Gene–Smoking Interactions Identify Several Novel Blood Pressure Loci in the Framingham Heart Study

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BACKGROUND
Cardiovascular diseases are among the most significant health problems in the United States. Blood pressure (BP) variability has a genetic component, and most of the genetic variance remains to be identified. One promising strategy for gene discovery is genome-wide analysis of interactions between single nucleotide polymorphisms (SNPs) and environmental factors related to cardiovascular diseases.

METHODS
We investigated SNP–smoking interaction effects on BP in genome-wide data in 6,889 participants from the Framingham Heart Study. We performed the standard 1 degree of freedom (df) test of the interaction effect and the joint 2 df test of main and interaction effects. Three smoking measures were used: cigarettes per day (CPD), pack years of smoking, and smoking status.

RESULTS
We identified 7 significant and 21 suggestive BP loci. Identified through the joint 2 df test, significant SBP loci include: rs12149862 ($P = 3.65 \times 10^{-9}$) in *CYB5B*, rs2268365 ($P = 4.85 \times 10^{-8}$) in *LRP2*, rs133980 ($P = 1.71 \times 10^{-8}$ with CPD and $P = 1.07 \times 10^{-8}$ with pack-years) near *MN1*, and rs12634933 ($P = 4.05 \times 10^{-8}$) in *MECOM*. Through 1 df interaction analysis, 1 suggestive SBP locus at SNP rs8010717 near *NRXN3* was identified using all 3 smoking measures ($P = 3.27 \times 10^{-7}$ with CPD, $P = 1.03 \times 10^{-7}$ with pack-years, and $P = 1.19 \times 10^{-7}$ with smoking status).

CONCLUSIONS
Several of these BP loci are biologically plausible, providing physiological connection to BP regulation. Our study demonstrates that SNP–smoking interactions can enhance gene discovery and provide insight into novel pathways and mechanisms regulating BP.

Keywords: blood pressure; gene–environment interaction; genome-wide association study; hypertension; single nucleotide polymorphisms; smoking.

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Cardiovascular diseases are among the most significant health problems in the United States. Genome-wide association studies (GWASs) have identified hundreds of common genetic variants associated with many common, complex disease traits (http://www.genome.gov). However, most identified variants confer relatively small increments in risk and explain only a small fraction of the heritability.[1] For example, the 29 common variants identified through 3 recent GWASs consortia[2–4] have shown to collectively explain <2.5% of systolic and diastolic blood pressure (BP) variance.[4] It is increasingly recognized that the near-exclusive focus on main effects has become a barrier to the identification of additional genes underlying these complex traits. Greater emphasis is being placed in recent years on gene–environment interaction analyses.[5] The identification of gene–environment interaction is important for many reasons. Gene–environment interaction or more complex pathways involving multiple genes and environments can explain part of the missing heritability.[1,6] They can further elucidate the biological networks underlying complex disease risk and enable "profiling" of individuals at highest risk for disease.[7]

Many lifestyle factors, including physical activity, tobacco use, excessive alcohol consumption, and dietary factors, influence BP.[8] These lifestyle factors may modulate the effect of genes on BP. This journal has recently published 3 articles that are related to environmental contribution to BP and hypertension. Dong *et al.*[9] presented the relationship between increasing trends in BP and body mass index among Chinese children and adolescents from 2005 to 2010. Xi *et al.*[10] presented a significant association of hypertension susceptibility loci in obese Chinese children, suggesting a likely influence of childhood obesity on the risk of hypertension. As nicely presented by Falkner,[11] obesity and dietary sodium intake are potentially modifiable environmental factors.

In this study, we focused on the role of smoking in the genetic and environmental architecture of BP. Cigarette smoking is a leading cause of preventable death, causing 5 million premature deaths worldwide each year, and current trends show that tobacco use will cause >8 million deaths annually by 2030, according to World Health Organization estimates. Smoking is a major risk factor for cancer, heart disease, stroke, and lung diseases. In the acute setting, cigarette smoking produces a rise in BP. Some epidemiologic

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studies have associated chronic smoking with lower BP, even after adjustment for other cardiovascular risk traits.[12] Therefore, genome-wide studies incorporating interactions between genetic variants and smoking may enhance BP gene discovery efforts and provide novel insights into the biological mechanisms and pathways underlying BP regulation.[7]

We examined the contribution of interactions between genetic variants and 3moking measures on BP traits: (i) cigarettes per day, measuring a smoking rate (per day); (ii) pack-years of smoking, measuring a volume of smoking exposure during a person's entire lifetime; and (iii) smoking status, a binary (yes/no) indicator of a current smoking status. We performed a genome-wide analysis of single nucleotide polymorphism (SNP)–smoking interactions on systolic BP (SBP) and diastolic BP (DBP) using 6,889 participants from the Framingham Heart Study (FHS). Our aim was to identify novel BP loci; discovery of such loci may facilitate smoking intervention strategies and achievement of BP goals in genetically susceptible individuals, thereby reducing the public health burden of hypertension.

METHODS

Study sample

In this study, we used the FHS SHARe (SNP Health Association Resource) data, as obtained through the Database of Genotypes and Phenotypes (dbGaP; http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000342.v8.p8). FHS is the oldest prospective longitudinal cohort study of cardiovascular risk factors in the United States. FHS began in 1948 with the recruitment of an original cohort of 5,209 men and women who were aged 28–62 years at entry. In 1971, a second generation of study participants, 5,124 children and spouses of children of the original cohort were enrolled. Enrollment of the third generation cohort of 4,095 children of the offspring cohort participants began in 2002. The study obtained informed consent from participants and approval from the appropriate institutional review boards. We analyzed a date-matched set of individuals aged 20–80 years using data from the 26th visit of the original cohort, the 7th visit of the offspring cohort, and the 1st visit of the third-generation cohort.

Genotype data

Genotype data from the FHS SHARe project include approximately 550,000 SNPs that were genotyped using Affymetrix GeneChip Human Mapping 500 k Array Set and the 50 k Human Gene Focused Panel by Affymetrix (Santa Clara, CA). Genotype calls were made with the Bayesian Robust Linear Model with Mahalanobis distance classifier (BRLMM) algorithm. Approximately 2.5 million autosomal SNPs were imputed with MACH (http://www.sph.umich.edu/csg/abecasis/MACH) using the HapMap Phase II (release 22) CEU reference panel from International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/). More detailed information is available elsewhere.[13]

For the genotyped SNPs, we excluded SNPs that have Hardy–Weinberg equilibrium P values $<10^{-6}$ and call rates <90%. Hardy–Weinberg equilibrium P values are computed based on founders only using PLINK,[14] as recommended for family studies. For the imputed SNPs, we excluded SNPs that had imputation quality measures <0.30, which resulted in 2,455,927 imputed SNPs. Finally, for both genotyped and imputed SNPs, we excluded SNPs with <30 copies of the minor allele from our interaction analysis. When the SNPs were available as both genotyped SNPs and imputed SNPs, we used genotyped SNPs. The number of SNPs after quality control and exclusion was 2,485,435 SNPs; our genome-wide interaction analysis was performed using these SNPs.

