Original Article

New Blood Pressure–Associated Loci Identified in Meta-Analyses of 475 000 Individuals

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†A list of all study participants is given in the Data Supplement.

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Background—Genome-wide association studies have recently identified >400 loci that harbor DNA sequence variants that influence blood pressure (BP). Our earlier studies identified and validated 56 single nucleotide variants (SNVs) associated with BP from meta-analyses of exome chip genotype data. An additional 100 variants yielded suggestive evidence of association.

Methods and Results—Here, we augment the sample with 140 886 European individuals from the UK Biobank, in whom 77 of the 100 suggestive SNVs were available for association analysis with systolic BP or diastolic BP or pulse pressure. We performed 2 meta-analyses, one in individuals of European, South Asian, African, and Hispanic descent (pan-ancestry, ≈475 000), and the other in the subset of individuals of European descent (≈423 000). Twenty-one SNVs were genome-wide significant ($P<5\times10^{-8}$) for BP, of which 4 are new BP loci: rs9678851 (missense, *SLC4A1AP*), rs7437940 (*AFAP1*), rs13303 (missense, *STAB1*), and rs1055144 (*7p15.2*). In addition, we identified a potentially independent novel BP-associated SNV, rs3416322 (missense, *SYNPO2L*) at a known locus, uncorrelated with the previously reported SNVs. Two SNVs are associated with expression levels of nearby genes, and SNVs at 3 loci are associated with other traits. One SNV with a minor allele frequency <0.01, (rs3025380 at *DBH*) was genome-wide significant.

Conclusions—We report 4 novel loci associated with BP regulation, and 1 independent variant at an established BP locus. This analysis highlights several candidate genes with variation that alter protein function or gene expression for potential follow-up. **(*Circ Cardiovasc Genet*. 2017;10:e. DOI: 10.1161/CIRCGENETICS.117.001778.)**

Key Words: blood pressure ■ exome ■ genetics ■ genotype ■ sample size

High blood pressure (BP) is a major risk factor for coronary artery disease, heart failure, stroke, renal failure, and premature mortality.[1] High BP has been estimated to cause 10.7 million deaths worldwide in 2015.[2,3] Pharmacological interventional trials of BP-lowering therapies in patients with hypertension have demonstrated reductions in cardiovascular complications, including mortality.[4] Although several antihypertensive drug classes exist, variability in treatment response by individual patients and ethnic/racial groups, and residual risks, suggests that identification of previously unrecognized BP regulatory pathways could identify novel targets and pave the way for new treatments for cardiovascular disease prevention.

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Genetic association studies have identified >400 loci at $P<5\times10^{-8}$ that influence BP.[5–11] Two recent reports independently performed discovery analyses, in sample sizes of up to ≈146 000 (CHARGE Exome BP consortium [The Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium]) and ≈192 000 individuals (the European-led Exome consortia [contributory consortia, CHD Exome+, ExomeBP, and GoT2D:T2DGenes]).[8,9] All samples were genotyped on the Illumina Exome array that was designed to interrogate rare and low frequency nonsynonymous and other putative functional variants and noncoding variants for association with biomedical traits. They each identified ≈80 promising single nucleotide variant (SNV) associations with systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), or hypertension and took them forward for replication in the reciprocal consortium[8,9] resulting in the identification of 56 novel BP-associated loci across the 2 reports, including associations with coding and rare SNVs. A total of 100 SNVs remained of interest, but did not achieve genome-wide significance. Increasing the sample size is likely to identify additional BP-associated SNVs among these variants.

In the current report, we augmented the sample size of these studies with up to 140 886 European individuals from the UK Biobank and analyzed 77 SNVs available in the UK Biobank for association with SBP, DBP, and PP, in a total sample size of up to ≈475 000 individuals (up to ≈423 000 European [EUR]).

