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### LifeLines Cohort Study

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# Multi-ancestry genome-wide association study accounting for gene-psychosocial factor interactions identifies novel loci for blood pressure traits

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## Summary

Psychological and social factors are known to influence blood pressure (BP) and risk of hypertension and associated cardiovascular diseases. To identify novel BP loci, we carried out genome-wide association meta-analyses of systolic, diastolic, pulse, and mean arterial BP, taking into account the interaction effects of genetic variants with three psychosocial factors: depressive symptoms, anxiety symptoms, and social support. Analyses were performed using a two-stage design in a sample of up to 128,894 adults from five ancestry groups. In the combined meta-analyses of stages 1 and 2, we identified 59 loci ( $p$  value  $< 5e-8$ ), including nine novel BP loci. The novel associations were observed mostly with pulse pressure, with fewer observed with mean arterial pressure. Five novel loci were identified in African ancestry, and all but one showed patterns of interaction with at least one psychosocial factor. Functional annotation of the novel loci supports a major role for genes implicated in the immune response (*PLCL2*), synaptic function and neurotransmission (*LIN7A* and *PPIA2*), as well as genes previously implicated in neuropsychiatric or stress-related disorders (*FSTL5* and *CHODL*). These findings underscore the importance of considering psychological and social factors in gene discovery for BP, especially in non-European populations.

## Introduction

High blood pressure (BP), or hypertension (MIM: 145500), is a leading risk factor for stroke, cardiovascular disease, end-stage renal disease, and mortality. By 2025, the number

of adults with hypertension is predicted to reach over 1.5 billion—approximately 30% of the world adult population.<sup>1</sup> Hypertension also contributes significantly to health disparities, with the highest age-adjusted prevalence in the world attributed to populations of African ancestry.<sup>2</sup>

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Both genetic and non-genetic influences have been implicated in the etiology of hypertension. In particular, genome-wide association studies (GWAS) have identified over 900 single nucleotide polymorphisms (SNPs) associated with BP traits, mostly in populations of European ancestry.<sup>3</sup> These explain only slightly more than a quarter of the estimated heritability of BP.<sup>3</sup> The remaining unexplained heritability may be due in part to gene-environment interactions (GxE).<sup>4</sup> Thus, incorporating GxE effects in GWAS of BP may yield novel loci

and reveal new insights about the biology of BP regulation and hypertension pathophysiology. Moreover, the detection of GxE effects may allow us to more precisely predict individual disease risk in the context of potentially modifiable environmental, lifestyle, and behavioral risk factors.

The role of psychological and social factors in the etiology of hypertension is supported by several epidemiological investigations<sup>5,6</sup> and animal model studies.<sup>7</sup> For example, anxiety and depressive symptoms have been consistently

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associated with a higher risk of hypertension.<sup>5,8</sup> In a systematic review of 15 prospective cohort studies, individuals with a high burden of psychological symptoms (anxiety, depression, and anger) had an 8% higher risk of hypertension compared to those reporting a low burden.<sup>9</sup> However, few studies have investigated potential effect modifications of genetic factors on BP traits by psychosocial factors.<sup>10</sup> To fill this gap in knowledge, we performed genome-wide association meta-analyses of systolic, diastolic, pulse, and mean arterial BP in the context of three psychosocial factors—depressive symptomatology, anxiety symptomatology, and social support—in a sample of up to 128,894 adults from five ancestry groups.

## Subjects and methods

### Study design and participating studies

The study was conducted in the setting of the Gene-Lifestyle Interactions Working Group of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.<sup>11, 12</sup> Study participants included adult men and women aged 18–80 years from five self-reported ancestry groups: African (AFR), Asian (ASN), Brazilian admixed (BRA), European (EUR), and His-

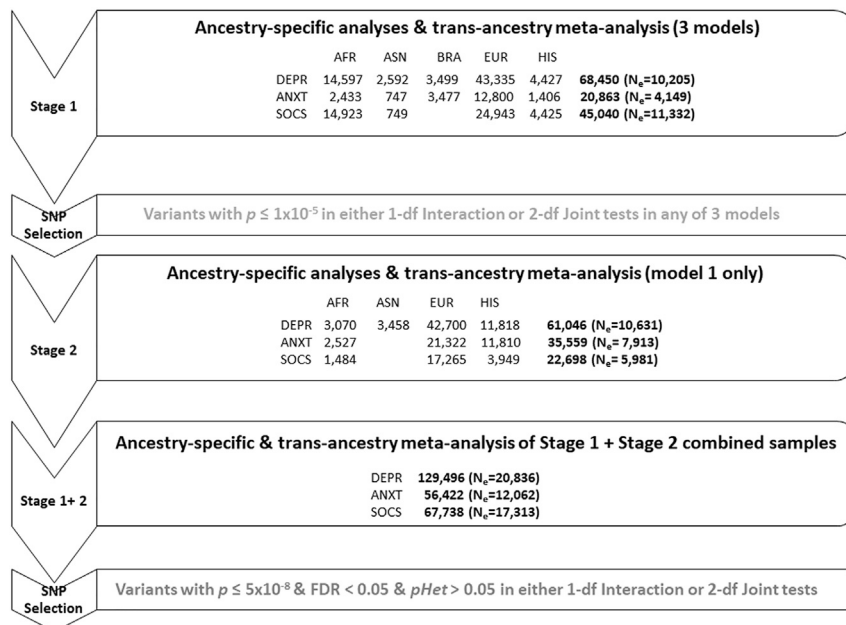
panic (HIS). Genome-wide association analyses accounting for gene-psychosocial factor interactions were carried out using a two-stage design (Figure 1). Stage 1 comprised up to 31 cohorts, including up to 68,450 individuals from the five self-reported ancestry groups. Stage 2 comprised up to 20 cohorts, including up to 61,046 individuals from four self-reported ancestry groups: AFR, ASN, EUR, and HIS. Not all studies or participants had data on all three psychosocial factors, so the number of participating studies and sample sizes varies for each exposure analysis (Figure 1). Details about the participating studies are provided in the [Supplemental subjects and methods](#). Procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national). Each study obtained written informed consent from the participants and approval from the appropriate institutional review boards.

