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

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BACKGROUND
Blood pressure (BP) variability has a genetic component, most of which has yet to be attributed to specific variants. One promising strategy for gene discovery is analysis of interactions between single-nucleotide polymorphisms (SNPs) and BP-related factors, including age, sex, and body mass index (BMI). Educational attainment, a marker for socioeconomic status, has effects on both BP and BMI.

METHODS
We investigated SNP–education interaction effects on BP in genome-wide data on 3,836 subjects in families from the Framingham Heart Study. The ABEL suite was used to adjust for age, sex, BMI, medication use, and kinship and to perform 1 degree-of-freedrom (df) and 2 df SNP–education interaction tests.

RESULTS
An SNP in *PTN* was associated with increased systolic BP (5.4 mm Hg per minor allele) in those without a bachelor's degree but decreased systolic BP (1.6 mm Hg per allele) in those with a bachelor's degree (2 df; $P = 2.08 \times 10^{-8}$). An SNP in *TOX2* was associated with increased diastolic BP (DBP; 4.1 mm Hg per minor allele) in those with no more educational attainment than high school but decreased DBP in those with education past high school (–0.7; 1 df; $P = 3.74 \times 10^{-8}$). Three suggestive associations were also found: in *MYO16* (pulse pressure: 2 df; $P = 2.89 \times 10^{-7}$), in *HAS2* (DBP: 1 df; $P = 1.41 \times 10^{-7}$), and in *DLEU2* (DBP: 2 df; $P = 1.93 \times 10^{-7}$). All 5 genes are related to BP, including roles in vasodilation and angiogenesis for *PTN* and *TOX2*.

CONCLUSIONS
PTN and *TOX2* are associated with BP. Analyzing SNP–education interactions may detect novel associations. Education may be a surrogate for unmeasured exposures and behaviors modifying SNP effects on BP.

Keywords: blood pressure; educational attainment; gene–education interaction; GWAS; hypertension; interaction.

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Elevated blood pressure (BP) is a major risk factor for cardiovascular disease and renal failure and was the leading global health burden in 2010,[1] annually costing tens of billions of dollars in the United States alone. Evidence suggests that systolic BP (SBP) and diastolic BP (DBP) have a heritability of at least 30%, but genetic variants identified thus far explain <3% of the total phenotypic variability.[2] Several explanations have been offered for this so-called missing heritability,[3] and diverse research strategies addressing these explanations have emerged to pursue it. In addition to meta-analyses in enormous genome-wide association study consortia,[2] investigators have focused on sequence data and the role of rare variants,[4] as well as subphenotypic distinctions within hypertension[5] (e.g., degree of salt sensitivity and of sympathetic nervous activation). Another recently expanding field explores the role of interactions amongst single nucleotide polymorphisms (SNPs)[6] and between SNPs and other factors known to influence BP such as age,[7] sex,[8] and body mass index (BMI).[8] Motivated by the observation that these variables are directly correlated with BP themselves, these studies of SNP–covariable interactions test the hypothesis that these covariables also modulate the effect of SNPs on BP and have reported compelling evidence supporting that hypothesis.

One such variable just beginning to receive attention for its role in BP is educational attainment. Noting that educational attainment is inversely associated with cardiovascular disease in developed countries,[9] Loucks *et al.* tested BP as an explanatory mechanism in the Framingham Heart Study. They reported that educational attainment (≤12 years vs. ≥17 years) was also inversely correlated with BP, both as a baseline effect and as a change in SBP over a 30-year life span (the latter particularly in female subjects).[10] The contrast between those with limited literacy and those with adequate

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literacy showed a complex effect on SBP, with the direction of effect varying depending on the health-care system used: those with limited literacy had lower SBP in the Veterans Affairs healthcare system but higher in the university healthcare system.[11] A study of low-income rural Mexican women showed no association between BP and educational attainment, although the study reported a negative association between BP and 3 other socioeconomic indicators: household income, housing, and assets.[12] Variance component modeling in the Strong Heart Family Study also indicated that educational attainment has a modifying effect on the contribution of SNPs to BP variance, with evidence suggesting distinct genetic effects on DBP among those with less than high school education as compared with those with at least high school education.[13] Although these studies provide evidence of an association between educational attainment and BP, to our knowledge this study is the first to report associations arising from SNP–education interactions on BP.

METHODS

Subjects

The data were drawn from the Framingham SNP Health Association Resource (SHARe) from the database of Genotypes and Phenotypes (dbGaP; http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000342.v8.p8). The Framingham Heart Study is a longitudinal study with 3 cohorts: the Original cohort, the Offspring cohort, and the Third Generation cohort. The Original cohort was recruited in 1948. The Offspring cohort was recruited in 1971, consisting of the offspring of the Original cohort, as well as the spouses and children of those offspring. The biological and adopted offspring of the Offspring cohort form the Third Generation cohort, recruited in 2002. The analysis data were taken from the Third Generation cohort's single visit because only this cohort provided the educational attainment data used in our analysis.

Phenotypes and genotypes

Three measurements (1 nurse/technician reading and 2 physician readings) were taken for each subject for SBP and DBP, and the averages of these 3 values were used for the analysis; mean arterial pressure (MAP; calculated as two-thirds of average DBP plus one-third of average SBP) and pulse pressure (PP; calculated as average SBP minus average DBP) were derived.

Subjects were genotyped on the GeneChip Human Mapping 500k Array Set (Affymetrix, Santa Clara, CA) containing 487,998 SNPs. Eight thousand four hundred seventy-seven subjects (across all 3 cohorts) had an additional 2.5 million SNPs imputed using MACH.[14] These SNPs were filtered by the absence of Mendelian errors, minor allele count ≥30 among all 3 cohorts in the most recent visit, Hardy–Weinberg equilibrium ($P \geq 10^{-6}$), and good quality for imputed SNPs ($r^2 \geq 0.3$).

All subjects had nonmissing genotype and imputed data, as well as complete data for SBP, DBP, both education covariables, age, sex, BMI, and hypertension medication use; this limited the analysis dataset to 3,386 Third Generation cohort subjects. Two education covariables were derived from the raw Framingham Heart Study education variable. This raw variable contains 9 categories: 0 = no schooling; 1 = grades 1–8; 2 = grades 9–12; 3 = high school graduate; 4 = some college; 5 = technical school; 6 = associate's degree; 7 = bachelor's degree; 8 = graduate/professional degree. We derived Comp_HS+ as a dichotomous variable distinguishing those with no more than high school education (categories 0–3) from those with at least some college (categories 4–8), and Comp_BA as a dichotomous variable distinguishing those without a bachelor's degree (categories 0–6) from those with a bachelor's degree (categories 7–8). Descriptive statistics for this sample are shown in Table 1.

Analysis

For each combination of BP trait and education covariable, we used the following linear mixed model:

Table 1. Study sample descriptive statistics

Trait	Value
Sample size	3,836
% Comp_HS+	84.7%
% Comp_BA	53.2%
% Male sex	47%
Age	40.2 ± 8.8
BMI	26.9 ± 5.5
% Taking antihypertensive meds	8.6%
SBP	116.3 ± 13.7
DBP	75.7 ± 9.3
MAP	89.2 ± 10.1
PP	40.6 ± 8.9

Data are mean ± SD unless otherwise stated.

Abbreviations: BMI, body mass index; Comp_BA, dichotomous variable distinguishing those without a bachelor's degree from those with a bachelor's degree; Comp_HS+, dichotomous variable distinguishing those with no more than high school education from those with at least some college; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure.