Phenotype data

SBP and DBP were measured using a consistent protocol and a standard mercury column sphygmomanometer (portable Baumanometer 300 Model or wall-mounted Baumanometer E98169, W.A. Baum Co., Copiague, NY) in the clinic (the protocol descriptions are publicly available on dbGaP). Participants were seated for at least 5 minutes before the first BP measurement. Our analysis phenotype was the average of 3 BP measurements (1 nurse/technician reading and 2 physician readings).

Smoking measures

We considered 3 smoking measures: cigarettes per day (CPD), pack-years of smoking, and smoking status. CPD represents the number of cigarettes that the subject smoked on average per day if he/she has ever smoked. Pack-years are calculated as the average number of packs smoked per day times the total number of years a subject smoked during his/her lifetime. Smoking status is a self-reported binary measure, coded as 1 if the subject smoked regularly in past year. All three smoking measures (CPD, pack-years, smoking status) were set to zero for nonsmokers. Smoking status was set to 0 for former smokers who quit smoking since last year, but their CPD and pack-years were used as they were in the

Table 1. Descriptive statistics of the blood pressure traits, covariables, and smoking measures used in the analysis

Characteristics	Descriptive statistics
Sample size	6,889
% Male	46.7
% Hypertensive	27.9
% Taking antihypertensive meds	19.4
Age, y	49.3 ± 13.7
BMI, kg/m^2	27.5 ± 5.5
SBP, mm Hg	120.5 ± 16.5
DBP, mm Hg	74.83 ± 9.4
Cigarettes per day	9.2 ± 12.8
Pack-years	9.9 ± 17.6
% Smoking status	15.76

Data are mean value ± SD or percentage.
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

analysis with CPD and pack-years. All smoking phenotype data were thoroughly checked, and any conflicting information regarding smoking responses were set to missing before analysis. In particular, if CPD and pack-years information was provided for nonsmokers, both values were simply deleted (set to missing) as part of routine quality control.

We note that the 3 smoking variables measure different aspects of nicotine smoking exposure. The current smoking status reflects the overall smoking behavior; the CPD reflects the rate/intensity of smoking; the pack-years information represents the total volume of smoking in one's life (up to that time), which therefore is a function of one's age. Our analysis sample included 6,889 genotyped individuals with at least 1 BP measure, 1 smoking measure, and nonmissing values of all covariables.

Statistical analyses

To identify SNP–smoking interactions, we performed the test proposed by Kraft *et al.*[15] that jointly tests the genetic main and G × E interaction effects. The expected response trait (Y) has the regression form

$$E[Y] = \alpha + \beta_g G + \beta_e E + \beta_{ge} GE,$$

where β_g and β_e, respectively, are the genetic and environmental (smoking) main effects and β_{ge} is their multiplicative interaction effect. In particular, we used a Wald test statistic that follows a χ^2 distribution with 2 degrees of freedom (df) under the H_0: $\beta_g=\beta_{ge}=0$. This Wald test statistic is based on estimates of β_g and β_{ge} and their corresponding 2×2 covariance matrix. We also performed the standard approach to identify G × E interactions by using the Wald test statistic that follows a χ^2 distribution with 1 df under the H_0: $\beta_{ge}=0$ (i.e., testing for the G × E interaction effect in the presence of the genetic main effect). Finally, we also tested the genetic main effect in the presence of G × E interaction effect by using Wald test statistic that follows a χ^2 distribution with 1 df under the H_0: $\beta_g=0$.

We used a linear mixed effect modeling framework, where a random effect is included to take account of phenotypic correlation across family members in the FHS family study; the covariance was determined by the kinship matrix based on the pedigree structure. In particular, we used GenABEL/MixABEL[16] that can provide estimates of β_g and β_{ge} and their corresponding 2×2 covariance matrix for the analysis of family data. Age, sex, body mass index, and antihypertensive medication use (yes/no) were included as covariables for our SNP–smoking interaction analysis.

We declared an SNP as genome-wide significant if $P \le 5\times10^{-8}$ and suggestive if $P \le 1\times10^{-6}$ following a standard