To detect novel loci with potentially underlying SNP by psychosocial factor (SNP × Psy) interaction effects, we used two complementary approaches: (1) both the SNP main effect and interaction effect on BP levels were jointly assessed using a two-degrees-of-freedom (2-df) test; (2) the effect of interaction alone was assessed using a 1-df test. When both the SNP main effect and interaction effect are present, the 2-df is more powerful<sup>13</sup> and, thus, may help identify BP-associated loci for which the 1-df test is underpowered.

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**Figure 1. Overall study design**

For each BP trait, association analyses were performed taking into account the interaction effects of genetic variants with each of three psychosocial factors: depressive symptoms (DEPR), anxiety (ANXT), and social support (SOCS). For each ancestry group, study-specific results were combined to perform a 1-degree of freedom (1-df) test for an interaction effect and a 2-df joint test of SNP main and interaction effects. Analyses were carried out separately in five self-reported ancestry groups: European (EUR), African (AFR), Asian (ASN), Hispanic (HIS), and Brazilian (BRA), and combined in a trans-ethnic meta-analysis. Sample sizes for each analysis are shown. N<sub>e</sub>, number of subjects in the exposed strata (E = 1).

### Blood pressure traits

Four BP measures were separately modeled: systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and pulse pressure (PP). SBP and DBP were adjusted for the BP-lowering effect of antihypertensive medication use by adding 15 and 10 mm Hg, respectively, to the observed BP readings.<sup>14</sup> After adjustment, MAP was calculated as two-thirds of DBP plus one-third of SBP. PP was calculated as the difference between SBP and DBP. Winsorizing was performed for each BP value that was more than six standard deviations away from the mean. Descriptive statistics for the four BP traits in each stage 1 and stage 2 cohort are shown in Table S1.

### Psychosocial exposures

Information on depressive symptomatology, anxiety symptomatology, and social support was collected in each participating study using validated screening questionnaires (Table S2). Each measure of psychosocial exposure was dichotomized. To harmonize psychosocial exposures assessed using different screening instruments, we used recommended standard cut points specific to the screening instrument to define high depressive symptoms and high anxiety symptoms, whereas low social support was defined based on the lowest quartile of the perceived social support score in each study (all coded as E = 1). Details about the screening instruments used to measure depressive and anxiety symptomatology and social support and the cut points used to define the dichotomous variables in each study are shown in Table S2. BP readings and psychosocial questionnaires were taken at the same examination.

### Genotype data

All cohorts performed genotyping on Illumina or Affymetrix arrays and imputed to the 1000 Genomes Project reference haplotypes.<sup>15</sup> Most studies used the Phase I Integrated Release Version 3 reference panel (2010-11 data freeze, 2012-03-14 haplotypes), which contains haplotypes for 1,092 individuals of all ethnic backgrounds.<sup>15</sup> Information on genotype and imputation for each study is presented in Table S3. Although we refer to the analyzed variants as SNPs, the imputed data also include indels (insertions and deletions).

### Study-specific statistical analysis

We considered three statistical models to satisfy slightly different purposes:<sup>12</sup>

Model 1 is a joint effect model and is our primary model. It represents the joint analysis of the effects of the SNP, psychosocial exposure, and their interaction:

$$E[BP] = \beta_0 + \beta_{SNP}SNP + \beta_E E + \beta_{SNP \times E} SNP * E + \beta_C C$$

where E is the psychosocial variable and C represents all covariates (including age, sex, cohort-specific variables, and principal components [PCs]). PCs were derived from the directly measured genotype data and adjusted as appropriate for each study population. Information on PCs and additional study-specific covariates included in each analysis is provided in Table S3. Each SNP was coded under the assumption of an additive model. The model incorporated a SNP × Psy interaction effect. A 2-df joint test was used to simultaneously evaluate the significance of SNP and SNP × Psy effects under the null hypothesis that  $\beta_{SNP} = \beta_{SNP \times E} = 0$ .<sup>16</sup> A 1-df test was also used to test for the interaction term alone under the null hypothesis that  $\beta_{SNP \times E} = 0$ .