Table 2. Genomic control λ values

Covariable (test)	SBP	DBP	MAP	PP
Comp_HS+ (1 df)	1.990	1.052	1.098	1.076
Comp_HS+ (2 df)	1.132	1.071	1.079	1.070
Comp_BA (1 df)	1.018	1.032	1.014	1.018
Comp_BA (2 df)	1.043	1.061	1.036	1.038

Abbreviations: Comp_BA, dichotomous variable distinguishing those without a bachelor's degree from those with a bachelor's degree; Comp_HS+, dichotomous variable distinguishing those with no more than high school education from those with at least some college; DBP, diastolic blood pressure; df, degrees of freedom; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure.

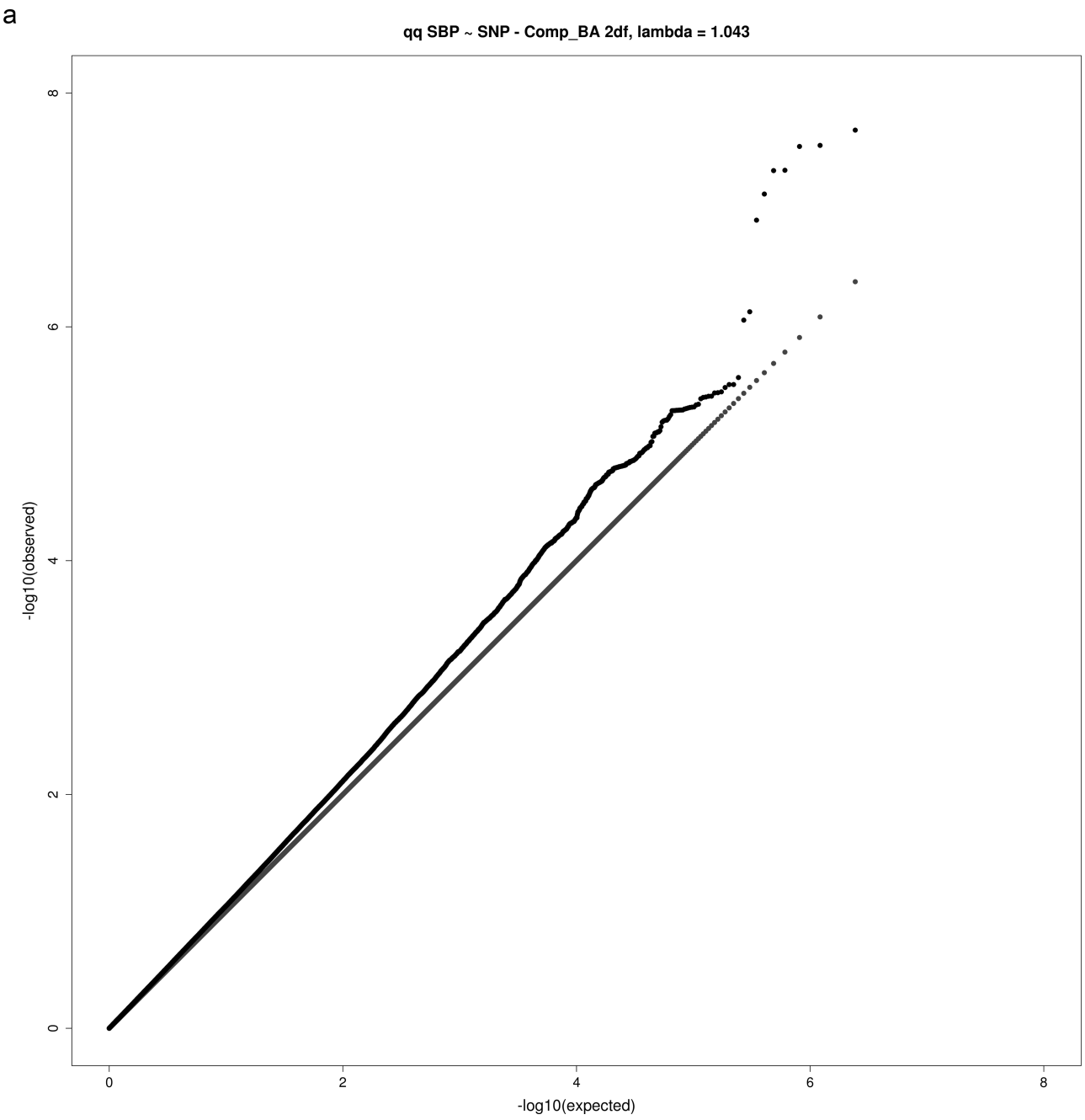
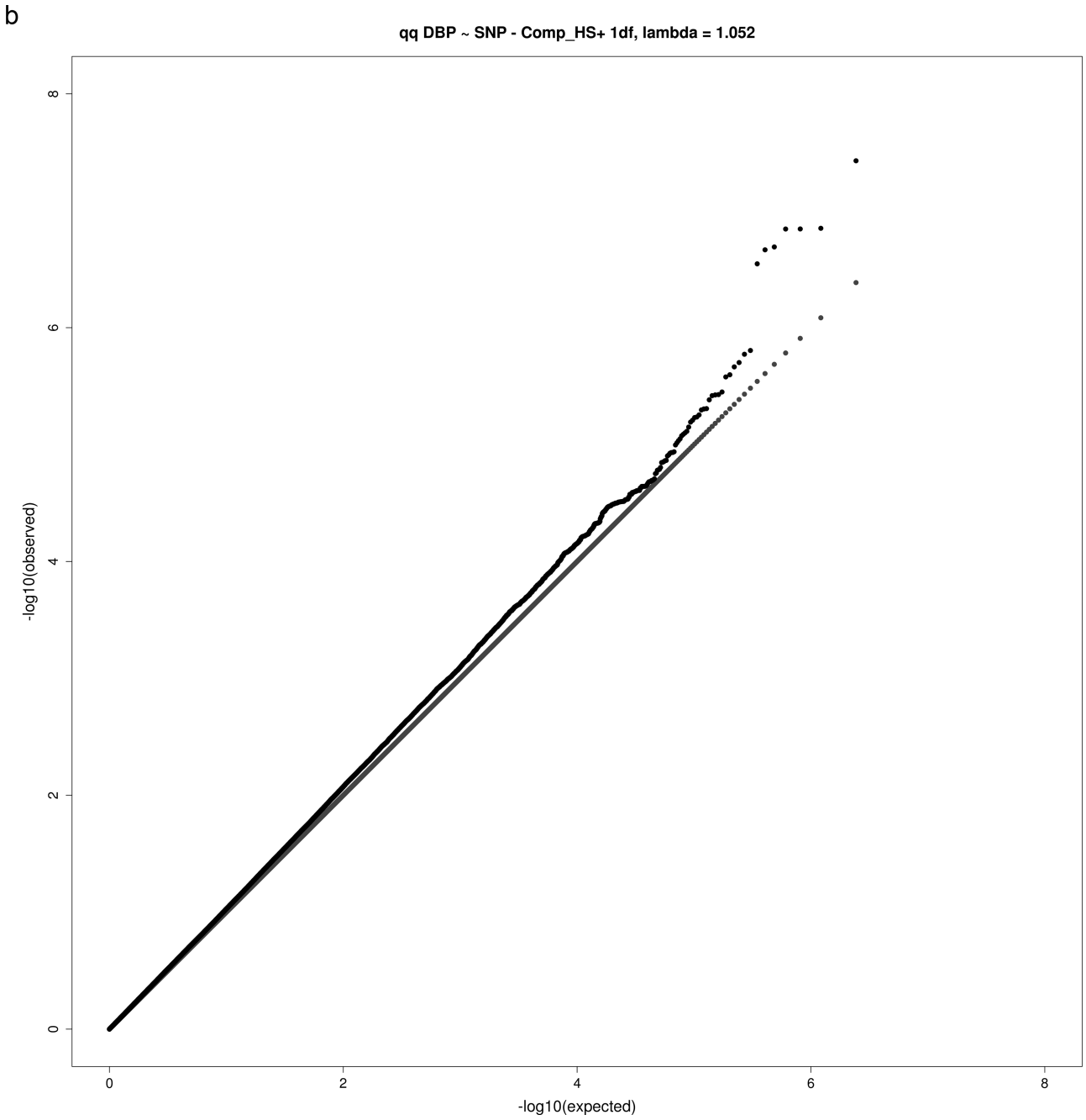


