1 Meta-analysis identifies common and rare variants influencing
2 blood pressure and overlapping with metabolic trait loci

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118 # Abstract

119 Meta-analyses of association results for blood pressure using exome-centric single-variants and
120 gene-based tests identified 31 novel loci in discovery among 146,562 individuals with follow-up
121 and meta-analysis in 180,726 additional individuals (N_{total}=327,288). These blood pressure loci
122 are enriched for known cardiometabolic trait variants. Associations were also observed for the
123 aggregation of rare/low-frequency missense variants in three genes, *NPR1*, *DBH,* and *PTPMT1*.
124 In addition, blood pressure associations at 39 previously reported loci were confirmed. The
125 identified variants implicate biological pathways related to cardiometabolic traits, vascular
126 function, and development. Several new variants are inferred to have roles in transcription or as
127 hubs in protein-protein interaction networks. Genetic risk scores constructed from the identified
128 variants were strongly associated with coronary disease and myocardial infarction. This large
129 collection of blood pressure loci suggests new therapeutic strategies for hypertension
130 emphasizing a link with cardiometabolic risk.

131 Hypertension (HTN) or high blood pressure (BP) is a major risk factor for cardiovascular
132 disease, chronic kidney disease, and mortality[1]. To date, in addition to rare mutations that cause
133 monogenic high or low BP disorders[2-4], candidate gene studies, genome-wide association studies
134 (GWAS), and admixture mapping approaches[5-15] have identified variants at more than 60 genetic
135 loci that are associated with BP or hypertension. Most of the known BP loci identified in large
136 population-based studies are common non-coding variants with small effects on BP.

137 The Human Exome BeadChip (Exome Chip; Illumina, Inc., San Diego, CA) was
138 designed to facilitate identification of functional variants that contribute to human traits, by
139 focusing on variants that alter amino acid sequence. The Exome Chip includes 247,039 markers
140 of which >90% are non-synonymous or splice modulating exonic variants that were not covered
141 by previous genotyping arrays. While variants on previous GWAS arrays are largely common
142 [minor allele frequency (MAF) ≥0.05], 83% of the Exome Chip variants are rare (MAF<0.01)
143 and another 6% are low frequency (MAF 0.01 to 0.05). Only 11% of the Exome Chip variants
144 are common, including a set of 5,542 (approximately 2% of overall array content) common
145 variants that were drawn from the associations reported in the NHGRI GWAS Catalog[16].

146 To identify functional coding variation associated with BP, we conducted a two-stage
147 study in up to 327,288 individuals who were genotyped with the Exome Chip (Figure 1) for
148 systolic and diastolic BP (SBP and DBP), pulse pressure (PP), mean arterial pressure (MAP),
149 and HTN. We identified single variant associations at 31 novel loci and gene-based associations
150 for three novel genes (two of which overlapped with the single variant loci) associated with BP
151 phenotypes. About half of the novel BP variants identified in this study reside in loci that were
152 previously reported in GWAS to be associated with lipids, immunologic diseases, and metabolic
153 phenotypes, suggesting common etiologies of BP and metabolic risk factors and an opportunity

154 to identify therapies that more broadly impact hypertension in the context of cardiometabolic
155 risk.

156 **New Loci Associated with BP by Single Variant Analyses**

157 In the discovery stage (Stage 1), a total of 15 distinct novel candidate loci were associated
158 (P<3.4x10^{-7}) with at least one BP trait in a primary meta-analysis among samples of all
159 ancestries and secondary meta-analyses among samples of European (EA) or African ancestry
160 (AA) (Supplementary Table 1, Supplementary Figure 1). Meta-analysis using individuals from
161 all ancestries identified 22 novel associations at 13 loci that met experiment-wide significance
162 (Supplementary Table 1). All associations with P<1x10^{-4} for at least one trait in the primary
163 analysis are listed in Supplementary Table 2. The sole locus that was identified in EA but not in
164 the all-ancestry analysis was a rare missense variant rs3025380 in *DBH* [MAF 0.005, 0.001, and
165 0.003 in EA, AA, and Hispanic ancestry (HA) samples, respectively]. Meta-analysis of AA
166 individuals identified a common missense variant rs12941884 in *SEZ6* (MAF=0.21 and 0.12,
167 respectively, in AA and EA) that was not identified in EA or all ancestry samples.

168 The Exome Chip contains 43 SNPs from loci previously identified in GWAS of BP[5-15].
169 Of these 43 loci, 39 were associated with at least one BP trait in Stage 1 analyses
170 (P<0.05/43~0.001) (Supplementary Table 3). Twenty-six of these SNPs met experiment-wide
171 significance (P<3.4x10^{-7}). Conditional analysis did not reveal any new independent variants at
172 any of these previously identified loci[5-15].