Model 2 is a SNP main effect model:

$$E[BP] = \beta_0 + \beta_{SNP}SNP + \beta_C C$$

which was analyzed among those measured for the relevant psychosocial factor. Model 2 is used to identify SNPs with main effects only.

Model 3 is a psychosocial context-dependent SNP main effect model:

$$E[BP] = \beta_0 + \beta_{SNP}SNP + \beta_E E + \beta_C C$$

which estimated the per-allele effect of the SNP on BP adjusting for an individual psychosocial factor. Model 3 is used to identify SNPs from the joint model that would be missed when the interaction term is not used.

Stage 1 cohorts performed ancestry-specific association analyses using all three models, while stage 2 cohorts performed analyses using Model 1 only. All association analyses within cohorts were

performed with various analytical software as described in Table S3.

#### Quality control and meta-analyses

Extensive quality control (QC) was performed for both the study-specific results and the meta-analyses results using EasyQC.<sup>17</sup> For each study, SNPs were filtered out if they had a minor allele frequency (MAF) less than 0.01, a low imputation quality (INFO score less than 0.5), a discrepancy in MAF compared with the 1000 Genomes reference panel greater than 0.3, or if the product of  $2 * \text{MAF} * N_{\text{exposed}}$  imputation quality score was less than 20. SNPs in the European-ancestry and multi-ancestry analyses had to be present in at least three cohorts and 3,000 participants to be reported. Due to the limited sample sizes, these criteria were relaxed for other ancestry-specific meta-analysis results, as shown in Table S4.

Inverse-variance weighted fixed-effect meta-analyses were conducted for all 3 models using METAL in stage 1.<sup>18</sup> Meta-analyses of the 2-df joint test and 1-df interaction test in the joint effect model (model 1) were carried out separately.<sup>13</sup> A 1-df Chi-square test was used to evaluate the 1-df interaction (model 1), SNP main effect (model 2), and psychosocial factor-adjusted SNP effect (model 3). A 2-df Wald test was used to jointly test the effects of both SNP and SNP  $\times$  Psy interaction. Meta-analyses were conducted within each ancestry separately, then combined in a trans-ancestry meta-analysis. Genomic control correction was applied to the study-specific results and to the ancestry-specific meta-analysis results.<sup>19</sup> The quantile-quantile (QQ) plots and the estimated genomic control inflation factors for both 2-df and 1-df tests in stage 1 are shown in Figures S1–S6. There was mild to moderate inflation across most analyses ( $\lambda \sim 1.1$ ). Variants with  $p < 1e-5$  in 1-df or 2-df tests in any meta-analysis and any of the three models were selected for stage 2 analyses.

In the focused discovery stage 2, only  $\sim 20,000$  variants were investigated, and we used the same approaches as in stage 1 to perform ancestry-specific and trans-ancestry meta-analyses but without genomic control correction or variant filtering. The  $\sim 20,000$  variants were examined for association with the 4 BP traits, in the context of the 3 psychosocial factors and in each of the 5 ancestry groups using the joint effect model (model 1).

Finally, we performed ancestry-specific and trans-ancestry meta-analyses of all the cohort-level data from stage 1 and stage 2 together (model 1 only). There was no variant filtering at that stage, and all available data from stage 1 and stage 2 were used. We computed false discovery rate (FDR) adjusted  $p$  values ( $q$ -values) for the 2-df test using the  $p.adjust$  function in R, correcting for the number of analyses performed in stage 1 (4 BP traits, 3 psychosocial factors, and 5 + 1 ancestry/trans-ancestry groups). SNPs with  $p < 5e-8$  and  $q < 0.05$  and without any evidence of heterogeneity ( $p_{\text{Het}} > 0.05$ ) in ancestry-specific meta-analyses for either the 1-df or 2-df test were considered statistically significant.

We defined a locus as the  $\pm 1$  Mbp region surrounding an index SNP and a novel locus as  $\pm 1$  Mbp away from an index SNP previously reported in the GWAS catalog and in Evangelou et al.<sup>3</sup>

#### Proportion of variance explained

We used the VarExp R package<sup>20</sup> to estimate the proportion of variance in each BP trait explained by previously reported BP variants and newly identified SNPs. The pruning threshold was set at  $r^2 = 0.2$  to trim off redundant contribution from SNPs in high linkage disequilibrium (LD). Summary statistics and BP-SNP association estimates were derived from the meta-analyses of stages 1 and 2.