Materials and Methods

Samples

These analyses consisted of a meta-analysis of results from 3 independent publications, the CHARGE Exome BP consortium,[8] European-led Exome consortia (contributory consortia, CHD Exome+, ExomeBP, and GoT2D:T2DGenes),[9] and the BP analyses from the UK Biobank Cardiometabolic consortium.[11]

The CHARGE Exome BP consortium included 120 473 individuals of EUR descent from 15 cohorts, 21 503 individuals of African descent from 10 cohorts, and 4586 individuals of Hispanic ancestry from 2 cohorts as described previously.[8] The European-led consortia included 165 276 individuals of EUR descent from 51 cohorts and 27 487 individuals of South Asian descent from 2 cohorts.[9] The UK Biobank data included 140 886 unrelated individuals of EUR descent.[11]

All samples from the CHARGE and European-led Exome consortia were genotyped on Exome arrays that includes ≈242 000 markers >90% of which are nonsynonymous or splice variants, with enrichment for variants with minor allele frequency (MAF)<0.05. The UK Biobank used the Affymetrix UK Biobank Axiom Array (approximately 100 000) or the Affymetrix UK BiLEVE Axiom Array (approximately 50 000) to genotype ≈800 000 SNVs with subsequent imputation based on UK10K sequencing and 1000 Genomes reference panels. SNVs with an imputation threshold INFO score of <0.10 were filtered by the Warren et al[11] UK Biobank Nature Genetics 2017 article, from which the SNV association statistics for UK Biobank were provided.[11] Imputation

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scores in the UK Biobank samples for the variants presented in the Table had INFO>0.6. SNVs that produced significant results are highlighted in green in Tables I and II in the Data Supplement, with a median INFO of 1. The studies by Surendran et al,[9] Liu et al,[8] and Warren et al[11] examined genomic inflation factors in the contributing studies and the combined meta-analyses for each of the traits analyzed. Genomic inflation ranged between 1.04 and 1.11 in these contributing studies and therefore did not suggest that there were significant issues with population stratification. In the current analyses, 77 nonvalidated BP-associated SNVs were available for analysis across all 3 data sets.

Institutional review board approval was obtained from each participating cohort, and informed consent was obtained from all subjects.[8,9] The UK Biobank study has approval from the North West Multi-Centre Research Ethics Committee and has Research Tissue Bank approval.

Phenotypes

Three BP traits were examined: SBP, DBP, and PP, where PP was calculated as the difference between SBP and DBP. For individuals taking antihypertensive therapies, 15 mm Hg and 10 mm Hg were added to the observed SBP and DBP, respectively, to estimate the BP that would be observed off antihypertensive therapy.[12,13] The traits were approximately normally distributed, and no transformations of the traits were performed.

Statistical Analyses

In the CHARGE Exome BP consortium, in cohorts of unrelated individuals, single SNV association tests were implemented via linear regression in R/PLINK/SNPTEST. For family-based cohorts linear mixed-effects models in R was used to estimate kinship via R KINSHIP2 package and using the LMEKIN function, to account for familial correlations (https://cran.r-project.org/web/packages/coxme/vignettes/lmekin.pdf; Supplemental Table 21 of Liu et al[8]). The component studies of the European-led consortia (CHD Exome+, ExomeBP, and GoT2D:T2D genes) used linear regression as implemented in PLINK[14] or linear mixed models as implemented in Genome-Wide Efficient Mixed Model Association[15] or EPACTS (the Efficient Mixed-Model Association eXpedited,[16] to test variants for association with BP traits. The UK Biobank study used linear regression models as implemented in SNPTEST.[17] All studies assumed an additive allelic effects model.

All studies adjusted for age, age^2, sex, body mass index, and additional cohort-specific covariates including (where appropriate) principal components of genetic ancestry, field centers, genotyping array, or case/control status for samples ascertained on case/control status for a non-BP trait. Both study-level QC and central QC were performed before the meta-analyses being performed. Full details are given in the reports from the component consortia.[8,9,11]

At the consortium level, meta-analyses of cohort-level association results were performed independently within CHARGE-Exome and the European-led Exome consortia using inverse variance-weighted fixed effects meta-analysis. These meta-analyses results were combined with the UK Biobank association results using fixed-effects inverse variance-weighted meta-analysis as implemented in METAL.[18] Two meta-analyses were performed, one pan-ancestry (PA; AA, European ancestry [EUR], Hispanic, South Asian) and the other of EUR ancestry. Statistical significance was set at genome-wide significance, $P<5\times10^{-8}$.