Figure 1. QQ plots for selected combinations of blood pressure phenotype, education covariable, and interaction test. (**a**) QQ plot for joint 2 degrees-of-freedom (df) test of single nucleotide polymorphism (SNP) main effect and interaction of SNP with a dichotomous variable distinguishing those without a bachelor's degree from those with a bachelor's degree (Comp_BA) for systolic blood pressure (SBP). (**b**) QQ plot for 1 df test of interaction of SNP with a dichotomous variable distinguishing those with no more than high school education from those with at least some college (Comp_HS+) for diastolic blood pressure (DBP). (**c**) QQ plot for 1 df test of SNP-Comp_HS+ interaction for pulse pressure (PP). (**d**) QQ plot for 2 df test of SNP main effect and SNP-Comp_BA interactions for DBP.

$$y = \beta_0 + \beta_{\text{cov}} * X_{\text{cov}} + \beta_1 * Education + \beta_2 * SNP + \beta_3 * SNP * Education + z_{polygene} + e.$$

where X_{cov} refers to the covariables age, age squared, sex, BMI, and use of hypertension medication, and $z_{polygene}$ is a random effect to account for family relationships using the kinship matrix. Using this model, we performed the 1 degree-of-freedom (df) test of the SNP–education interaction (β_3), and the 2 df joint test of the SNP main effect and SNP–education interaction (β_2 and β_3).[15] These analyses were performed using the ABEL suite, including GenABEL (c 1.6–6), ProbABEL (v 0.1-9e), and MixABEL (v 0.0–9).[16,17] MixABEL is one of few packages that can provide the 2 df joint test for family data; we also



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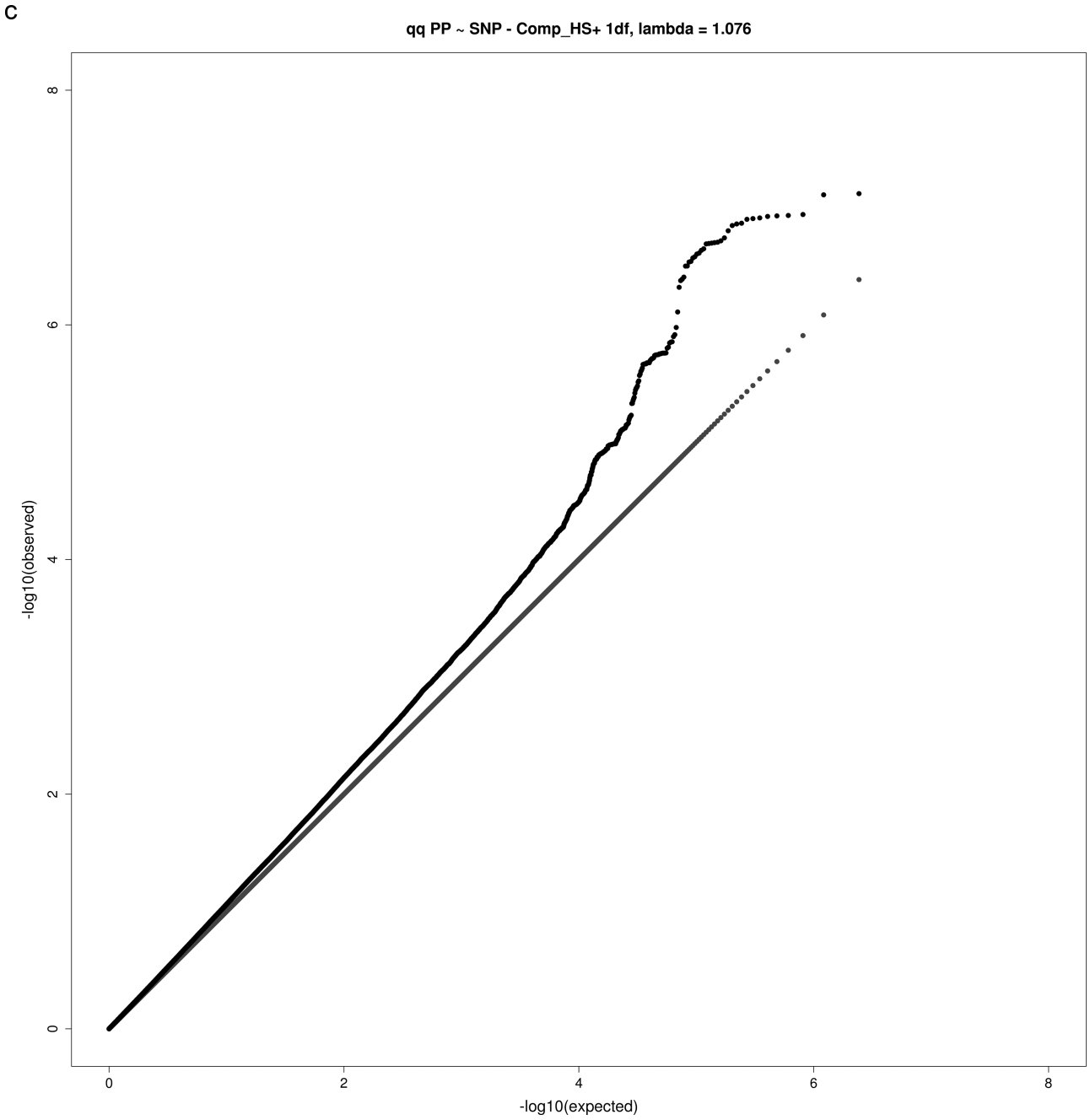
found the ABEL suite to be faster than other options, which was an important practical consideration for this analysis of approximately 2.5 million imputed SNPs on multiple combinations of phenotype and education covariable.

For both the 1 df and 2 df SNP–education interaction tests, the genomic inflation factor λ was calculated as the ratio of the observed median χ^2 value to the expected median χ^2 value. None of the analyses yielding significant or suggestive loci exhibited substantial evidence of inflation, with λ values in the range 1.02–1.08 (Table 2), as expected for highly polygenic traits.[18] Figure 1a–d show QQ plots for these analyses.