#### Bioinformatics and functional annotation

We assessed the functional potential of identified SNPs in the meta-analyses using multiple tools. We first used HaploReg

v4.1<sup>21</sup> and the Functional Mapping and Annotation (FUMA)<sup>22</sup> to annotate the functional features of our novel BP loci. HaploReg was used to evaluate the effect of the identified SNPs on transcription factor binding site motifs and to perform enhancer enrichment analysis. Specifically, we assessed the overlap of our novel BP-associated SNPs with predicted enhancers using the ChromHMM 15-state core model and a binomial test of enrichment relative to the background frequencies of all common variants in 127 cell types. FUMA was used to prioritize candidate genes at each of the novel BP loci by incorporating three mapping strategies (positional, expression Quantitative Trait Locus (eQTL), and chromatin interaction mappings), MAGMA gene-set analyses, and several other annotation tools, such as the Combined Annotation Dependent Depletion (CADD) score.<sup>23</sup> We also used the Phenoscanner v2<sup>24</sup> database to evaluate our novel BP-associated SNPs for association with diseases and traits, metabolites (metabolite quantitative trait loci, mQTL), gene expression (eQTL), proteins (protein quantitative trait loci, pQTL), and DNA methylation (methylation quantitative trait loci, methQTL). Finally, we carried out protein-protein interaction networks and pathways enrichment analyses using STRING v.11.<sup>25</sup>

## Results

Stage 1 analyses comprised up to 68,450 participants from five ancestry groups (Figure 1). Descriptive statistics of the studies participating in stage 1 are shown in Table S1. The proportion of individuals with psychological symptoms varied by cohort and ancestry group. On average, 16% (range: 5%–41%) of individuals reported depressive symptoms and 24% (range: 6%–75%) reported anxiety symptoms.

In stage 1 genome-wide interaction meta-analyses, we identified 20,323 unique variants with suggestive evidence ( $p < 1e-5$ ) of any BP trait association with at least one of the three models tested. These were then evaluated for BP association in stage 2 in an independent sample of up to 61,046 individuals from four race/ethnicity groups (AFR, ASN, EUR, and HIS) (Figure 1).

In meta-analyses combining stage 1 and 2 cohorts, we identified 1,624 SNPs in 59 loci with genome-wide significant BP associations ( $p < 5e-8$ ) (Table S5). A total of 597 SNPs in 28 loci were associated with SBP, 1,261 SNPs in 26 loci were associated with DBP, 570 SNPs in 26 loci were associated with MAP, and 150 SNPs in 19 loci were associated with PP. There were 604 SNPs associated with more than one BP trait (Figure S7). Almost all (1,614) SNPs were identified through the 2-df joint test only. Additionally, six SNPs in four loci were identified through the 1-df interaction test only, and 4 SNPs in 3 loci were identified through both. A total of 1,316 SNPs reached genome-wide significance in more than one association test (Table S5).

#### Novel BP loci

Among the 59 genome-wide significant loci, 15 SNPs in 9 loci were at least 1 Mbp away from any previously reported BP locus and therefore considered novel (Table 1; Table S6). All of them reached genome-wide significance in the 2-df

**Table 1. Novel loci associated with BP traits discovered in the combined analysis of stages 1 and 2**

Locus	Nearest gene	rsID	CHR: position	EA	EAF	MAF AA/EA/HIS/BR/ASN	Effect <sup>a</sup>	SE	IntEffect <sup>a</sup>	IntSE	P.2 df	Q.2 df	P.1 df	HetPVal <sup>b</sup>	Most significant 2-df model	n
1	<i>CSF3R</i>	rs77010007	1:37049595	C	0.97	0.03/0/0/0/0	1.721	0.672	3.920	1.167	2.34E-08	4.81E-04	7.82E-04	0.338	AA-MAP-DEPR	14,865
	<i>CSF3R</i>	rs112421395	1:37056662	A	0.03	0.03/0/0/0/0	-1.864	0.664	-3.712	1.166	2.05E-08	4.24E-04	1.45E-03	0.364	AA-MAP-DEPR	14,865
2	<i>PLCL2</i>	rs60884297	3:17115469	A	0.98	0.02/0/0/0/0	-0.106	0.638	5.125	1.049	1.39E-08	2.96E-04	1.03E-06	0.794	AA-PP-SOCS	16,406
	<i>PLCL2</i>	rs111333873	3:17123818	T	0.97	0.03/0/0/0/0	-0.004	0.587	4.970	0.986	3.01E-09	7.16E-05	4.58E-07	0.753	AA-PP-SOCS	16,406
	<i>PLCL2</i>	rs73153364	3:17135437	T	0.03	0.03/0/0/0/0	-0.005	0.533	-4.720	0.941	9.11E-09	1.99E-04	5.26E-07	0.576	AA-PP-SOCS	16,406
3	<i>FSTL5</i>	rs138187213	4:162397256	D	0.90	0.10/0.16/0.26/0.19/0.41	0.105	0.301	3.311	0.652	3.63E-08	7.08E-04	3.75E-07	0.665	AA-PP-DEPR	14,534
	<i>FSTL5</i>	rs5863461	4:162403550	D	0.89	0.11/0.16/0.26/0.19/0.41	0.074	0.306	3.321	0.646	2.87E-08	5.78E-04	2.72E-07	0.675	AA-PP-DEPR	14,534
4	<i>CASP8AP2</i>	rs9342214	6:90593029	A	0.91	0.01/0.01/0.12/0.05/0.42	0.055	0.234	2.430	0.453	4.72E-09	4.31E-05	7.97E-08	0.000	TRANS-PP-ANXT	23,157
5	<i>ACA59</i>	rs201673188	11:115004812	D	0.06	0.06/0.25/0.07/0.18/0	0.249	0.440	-3.570	0.708	3.86E-08	7.48E-04	4.53E-07	0.169	AA-PP-DEPR	12,882
6	<i>ACSS3</i>	rs140203359	12:81590456	A	0.99	0.01/0.01/0.11/0/0	0.400	0.578	4.444	0.969	3.23E-09	3.27E-05	4.46E-06	0.575	EA-PP-SOCS	32,600
	<i>SNORD38</i>	rs142313940	13:90434805	A	0.02	0.20/0.02/0.06/0.05/0.25	-0.405	0.279	-2.586	0.552	4.01E-09	3.90E-05	2.75E-06	0.792	EA-PP-DEPR	76,812
7	<i>SNORD38</i>	rs150161168	13:90434806	A	0.02	0.21/0.02/0.06/0.05/0.25	-0.401	0.279	-2.594	0.551	3.87E-09	3.80E-05	2.55E-06	0.787	EA-PP-DEPR	76,812
	<i>7SK</i>	rs202048896	18:36191432	D	0.96	0.04/0.04/0/0.02/0	0.926	0.520	4.393	0.936	9.86E-11	2.81E-06	2.67E-06	0.241	AA-MAP-DEPR	14,534
9	<i>CHODL</i>	rs73321585	21:19312167	T	0.96	0.08/0/0.02/0.01/0	0.025	0.289	2.670	0.532	1.19E-08	9.51E-05	5.17E-07	0.003	TRANS-MAP-DEPR	37,392
	<i>CHODL</i>	rs73321586	21:19312525	T	0.04	0.08/0/0.02/0.01/0	0.101	0.300	-2.773	0.548	3.67E-08	2.46E-04	4.25E-07	0.002	TRANSC-MAP-DEPR	34,421