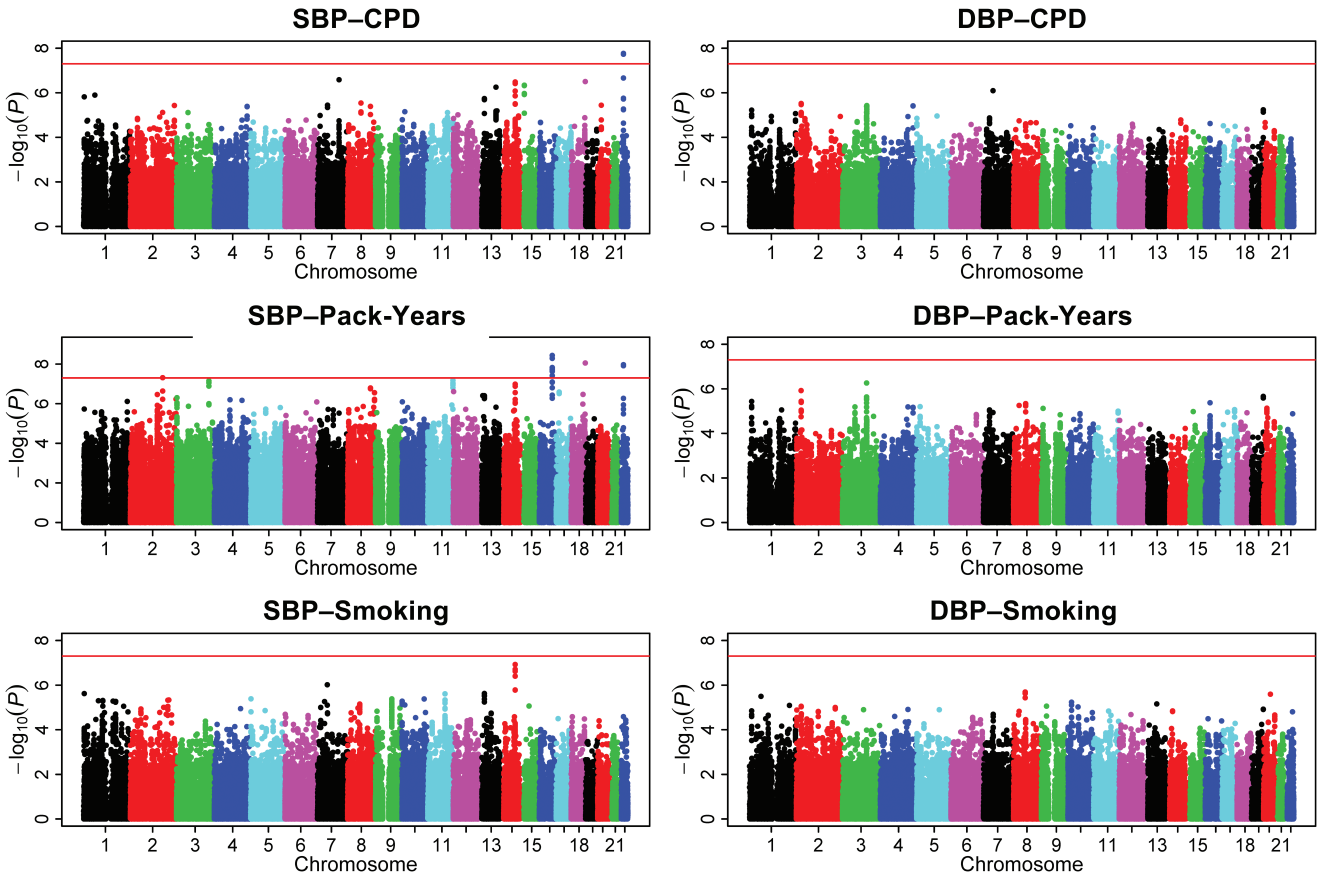

Figure 1. Manhattan plots of the joint 2 degree of freedom (df) test of the single nucleotide polymorphism (SNP) main effect and SNP–smoking interaction effect for each combination of 2 blood pressure (BP) traits (systolic BP (SBP) and diastolic BP (DBP)) and 3 smoking measures cigarettes per day (CPD), pack-years, and smoking status). The *P* value of the joint 2 df test of each SNP was plotted vs. the chromosomal location for all SNPs genome-wide.

Table 2. Index single nucleotide polymorphisms representing 28 blood pressure loci with $P < 1 \times 10^{-6}$ from the 2 degrees of freedom test

						SNP Main	SNP–smoking interaction		Joint 2 df test	
Chr	Position	SNP	Genomic location	MAF	Smoking measure	*P* value	*P* value	GC *P* value	*P* value	GC *P* value
Seven significant and 19 suggestive systolic blood pressure loci										
1	230,735,895	rs11589828	Intron *LOC729336*	14.3	Pack-years	4.63×10^{-3}	1.21×10^{-7}	1.17×10^{-5}	7.66×10^{-7}	9.39×10^{-6}
2	141,638,258	rs1033284	Intron *LRP1B*	29.2	Pack-years	0.09	7.49×10^{-8}	8.39×10^{-6}	3.42×10^{-7}	4.84×10^{-6}
2	169,802,415	**rs2268365**	Intron *LRP2*	14.3	Pack-years	0.33	**3.49×10^{-8}**	4.94×10^{-6}	**4.85×10^{-8}**	9.72×10^{-7}
2	240,109,156	rs11679072	Intergenic near *FLJ45964*	5.1	Pack-years	0.12	1.55×10^{-7}	1.39×10^{-5}	5.86×10^{-7}	7.54×10^{-6}
3	2,460,969	rs9878978	Intron *CNTN4*	34.4	Pack-years	0.46	3.69×10^{-7}	2.54×10^{-5}	4.95×10^{-7}	6.56×10^{-6}
3	170,512,673	**rs12634933**	Intron ***MECOM***	8.5	Pack-years	0.27	**4.05×10^{-8}**	5.47×10^{-6}	7.04×10^{-8}	1.32×10^{-6}
4	82,345,145	rs17484474	Synonymous *PRKG2*	6.9	Pack-years	0.18	2.17×10^{-7}	1.76×10^{-5}	6.31×10^{-7}	8.01×10^{-6}
4	145,477,389	rs6537278	Intergenic *GYPA-KRT18P51*	27.6	Pack-years	3.50×10^{-7}	2.35×10^{-4}	2.32×10^{-3}	6.78×10^{-7}	8.50×10^{-6}
6	167,184,091	rs4710117	Intron *RPS6KA2*	0.4	Pack-years	0.11	1.38×10^{-4}	1.60×10^{-3}	8.12×10^{-7}	9.86×10^{-6}
7	51,833,102	rs4947642	Intergenic	29.8	Smoking	0.53	4.60×10^{-6}	5.91×10^{-6}	9.58×10^{-7}	1.23×10^{-6}
7	113,785,482	rs12705959	Intergenic *PPP1R3A-FOXP2*	47.4	CPD	0.18	2.34×10^{-7}	1.09×10^{-6}	2.59×10^{-7}	6.91×10^{-7}
8	120,212,220	**rs6989684**	Intergenic *COLEC10-MAL2*	7.7	Pack-years	0.05	**3.09×10^{-8}**	4.54×10^{-6}	1.60×10^{-7}	2.59×10^{-6}
8	141,473,511	**rs7823724**	Intron *TRAPPC9*	1.3	Pack-years	0.07	**4.28×10^{-8}**	5.69×10^{-6}	2.77×10^{-7}	4.07×10^{-6}
10	1,627,136	rs6560743	Intron *ADARB2*	25.6	Pack-years	0.09	1.54×10^{-7}	1.38×10^{-5}	8.03×10^{-7}	9.76×10^{-6}
11	132,544,409	rs7104871	Intron ***OPCML***	42.8	Pack-years	0.25	7.65×10^{-6}	2.10×10^{-4}	6.65×10^{-8}	1.26×10^{-6}
12	1,772,425	rs2286379	3' UTR *CACNA2D4*	41.6	Pack-years	2.76×10^{-4}	8.25×10^{-8}	8.97×10^{-6}	2.44×10^{-7}	3.67×10^{-6}
13	22,942,344	rs2297585	Intergenic *SACS-TNFRSF19*	6.0	Pack-years	0.03	5.79×10^{-8}	7.02×10^{-6}	3.90×10^{-7}	5.39×10^{-6}
13	31,525,648	rs9533282	Intron *FRY*	4.3	Pack-years	7.71×10^{-4}	0.06	0.06	3.93×10^{-7}	5.43×10^{-6}
13	92,527,286	rs9561252	Intergenic *GPC5-GPC6*	7.7	CPD	0.39	1.18×10^{-6}	4.62×10^{-6}	5.59×10^{-7}	1.42×10^{-6}
14	79,480,194	rs8010717	Intron *LOC730007*	28.0	CPD	0.13	4.82×10^{-4}	9.96×10^{-4}	3.27×10^{-7}	8.58×10^{-7}
			Near ***NRXN3***		Pack-years	0.13	4.52×10^{-5}	7.29×10^{-4}	1.03×10^{-7}	1.80×10^{-6}
					Smoking	0.01	2.31×10^{-4}	2.72×10^{-4}	1.19×10^{-7}	1.59×10^{-7}
15	21,198,852	rs937741	Intergenic near *HERC2P6*	32.6	CPD	9.25×10^{-4}	6.96×10^{-8}	3.69×10^{-7}	4.57×10^{-7}	1.17×10^{-6}
16	68,054,704	**rs12149862**	3' UTR ***CYB5B***	20.0	Pack-years	0.01	**4.76×10^{-10}**	2.51×10^{-7}	**3.65×10^{-9}**	1.16×10^{-7}
17	15,840,400	rs7211756	Intron *ZSWIM7*	0.8	Pack-years	0.33	2.30×10^{-7}	1.83×10^{-5}	2.57×10^{-7}	3.83×10^{-6}
18	62,721,365	rs7234531	Intergenic *CDH19-DSEL*	1.6	Pack-years	0.14	9.85×10^{-8}	1.02×10^{-5}	3.38×10^{-7}	4.80×10^{-6}