RESULTS

Figure 2a–d show Manhattan plots for the indicated combination of BP trait (SBP, DBP, or PP), educational attainment covariable (Comp_HS+ or Comp_BA), and test (1 df interaction or 2 df joint test). There were 2 loci reaching significance at $\alpha = 5 \times 10^{-8}$ and 3 suggestive loci with $P < 5 \times 10^{-7}$. These SNPs are summarized in Table 3 (significant loci) and Table 4 (suggestive loci).

The most significant interaction (2 df; $P = 2.08 \times 10^{-8}$) was between rs7792298 and the Comp_BA covariable on SBP



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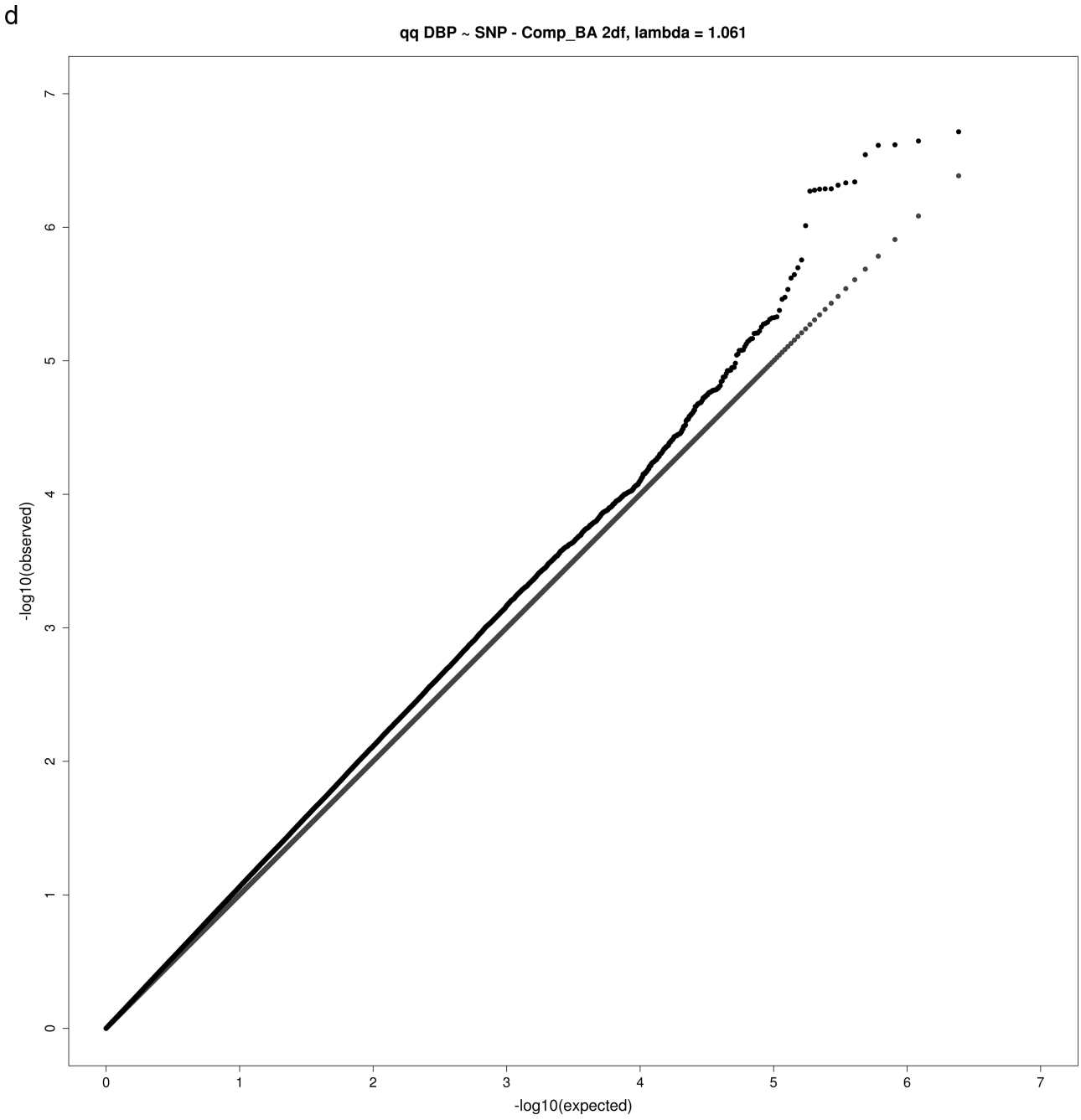
(Figure 2a). This is one of several SNPs in the chromosome 7 locus with similar effects, minor allele frequencies, and *P* values (see Table 3). Subjects with 1 minor allele and no bachelor's degree showed a large increase in average adjusted SBP (5.5 mm Hg) relative to those without a minor allele, whereas those with a bachelor's degree and a minor allele showed a decrease (1.7 mm Hg) (Figure 3a). All of the significant SNPs at this locus are located in intron 4 of pleiotrophin (*PTN*).

A single SNP in an intron of TOX high mobility group box family member 2 (*TOX2*) on chromosome 20 also reached significance for interaction with Comp_HS+ on DBP (1 df; $P = 3.74 \times 10^{-8}$) (Figure 2b). Subjects with no education beyond high school showed an increase in DBP of 4.1 mm Hg for each minor allele of this genotyped SNP (rs11086907), whereas those with some post–high school education and 1 or 2 minor alleles showed a modest decrease in DBP (0.7 mm Hg per allele) (Figure 3b).

Three other loci showed suggestive association with a BP trait (Table 4). An SNP in an intron of myosin XVI (*MYO16*) on chromosome 13 had an interaction with Comp_HS+ associated with PP. Pulse pressure was higher



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by 0.2 mm Hg per minor allele among those with no more than high school education but lower by 0.1 mm Hg per minor allele among those with more than high school education (1 df; $P = 7.61 \times 10^{-8}$) (Figure 2c). Several SNPs on chromosome 8 near hyaluronan synthase 2 (*HAS2*) had interactions with Comp_HS+ suggestively associated with DBP. The strongest association was with rs1994404, which showed an increase of 2.6 mm Hg DBP per minor allele for those with no more than high school education and a decrease of 0.4 mm Hg DBP per minor allele for those with at least some college education (1 df; $P = 1.41 \times 10^{-7}$) (Figure 2b). Finally, several SNPs on chromosome 13 near deleted in lymphocytic leukemia 2 (*DLEU2*) had interactions with Comp_BA suggestively associated with DBP. The strongest association was with rs12100048, which showed an increase of 2.4 mm Hg per minor allele for those without a bachelor's degree and a decrease of 0.6 mm Hg per minor allele for those with a bachelor's degree (2 df; $P = 1.93 \times 10^{-7}$) (Figure 2d).