SNPs with  $p < 5 \times 10^{-8}$  in the 2-df test or 1-df interaction test and at least 1 Mbp away from any previously reported BP locus are shown. EA, effect allele; EAF, effect allele frequency; MAF, minor allele frequency; SE, standard error; HetPVal, heterogeneity p value, AA, African ancestry; EUR, European ancestry; HIS, Hispanic ancestry; BR, Brazilian ancestry; ASN, Asian ancestry; df, degrees of freedom; P.2df, P value of the joint test of SNP main effect and interaction effect with 2 df; Q.2df, false discovery rate q value of the joint test with 2 df; P.1 df, P value of the interaction test with 1 df; TRANS, transethnic meta-analysis; DEPR, depressive symptomatology; ANXT, anxiety symptomatology; SOCS, social support; PP, pulse pressure; MAP, mean arterial pressure; n, total sample size.

<sup>a</sup>SNP main (Effect) and interaction (IntEffect) effects estimated in the joint model. Effect is in mm Hg.

<sup>b</sup>p Value for heterogeneity in the stage 1 + 2 in the most significant 2-df model.

test, and all but one showed suggestive evidence of interaction (1-df  $p < 0.05/59 = 8.5e-4$ ). Indeed, as shown in forest plots (Figures S8–S12), associations at these novel loci were predominantly driven by interaction effects. Ten of the newly identified variants were discovered through modeling of interaction effects with depressive symptomatology, another four with social support, and only one with anxiety. Except for the two variants in the *FSTL5* gene on chromosome 4, the novel variants were of low frequency (MAF, 0.01–0.05) in the population in which they were identified. Nine of the 15 variants were discovered in analyses of populations of AFR ancestry. The enhanced discovery of novel loci in AFR ancestry may be due to differences in allele frequencies among ancestry groups. Indeed, five of the nine variants discovered in analyses of populations of AFR ancestry were not observed in any other ancestry. Seven of the 15 novel variants were not observed in individuals of EUR ancestry. Alternatively, SNP  $\times$  Psy interaction effect sizes may be greater in AFR ancestry. For example, while rs201673188 is more common in EUR than AFR, the interaction of SNP with depressive symptomatology was associated with a decrease in PP of 3.57 mm Hg compared to 0.00 mm Hg in EUR (Table S5). The two low-frequency variants on chromosome 13 identified in EUR ancestry showed some evidence of BP association in AFR ancestry, where they were more common (MAF = 0.20) (2-df  $p = 3.5e-3$ ; 1-df  $p = 2.3e-3$ ). Three novel SNPs were identified in the trans-ancestry meta-analyses. However, these exhibited significant heterogeneity by ancestry ( $p_{\text{Het}} < 0.016$ ), with significance being driven by results from a single ancestry group comprising stage 1 cohorts only (Table S7). Further replication of the association of these 3 SNPs with BP is therefore warranted.

### Known BP loci

The remaining 1,609 SNPs reaching genome-wide significance mapped within 1 Mbp of 50 previously reported BP SNPs. These were mostly identified in European ancestry samples. Of the 1,609 SNPs, 117 showed nominally significant interaction effects (1-df  $p < 0.05$ ), and these were mostly observed in African ancestry samples or trans-ancestry meta-analyses (Table S5).