Functional Annotation

Associated variants were annotated using Human Genome Build 38 dbSNP and Entrez Gene (The National Center for Biotechnology Information). We interrogated publically available gene expression regulatory features from the Encyclopedia of DNA Elements consortium and ROADMAP Epigenome projects using HaploReg[19] and RegulomeDB.[20] Expression quantitative trait loci (eQTLs) were assessed using data from Genotype-Tissue Expression consortium,[21] GRASP,[22] Westra et al,[23] Lappalainen et al,[24] and STARNET.[25] In addition, we used the FHS eQTL results from microarray-based gene and exon expression levels in whole blood from 5257 individuals.[26] We queried whether any of the 5 BP-associated SNVs were eQTLs for genes in the 5 BP-associated regions or whether they were in LD (r^2>0.8) with any of the eQTLs for genes in these regions. Where putative eQTLs were identified, we verified the BP-associated SNVs were in LD (r^2>0.8) with the top eQTL for that gene.

We interrogated publicly available GWAS databases through PhenoScanner,[27] a curated database holding publicly available results from large-scale genome-wide association studies facilitating phenome scans. We report results for SNVs with P value≤5×10^{-8}.

Capture HiC interactions were accessed from the Capture HiC Plotter (www.CHiCP.org). Javierre et al[28] used an interaction confidence score derived using CHiCAGO software.[29] The interactions with a CHiCAGO score ≥5 in at least 1 cell type were considered as high-confidence interactions.

Results

Association results for the 77 SNVs with the 3 BP traits are shown in Table I in the Data Supplement for the PA (European, South Asian, African, and Hispanic descent) meta-analysis and in Table II in the Data Supplement for the EUR meta-analysis. Twenty-one of the 77 SNVs were associated with at least 1 BP trait with genome-wide significance, $P<5\times10^{-8}$ and concordant directions of effects across the results from all contributing data sets (Table). Sixteen SNVs (*PKN2*, *ARHGEF3*, *AFAP1*, *ANKDD1B*, *LOC105375508*, *ZFAT*, *RABGAP1*, *DBH*, *SYNPO2L*, *BDNF-AS*, *AGBL2*, *NOX4*, *CEP164*, *HOXC4*, *CFDP1,* and *COMT*) were genome-wide significant in both PA and EUR samples. Two SNVs at *SLC4A1AP* and 7p15.2, respectively, were significant only in the PA sample, and 3 SNVs at *STAB1/NT5DC2*, *KDM5A,* and *LACTB* only in the EUR sample. All the significant SNVs were common (MAFs≥0.19), except the SNV at the *DBH* locus (PA, MAF=0.0043). While this report was in preparation, 17 of these loci were published elsewhere.[7,10,11] Four loci remain novel: rs9678851 (*SLC4A1AP*, missense), rs7437940 (*AFAP1*, intron), rs13303 (*STAB1*, missense), and rs1055144 (7p15.2, noncoding transcript; Figure IA through ID in the Data Supplement). The *SLC4A1AP* (rs9678851) was associated with SBP, and *AFAP1* (rs7437940) and 7p15.2 (rs1055144) were associated with PP. We also observed a potentially new independent BP association (r^2=0.001 in 1000G EUR and PA samples) at a recently published locus rs34163229 (*SYNPO2L*, missense; Table; Figure IE in the Data Supplement). We used a conservative r^2<0.1 threshold to minimize the possibility of an association because of correlation with a strongly associated established BP variant. Furthermore, conditional analyses within the ≈140 000 UK Biobank participants with comprehensive genomic coverage suggested that the association with SBP of rs34163229 was independent of the established SNV, rs4746172. Regional association plots in UK Biobank are provided in Figure IIA through IIE in the Data Supplement. Conditional analyses within the full data set was not possible given the targeted nature of the Exome array that makes claims of independence provisional. Twenty-two of the 77 SNVs had MAF≤0.01, and 1 rs3025380, a missense variant in DBH, was confirmed as a BP-associated locus.