(*Continued*)

Table 2. Continued

Chr	Position	SNP	Genomic location	MAF	Smoking measure	SNP Main	SNP–smoking interaction		Joint 2 df test	
						P value	*P* value	GC *P* value	*P* value	GC *P* value
18	74,595,580	**rs4573996**	Intergenic	3.7	CPD	0.32	5.79×10^{-7}	2.44×10^{-6}	3.13×10^{-7}	8.25×10^{-7}
					Pack-years	0.55	$\mathbf{1.93\times10^{-8}}$	3.27×10^{-6}	$\mathbf{8.77\times10^{-9}}$	2.38×10^{-7}
22	26,352,728	**rs133980**	Intergenic near ***MN1***	49.5	CPD	0.21	3.16×10^{-5}	8.71×10^{-5}	$\mathbf{1.71\times10^{-8}}$	5.44×10^{-8}
					Pack-years	0.07	1.90×10^{-5}	3.98×10^{-4}	$\mathbf{1.07\times10^{-8}}$	2.82×10^{-7}
2 suggestive diastolic blood pressure loci										
3	127,132,093	rs7615952	*LOC200810*	16.4	Pack-years	0.03	8.20×10^{-4}	1.31×10^{-3}	5.45×10^{-7}	1.05×10^{-6}
7	50,765,179	rs10275663	Intron *GRB10*	0.2	CPD	3.11×10^{-7}	9.01×10^{-6}	9.00×10^{-6}	8.02×10^{-7}	9.47×10^{-7}

Bold-faced SNPs are genome-wide significant (with $P < 5\times10^{-8}$), and bold-faced genes are biologically plausible, providing physiological connection to BP regulation (as in the Discussion). Physical positions are listed according to National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) build 36.3.
Abbreviations: Chr, chromosome; CPD, cigarettes per day; df, degree of freedom; GC = genomic controlled; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

GWAS practice. A consensus using 5×10^{-8} corresponds to a conservative Bonferroni correction based on roughly 1 million "effectively independent" common SNPs throughout the genome, given the pattern of linkage disequilibrium among common variants across the genome.[17] For each significant/suggestive association, a locus was defined as a cluster of SNPs within 100 kb of the SNP with the lowest *P* value in the region (called an index SNP). We plotted quantile–quantile (QQ) plots and computed the genomic inflation factor λ, the degree of inflation of the median test statistic, for each analysis. We also computed the genomic controlled *P* values by dividing test statistics by λ, as they are widely used to correct for minor substructure problems.[18] Manhattan plots were created with the y-axis indicating $-\log_{10}(P)$ values and the x-axis plotting the physical position of the SNPs. Regional association plots were generated to highlight chromosomal regions with a clustering of SNPs with significant association using LocusZoom software (available at http://csg.sph.umich.edu/locuszoom/). All other plots were generated in R, a freely available language and environment for statistical computing and graphics (available from cran.r-project.org).

RESULTS

Table 1 displays the descriptive statistics for the FHS subjects used in the interaction analysis of 3 smoking measures. All 6,889 subjects with GWAS and BP measures had current smoking status (yes/no). CPD and pack-years were available for 6,796 and 6,686 subjects, respectively. We performed 3 genome-wide tests (1 df main effect, 1 df interaction effect test, joint 2 df test) using 2 BP traits (SBP and DBP) and 3 smoking measures (CPD, pack-years, and smoking status). The Manhattan plots in Figure 1 display the results for the joint 2 df test of the SNP main effect and SNP–smoking interaction effect for all 6 combinations of trait and smoking measure. We computed genomic inflation factors λ for each BP trait and smoking measure. The genetic main effect test exhibited no genomic inflation (all $\lambda \le 1.02$). However, the 1 df interaction effect test exhibited substantial inflation (λ up to 1.46), and the joint 2 df test also exhibited inflation (λ up to 1.22). Therefore, we computed the genomic controlled (gc) *P* values for both 1 df interaction and joint 2 df tests to achieve the expected distribution of *P* values. QQ plots for these original and genomic controlled *P* values are displayed in the Supplementary Materials.

Using the joint 2 df test, we found 110 signals with $P \le 1\times10^{-6}$ across the 2 BP traits and 3 smoking measures. These signals were grouped into 28 loci. For each BP trait, we selected an index SNP to represent each significant ($P \le 5\times10^{-8}$) and suggestive ($P \le 1\times10^{-6}$) locus. Association results for the index SNPs are displayed in Table 2. We found 7 significant and 19 suggestive SBP loci. In particular, interaction analysis with pack-years enabled the discovery of 7 significant loci and 15 suggestive SBP loci. Six loci achieved significant or suggestive evidence when using CPD, whereas only 2 loci reached suggestive evidence with smoking status. Except for 1 locus (represented by rs9533282) on chromosome 13 that was driven mostly by main effect (with $P_{\text{interaction}} = 0.06$), all 25 loci were identified by interaction analysis with smoking measures.

Using the 2 df joint test, we found 4 loci that significantly interacted with pack-years to influence SBP. Figure 2 displays the regional association plots for 3 of these 4 SBP loci. The highest evidence of association ($P = 3.65 \times 10^{-9}$; gc $P = 1.15 \times 10^{-7}$) was observed at SNP rs12149862, which lies within cytochrome b5 type B (*CYB5B*) on chromosome 16. Three other significantly associated loci are SNP rs2268365 ($P = 4.85 \times 10^{-8}$; gc $P = 9.72 \times 10^{-7}$) intronic to low-density lipoprotein receptor-related protein 2 (*LRP2*) on chromosome 2, SNP rs4573996 ($P = 8.77 \times 10^{-9}$; gc $P = 2.38 \times 10^{-7}$) on chromosome 18, and SNP rs133980 ($P = 1.07 \times 10^{-8}$; gc $P = 2.82 \times 10^{-7}$) near meningioma 1 (*MN1*) on chromosome 22. The latter two loci were identified also using CPD interaction analysis ($P = 3.13 \times 10^{-7}$, gc $P = 8.25 \times 10^{-7}$ at rs4573996; P=1.71×10^{-8}, gc $P = 5.44 \times 10^{-8}$ at rs133980).