173 The 15 newly identified variants (P<3.4x10^{-7}, Supplementary Table 1) and 62 additional
174 variants (P <1x10^{-5} for at least one BP phenotype, Supplementary Table 2) from Stage 1 were
175 selected for follow up in 180,726 independent individuals (Supplementary Methods). Of the 15
176 newly identified variants, 11 replicated (P<0.05/15~0.0033) in the follow-up samples

177 (Supplementary Tables 4, and 5). In Stage 2 analyses (i.e. joint meta-analysis of results from the
178 Stage 1 and follow-up samples), we identified 48 novel BP variants at 31 loci (including the 11
179 replicated loci) associated with SBP, DBP, PP, or HTN at $P<3.4x10^{-7}$ (MAP was not available in
180 the follow-up analyses; Supplementary Tables 4 and 5). Among the top variants at the 31 loci, 13
181 were missense (Table 1). In Stage 2 analyses restricted to EA samples (Supplementary Table 4),
182 all newly identified associations in EA samples meeting the significance threshold were also
183 statistically significant in meta-analysis combining all ancestries (Supplementary Table 5) with
184 the exception of rs1925153 in *COL21A1*. In addition, all of the variants except for the four that
185 were nominated for follow up based on PP (SBP minus DBP) showed concordant directions of
186 effects for SBP and DBP (Supplementary Table 6).
187 Three of the 31 significant novel SNPs were low-frequency (MAF 0.01 to 0.05). These
188 SNPs encode non-synonymous substitutions in the genes *NPR1* (rs35479618), *SVEP1*
189 (rs111245230), and *PTPMT1* (rs11537751). *NPR1* encodes natriuretic peptide receptor 1 and has
190 been reported to be associated with BP regulation in animal models[17,18] but not previously in
191 humans; *SVEP1* and *PTPMT1* are novel BP genes. The minor alleles of all three SNPs were
192 associated with increased BP and had larger absolute effects on BP than the alleles of any of the
193 newly identified common variants. For example, each minor allele of rs35479618 was associated
194 with an increase of 0.85 mm Hg in SBP in the follow-up samples compared with a maximum
195 absolute difference (per minor allele) among the novel common variants of 0.43 mm Hg in SBP
196 (for rs8068318 in *TBX2*; Supplementary Table 5).
197 Of the 28 newly identified common variants for BP, 14 were genome-wide significant in
198 prior GWAS of lipids[19], immunologic disease[20-22], diabetes[23-25], kidney function[26], age at
199 menarche[27], resting heart rate[28], waist-hip ratio[29], and homocysteine concentration[30], but not BP

200 (Table 2 and Supplementary Table 7). Six additional variants were reported for several
201 phenotypes (Table 2) in previous candidate gene, patent filing or GWAS studies, but their *P*
202 values were not specified or did not reach the genome-wide significance level[31-36]. By contrast,
203 the remaining eight variants were missense SNPs that have not been reported in the NHGRI
204 GWAS Catalog for any trait (Table 2). Several genes in Table 2 contain multiple variants
205 showing distinct allelic roles. *HOXA3* and *NOS3*, harbor variants rs17428471 (*HOXA3*)[12] and
206 rs3918226 (*NOS3*)[10] with genome-wide significant BP association that are independent of the
207 Exome Chip variants (r^2=0.007 for rs17428471 with rs6969780 and r^2=0.007 for rs3918226 with
208 rs891511, respectively, in the 1000 Genomes data). A variant rs2651899 in *PRDM16* has been
209 reported to be associated with migraine[37], but this variant is not in LD with the new BP variant
210 rs2493292 (r^2=0.01 in the 1000 Genomes data), suggesting predisposition to distinct vascular
211 consequences for different variants at this locus. In addition, *PRDM16* has been shown to play a
212 critical role in vascular development[38], adipocyte function in subcutaneous fat, and development
213 of diabetes[39]. Finally, several variants in *DOT1L* were reported to be associated with cartilage
214 thickness and hip osteoarthritis[40]. The new BP variant rs2302061, however, was not in LD with
215 any of the prior identified signals at this locus[40].

216 Together, the 31 newly identified single variants explain 0.7% and 1.3% of inter-
217 individual variation in SBP and DBP, respectively. The previously established and newly
218 identified variants together explain 2.8% and 2.9% of phenotypic variation in SBP and DBP,
219 respectively.

220 **Gene Level Analyses**

221 We considered the possibility that an aggregation of rare or low-frequency coding alleles at
222 individual genes contributes to BP variation and tested specifically for effects of non-

synonymous, stop codon, and splicing coding variants with MAF<0.05 (T5 test) or MAF<0.01 (T1 test) using the seqMeta package. The standard burden test[41,42], which is sensitive for detecting association when all variants contribute effects on BP in a concordant direction, identified an aggregation of rare and low-frequency coding alleles in *PTPMT1* that contribute to higher odds of HTN (experiment wide significance $P<1\text{x}10^{-6}$, Table 3, Supplementary Table 8A). The SKAT test[43], which is designed to detect effects of alleles that collectively contribute to higher and lower BP effects, identified significant BP associations for *DBH* (T1) and *NPR1* (T5; Table 3, Supplementary Table 8A). Among additional individuals of European ancestry (up to 154,543 individuals) who were used for follow-up analysis, gene-based SKAT (with the RAREMETAL package) was performed for inverse normal transformed DBP, SBP, PP, and HTN (see Methods). The gene-based associations replicated in the follow-up samples at $P<0.05/3\sim0.017$ for *NPR1* ($P=4.4\text{x}10^{-5}$ for SBP) and were marginally significant for *PTPMT1* ($P=0.019$ for HTN) and *DBH* ($P=0.053$ for DBP) (Supplementary Table 8B).

Twenty-eight previously reported genes associated with monogenic BP disorders[3] contained at least two non-synonymous, stop codon, or splice-site coding variants with MAF <0.05 on the Exome Chip. Burden testing of these 28 genes identified a statistically significant association of *SLC12A1* (26 variants all having MAFs<0.005) with SBP ($P=0.0006<0.05/28$; T1 test; Supplementary Table 9). Mutations in *SLC12A1*, the Na-K-2Cl co-transporter, cause Bartter's syndrome, a Mendelian salt-wasting condition associated with hypotension[44]. The 26 variants in *SLC12A1*, however, did not overlap with the previously reported Bartter's syndrome variants[44]. The other 27 monogenic BP genes did not reach statistical significance in standard burden testing. Additionally, none of the 28 genes showed significant association with BP using the SKAT test[43] (all $P>0.0006$; Supplementary Table 9).