One additional SNP, rs6936075, yielded a significant interaction with Comp_BA associated with SBP (2 df; $P = 2.8 \times 10^{-8}$) (Figure 2a). This SNP had a minor allele

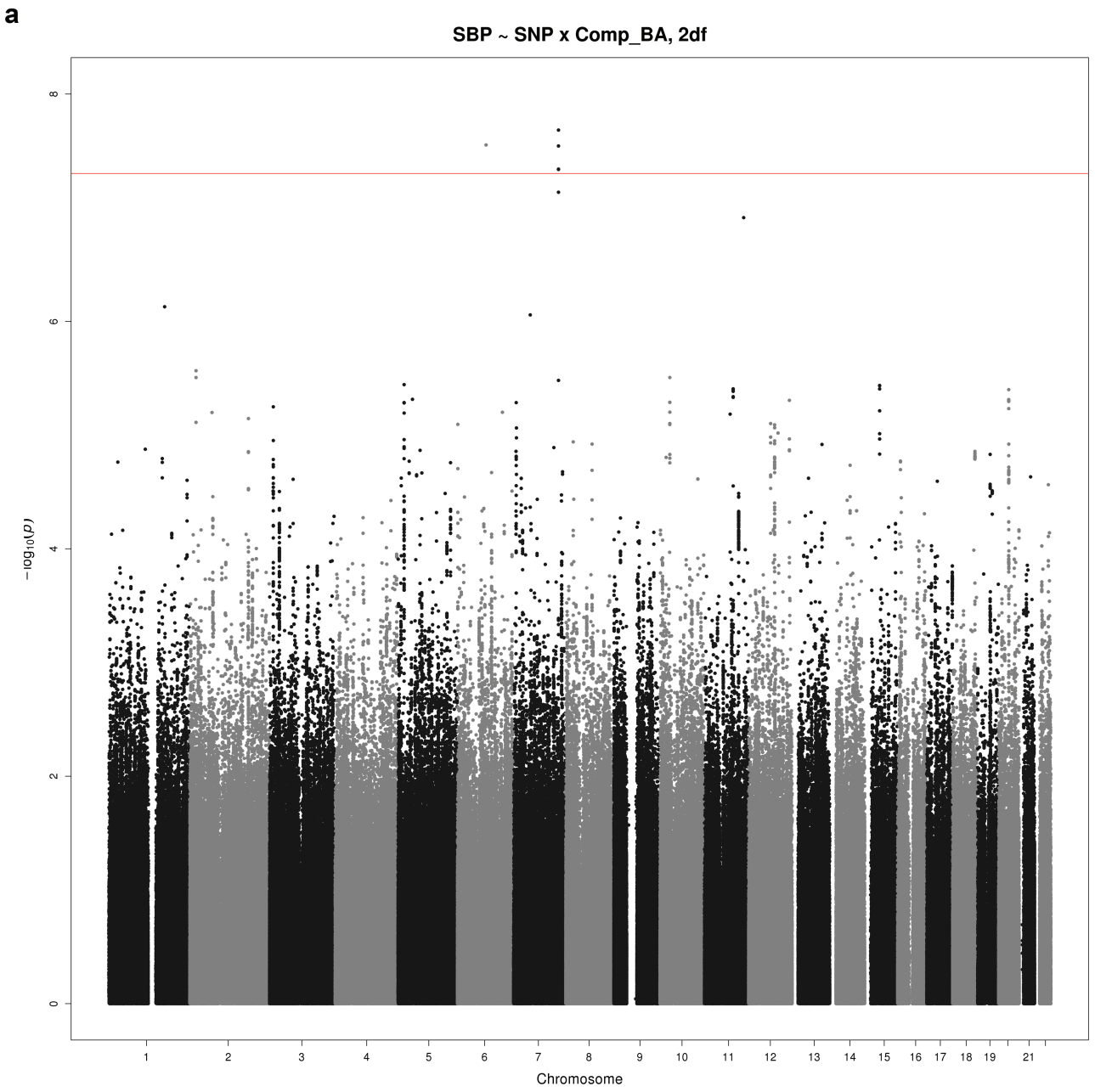
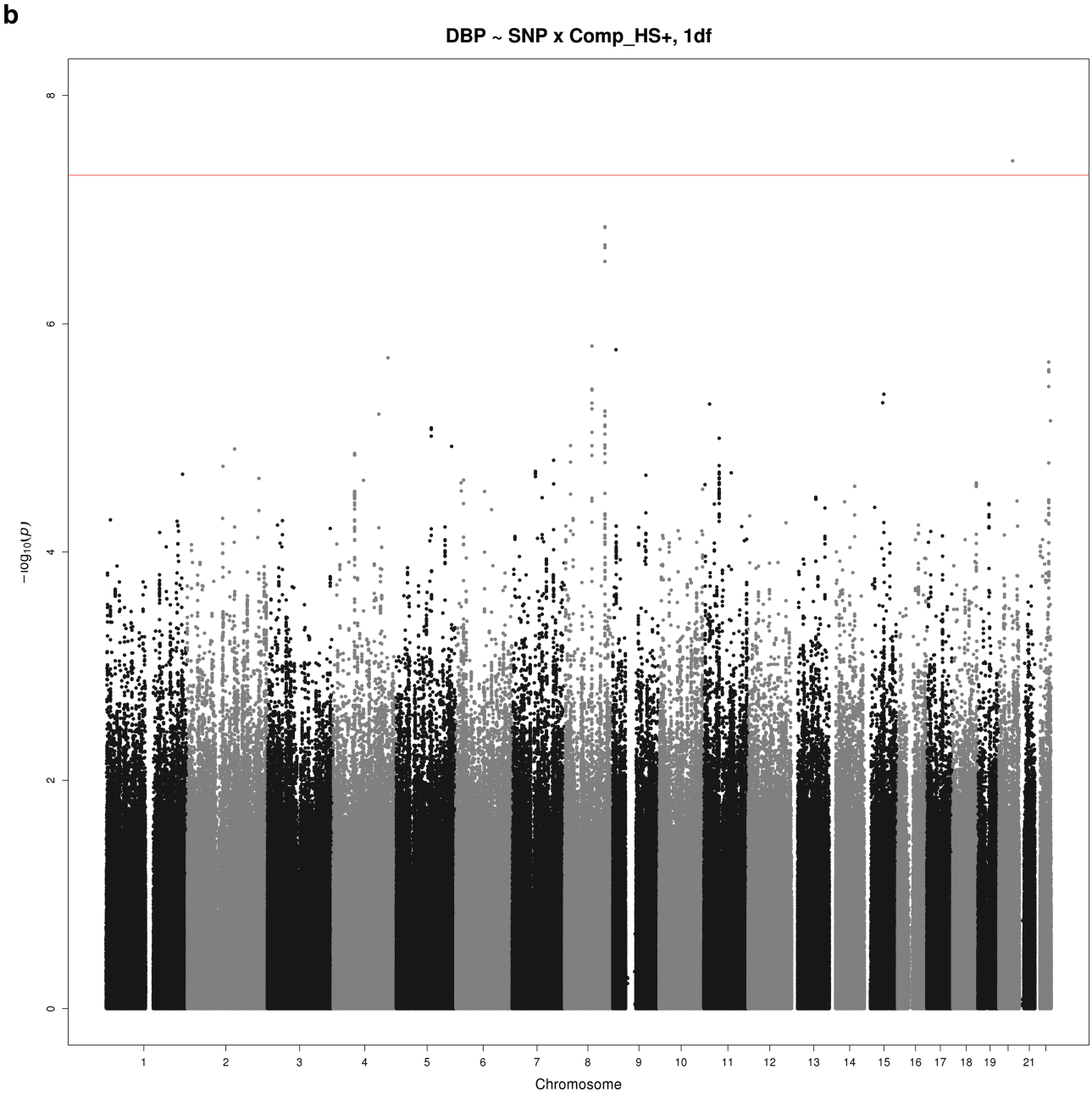