Using the 1 df interaction test, we found the 3 additional loci that significantly interacted with pack-years to influence SBP. Figure 3 displays the regional association plots for these 3 SBP loci. They are rs12634933 (1 df P =4.05×10^{-8}; 1 df gc $P = 5.47 \times 10^{-6}$) intronic to *MDS1* and *EVI1* complex locus (*MECOM*) on chromosome 2; rs6989684 (1 df $P = 3.09 \times 10^{-8}$; gc $P = 4.54 \times 10^{-6}$) near collectin subfamily member 10 (*COLEC10*), T-cell differentiation protein 2 (*MAL2*); and rs7823724 (1 df $P = 4.28 \times 10^{-8}$; 1 df gc $P = 5.69 \times 10^{-6}$), intronic to trafficking protein particle complex 9 (*TRAPPC9*) on chromosome 8.

Two of the 28 loci gave suggestive evidence for DBP. The first locus on chromosome 3 had joint 2 df $P = 8.0 \times 10^{-7}$ and gc $P = 9.5 \times 10^{-7}$, mostly driven by interaction with pack-years, whereas the second locus chromosome 7 had $P = 5.5 \times 10^{-7}$ (gc $P = 1.1 \times 10^{-6}$), which was driven by both SNP main effect ($P = 3.1 \times 10^{-7}$) and interaction with CPD ($P = 9.0 \times 10^{-6}$).

We found the suggestive SBP locus at SNP rs8010717 near neurexin 3 (*NRXN3*) on chromosome 14 using all 3 smoking measures ($P = 3.27 \times 10^{-7}$ using CPD; $P = 1.03 \times 10^{-7}$ using pack-years; $P = 1.19 \times 10^{-7}$ using smoking status). To evaluate consistency across 3 smoking measures, we present scatterplots of $-\log_{10}(P)$ values for the analysis of SBP at all 2.5 million SNPs in Figure 4. Supplementary Table S1 presents P values at the 28 SNPs listed in Table 2. We found that the 2 quantitative measures CPD and pack-years were more consistent with each other than with the smoking status (with correlation = 0.87, 0.71, and 0.82 for 1 df main effect, 1 df interaction effect, and joint 2 df test, respectively). Smoking status was less consistent with either CPD or pack-years, as shown in the 2nd and 3rd rows in Figure 4. As described in the Methods, the 3 smoking variables measure very different

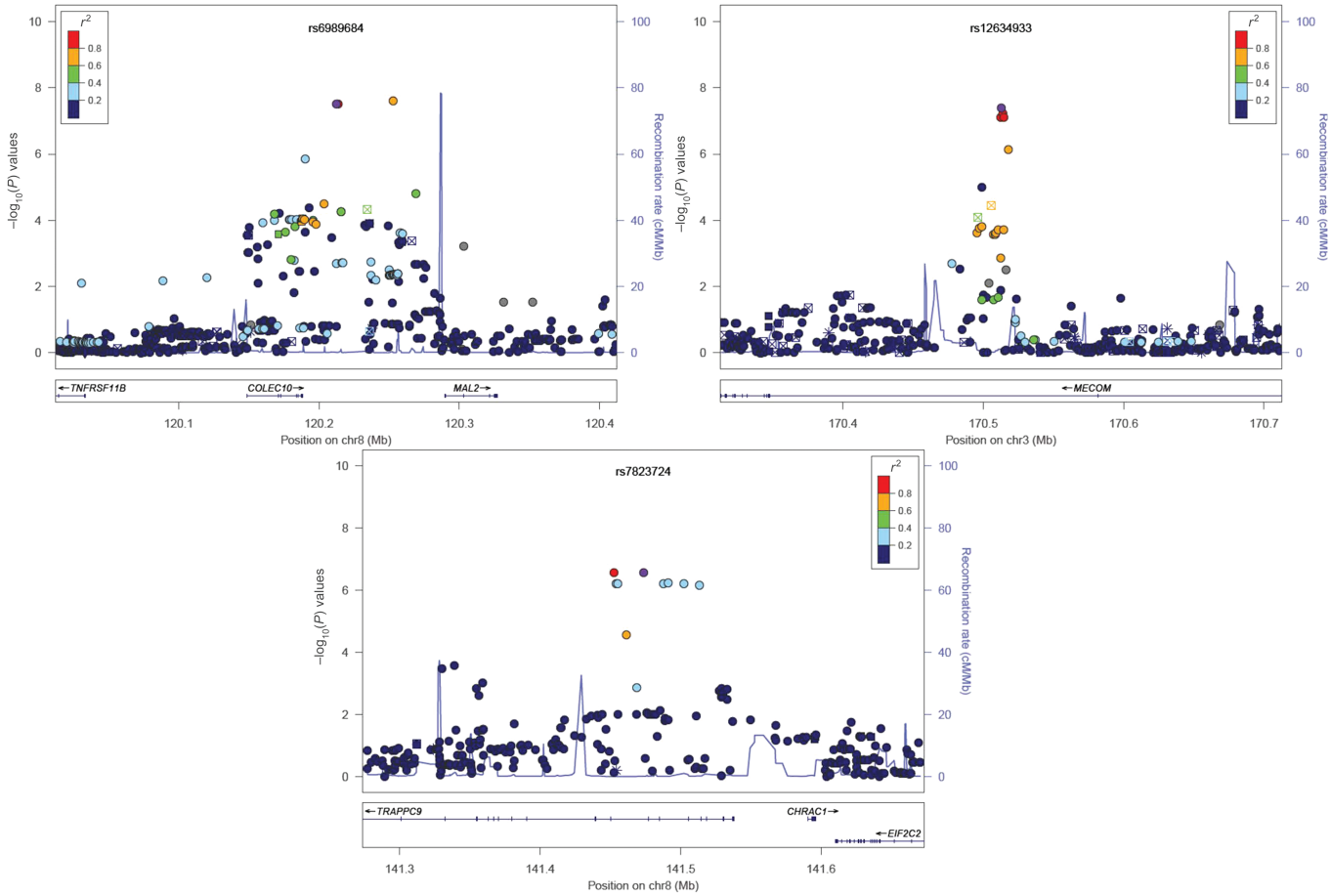

Figure 2. Regional association plots of the 3 systolic blood pressure (SBP) loci showing genome-wide significant associations ($P < 5 \times 10^{-8}$) using the joint 2 degree of freedom (df) test of single nucleotide polymorphism (SNP) main effect and SNP–pack-years interaction effect. These regional plots were generated using LocusZoom (http://csg.sph.umich.edu/locuszoom/).

aspects of smoking exposure. Based on nicotine biology, we do not necessarily expect highly consistent results across the 3 smoking variables.