246 **Inferred Function of the Identified BP Loci**

247 We applied several computational strategies and conducted *cis* expression quantitative locus
248 (eQTL) analysis to infer biological functions associated with genes at the 31 significant single
249 variant BP loci (see details in Supplementary Methods).

250 *Disease and pathway enrichment analysis*: We examined functional annotations derived from
251 pre-compiled gene sets in GeneGO and literature-based inference in Literature Lab[45]. In
252 GeneGO biological processes, the 31 novel loci were enriched for cell signaling and
253 development functions (e.g. "regulation of signaling", "regulation of growth") compared with
254 largely cardiovascular functions (e.g. "negative regulation of [smooth] muscle contraction",
255 "blood circulation") for the 39 validated BP loci (Supplementary Table 10). The novel loci were
256 also enriched for several conditions related to cardiovascular and metabolic disease (e.g.
257 "myocardial ischemia", "congenital hyperinsulinism", "acid-base imbalance") whereas the
258 validated loci were enriched for conditions more directly related to BP or cardiovascular
259 conditions (e.g. "arrhythmias, cardiac", "hypertension", "hypotension"). Significant Literature
260 Lab[45] (Supplementary Table 11) pathways and disease MeSH headings were enriched for
261 insulin-related terms (e.g. "IGF-1", "type II diabetes", "hyperinsulinism") for the novel loci
262 compared to BP-related terms (e.g. "cardiac muscle contraction") and cardiovascular
263 electrophysiology (e.g. "antiarrhythmics") for the validated loci; both sets of loci were
264 significant for "heart development". In the Literature Lab[45] anatomical annotations, the
265 cardiovascular system (e.g. "myocardium", "heart ventricles") was highlighted for both the novel
266 and validated SNPs, while the validated SNPs also associated with the renal system (e.g.
267 "nephron", "urinary tract"). Almost no annotations for either GeneGO or Literature Lab[45] were

268 unique to the set of combined novel and validated loci with the exception of a few terms
269 predominantly related to BP or the renal system.
270 *Protein-Protein Interaction Analysis*: Using NCBI's protein-protein interaction (PPI) network
271 resources (Supplementary Methods), a total of 399 genes were found to be connected to at least
272 one of the 31 novel BP genes (Supplementary Figure 2). Ordered on the basis of connectivity
273 ("degree"; Supplementary Table 12), a measure that signifies a hub disposition in the PPI
274 network, the top five BP candidate genes were *INSR, PABPC4, NOS3, IGFBP3,* and *DOT1L.*
275 Based on "Google" page-rank, a connectivity measure that recognizes degree of connectivity
276 while also emphasizing connections between highly connected nodes, the five top genes differed
277 from ordering based on connectivity alone by the replacement of *IGFBP3* by *PTPMT1*
278 (Supplementary Table 12).
279 *ENCODE and Roadmap Epigenomics Analyses:* RegulomeDB[46] and HaploReg[47] evaluations of
280 potential *cis* regulatory functions identified rs8068318 (intronic to *TBX2*) as having the highest
281 score among loci (or their LD proxies) that showed relatively strong evidence for a role in
282 transcription (Supplementary Table 13). This SNP maps to an active *TBX2* promoter histone
283 mark in lung fibroblast and DNAse I hypersensitivity marks in seven cell types, while
284 overlapping with five transcriptional regulatory motifs. *TBX2* is a member of a highly conserved
285 T-box family of transcription factors and has been implicated in cardiac developmental
286 abnormalities[48,49] and kidney function[26].
287 *cis-eQTL Analysis:* The 31 newly identified BP variants were queried for *cis*-eQTL association
288 (Supplementary Table 14) in over 5,000 participants from the Framingham Heart Study (FHS),
289 using microarray-based transcriptomic profiling of RNA from whole blood. A total of 720 SNP-
290 transcript pairs were tested. Forty-three pairs (representing 17 variants) were significant at

291 FDR<10%, among which eight variants were *cis*-eQTLs for multiple gene transcripts. For
292 example, rs1953126 (near the 5'-UTR of *PHF19)* is a *cis*-eQTL for *PHF19* and for multiple
293 nearby genes including *C5, GSN, PSMD5, RAB14, FBXW2,* and *TRAF1*. Query of publicly
294 available eQTL databases via GRASP[50] and recent publications[51,52] based on profiling of whole
295 blood or other tissue types[51-58] yielded eQTL assignments that were concordant with the FHS
296 findings for most variants listed in Supplementary Table 14.