Three of the five newly discovered BP-associated SNVs are missense variants, mapping to *SLC4A1AP, STAB1,* and *SYNPO2L* (Table and Table III in the Data Supplement). At

SLC4A1AP, rs9678851 (C>A, Pro139Thr) has MAF=0.46 and the C allele is associated with an increase of 0.23 mm Hg in SBP. This variant is correlated with 2 other missense variants in *C2orf16* (rs1919126 and rs1919125, r^2=0.81 [EUR] based on 1000G,[30] for both). At *STAB1*, the C allele of rs13303 (T>C, Met2506Thr, with MAF=0.44) is associated with an increase of 0.15 mm Hg in PP per minor allele in EUR. This residue is located in a conserved region of the protein[31] (Table IV in the Data Supplement). The T allele of rs34163229, the new association at the *SYNPO2L* locus (G>T, Ser833Tyr, with MAF=0.15), is associated with an increase of 0.36 mm Hg in SBP per allele. This variant is in LD with another missense variant in *SYNPO2L* (rs3812629 r^2=1, 1000G EUR).[30] Using Polyphen2 (http://genetics.bwh.harvard.edu/pph2/index.shtml), the SNVs rs9678851 in *SLC4A1AP* and rs13303 in *STAB1* were predicted to be benign, whereas rs34163229 in *SYNPO2L* was predicted to have a possible damaging impact on the corresponding human proteins' structure and function.

We interrogated publicly available eQTL data sets through Genotype-Tissue Expression consortium, the Encyclopedia of DNA Elements consortium, RoadMap projects, PhenoScanner,[27] STARNET,[25] and Framingham Heart Study[26] to further highlight potential causal genes and mechanisms at each of the newly identified BP loci (Table III in the Data Supplement). The PP-associated SNV, rs13303, at *STAB1* is correlated (r^2>0.8 1000G EUR) with the top eQTLs for *NT5DC2* in atherosclerotic lesion-free internal mammary artery, atherosclerotic aortic root, subcutaneous adipose, visceral abdominal fat, and liver tissues (all $P<1\times10^{-11}$).[25] The rs13303 was also associated with expression levels of *NT5DC2* in EBV-transformed lymphocytes, transformed fibroblasts,[25] and thyroid cells (Table III in the Data Supplement).[21] The SBP-associated SNV at *SYNPO2L* (rs34163229) is correlated (r^2=0.86 in 1000G EUR) with the top eQTL (rs2177843) for *MYOZ1* in heart atrial appendage tissue (Table III in the Data Supplement).[21] The 5 new BP associated SNVs were not in LD with the top eQTLs for these gene regions in whole blood in the Framingham Heart Study eQTL data. We also took the opportunity to assess whether the additional 15 recently established genome-wide significant BP-associated SNVs were eQTLs in the Framingham sample. Among the genome-wide significant BP SNVs, 3, rs4680 at *COMT*, rs12680655 at *ZFAT,* and rs10760260 at *RABGAP1*, were the top eQTL for the corresponding genes in whole blood (Table V in the Data Supplement). We also examined the 5 BP-associated SNVs in endothelial precursor cell Hi-C data (www.chicp.org) [28,32] to explore long-range chromatin interactions. rs13303 was found to contact *NISCH* (score 17.34) and rs34163229 contacts *USP54* (score 33.89)

Finally, we assessed the association of the new BP-associated variants and their close proxies (r^2>0.8) with cardiovascular disease risk factors, molecular metabolic traits, and clinical phenotypes using PhenoScanner, the NHGRI-EBI GWAS catalog and GRASP.[27] We observed 5 of the newly discovered BP-associated SNVs to have genome-wide significant associations with other traits, including height (7p15.2),[33] waist-to-hip ratio (*STAB1* and 7p15.2),[34,35] triglycerides (*SLC4A1P*), adiponectin levels (*STAB1*),[36] and atrial fibrillation (rs7915134 which has r^2=0.92 in the EUR 1000G samples with rs34163229 in *SYNPO2L*[37]; Table III in the Data Supplement).