Our interaction analysis used all subjects with smoking status. In particular, we used 6,796 and 6,686 subjects for CPD and pack-years by including nonsmokers, whose values were set to 0. Therefore, we also performed our interaction analysis using 3,329 smokers only after excluding nonsmokers for the analysis of SBP with pack-years at the 28 SNPs listed in Table 2. Scatterplots in Figure 5 show effect sizes, standard errors (SEs), and $-\log_{10}(P)$ values at 28 SNPs between 2 sets of analysis. For both SNP main effect and interaction effects, analysis using all subjects provided smaller SE, as shown in the 2nd column of Figure 5. This leads to smaller P values, as the red dashed regression line was below the blue diagonal line. Our most significantly associated locus in *CYB5B* on chromosome 16 was identified using both samples (1 df interaction $P = 7.43 \times 10^{-10}$, 2 df joint $P = 3.09 \times 10^{-9}$ using smokers only; 1 df $P = 4.76 \times 10^{-10}$, 2 df $P = 3.65 \times 10^{-9}$ using all subjects).

DISCUSSION

We identified 7 significant and 21 suggestive BP loci by exploiting gene–smoking interactions in the analysis of 6,889 participants from FHS. Our results demonstrated the advantage of including G × E interactions for gene discovery. The joint 2 df test can be more powerful than either the 1 df test of the genetic main effect only or the 1 df test of the interaction effect alone.[15] The increase in power for the 2 df over either 1 df test can be dramatic when the type I error rate is controlled at low levels as it is common in GWASs.[19] Because the joint 2 df test supplements standard marginal tests of genetic main effects with additional information from G × E interactions, the joint test can detect loci that are missed in marginal scans. Manning *et al.* used this approach and demonstrated power enhancement for detecting G × E interactions.[20]

Our significant association in the *MECOM–MDS1–EVI1* sequence complex on chromosome 2 (1 df $P = 4.05 \times 10^{-8}$) is supported by several GWASs with BP traits. The Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) Consortium identified a marginal association with SBP ($P = 1.28 \times 10^{-6}$), which strengthened ($P = 1.18 \times 10^{-7}$) when combined with the top SNPs replicated in cohorts of the Global BPgen Consortium.[3] In a companion article published simultaneously, another SNP in *MDS1* was associated with DBP in the Global BPgen Consortium; the association improved with joint analyses also using data from the CHARGE Consortium ($P = 8 \times 10^{-8}$).[2] The Women's Genome

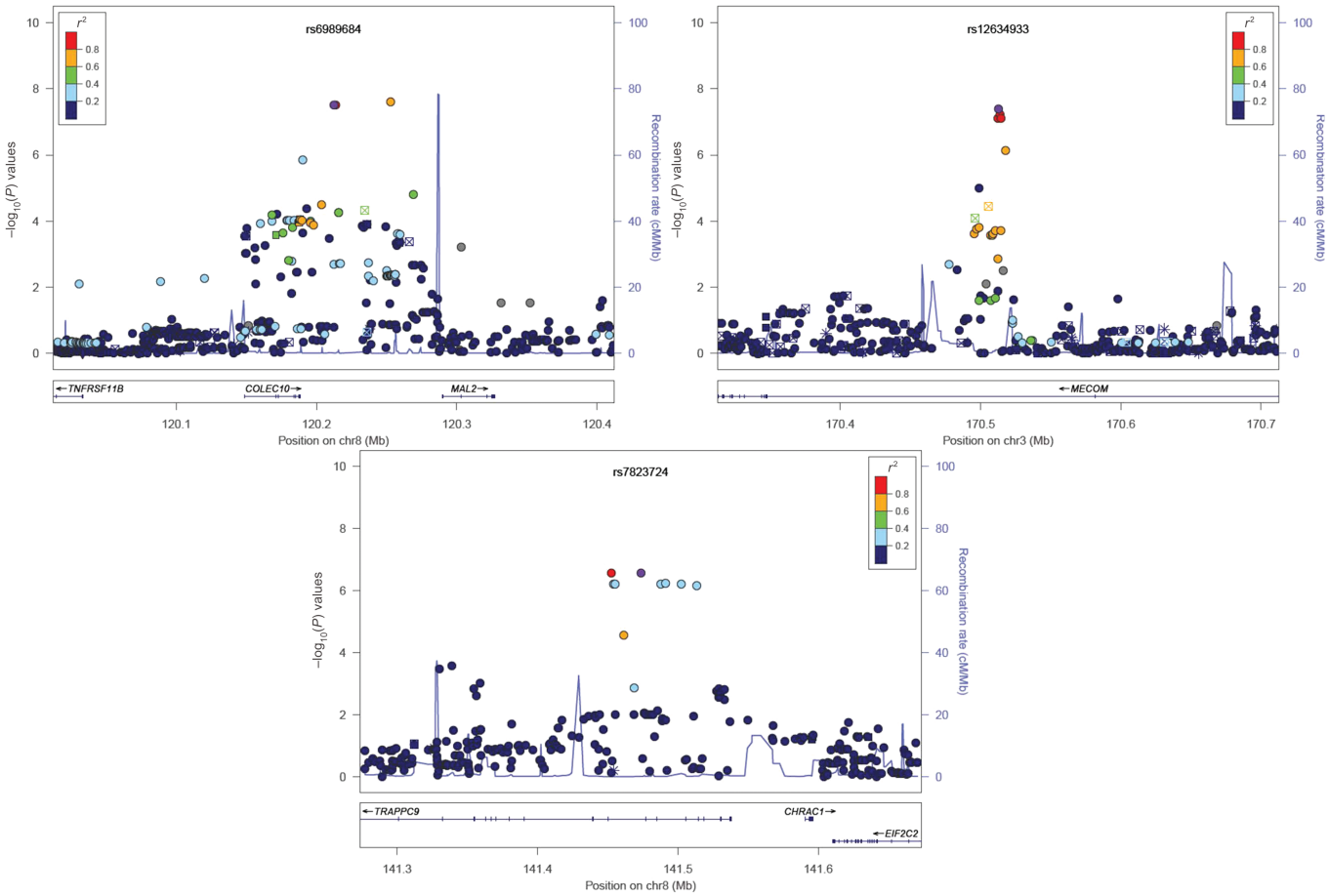

Figure 3. Regional association plots of the 3 additional systolic blood pressure (SBP) loci showing genome-wide significant associations ($P < 5 \times 10^{-8}$) using the 1 degree of freedom (df) single nucleotide polymorphism (SNP)–pack-years interaction test.