297 **Effects of BP-associated Variants on Clinical Outcomes**

298 We considered the aggregate effects of the BP loci on BP-related clinical outcomes using new
299 Exome Chip-based results for coronary artery disease/myocardial infarction (CAD/MI),
300 including 42,335 cases and 78,239 controls[59], and for renal function measured by glomerular
301 filtration rate (GFR) in up to 111,655 individuals. For 59 of the 70 BP associated SNPs, alleles
302 that were associated with higher BP were also associated with increased odds of CAD/MI
303 (Supplementary Tables 15 and 16), a highly significant concordance with the known influence of
304 BP on CAD/MI (sign test, binomial P=4.5x10^{-9}). Similarly, genetic risk scores (GRS)
305 constructed from the 70 BP SNPs using weights derived from their effects on SBP, DBP, and
306 MAP were highly significantly associated with CAD/MI with odds-ratios (per 1 mm Hg
307 increment in SNP-based BP) of 1.05 (P=8.6x10^{-44}), 1.08 (P=1.9x10^{-41}), and 1.06 (P=1.1x10^{-45})
308 respectively (Supplementary Table 17, Supplementary Methods). GRSs constructed solely from
309 the rare/low-frequency variants at the three loci with significant gene-based tests (*DBH*, *NPR1*,
310 *PTPMT1)* were significant for CAD/MI using MAP-based weightings for *DBH* (P=0.026) and
311 HTN-based weightings for *PTPMT1* (P=0.003) with a non-significant concordant trend using
312 MAP-based weightings for *NPR1* (P=0.13; Supplementary Table 18). By contrast, BP-raising
313 alleles for only 39 of the 70 BP associated SNPs were associated with diminished kidney

314 function (CKD) as reflected by lower GFR, indicating a degree of concordance that was not
315 significant (sign test, binomial P=0.40). A similar lack of association was observed for the BP
316 GRS associations with GFR using weights for SBP (P=0.18), DBP (P=0.63), and MAP
317 (P=0.31).

318 **Discussion**

319 Through a two-stage study design of discovery (n=146,562) followed by external look ups
320 (n=180,726) and joint analysis (n=327,288), we identified single variant associations at 31 novel
321 loci and gene-based associations for three novel genes (two of which overlapped with the single
322 variant loci) associated with BP phenotypes. We also confirmed common variants at 39
323 previously reported BP loci, raising the number of statistically significant BP loci in our study to
324 71 and extended the number of non-monogenic BP-associated loci[5-15] to over 90. The sample
325 size for the joint analysis in this study is far larger than any prior genetic study of BP[5-15]. This
326 large increase in sample size is an important reason for the discovery of many new BP loci and
327 likely explains why some of the newly identified common loci were not discovered in previous
328 BP GWAS. In addition, direct genotyping of coding variants likely added incremental power
329 over imputed genotypes and tagging SNPs that were the basis of prior GWAS, suggesting that
330 novel common variants will continue to be identified for BP phenotypes using the same set or
331 similar set of samples with exome sequencing and whole genome sequencing. Furthermore,
332 phenotypic and possibly genetic heterogeneity (due to additional samples in this study),
333 differences in analysis plans, and the play of chance may be additional explanations of why some
334 of the common variants identified in this study were not identified in prior BP GWAS.

335 Fourteen of the novel BP variants identified in the present study reside in loci that were
336 previously reported in GWAS to be associated with lipids[19], immunologic diseases[20-22], and

337 metabolic phenotypes[23-25, 29] (Table 2 and Supplementary Table 7). Thirteen of the previously
338 identified BP variants were also linked to non-BP traits/diseases (Supplementary Table 19).
339 Considerable evidence has accumulated linking high BP to insulin resistance, altered lipid levels,
340 inflammation, and other features of the metabolic syndrome[60-65]. Gene set enrichment, regulatory
341 sequence variation, and PPI annotations of the new BP loci implicate genes that contribute to
342 cardiac structure and function as well as insulin signaling and type 2 diabetes. In addition, among
343 the previously reported BP genes that were confirmed in our study, *ATXN2*, *GRB14*, *HECTD4*,
344 *PTPN11,* and *SLC39A8* (Supplementary Table 3) have been proposed as candidate genes for
345 metabolic syndrome based on their associations with metabolic traits and inflammatory
346 biomarkers[65].

347 The *NPR1* gene was associated with BP in both single variant and gene-based tests. This
348 gene encodes the receptor for atrial and B-type natriuretic peptides, which regulate blood volume
349 and BP[17,18]. The functional consequences of the Glu967Lys amino acid substitution that is
350 encoded by rs35479618 (the significant *NPR1* SNP in single variant analysis) is unknown, but
351 the change results in opposite charge and a large difference in side chain volume, and is
352 predicted to be possibly damaging (score=0.513) by Polyphen-2[66]. The effects of the 13 rare and
353 one low-frequency variants in *NPR1* varied in directions, explaining why gene-based testing was
354 significant using SKAT[43], which is sensitive to BP-raising and lowering effects, rather than
355 burden[41,42] testing, which requires a consistent direction of BP effect, (Figure 2, Supplementary
356 Figure 3). Of note, *Npr1* knockout mice have hypertension, cardiac hypertrophy, and sudden
357 death phenotypes[17,18,67] and mice with only one copy of the *Npr1* gene have salt-sensitive
358 hypertension compared to wild type mice[17]. Future studies are warranted to determine if humans
359 carrying the rare BP-increasing alleles of *NPR1* also have salt-sensitive hypertension. We have

360 previously demonstrated that common variation that raises atrial natriuretic peptides level lowers
361 BP[13], suggesting the potential for BP-lowering strategies that target natriuretic peptide
362 interaction with natriuretic peptide receptors. Similarly, molecular mimicking of the action of
363 BP-lowering alleles in *NPR1* may be worth exploring as a novel BP treatment.