Figure 2. Manhattan plots for selected combinations of blood pressure phenotype, education covariable, and interaction test. (**a**) Manhattan plot of the joint 2 degrees-of-freedom (df) test of the singlue nucleotide polymorphism (SNP) main effect and interaction of SNP with a dichotomous variable distinguishing those without a bachelor's degree from those with a bachelor's degree (Comp_BA) for systolic blood pressure (SBP). The $-\log_{10}$(*P* value) is plotted by genomic position. One novel locus reaching significance is located on chromosome 7 (*PTN*). (**b**) Manhattan plot of the 1 df test of interaction of SNP with a dichotomous variable distinguishing those with no more than high school education from those with at least some college (Comp_HS+) for diastolic blood pressure (DBP). The $-\log_{10}$(*P* value) is plotted by genomic position. One novel locus reaching significance is located on chromosome 20 (*TOX2*), and a suggestive locus is located on chromosome 8 (*HAS2*). (**c**) Manhattan plot of joint 1 df test of the SNP main effect and SNP-Comp_HS+ interaction for pulse pressure (PP). The $-\log_{10}$(*P* value) is plotted by genomic position. A suggestive locus is located on chromosome 13 (*MYO16*). (**d**) Manhattan plot of joint 2 df test of the SNP main effect and SNP-Comp_BA interaction for DBP. The $-\log_{10}$(*P* value) is plotted by genomic position. A suggestive locus is located on chromosome 13 (*DLEU2*).

frequency of 0.16%, barely surviving the cutoff based on minor allele count in all 3 cohorts in the most recent visit (see Methods). In the analysis sample (Third Generation cohort only), the total number of minor alleles present in the sample dropped to <30. Because no other SNP supported its association with $P < 5 \times 10^{-6}$, this locus was not further considered.

DISCUSSION

The effects of interactions between SNPs and educational attainment on SBP, DBP, MAP, and PP were analyzed. Two loci showed significant interactions: intronic SNPs in pleiotrophin (*PTN*) and Comp_BA showed significant interaction effects on SBP using either the 1 or 2 df tests (Table 3)



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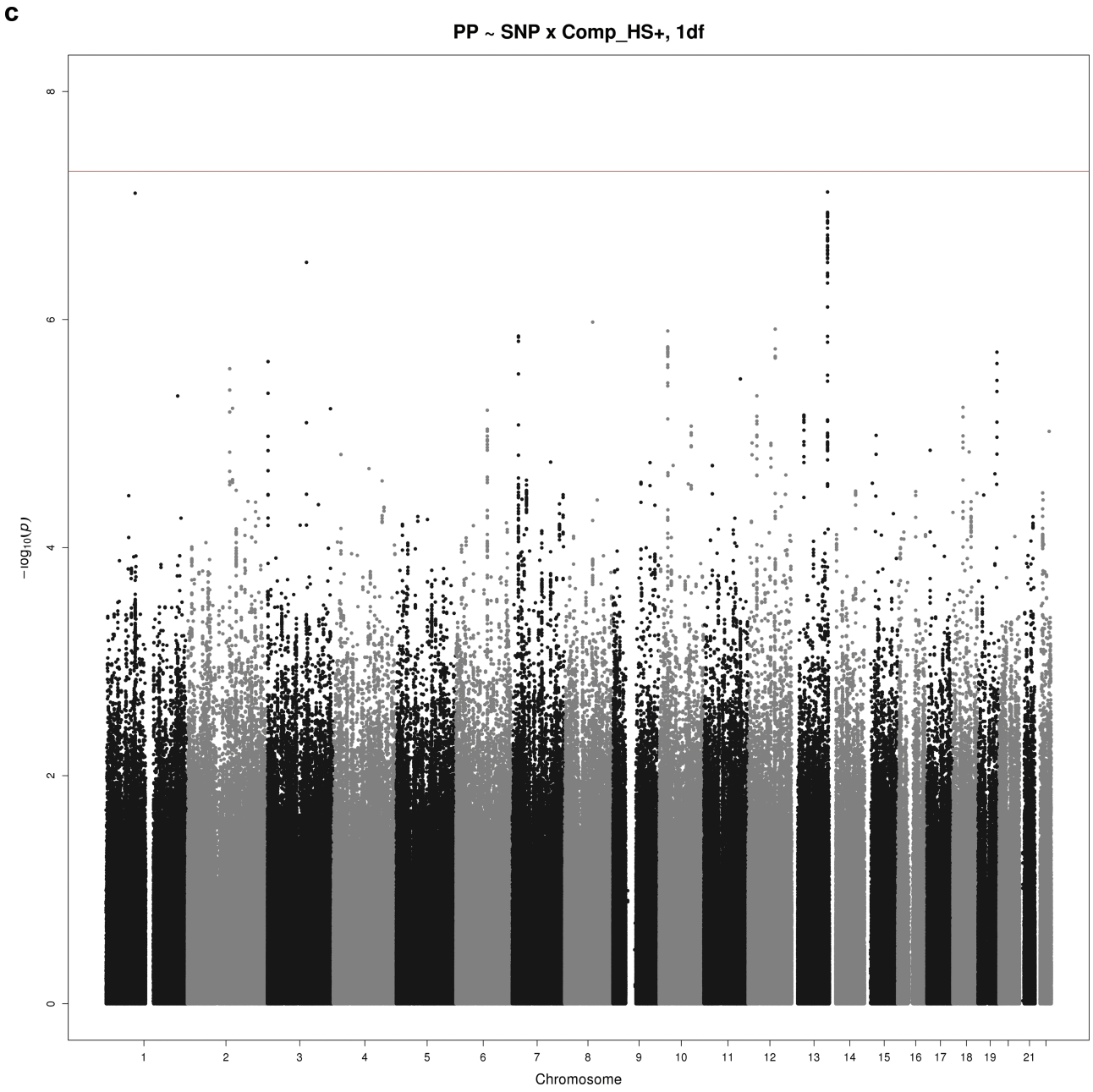
and an intronic SNP in *TOX2* and Comp_HS+ showed a significant interaction effect on DBP. Loci in 3 genes also suggested interaction effects (Table 4): *MYO16* and Comp_HS+ affecting PP, *HAS2* and Comp_HS+ affecting DBP, and *DLEU2* and Comp_BA affecting DBP.

Previous analyses of the Framingham Heart Study based on SNP main effects did not show significant associations at the *PTN* locus associated with SBP in this study.[19] Modest associations ($2 \times 10^{-5} < P < 6 \times 10^{-5}$) with other SNPs in *PTN* have been reported in dbGaP for several related phenotypes, including high-density lipoprotein cholesterol, BMI, and coronary artery disease,[20] but they were all based on main effects only. This growth factor has numerous biological functions, including roles in angiogenesis and kidney development, and it critically regulates both the renin-angiotensin system and catecholamine synthesis in mice.[21] *PTN* is expressed in vascularized atherosclerotic plaques and mediates migration of endothelial cells following upregulation by the vasodilator nitric oxide.[22] It is also involved in inflammation and has been shown to induce expression of inflammatory cytokines including tumor necrosis factor alpha, interleukin 1 beta, and interleukin 6 in peripheral blood mononuclear cells.[23] Collectively, these studies point to a multifaceted role for *PTN* in