Of the 77 analyzed SNVs, from the original Exome array analyses, 56 SNVs were not genome-wide significant in the current analysis. With ≈300 BP loci reported since the time of our analysis, we investigated whether any of the 56 SNVs that were not genome-wide significant in our meta-analysis have been reported as new BP-associated loci in any of the 3 recent publications.[7,10,11] Twelve SNVs in our data set were located within 1 Mb of a recently reported BP locus: *CACNA1S, TSC22D2, RPL26L1, EDN1, GPRC6A, ACHE, CAV1, NOX5, PGLYRP2, NAPB, EDEM2,* and *KCNB1* (Tables I and II in the Data Supplement) although none of the SNVs were in LD (r^2>0.1 in all 1000G populations) with the published variants at these loci.

Discussion

We identified genome-wide significant associations with BP for 21 additional SNVs from our original Exome array analyses[8,9] by including UK Biobank participants to augment our sample size to ≈475 000 individuals. Four of the 21 BP-related loci we identified were novel, of which 2 were missense variants and 1 was a putative new independent signal at an established locus and was a missense variant.

A missense SNV in *SLC4A1AP* (rs9678851) marks the PP-associated locus on chromosome 2. *SLC4A1AP*, encodes a solute carrier also known as kidney anion exchanger adapter protein although it is widely expressed in most Genotype-Tissue Expression consortium tissues.

At the new locus on chromosome 3 (rs13303), 3 potential candidate genes are highlighted: *STAB1*, *NT5DC2,* and *NISCH*. *STAB1* encodes stabilin1, a protein known to endocytose low-density lipoprotein cholesterol, Gram-positive bacteria and Gram-negative bacteria, and advanced glycosylation end products.[38,39] The gene product is also referred to as CLEVER-1, a common lymphatic endothelial and vascular endothelial receptor-1,[40] which is expressed in macrophages.[41] SNX17 interacts with STAB1 and is a trafficking adaptor of STAB1 in endothelial cells.[38,42] The rs13303 is located 500-bp downstream of *NT5DC2*. This additional gene is highlighted through the association of rs13303 with expression of *NT5DC2* in multiple tissues (Table III in the Data Supplement). *NT5DC2* encodes the 5′-nucleotidase domain containing 2 protein. The gene is widely expressed, with higher levels observed in the heart and coronary artery, although its function is unknown. Finally, exploration of long-range chromatin interaction identified contact of the SNV region with the genetic sequence including the gene *NISCH,* which encodes the nonadrenergic imidazoline-1 receptor protein localized to the cytosol and anchored to the inner layer of the plasma membrane. This protein binds to the adapter insulin receptor substrate 4 (*IRS4*) to mediate translocation of α5 integrin from the cell membrane to endosomes. In human cardiac tissue, this protein has been found to affect cell growth and death.[43]

The PP-associated variant, rs7437940, on chromosome 4 is intronic to *AFAP1* and is located in promoter histone marks

Table. Variants Associated With Systolic Blood Pressure, Diastolic Blood Pressure, or Pulse Pressure in the Pan-Ancestry or European-Ancestry Meta-Analyses in up to ≈475 000 Individuals