364 Both single variant and gene-based (T1) analysis in Stage 1 identified *DBH* as a BP gene
365 (Figure 3). *DBH* codes the enzyme dopamine beta hydroxylase, which catalyzes the
366 transformation of dopamine to norepinephrine. Both dopamine and norepinephrine act on the
367 sympathetic nervous system, influencing a variety of complex traits including BP. Impaired
368 dopamine beta hydroxylase activity has been identified in individuals with severe autonomic
369 failure, including orthostatic hypotension[68,69] , and mutation of *DBH* has been identified in two
370 individuals with autonomic dysfunction[70]. The rare minor allele of rs3025380, encoding the
371 Gly88Ala non-synonymous substitution, was associated with a comparatively large reduction of
372 1.81 mm Hg in MAP even though the amino acid change is predicted to be remote from the
373 active site[71]. Inhibition of DBH has long been considered a potential target for anti-hypertensive
374 therapy[72] but these efforts have been undermined due to the broad involvement of
375 catecholamines in a variety of critical biologic processes[73,74] and the potential for undesirable
376 side effects.

377 The remaining significant gene in gene-based testing was *PTPMT1,* which codes for
378 mitochondrial protein tyrosine phosphatase 1. Knockdown of *PTPMT1* expression in a rat
379 pancreatic insulinoma cell line was found to enhance ATP production and insulin secretion[75],
380 which is closely aligned with the insulin and cardiometabolic regulatory features of many of the
381 novel BP loci identified in this study. In addition, targeted burden testing of uncommon and rare
382 variants in genes that cause monogenic BP disorders identified a significant BP association with

383 *SLC12A1,* the Na-K-2Cl co-transporter that is well established to harbor rare mutations that
384 cause Bartter's syndrome, a salt wasting condition associated with hypotension[44].
385 The Exome Chip array was designed to aid in the search for rare functional variants with
386 large effect sizes. This study did not, however, identify any rare variants associated with BP
387 phenotypes through single variant analyses, suggesting that rare variants with large effects on BP
388 are an uncommon occurrence. With the current sample size, this study was not adequately-
389 powered to identify rare variants with only modest effect sizes. Within the predominant class of
390 variants studied (i.e. low-frequency and rare non-synonymous SNPs), there may not be a large
391 enough number of variants or effects of sufficient size to account for a substantial proportion of
392 the remaining missing heritability of BP. Nevertheless, this study greatly extends the number of
393 known BP-associated loci and moreover demonstrates their potential relevance to cardiovascular
394 disease. The discovery of a total of 32 new BP loci (31 from single variant tests, 1 from gene-
395 based tests) and their overlap with other disease-related phenotypes suggest common etiologies
396 of BP and metabolic risk factors and an opportunity to identify therapies that more broadly
397 impact hypertension in the context of cardiometabolic risk.

398 **URLs**
399 BIND, http://www.bind.ca
400 BioGRID, http://thebiogrid.org/
401 CHARGE+ Exome Chip, http://www.chargeconsortium.com/main/exomechip
402 EcoCys, http://www.ecocyc.org
403 GeneGO, http://lsresearch.thomsonreuters.com/
404 Literature Lab, http://www.acumenta.com/acumenta/overview/index.php
405 HaploReg, http://www.broadinstitute.org/mammals/haploreg/haploreg_v3.php
406 HPRD, http://www.hprd.org
407 NCBI PPI, ftp://ftp.ncbi.nih.gov/gene/GeneRIF/
408 NHGRI GWAS Catalog, http://www.genome.gov/gwastudies
409 Polyphen-2, http://genetics.bwh.harvard.edu/ggi/pph2/
410 RAREMETAL, http://genome.sph.umich.edu/wiki/RAREMETAL_Documentation
411 RegulomeDB, http://regulomedb.org/
412 Recode alleles, http://depts.washington.edu/chargeco/wiki/cgi_img_auth.php/c/c6/Recode_all.txt
413 Roadmap Epigenomics, http://www.roadmapepigenomics.org/

414 seqMeta package, http://cran.r-project.org/web/packages/seqMeta/index.html
415
416 **Accession codes**
417 The meta-analysis results at single variant level for SBP, DBP, MAP, PP and HTN can be
418 downloaded at the dbGaP CHARGE Summary site phs000930.

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629 **Figure Legends for main text**

630

631 **Figure 1. Overall study design**. In the discovery phase, single variant and gene-based analyses
632 were performed for systolic and diastolic blood pressure, pulse pressure, mean arterial pressure,
633 and hypertension among 146,562 individuals from the Cohorts for Heart and Aging Research in
634 Genomic Epidemiology Plus (CHARGE+) Exome Chip Blood Pressure Consortium. Fifteen
635 variants were significant ($P<3.4x10^{-7}$) and 62 displayed $P<1x10^{-5}$. In the follow-up phase, meta-
636 analysis was performed for 77 variants with results from 180,726 individuals from the CHD
637 Exome+ Consortium, ExomeBP Consortium, GoT2DGenes Consortium, T2D-GENES
638 consortium.

639

640 **Figure 2. *NPR1* Gene: Low-frequency and rare variants associated in aggregate with mean**
641 **arterial pressure**. The *NPR1* protein (1,061 amino acids) is comprised of three domains:
642 extracellular domain, kinase homology domain, and guanylate cyclase domain. The effects of the
643 14 low-frequency and rare variants after adjustment for age, age^2, sex, and body mass index on
644 mean arterial pressure are shown for higher (tan) or lower (purple) values in mm Hg; dot area is
645 proportional to the number of minor allele carriers. The minor allele of rs35479618 (MAF ~
646 0.012, E967K), was carried by 3,164 participants. The minor allele of rs201787421 (MAF ~
647 $2.6x10^{-5}$, R782Q), was carried by 5 participants.