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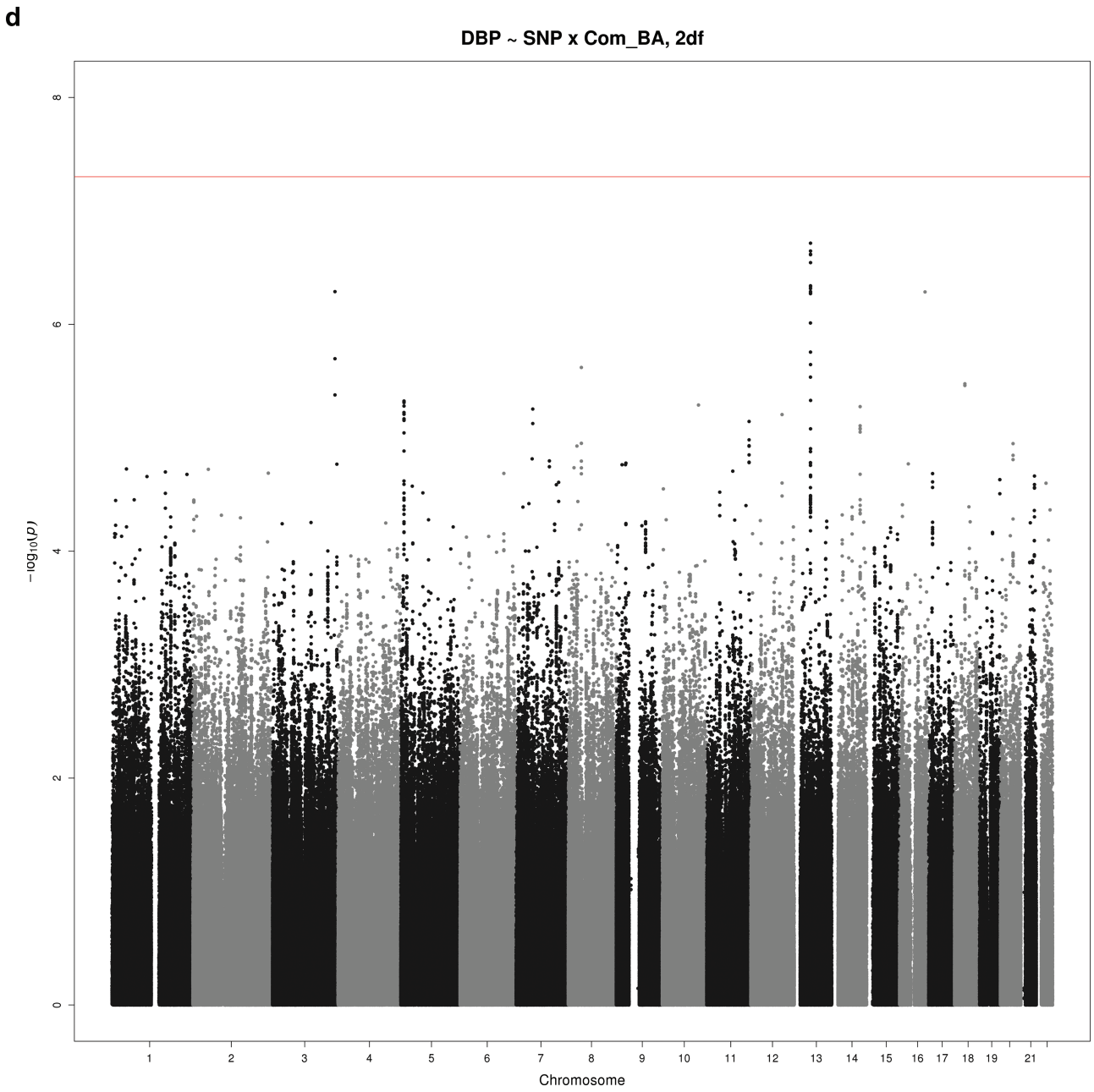
cardiovascular and metabolic physiology with effects on inflammation and BMI, as well as cholesterol. Our results extend this role to BP in particular. In addition, the interaction with educational attainment may reflect *PTN*'s role in learning and memory, as it has been shown to inhibit long-term potentiation (a crucial neural mechanism of memory) in the hippocampus.[24]

The second genome-wide significant locus contains a single SNP (Table 3) located in an intron of *TOX2* coding a DNA-binding protein about which relatively little is known. As with the *PTN* locus, the SNP–Comp_HS+ interaction reached significance in the absence of significant associations of SNP main effects at this locus in previous analyses of the Framingham Heart Study.[19] The expression of several hundred genes was affected by small interfering RNA–mediated knockdown of *TOX2* in 2 cell lines normally expressing *TOX2*.[25] These genes represented multiple pathways, including vasodilation, blood clotting, angiogenesis, and inflammatory response.[25] A meta-analysis of the Family Blood Pressure Program reported linkage evidence with BP for which the confidence interval (logarithm of the odds ratio ≥ 2; approximately 10 Mb wide) included *TOX2*.[26] In addition, an association between *TOX2* SNPs and conduct disorder[27] and a suggestive association with alcoholism[28] have been reported,



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identifying possible mechanisms linking BP regulation with low educational attainment.

The suggestive interaction effect involving *MYO16* on PP (Table 4) is supported by previous association results. Zhang *et al.*[29] reported an association with PP in Chinese twins ($P = 7.21 \times 10^{-7}$). Associations with other cardiovascular and metabolic traits, including high-density lipoprotein cholesterol, diabetic nephropathies, and coronary artery disease, have also been reported in dbGaP.[30] In addition, a suggestive association ($P = 3.3 \times 10^{-6}$) has been reported with urinary free dopamine in a Hispanic population.[31] Expression analysis contrasting 3 strains of spontaneously hypertensive rats with Wistar Kyoto rats also implicated *MYO16* in hypertension.[32] *MYO16* is expressed predominantly in the central nervous system, as well as in the kidney, and collectively these results suggest a role for *MYO16* in BP, possibly through involvement in sympathetic nervous activity, which regulates both vasodilation and renal sodium transport. In addition to its role in cardiovascular and metabolic traits, *MYO16* has also been implicated in brain development, neurological morphogenesis, and autism,[33] traits which may be associated with educational attainment.

The chromosome 8 locus suggestive of an interaction effect on DBP (Table 4) is located in an intron of *HAS2*, an

Table 3. Significant single nucleotide polymorphism–education interactions

SNP rs no.	Chr	Location	Type	MAF	Trait	Covariable	Beta (Main)	SE (Main)	Beta (Int)	SE (Int)	*P* value 1 df interaction	*P* value 2 df interaction
rs12113527	7	*PTN* intron	Imp	0.044	SBP	Comp_BA	−5.40	0.98	7.05	1.29	**4.28E-08**	**2.86E-08**
rs12536916	7	*PTN* intron	Imp	0.044	SBP	Comp_BA	−5.30	0.98	6.97	1.29	5.83E-08	**4.61E-08**
rs6979228	7	*PTN* intron	Imp	0.044	SBP	Comp_BA	−5.30	0.98	6.97	1.28	5.81E-08	**4.58E-08**
rs7792298	7	*PTN* intron	Gen	0.047	SBP	Comp_BA	5.50	0.99	−7.18	1.29	**2.90E-08**	**2.08E-08**
rs11086907	20	*TOX2* intron	Gen	0.102	DBP	Comp_HS+	4.12	0.81	−4.81	0.87	**3.74E-08**	2.61E-07

Bolded values are those with $P < 5.00E-08$.

Abbreviations: Comp_BA, dichotomous variable distinguishing those without a bachelor's degree from those with a bachelor's degree; Comp_HS+, dichotomous variable distinguishing those with no more than high school education from those with at least some college; DBP, diastolic blood pressure; df, degrees of freedom; Gen, genotyped; Int, interaction effect; Imp, imputed; MAF, minor allele frequency; Main, main effect; SBP, systolic blood pressure; SNP, single nucleotide polymorphism.