We further assessed gene-psychosocial factor interactions on BP at previously known loci<sup>3</sup> in our dataset. Of 983 previously reported BP loci, 976 were present in our meta-analyses of stages 1 and 2. After harmonization of risk alleles against the previously published results, we tested for interaction in the 976 index SNPs. A Bonferroni-corrected  $p$  value threshold controlling for the number of SNPs tested (976), the number of BP traits (4), the number of Psy traits (3), and the number of ancestry groups (5) was used. There was evidence of gene-psychosocial interaction for 14 known independent SNPs (1-df  $p < 0.05/976 \times 4 \times 3 \times 5 = 8.5e-7$ ), including 9 SNPs reaching genome-wide significance (Table S8). Notably, while these BP loci were identified from populations of EUR ancestry, the most significant evi-

dence of interaction with psychosocial factors was obtained in samples of non-EUR ancestry.

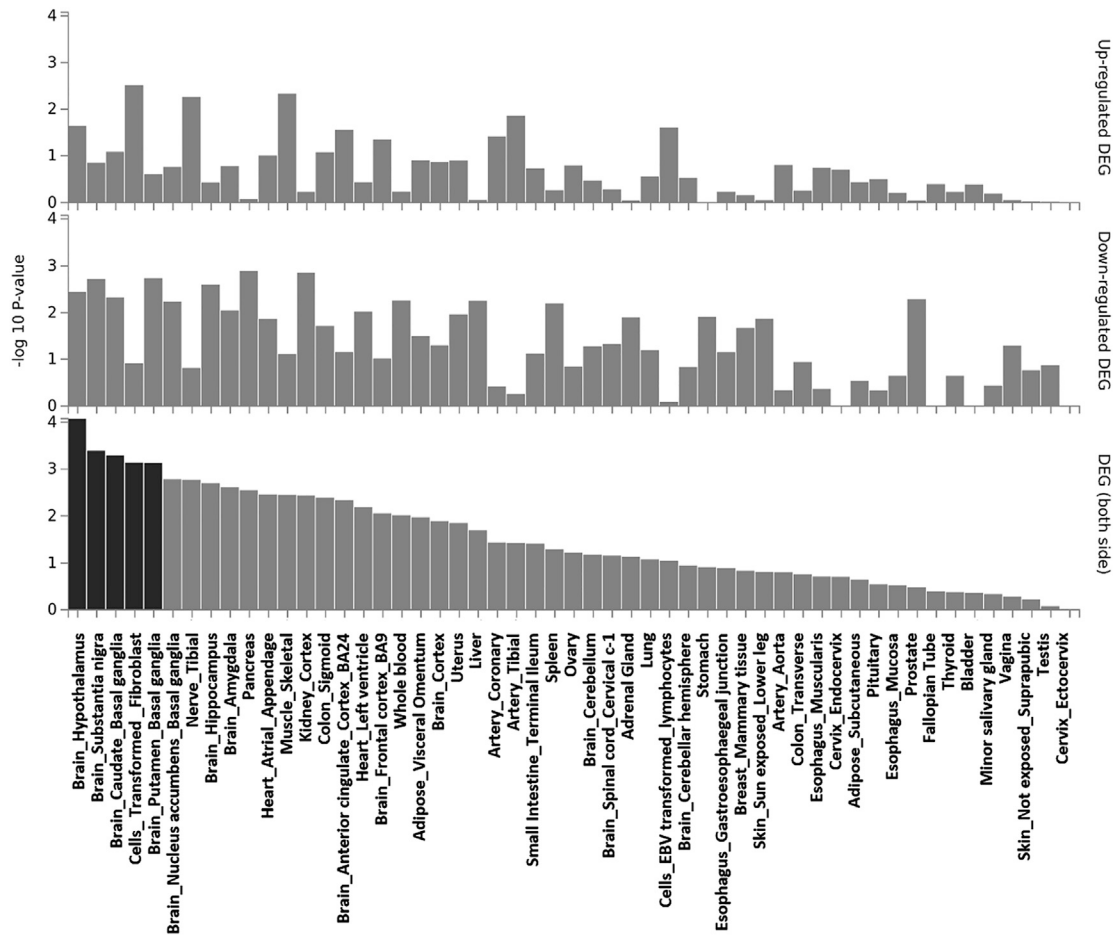
### Proportion of variance explained

We used ancestry-specific LD-pruned ( $r^2 < 0.2$ ) known and novel BP SNPs to calculate the percent variance explained by the SNP main effect and the SNP-psychosocial factor interaction effect. The highest percent variance explained was 10.4% for DBP among Asian individuals when the SNP main effect and anxiety symptoms interactive effects were jointly modeled (Table S9). Notably, except for populations of EUR ancestry, the percent variance explained by interaction effects at the identified SNPs was at least equal to or greater than that explained by the SNP main effects. This was especially striking in the AFR ancestry group. Consistently, the percent variance explained by the joint effects of SNPs and psychosocial factors was 1.3- to 3.7-fold greater than that of the SNP main effects across the 4 BP traits, with the greatest difference observed for the joint effect of SNP  $\times$  depressive symptoms (DEPR) in AFR ancestry (Table S9).