648

649 **Figure 3. *DBH* Gene: Rare variants associated in aggregate with mean arterial pressure.**
650 The *DBH* protein (617 amino acids) contains the dopamine β-monooxygenase N-terminal
651 (DOMON) domain, the catalytic core (the Cu_H and Cu_M domains) and the C-terminal (C-T)
652 domain. The effects of the 27 rare variants after adjustment for age, age^2, sex, and body mass
653 index on mean arterial pressure are shown for higher (tan) or lower (purple) values in mm Hg.
654 The minor allele of rs74853476 (MAF ~ 0.0015), a splicing variant, was carried by 291
655 participants. The minor allele of rs201681337 (MAF ~ $7.9x10^{-5}$, A301T), was carried by 4
656 participants.

657

Table 1. **The newly identified significant blood pressure loci in meta-analysis of the discovery and follow-up samples (P<3.4 x 10^{-7})**

								Discovery (n=146,562)		Follow-up (n=180,726)		Combined (n=327,288)		ICBP Discovery (n=69,395)	
Trait	Locus[*]	dbSNPID	Chr	Position	CA/ NCA	CAF	Function[†]	Beta (SE) /Z score[‡]	*P* value	Beta (SE) /Z score[‡]	*P* value	Beta (SE) /Z score[‡]	*P* value	IQ[ǁ] or r^2/IQ	*P* value SBP/DBP
Low-frequency variants (0.01 < MAF<0.05)															
SBP	*NPR1*	rs35479618	1	153662423	A/G	0.014	E967K	1.34(0.28)	2.1 x 10^{-6}	0.85(0.30)	3.9 x 10^{-3}	1.11(0.20)	5.7x10^{-8}	n.a.	n.a.
SBP	*SVEP1*	rs111245230	9	113169775	C/T	0.032	D2702G	0.94(0.18)	2.9 x 10^{-7}	0.44(0.19)	2.2x10^{-2}	0.70(0.13)	1.2x10^{-7}	1/0.91	0.009/0.003
HTN	*PTPMT1*	rs11537751	11	47587452	T/C	0.048	S93L	5.09	3.6 x 10^{-7}	2.72	0.006	5.40	6.9x10^{-8}	1/0.97	0.13/0.11
Common variants (MAF>0.05)															
SBP	*PRDM16*	rs2493292	1	3328659	T/C	0.151	P633L	0.42(0.09)	4.0 x 10^{-6}	0.32(0.09)	7.2x10^{-4}	0.37(0.07)	1.4x10^{-8}	n.a.	n.a.
DBP	*PABPC4*	rs4660293	1	40028180	G/A	0.208	IN	0.27(0.05)	1.1 x 10^{-7}	0.11(0.04)	0.016	0.18(0.03)	9.6x10^{-8}	1[ǁ]	0.0030/0.0018
SBP	*SULT1C3*	rs6722745	2	108875244	C/T	0.338	M194T	0.28(0.08)	3.3 x 10^{-4}	0.26(0.07)	9.0x10^{-5}	0.27(0.05)	1.1x10^{-7}	0.99[ǁ]	0.37/0.37
PP	*CSNK1G3*	rs4530754	5	122855416	G/A	0.411	IN	0.22(0.05)	4.5x10^{-6}	0.13(0.04)	2.5x10^{-3}	0.17(0.03)	9.9x10^{-8}	1[ǁ]	0.03/0.46
DBP	*C5orf56*	rs2188962	5	131770805	T/C	0.366	ncRNA_IN	-0.2(0.04)	4.2x10^{-6}	-0.19(0.04)	1.6x10^{-6}	-0.20(0.03)	3.0x10^{-11}	1[ǁ]	0.86/0.05
DBP	*SNORD32B*	rs926552	6	29548089	T/C	0.111	ITG	-0.31(0.07)	8.5x10^{-6}	-0.22(0.07)	1.6x10^{-3}	-0.26(0.05)	7.2x10^{-8}	0.88[ǁ]	0.44/0.45
PP	*MSH5-SAPCD1*	rs409558	6	31708147	G/A	0.176	ncRNA_IN	-0.22(0.06)	3.7x10^{-4}	-0.29(0.06)	1.4x10^{-6}	-0.26(0.04)	2.7x10^{-9}	1/0.98	0.0019/0.10
SBP	*SLC22A7*	rs2270860	6	43270151	T/C	0.367	SYN, splicing	0.33(0.07)	2.6x10^{-6}	0.31(0.07)	2.4x10^{-6}	0.32(0.05)	2.9x10^{-11}	0.9[ǁ]	0.00013/0.037
PP	*COL21A1*	rs1925153[§]	6	56102780	T/C	0.445	IN	-0.21(0.05)	1.9x10^{-5}	-0.17(0.05)	5.9x10^{-4}	-0.19(0.04)	4.9x10^{-8}	0.71[ǁ]	0.16/0.42
DBP	*PHIP*	rs10943605	6	79655477	A/G	0.462	IN	0.18(0.04)	1.2x10^{-5}	0.15(0.04)	5.4x10^{-5}	0.16(0.03)	3.3x10^{-9}	1[ǁ]	0.05/0.01
DBP	*HOXA3*	rs6969780	7	27159136	C/G	0.125	5'UTR, splicing	0.32(0.06)	7.8x10^{-7}	0.21(0.07)	2.0x10^{-3}	0.26(0.05)	1.1x10^{-8}	0.98[ǁ]	0.02/0.1