Table 4. Suggestive single nucleotide polymorphism–education interactions

SNP rs no.	Chromosome	Location	Type	MAF	Trait	Covariable	Beta (Main)	SE (Main)	Beta (Int)	SE (Int)	*P* value 1 df interaction	*P* value 2 df interaction
rs4771625	13	*MYO6* intron	Imp	0.37	PP	Comp_HS+	0.23	0.05	−0.29	0.05	7.61E-08	2.89E-07
rs13274659	8	Near *HAS2*	Imp	0.49	DBP	Comp_HS+	−2.65	0.54	3.01	0.58	2.04E-07	1.22E-06
rs1994404	8	Near *HAS2*	Imp	0.49	DBP	Comp_HS+	2.65	0.54	−3.05	0.58	1.41E-07	9.11E-07
rs2171348	8	Near *HAS2*	Imp	0.5	DBP	Comp_HS+	−2.63	0.55	3.03	0.59	2.84E-07	1.79E-06
rs6990539	8	Near *HAS2*	Imp	0.49	DBP	Comp_HS+	−2.65	0.54	3.05	0.58	1.43E-07	9.21E-07
rs6991390	8	Near *HAS2*	Imp	0.49	DBP	Comp_HS+	−2.65	0.54	3.05	0.58	1.43E-07	9.19E-07
rs7839349	8	Near *HAS2*	Imp	0.49	DBP	Comp_HS+	−2.72	0.55	3.09	0.60	2.16E-07	1.28E-06
rs12100048	13	*DLEU2* intron	Imp	0.12	DBP	Comp_BA	−2.36	0.43	2.01	0.58	5.60E-04	1.93E-07
rs17073826	13	*DLEU2* intron	Imp	0.13	DBP	Comp_BA	−2.30	0.42	1.88	0.57	1.04E-03	2.41E-07
rs17073834	13	*DLEU2* intron	Imp	0.13	DBP	Comp_BA	−2.27	0.42	−1.82	0.57	1.45E-03	2.86E-07
rs6561575	13	*DLEU2* intron	Imp	0.13	DBP	Comp_BA	2.31	0.42	−1.92	0.58	8.53E-04	2.26E-07
rs7981648	13	*DLEU2* intron	Imp	0.13	DBP	Comp_BA	−2.30	0.42	1.89	0.57	9.92E-04	2.43E-07

Abbreviations: Comp_BA, dichotomous variable distinguishing those without a bachelor's degree from those with a bachelor's degree; Comp_HS+, dichotomous variable distinguishing those with no more than high school education from those with at least some college; DBP, diastolic blood pressure; df, degrees of freedom; Int, interaction effect; Imp, imputed; MAF, minor allele frequency; Main, main effect; PP, pulse pressure; SNP, single nucleotide polymorphism.

important member of the extracellular matrix in atherosclerotic vascular lesions,[34] which provides a framework for blood vessel growth. The Framingham Heart Study linked *HAS2* with microalbuminuria,[35] a risk factor for cardiac and vascular abnormalities, and a SNP near *HAS2* has been associated with survival among female black diabetic dialysis patients.[36] Rat studies have shown a role for hyaluronan in renal water handling, and response to water deprivation and an F2 intercross (between normotensive and spontaneously hypertensive rats) showed a linkage peak for both MAP and left ventricular mass that contained *HAS2*.[37]

The final suggestive locus contains both *DLEU1* and *DLEU2* (Table 4), 2 noncoding RNAs that harbor 2 microRNAs (*MIR15A* and *MIR16-1*). Little is known about these genes, and most studies targeting them are focused on cancers (the name refers to their being frequently downregulated in lymphocytic leukemia). However, epigenetic regulation of *DLEU2* has been linked with downregulation of a group of genes regulating the nuclear factor kappa B (NF-κB) complex, a core mediator of inflammation signaling.[38] Recent unpublished work (of J. J. Basson) suggests a possible association between BP and SNP–SNP interactions between NF-κB genes and members of the NF-κB–inhibiting IκK complex. Another major inflammatory mediator, transforming growth factor β, is regulated by miRNA-16, which also regulates the renal sodium channel *SCNN1B* and a serotonin transporter.[39] Serotonin is widely expressed in the brain, heart, kidney, and vasculature, organs which play a key role in BP regulation. SNPs in this locus have also been found to have suggestive associations with bipolar disorder;[40] expression levels of miRNA-16 have been found to be dysregulated in brains from schizophrenic individuals.[41] Once again, a

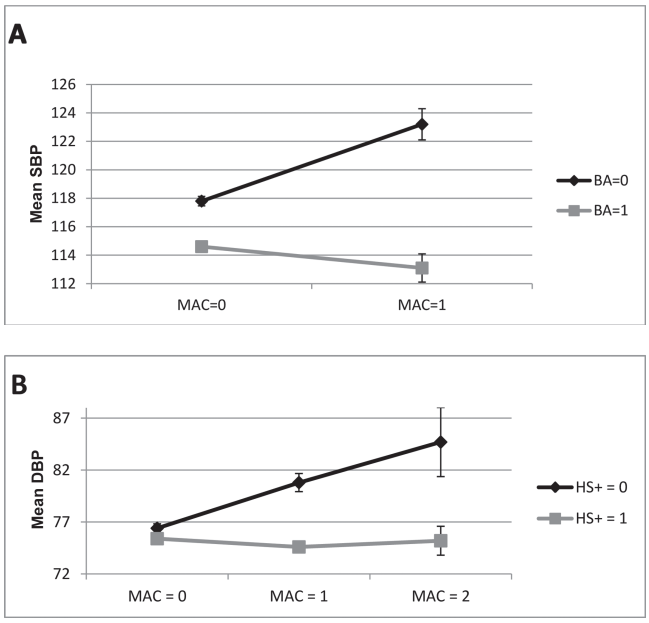

Figure 3. Mean blood pressure by minor allele count (MAC) for selected combinations of blood pressure phenotype and SNP. (**a**) Mean systolic blood pressure (SBP) by *PTN* genotype and a dichotomous variable distinguishing those without a bachelor's degree from those with a bachelor's degree (Comp_BA). (**b**) Mean diastolic blood pressure (DBP) by *TOX2* genotype and a dichotomous variable distinguishing those with no more than high school education from those with at least some college (Comp_HS+).

common theme has appeared whereby genes associated with BP regulation are also found to be associated with neuropsychiatric disorders.

SNP interactions with educational attainment have thus revealed 2 significant and 3 suggestive loci associated with BP, none of which were previously implicated in BP by SNP main effects in the Framingham Heart Study or any other study. Although the identified loci are in or near genes not previously associated with BP, biological plausibility, in some cases substantial, provides physiological connection to BP regulation. In this study, as in others, educational attainment likely serves as a surrogate for other unmeasured innate and environmental exposures and behaviors, including neuropsychiatric disorders, smoking, alcohol consumption, physical activity, and stress;[42] thus, the specific mechanisms of the identified SNP–education interactions remain uncertain. Although further work is necessary to validate these findings and elucidate their mechanisms, these data provide evidence that educational attainment (or other factors for which educational attainment serves as a surrogate) modifies the effect of SNPs on BP and that consideration of SNP–education interactions can improve gene discovery.

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DISCLOSURE

The authors declared no conflict of interest.

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