rsID	Gene	Annotation	chr-pos	Trait	Meta	a1/2	Freq1	β (SE)	*P* Value	Dir	Het*P*	N	UK-BioBank INFO
New loci													
rs9678851	*SLC4A1AP*	**Missense**	2-27664167	S	PA	a/c	0.54	−0.23 (0.04)	1.07E−09	−−−	0.09	474 569	1.0000
rs13303*	*STAB1*	**Missense**	3-52523992	P	EUR	t/c	0.44	−0.15 (0.03)	3.72E−08	−−−	0.11	418 405	1.0000
rs7437940	*AFAP1*	Intronic	4-7885773	P	**EUR**, PA	t/c	0.47	−0.15 (0.03)	2.88E−08	−−−	0.007	420 616	0.9974
rs1055144	7p15.2	Nc-transcript	7-25831489	P	PA	a/g	0.19	0.19 (0.03)	3.47E−08	+++	0.18	453 880	1.0000
Recently reported loci													
rs786906	*PKN2*	Synonymous	1-88805891	S, **P**	**EUR**, PA	t/c	0.44	0.19 (0.03)	1.29E−12	+++	0.08	422 556	1.0000
rs3772219	*ARHGEF3*	Missense	3-56737223	**S**, D	EUR, **PA**	a/c	0.68	0.25 (0.04)	2.00E−10	+++	0.25	474 558	1.0000
rs40060	*ANKDD1B*	3'UTR	5-75671561	D	**EUR**, PA	t/c	0.65	−0.17 (0.02)	3.47E−12	−−−	0.46	422 598	0.9938
rs972283	*LOC105375508*	Intronic	7-130782095	**S**, D	EUR, **PA**	a/g	0.47	−0.23 (0.04)	9.12E−10	−−−	0.1	474 569	1.0000
rs12680655	*ZFAT*	Intronic	8-134625094	**S**, D	**EUR**, PA	c/g	0.6	−0.29 (0.04)	1.62E−12	−−−	0.18	402 962	1.0000
rs10760260	*RABGAP1*	Intronic	9-122951247	P	EUR, **PA**	t/g	0.14	−0.25 (0.04)	2.88E−10	−−−	0.12	421 223	0.9975
rs3025380	*DBH*	Missense	9-133636634	S, **D**	**EUR**, PA	c/g	0.004	−1.14 (0.19)	1.23E−09	−−−	0.05	400 891	0.8763
rs34163229*	*SYNPO2L*	**Missense**	10-73647154	**S**, P	EUR, **PA**	t/g	0.15	0.36 (0.05)	1.15E−11	+++	0.32	448 759	1.0000
rs925946	*BDNF-AS*	Intronic	11-27645655	D	EUR, **PA**	t/g	0.31	−0.16 (0.02)	7.08E−12	−−−	0.25	474 564	1.0000
rs12286721	*AGBL2*	Missense	11-47679976	S, **D**	**EUR**, PA	a/c	0.56	−0.17 (0.02)	3.39E−13	−−−	0.05	422 593	1.0000
rs10765211	*NOX4*	Intronic	11-89495257	P	EUR, **PA**	a/g	0.38	−0.19 (0.03)	6.46E−12	−−−	0.05	474 550	0.9964
rs8258	*CEP164*	3'UTR	11-117412960	P	EUR, **PA**	a/g	0.37	0.22 (0.03)	1.95E−15	+++	0.003	422 546	1.0000
rs11062385	*KDM5A*	Missense	12-318409	P	EUR	a/g	0.73	−0.17 (0.03)	2.69E−08	−−−	0.84	422 563	1.0000
rs7136889†	*HOXC4*	Intronic	12-54043968	**S**, P	**EUR**, PA	t/g	0.69	0.36 (0.05)	1.58E−13	+++	0.33	419 905	0.6070
rs2729835*	*LACTB*	Missense	15-63141567	S	EUR	a/g	0.68	−0.24 (0.04)	1.29E−08	−−−	0.25	394 656	1.0000
rs2865531	*CFDP1*	Intronic	16-75356418	**S**, P	EUR, **PA**	a/t	0.6	0.42 (0.06)	2.14E−13	+++	0.51	217 419	0.9998
rs4680	*COMT*	Missense	22-19963748	P	**EUR**, PA	a/g	0.51	0.16 (0.03)	2.24E−09	+++	0.005	418 385	1.0000