PP	*IGFBP3*	rs11977526	7	46008110	A/G	0.397	ITG	-0.41(0.05)	3.8x10^{-18}	-0.32(0.04)	3.9x10^{-13}	-0.36(0.03)	2.9x10^{-29}	0.87[ǁ]	0.62/0.004
DBP	*NOS3*	rs891511	7	150704843	A/G	0.373	IN	-0.25(0.04)	1.8x10^{-8}	-0.26(0.04)	2.0x10^{-9}	-0.26(0.03)	2.0x10^{-16}	n.a.	n.a.
DBP	*HRCT1*	rs76452347	9	35906471	T/C	0.191	R63W	-0.25(0.05)	1.1x10^{-6}	-0.20(0.05)	1.1x10^{-4}	-0.23(0.04)	6.8x10^{-10}	n.a.	n.a.
PP	*PHF19*	rs1953126	9	123640500	T/C	0.331	ITG	0.27(0.05)	6.3x10^{-8}	0.10(0.05)	0.035	0.17(0.03)	1.8x10^{-7}	0.99[ǁ]	0.11/0.86
DBP	*ADO*	rs10995311	10	64564934	G/C	0.381	P39A	-0.20(0.04)	2.4x10^{-6}	-0.20(0.04)	1.9x10^{-6}	-0.20(0.03)	2.1x10^{-11}	n.a.	n.a.
DBP	*CYP2C19*	rs4494250	10	96563757	A/G	0.319	IN	0.21(0.05)	5.2x10^{-6}	0.11(0.04)	5.1x10^{-3}	0.15(0.03)	3.4x10^{-7}	0.93/0.98	0.017/0.0030
DBP	*ARNTL*	rs900145	11	13293905	G/A	0.336	ITG	-0.25(0.05)	9.1x10^{-7}	-0.15(0.05)	0.002	-0.20(0.03)	1.8x10^{-8}	1[ǁ]	0.0041/0.00087
SBP	*KCNJ11*	rs5219	11	17409572	T/C	0.320	K23E	0.48(0.07)	1.8x10^{-11}	0.21(0.06)	9.4x10^{-4}	0.32(0.05)	4.9x10^{-12}	0.94/1	0.00018/0.0023
DBP	*CERS5*	rs7302981	12	50537815	A/G	0.338	C75R	0.23(0.04)	1.8x10^{-7}	0.27(0.04)	6.5x10^{-13}	0.25(0.03)	9.4x10^{-19}	1[ǁ]	7.7x10^{-5}/0.0053
PP	*MYH6*	rs452036	14	23865885	A/G	0.400	IN	-0.23(0.05)	1.6x10^{-6}	-0.31(0.05)	1.4x10^{-11}	-0.27(0.03)	2.4x10^{-16}	0.89[ǁ]	0.64/0.094
SBP	*TNRC6A*	rs11639856	16	24788645	A/T	0.193	N185K	-0.37(0.08)	7.7x10^{-6}	-0.30(0.08)	3.6x10^{-4}	-0.34(0.06)	1.3x10^{-8}	0.99[ǁ]	0.068/0.54
DBP	*DPEP1*	rs1126464	16	89704365	C/G	0.215	E351Q	0.23(0.05)	6.4x10^{-6}	0.26(0.04)	7.0x10^{-9}	0.24(0.03)	2.4x10^{-13}	1/0.39	0.050/0.077
DBP	*TBX2*	rs8068318	17	59483766	C/T	0.350	IN	-0.23(0.05)	2.2x10^{-7}	-0.28(0.04)	1.8x10^{-12}	-0.26(0.03)	3.0x10^{-18}	1[ǁ]	0.00080/9.0x10^{-6}
PP	*DOT1L*	rs2302061	19	2226772	C/G	0.163	V1418L	0.30(0.07)	5.1x10^{-6}	0.28(0.06)	1.0x10^{-5}	0.29(0.05)	2.2x10^{-10}	0.64[ǁ]	0.019/0.88
PP	*INSR*	rs7248104	19	7224431	A/G	0.395	IN	-0.20(0.05)	1.8x10^{-5}	-0.20(0.04)	3.3x10^{-6}	-0.20(0.03)	2.6x10^{-10}	1[ǁ]	0.16/0.43
DBP	*RGL3*	rs167479	19	11526765	T/G	0.448	P162H	-0.26(0.04)	6.4x10^{-10}	-0.33(0.04)	3.8x10^{-20}	-0.30(0.03)	4.2x10^{-28}	n.a.	n.a.
SBP	*ZNRF3*	rs4823006	22	29451671	G/A	0.424	3'UTR	-0.33(0.07)	8.7x10^{-7}	-0.20(0.06)	9.2x10^{-4}	-0.26(0.05)	7.9x10^{-9}	0.98[ǁ]	0.29/0.093

CA/NCA, coded allele/non-coded allele; CAF: coded allele frequency; SYN, synonymous; IN, intronic; ITG, intergenic; UTR3, 3' untranslated region; The discovery meta-analysis was performed in CHARGE+ Exome Chip BP Consortium samples (n=146,562); The follow-up meta-analysis was performed with samples from the CHD Exome+ Consortium, ExomeBP Consortium, GoT2DGenes Consortium, T2D-GENES consortium samples (n=180,726); The "combined" or joint meta-analysis was performed with both discovery and follow-up samples (n = 327,288); ICBP Discovery, the discovery sample for International Consortium for Blood Pressure; n.a, not available; IQ, Imputation quality; r^2/IQ, linkage disequilibrium between the best proxy in ICBP and the one in "dbSNPID" column and imputation quality for the best proxy.

