 top SNPs (one per gene) for their association with MetS

Number	Hugo	SNP	Chrom.	Position (bps)	Meta- β coefficient	Meta- β SE	χ^2 value	P value	Homogeneity P value	Number of samples
1	GCKR	rs780093	2	27596107	0.042	0.030	1.94	1.6E-01	0.222	8
2	C2orf16	rs1919128	2	27655263	-0.055	0.035	2.51	1.1E-01	0.186	8
3	ZNF512	rs13022873	2	27669014	-0.054	0.036	2.24	1.3E-01	0.154	8
4	CCDC121	rs3749147	2	27705422	-0.050	0.039	1.62	2.0E-01	0.110	8
5	ABCB11	rs569805	2	169491126	0.059	0.024	5.91	1.5E-02	0.411	8
6	TFAP2B	rs2206277	6	50906485	0.134	0.046	8.57	3.4E-03	0.073	6
7	LOC100129150	rs9987289	8	9220768	0.186	0.038	23.83	1.1E-06	0.703	8
8	LPL	rs13702	8	19868772	0.152	0.029	27.45	1.6E-07	0.343	8
9	TRIB1	rs2954026	8	126562928	-0.084	0.035	5.74	1.7E-02	0.132	8
10	LIPC	rs2043085	15	56468246	-0.059	0.023	6.40	1.1E-02	0.734	8
11	LOC100128354	rs10830956	11	92320661	-0.070	0.031	5.03	2.5E-02	0.254	8
12	BUD13	rs10790162	11	116144314	0.243	0.060	16.65	4.5E-05	0.172	8
13	ZNF259	rs2075290	11	116158506	0.259	0.059	19.52	1.0E-05	0.196	7
14	APOA5	rs2266788	11	116165896	0.265	0.044	36.07	1.9E-09	0.663	6
15	CETP	rs9939224	16	55560233	-0.168	0.036	21.84	3.0E-06	0.810	8
16	LOC100129500	rs439401	19	50106291	0.053	0.056	0.89	3.5E-01	0.071	6

Number of samples represents study results that were included in the meta-analysis for these SNPs. A few of them were not included in the analysis if they had MAF less than 5%, or Hardy-Weinberg Equilibrium P value less than 10^{-6} , or if the quality of imputation was less than the required thresholds (see RESEARCH DESIGN AND METHODS and Supplementary Data).

LD with the *MTNR1B* gene, although they reside in an LD block physically closer to the *LOC1001128354* gene (Supplementary Fig. 4). A recent large study with more than 36,000 participants of European descent showed that *rs13887153* associated with glucose. They reported that each copy of the *rs13887153* 'G' allele was associated with increased levels of glucose, reduced β -cell function measured by HOMA-B, and an increased risk for type 2 diabetes (48). Similar findings for fasting glucose were described by others (49). Our findings support the hypothesis that variants *rs10830956* and *rs13887153* may serve as proxies for variants in nearby genes *LOC100128354/MTNR1B* (melatonin receptor) involved in insulin/glucose metabolism.

Several variants were associated with only a single bivariate trait. The *rs569805* in the *ABCB11* significantly associated with HDLC-GLUC. This intronic SNP is located within 20 Kb and is in LD ($r^2 = 0.67$) with intronic SNP *rs560887* in *G6PC2*, the most significant variant in association with fasting GLUC ($P = 4E-75$) in a meta-analysis conducted by the MAGIC consortium (36). By contrast, *rs560887* was not associated with HDLC levels in the ENGAGE consortium (21). Another significant association for TG-HDLC was identified on chromosome 19, with *rs439401*, in an intron of the hypothetical protein *LOC100129500*. This SNP demonstrated association ($P = 2E-9$) with TG levels and nominal association ($P = 3E-3$) with HDLC levels in a recent meta-analysis of GWA studies in 16 European cohorts (21). This SNP is in *APOC1* (with relative position of -3.7 Kb) located within the larger *APOE/C1/C4/C2* gene cluster, known to have pleiotropic effects on lipid metabolism (50). A number of our lipid findings are also reported by Teslovich et al. (20) in a GWAS meta-analysis for plasma lipid traits with more than 123,000 subjects. In that study, 26 out of the 95 loci associated with more than one lipid trait at genome-wide significance.

We set out to test the hypothesis that common variants explain the correlated architecture among MetS traits, at least in part. These common top SNPs (16 variants selected one per gene) together explained a total average

variance ranging from 1.4% for SBP to 9.0% for TG. The weak effects on SBP are consistent with a large GWAS on BP with more than 84,000 participants, able to explain less than 2% of BP variation (17). Explicit tests of pleiotropy of our study's top SNPs on the relevant quantitative traits validated 11 pleiotropic relationships summarized in Fig. 3. Because they explained a small fraction of the correlation among metabolic syndrome traits, other factors must also be at play: variants with rare alleles; nonadditive effects, such as dominance and epistasis; furthermore, cascade effects of a phenotype, e.g., obesity directly influencing glycemic, lipid and blood pressure traits; as well as common environmental factors in defining the correlated architecture of these traits. For tests of pleiotropy carried out on the quantitative phenotypes, we imputed lipid- and SBP trait values for treated subjects based on summaries of a large number of clinical trials. However, we acknowledge that this approach is not ideal and may have limited our ability to detect true pleiotropic effects.