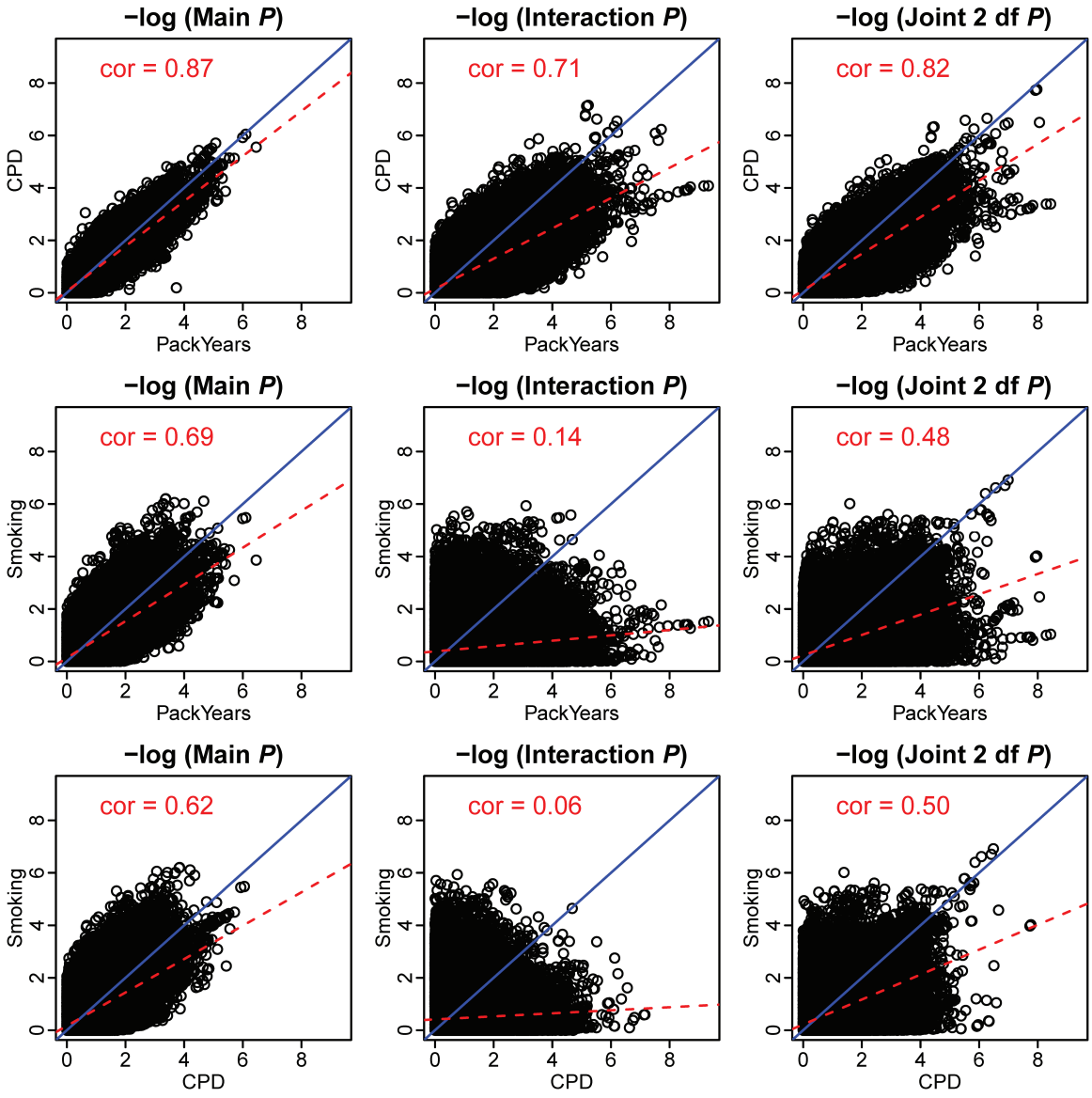

Figure 4. Scatterplots of $-\log_{10}(P)$ values using 3 smoking measures for the analysis of systolic blood pressure (SBP). The 1st row compares pack-years vs. cigarettes per day (CPD); the 2nd row compares pack-years vs. smoking status; the 3rd row compares CPD vs. smoking status. The 1st column is *P* values using 1 degree of freedom (df) main effect; the 2nd column is *P* values using 1 df interaction effect test; and the 3rd column is using the joint 2 df test. The dashed line is the regression line, and the solid line indicates where the values on the two axes are equal.

Health Study also found suggestive association ($P = 9.1 \times 10^{-8}$),[21] which was replicated in the International Consortium for Blood Pressure Genome-Wide Association Studies (SBP: $P = 1.8 \times 10^{-13}$; DBP: $P = 2.1 \times 10^{-12}$).[4] The *MECOM–MDS1–EVI1* complex is an oncoprotein that is located in a region often fused with *AML1* (3;21 translocation) in patients with a variety of hematologic disorders.[22] The mechanism by which *MDS1* can regulate BP remains uncertain, although this locus may be involved in the regulation of apoptosis stimulated by DNA damage.[23] Genetic variants in the *MECOM–MDS1–EVI1* complex have also been associated with nasopharyngeal cancers in individuals of Chinese and Thai descent.[24,25] Smoking has shown to be a key risk factor for nasopharyngeal cancers, particularly among populations of Asian descent.[25,26]

Our most significantly associated locus in *CYB5B* on chromosome 16 (1df $P = 4.76 \times 10^{-10}$; 2df $P = 3.65 \times 10^{-9}$) is biologically plausible. *CYB5B* is a member of the mitochondrial cytochrome P450 enzyme complex that is integral to the synthesis of steroid sex hormones by the adrenal glands. Cytochrome b5 is also overexpressed in the adrenal tissue from 2 distinct murine models of hypertension.[27] However, the mechanism by which *CYB5B* influences BP may be more directly related to its role in the kidney and the vasculature, where the cytochrome P450 complex has been shown to metabolize arachidonic acid into a variety of substances that modulate renal and system arterial tone.[28] Cytochrome P450 enzymes are also responsible for the oxidation of nicotine to its long-acting, active metabolite, cotinine.[29] Because cotinine levels have been inversely association with BP,[30] our