### Functional annotation and gene prioritization

Most newly identified SNPs were annotated as either intronic or intergenic. This suggests evidence of regulatory mechanisms by which the identified SNPs may influence BP. Indeed, functional annotation using HaploReg v.4.1<sup>21</sup> and FUMA<sup>22</sup> showed evidence of regulatory motif disruption and overlap with predicted enhancers in major tissue types for the identified SNPs (Table S10). Enhancer enrichment analysis in HaploReg v.4.1<sup>21</sup> showed that the strongest signal was in primary natural killer cells from peripheral blood ( $p = 0.01$ ), suggesting a possible role of innate immunity as a mechanism underlying these novel associations. Functional annotation was also conducted on all SNPs in moderate LD ( $r^2 > 0.6$ ) with the identified novel SNPs (Table S11). Among the 125 SNPs that encompass the index SNPs and variants in LD, only two were exonic. These include a stop-loss variant (rs60111091) in *PLCL2* in high LD with the index variant rs111333873 on chromosome 3 (Locus #2). Several SNPs in LD with the novel index SNPs exhibited high CADD scores, suggesting that they are likely pathogenic. One SNP with a CADD score of 16.2 and in complete LD with rs140203359 (Locus #6) was located in an intron of *LIN7A* (MIM: 603380). Interestingly, rs140203359 was also identified as an eQTL for *LIN7A* in whole blood (Table S12). Two SNPs with similarly high CADD scores and in moderately high LD with rs73321585 (Locus #9) were located in an intron of *CHODL*. Chromatin interaction mapping showed significant evidence of long-range interactions of rs60884297 and nearby SNPs (Locus #2) with the promoter of *ANKRD28* (MIM: 611122), *DAZL* (MIM: 601486), and *PLCL2* (MIM: 614276) in aortic and left ventricular tissues (Table S13). These three genes were predicted to be highly intolerant to a loss-of-function mutation (probability of loss-of-function intolerance (pLI) score  $> 0.9$ ).





**Figure 2. Enrichment of the prioritized genes mapped to the novel loci in Differentially Expressed Gene (DEG) sets from GTEx v7 data from 53 tissue types**  
Significantly enriched DEG sets (Bonferroni-corrected  $p < 0.05$ ) are highlighted in red.

A total of 72 genes were prioritized by FUMA based on their physical position and their potential role in 3D chromatin interactions (Table S14). Two additional genes were prioritized via eQTL analysis using PhenoScanner v2 (Table S12). The 74 prioritized genes showed gene expression enrichment in brain tissue, notably the hypothalamus (Figure 2), and enrichment in 12 Gene Ontology terms, including several related to synaptic function (Table S15). We also used STRING v.11<sup>25</sup> to investigate protein-protein interaction networks and pathway enrichment analyses among the 74 prioritized genes. There was significant evidence of protein-protein interaction among the prioritized genes ( $p = 2.5e-12$ ). A total of 46 protein-protein interactions were predicted. These showed enrichment in 4 major Reactome Pathways, including Neuronal System, Transmission across Chemical Synapses, Dopamine Neurotransmitter Release, and Protein-protein Interactions at Synapses (Table S15).

## Discussion

This genome-wide association study systematically evaluated the joint effect of SNPs and SNP  $\times$  Psy interactions

on BP in a large and diverse sample and identified 59 genome-wide significant loci, of which nine were novel. Most novel loci were identified in non-European ancestry, and all but one showed patterns of interaction with at least one psychosocial factor. The enhanced discovery of novel loci in non-EUR ancestry was due to population differences in allele frequencies, with multiple novel variants not observed in EUR, and/or to population differences in SNP  $\times$  Psy interaction effect sizes.

PP estimates the pulsatile component of BP and is influenced by the stiffness of large arteries and the pattern of wave reflections.<sup>26</sup> In a recent meta-analysis comprising 5,060 white and 3,225 African American healthy adults from 11 studies, measures of arterial stiffness and wave reflection were consistently higher in African Americans than in whites.<sup>27</sup> Intriguingly, the majority of the newly discovered loci were identified from analyses of African ancestry, with several of the identified variants not observed in European ancestry. The burden of hypertension in populations of African ancestry is among the highest in the world and is a primary cause of disparities in cardiovascular health and life expectancy between African Americans and whites.<sup>28</sup> Psychological and social stressors have been

associated with hypertension and are thought to play a major role in racial/ethnic differences in hypertension.<sup>29</sup> In particular, several lines of evidence indicate that psychosocial stressors may uniquely impact heart rate variability among African Americans.<sup>29</sup> The findings reported here underscore the value of including diverse populations in discovery of novel BP loci and may provide clues about possible biological mechanisms underlying the relationships between genes, psychosocial factors, and BP.