We explored also the relationships among the genes identified in this study. A hypothesized network of interactions among identified genes was constructed using GeneGO software (Fig. 4). Five of the selected genes interact with many genes, represented in Fig. 4 as hidden links. Even this consideration suggests the possibility that many other genes may act in the context of their respective pathways, rather than independently. For example, *LPL* interacts with *INS*, *APOE*, *APOB*, *APOA1*, *APOA4*, *APOC3*, *APOC4*, *LRP1*, and *NETO1*; and *CETP* has at least 35 interactions, based on Sigma-Aldrich and Ingenuity database (<http://www.sigma-aldrich.com/yfg>). It is possible that cross-talk among these pathways via intermediate activator/suppressor molecules contributes to the clustering of disorders in MetS. Thus one possible fruitful direction of study is to examine MetS and its correlated structure in a systems biology context. In summary, none of these variants associated simultaneously with most or all the traits, supporting the concept that MetS is not a consequence of any single pathway or factor but rather a consequence of interactions among different pathways.

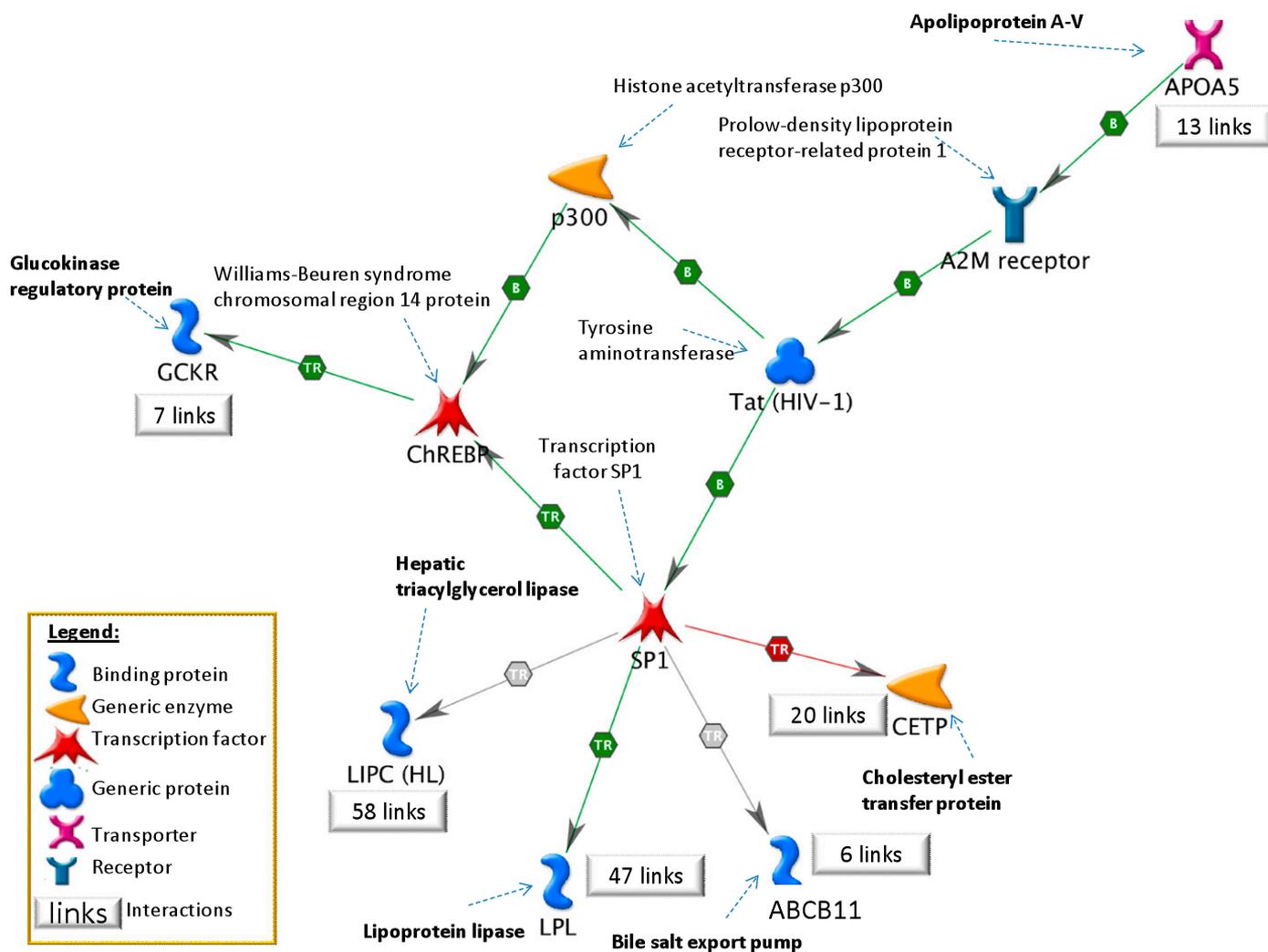


FIG. 4. One of the simplified networks of the genes *LPL*, *CETP*, *APOA5*, *GCKR*, *LIPC*, and *ABCB11* among 16 genes reported in this article with variants significantly associated to MetS and/or its bivariate traits. This network was built from curated publications based on GeneGO database. (Green arrows show activation; red arrows show suppression.) (A high-quality color representation of this figure is available in the online issue.)

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