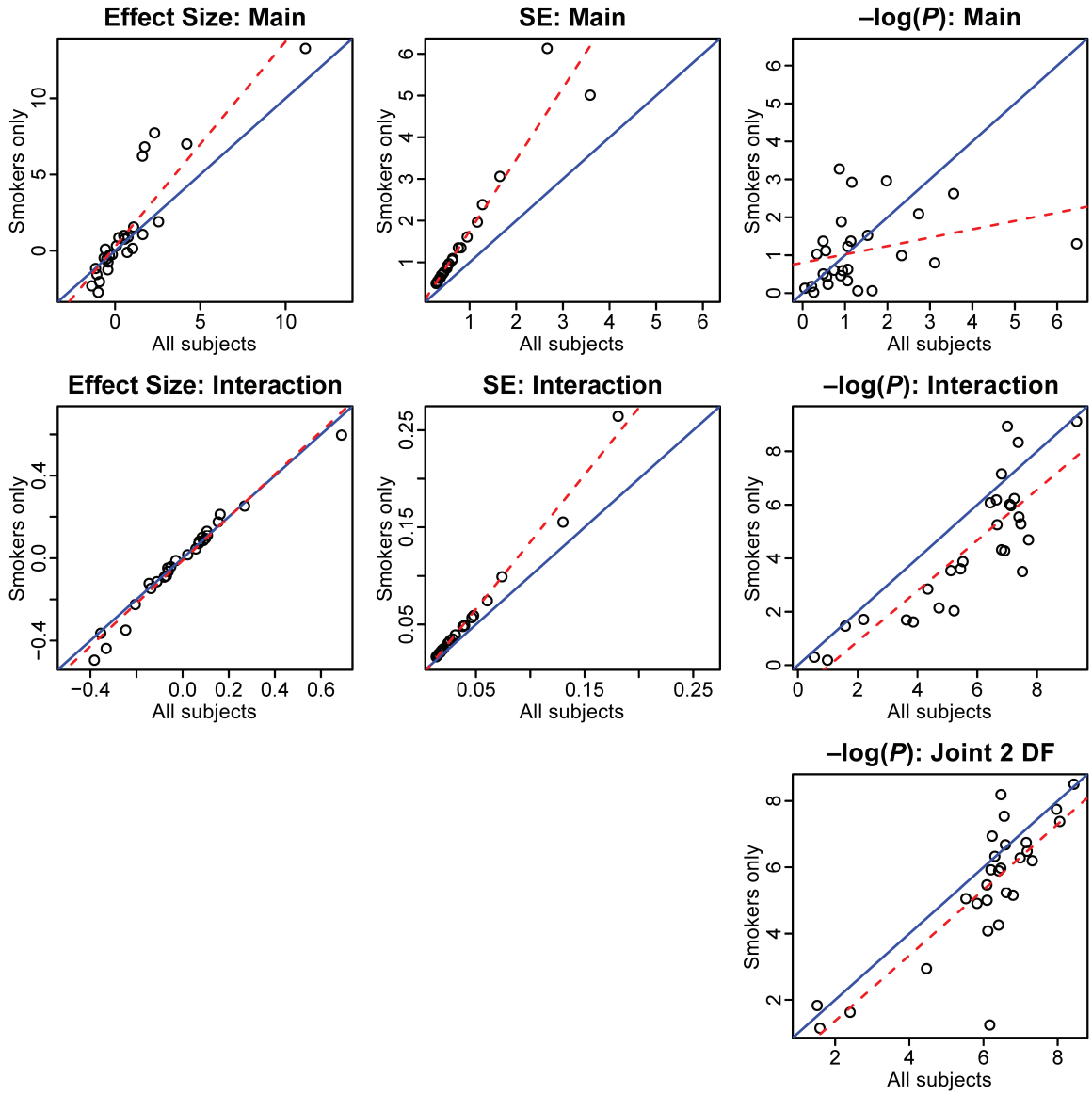

Figure 5. Scatterplots showing effect sizes, standard errors (SEs), and $-\log_{10}(P)$ values at the 28 SNPs (listed in Table 2) between analysis using all 6,686 subjects and the analysis using 3,329 smokers only for the analysis of systolic blood pressure (SBP) with pack-years. The dashed line is the regression line, and the solid line indicates where the values on the two axes are equal.

finding of association may point to a role for tobacco use in modulating *CYB5B*'s genetic contributions to BP regulation.

Our suggestive SBP association near neurexin 3 (*NRXN3*) on chromosome 14 was consistently found using all 3 smoking measures (2df $P = 3.27 \times 10^{-7}$using CPD; $P = 1.03 \times 10^{-7}$ using pack-years; $P = 1.19 \times 10^{-7}$ using smoking status). *NRXN3* belongs to a class of transmembrane adhesion proteins widely expressed in the central nervous system where they play roles in modulating nerve signaling.[31] *NRXN3* has been associated with a wide range of neuropsychiatric and addiction disorders,[32] including tobacco[33] and alcohol use[34] and autism spectrum disorders.[35] Recently, neurexins have also been shown to be widely expressed by endothelial and vascular smooth muscle cells, where they influence blood vessel tone, a key determinant in BP regulation.[31] *NRXN3* has also been directly associated with BP traits in GWASs. For example, a suggestive association with DBP was identified in a relatively small cohort of blacks (n = 1,017; $P = 4.47 \times 10^{-6}$).[36] Although this locus failed to replicate in an independent black cohort (n = 2,474; $P = 0.21$),[37] it did replicate for hypertension as a binary trait in a larger Korean cohort (n = 8,842; $P = 0.03$).[38] We believe that our association between SNPs in *NRXN3* and SBP may have been strengthened by consideration of smoking interactions.

A suggestive SBP locus in *OPCML* (opioid binding protein/cell adhesion molecule-like) on chromosome 11 (2df $P = 6.65 \times 10^{-8}$) also appears to be biologically plausible. OPCML is a tumor suppressor gene that is also believed to play an accessory role in opioid receptor function.[39]

Association with an *OPCML* SNP and smoking initiation has been also identified elsewhere (with $P = 9.74 \times 1\ 0^{-5}$).[40] This region of chromosome 11 has also been linked to cardiometabolic traits such as glucose homeostasis in black and Hispanic families,[41] and SNPs in *OPCML* have been associated with body fat distribution in blacks.[42] However, our suggestive association with BP can be considered novel.

A significantly associated SBP locus on chromosome 22 (2df $P = 1.07 \times 10^{-8}$) is also considered novel. It is approximately 100–200 kb upstream from *MN1* (meningioma (disrupted in balanced translocation) 1) and *PITPNB* (phosphatidylinositol transfer protein, β). *PITPN* is a member of a family of lipid-binding proteins that shuttle lipid messengers between membrane compartments.[43] As the name suggests, *MN1* is an oncogene that has been identified in several forms of malignancy, including meningioma[44] and myeloproliferative disorders such as leukemia.[45] No clear physiologic links to BP, addiction, or tobacco use were identified for either gene. It is possible that this region contains regulatory elements for more distant genes.

In summary, we identified 7 significant and 21 suggestive BP loci by exploiting genome-wide gene–smoking interactions in the analysis of 6,889 participants from the FHS. One significant locus corresponds to one of 29 BP loci identified through the International Consortium for Blood Pressure Genome-Wide Association Studies.[4] We found that 26 (of 28) loci were identified through interaction effects. Although genomic control lowers the levels of significance, several of these BP loci are biologically plausible, providing physiological connection to BP regulation. Given that published GWASs with sample sizes up to 200,000 individuals have collectively identified fewer than 50 BP-associated loci, the identification of 28 candidate loci using interactions in a modest-sized sample demonstrates the potential advantage of including G × E interactions in association analysis. Although we restricted this analysis to a single visit from each participant, we plan to follow up with a longitudinal analysis of gene–smoking interactions using the FHS SHARe data. In addition, the validity of our findings is somewhat limited because they are based on a single study. We acknowledge that further validation and replication in an independent sample would strengthen our findings.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

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DISCLOSURE

The authors declared no conflict of interest.

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