Functional annotation of the newly identified loci provides support for a major role of genes implicated in synaptic function, neurotransmission, and innate immunity. One of the newly identified loci for PP mapped to the *PLCL2* gene region on chromosome 3p24. Three variants in moderately high LD and polymorphic in samples of African ancestry only were associated at the genome-wide significance level with PP in the context of social support and at nominal significance level ( $p < 0.05$ ) with SBP and MAP in the context of social support and anxiety. These variants are in strong LD with a rare stop-loss variant in the *PLCL2* gene and map to a region of long-range chromatin interaction with the *PLCL2* promoter in aortic and left ventricular tissues. *PLCL2* encodes a phospholipase C-like protein that lacks phospholipase catalytic activity.<sup>30</sup> *PLCL2* is expressed in hematopoietic cells, including B cells and T cells, and is a negative regulator of B cell receptor signaling and immune responses.<sup>31</sup> Genetic variants in the *PLCL2* gene have been associated with several autoimmune disorders<sup>32–34</sup> and myocardial infarction<sup>35</sup> in GWAS. The newly identified associations are consistent with the well-documented role of inflammation and the immune system in hypertension.<sup>36,37</sup>

Another newly identified association for PP mapped to a region on chromosome 12q21 that harbors several genes involved in synaptic function and plasticity. Lin-7 homolog A (*LIN7A*) is part of a family of scaffolding proteins that function as part of a tripartite complex and play a major role in synaptic function.<sup>38</sup> This evolutionarily conserved complex couples synaptic vesicle exocytosis to cell adhesion in the brain<sup>39</sup> and participates in NMDA receptor-containing vesicle transport.<sup>40</sup> *PPFIA2* (MIM: 603143) encodes liprin  $\alpha 2$ , which organizes pre-synaptic ultrastructure and controls synaptic output by regulating synaptic vesicle release.<sup>41</sup> *SYT1* (MIM: 185605) encodes synaptotagmin 1. The synaptotagmins are integral membrane proteins of synaptic vesicles thought to serve as calcium sensors in the process of vesicular trafficking and exocytosis.<sup>42</sup>

The newly identified locus on chromosome 4 associated with PP through depressive symptomatology mapped to an intronic region of the follistatin-like 5 (*FSTL5*) gene. This gene encodes a secretory glycoprotein with calcium-binding function. Gene expression analysis of mouse brain tissue shows that *Fstl5* is expressed in the olfactory system, hippocampal CA3 area, and granular cell layer of the cerebellum.<sup>43</sup> Variants in or near this gene have been associated with alcohol-related life events,<sup>44</sup> schizophrenia,<sup>45</sup>

and the clustering of bipolar disorder, major depression, and schizophrenia.<sup>46</sup>

The locus on chromosome 21 mapped to an intronic region of the *CHODL* gene (MIM: 607247), which encodes a membrane-bound C-type lectin expressed in heart and skeletal muscle and is involved in muscle organ development. Rare copy number variants in this gene have been implicated in stress cardiomyopathy, also known as “broken heart syndrome,” a sporadic condition precipitated by psychological or physical stress.<sup>47</sup>

Strengths of our study include a large sample of community-based cohorts with diverse ancestral backgrounds. Several limitations must also be acknowledged. First, while sample size was relatively balanced in the two analysis stages for populations of European and Asian ancestry, this was not the case for the other populations. Moreover, Asian and Brazilian populations were underrepresented in the overall sample. A more balanced population representation across stages 1 and 2 and a more diverse sample may have identified additional loci. Second, not all studies used the same validated instrument to capture depressive symptomatology, anxiety symptoms, and social support. This may have introduced some degree of heterogeneity and thus reduced power of our study. Finally, numerous studies show that psychological and social stressors are associated with poor health behaviors, such as cigarette smoking, excess alcohol consumption, low physical activity, and poor diet.<sup>48–50</sup> Thus, it is possible that the associations identified here were mediated at least in part by these factors. Indeed, although none of the nine novel loci identified here overlap with loci reported in previous GWAS of gene-by-alcohol or gene-by-smoking interaction on BP,<sup>51,52</sup> several known loci show such an overlap (Table S16).

In conclusion, we identified nine novel loci associated with BP traits, which harbor genes implicated in the neuronal system, synaptic function, and the immune response. Associations of these loci with BP were driven by interaction effects with at least one of three psychosocial factors. Moreover, our data highlight the potential for psychosocial factors to modify genetic associations of BP traits at previously reported loci. These findings underscore the importance of considering psychological and social factors in gene discovery for BP, especially in African ancestry.

## Data and code availability

The summary statistics of the meta-analyses generated in this project are available at the CHARGE Consortium Summary Results at the database of Genotypes and Phenotypes (dbGaP) under accession number dbGaP: phs000930 or directly from the authors upon request.

## Supplemental Information

Supplemental Information can be found online at <https://doi.org/10.1016/j.xhgg.2020.100013>.

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## Declaration of Interests

The authors declare no competing interests.

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## Web resources

OMIM, <https://www.omim.org/>

FUMA GWAS, <https://fuma.ctglab.nl/>

HaploReg v4.1, <https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>

PhenoScanner V2, <http://www.phenoscaner.medschl.cam.ac.uk/>

STRING V11, <https://string-db.org/>

dbGaP, <https://www.ncbi.nlm.nih.gov/gap/>

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