rsID, SNV name; gene, name of the closest gene or cytogenetic band based on Gene Entrez of NCBI; annotation, SNV annotation based on dbSNP of NCBI; chr-pos, chromosome-bp position in Human Genome build 38; trait, the blood pressure trait (diastolic blood pressure, systolic blood pressure, or pulse pressure) the variant is associated with; meta, the meta-analysis the variant is associated in, Pan-Ancestry or EURopean; A1/2, allele 1/allele 2; freq1, allele frequency for allele 1; β (SE), effect estimate, β and its SE for allele 1 from the corresponding meta-analysis; *P* value, *P* from meta-analysis; dir, direction of effect in each of the contributing consortia in the following order: EUROPEAN led Exome Consortia, UK-BIOBANK, and CHARGE-BP Consortium; HetP, *P* value of heterogeneity across the 3 contributing consortia; N, sample size for the trait and meta-analysis with the lowest *P* value; UK-BIOBANK INFO, a quality of imputation score in UK BIOBANK. For more details, see Tables I and II in the Data Supplement. D indicates diastolic blood pressure; P, pulse pressure; S, systolic blood pressure; and SNV indicates single nucleotide variant.

*Potential new signal at a recently reported locus (LD, r^2<0.1 with a published BP SNV).

†First report of this variant as genome-wide significant.

in right atrial tissue, based on regulatory chromatin states from DNAse and histone ChIP-Seq in Roadmap Epigenomics Consortium (identified with HaploReg, Table IV in the Data Supplement).[44] *AFAP1* encodes actin filament–associated protein 1. This protein is thought to have a role in the regulation of actin filament integrity, and formation and maintenance of the actin network.[45]

At the locus on chromosome 10 (rs34163229), 2 candidate genes were highlighted (*SYNPO2L* and *MYOZ1*). *SYNPO2L* encodes synaptopodin like 2, which is not well characterized, but may play a role in modulating actin-based shape. The lead SNV is also associated with expression levels of *MYOZ1* in heart appendage tissues. *MYOZ1* encodes myozenin 1, an α-actinin and gamma filamin binding Z line protein predominantly expressed in skeletal muscle.[46]

At 2 loci (*SLC4A1AP* and *SYNPO2L*), we observed >1 missense variant in high LD (r^2>0.8). Functional follow-up of these variants are needed to disentangle the causal variants. At the *SLC4A1AP* locus, there are 3 misssense variants, none of which are predicted to be damaging. Two of these are in *C2orf16* that is predicted to encode an uncharacterized protein. Current evidence is at the transcriptional level. Cellular assays comparing the function of *SLC4A1AP* with the missense variant may be developed or an animal model could be created and BP can be measured. In the first instance, a knockout model may be required, because of the predicted weak effects of the BP variants. At the *SYNPO2L* locus, the 2 missense variants are both in *SYNPO2L*, of which 1 is predicted damaging, cellular experiments testing functional effects of this variant alone or part of a haplotype maybe a good starting point.

In conclusion, we identified 4 new loci and 1 potential new SNV in a known locus, which influence BP variation and highlight specific genes and pathways that could potentially facilitate an improved understanding of BP regulation, and identify novel therapeutic targets to reduce the burden of cardiovascular disease.

Appendix

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CLINICAL PERSPECTIVE

We analyzed 77 single nucleotide variants that remained of interest, but did not achieve genome-wide significance with blood pressure (BP) traits from a prior analysis of Exome chip genotypes. A meta-analysis of results from the CHARGE Exome BP and European led consortia in combination with association results from UK Biobank samples (pan-ancestry sample of ≈475 000 and European only sample of ≈423 000) indicated 21 genome-wide significant loci. Four of these are novel BP loci: rs9678851 (missense, *SLC4A1AP*), rs7437940 (*AFAP1*), rs13303 (missense, *STAB1*), and rs1055144 (7p15.2). We also identified a potentially independent novel BP-associated single nucleotide variant, rs3416322 (missense, *SYNPO2L*) at a known locus. Two of the BP-associated single nucleotide variants influence expression levels of nearby genes. These new findings add to the growing number of BP loci and could potentially facilitate an improved understanding of BP regulation, and identify novel therapeutic targets to reduce the burden of cardiovascular disease.