[*] Loci are named according to the closest gene based on the position of the lead SNP.

[†] Amino acid substitution is provided for a missense variant.

[‡] Meta-analysis used the inverse variance method for DBP, PP, and SBP and used the optimal Z score method for HTN.

[§] rs1925153 was significant from joint meta-analysis of EA only samples, the rest were from samples of all ancestries.

[ǁ] The same variants in "dbSNPID" column were analyzed in ICBP.

Table 2. Novel common BP SNPs associated with non-BP traits

Locus* (Function)	dbSNPID	Chr:Position	CA/NCA	CAF	GWAS Trait†	Amino Acid Substitution	Literature Lab Term(s)‡
SNPs not previously reported in GWAS							
PRDM16 (NS)	rs2493292	1:3328659	T/C	0.15	n.a.	Pro633Leu	
SULT1C3 (NS)	rs6722745	2:108875244	C/T	0.34	n.a.	Met194Thr	
HRCT1 (NS)	rs76452347	9:35906471	T/C	0.19	n.a.	Arg63Trp	
ADO (NS)	rs10995311	10:64564934	G/C	0.38	n.a.	Pro39Ala	
CERS5 (NS)	rs7302981	12:50537815	A/G	0.34	n.a.	Cys75Arg	
TNRC6A (NS)	rs11639856	16:24788645	A/T	0.19	n.a.	Asn185Lys	
DOT1L (NS)	rs2302061	19:2226772	C/G	0.16	n.a.	Val1418Leu	
RGL3 (NS)	rs167479	19:11526765	T/G	0.448	n.a.	Pro162His	
SNPs previously reported to be significant in GWAS of other traits§							
PABPC4 (IN)	rs4660293	1:40028180	G/A	0.21	HDL		
CSNK1G3 (IN)	rs4530754	5:122855416	G/A	0.41	LDL and TC		
C5orf56 (IN)	rs2188962	5:131770805	T/C	0.35	Crohn's Disease		
	rs926552	6:29548089	T/C	0.11	T1D		
MSH5-SAPCD1 (IN)	rs409558	6:31708147	G/A	0.18	SLE		
IGFBP3	rs11977526	7:46008110	A/G	0.40	IGFBP3		Insulin, 9%, IGF-1 signaling, 55%
PHF19 (5' near gene)	rs1953126	9:123640500	T/C	0.33	RA		
	rs900145	11:13293905	G/A	0.34	Age at Menarche		
KCNJ11 (NS)	rs5219	11:17409572	T/C	0.32	T2D	Lys23Glu	Insulin, 0.6%, T2D, 2.5%
MYH6 (IN)	rs452036	14:23865885	A/G	0.40	Resting Heart Rate		Heart Development, 73%, Hypertrophy model, 83%, Cardiac muscle contraction, 84%
DPEP1 (NS)	rs1126464	16:89704365	C/G	0.22	Homocysteine Concentration	Glu351Gln	
TBX2 (IN)	rs8068318	17:59483766	C/T	0.35	Creatinine and eGFR		Heart development, 17.5%
INSR (IN)	rs7248104	19:7224431	A/G	0.395	TG		Insulin, 90%, IGF-1 signaling, 45%, T2D, 93%, Hypertrophy model, 5.4%
ZNRF3 (UTR3)	rs4823006	22:29451671	G/A	0.424	WHR		
SNPs previously reported in patent filing, candidate gene or GWAS‖							
SLC22A7 (SYN)	rs2270860	6:43270151	T/C	0.37	HTN (patent filing)		
COL21A1 (IN)	rs1925153	6:56102780	T/C	0.45	Bipolar disease traits		
PHIP (IN)	rs10943605	6:79655477	A/G	0.46	Colon cancer (patent filing)		
HOXA3 (UTR5)	rs6969780	7:27159136	C/G	0.13	Hypospadias		
NOS3 (IN)	rs891511	7:150704843	A/G	0.37	Endothelium-dependent vasodilation		Heart Development, 6.7%, T2D, 3.9%, Cardiac muscle contraction, 14.5%
CYP2C19 (IN)	rs4494250	10:96563757	A/G	0.32	Breast cancer		

SNPs included in this table are common SNPs in Table 1. CA/NCA, coded allele/non-coded allele; CAF, coded allele frequency; IN, intron; NS, nonsynonymous; UTR3, 3' upstream; UTR5, 5' upstream; HDL/LDL, high/low- density cholesterol; TC, total cholesterol; T1D/T2D, Type I/Type 2 diabetes; SLE, systemic lupus erythematosus; IGFBP3, insulin-like growth factor-binding protein 3; RA, rheumatoid arthritis; TG, triglyceride; WHR, waist/hip ratio.

* Loci are named according to closest gene based on the position of the index SNP.

† Indicates whether a SNP was reported in previous genome-wide association studies (GWAS). n.a., not available.

‡ Reported results were part of identifying biological and biochemical terms that were significantly associated with the investigated gene set using Literature Lab database. Percent shows relative weight of references to a BP candidate gene in relation to associated pathways / terms for the full gene set. Out of three classes of significances (STRONG, MODERATE and POSITIVE) above we reported only STRONG class.

§ Reported to be significant in GWAS using $P<5 \times 10^{-8}$ or pre-specified significance levels in the reported study. Details of association direction were included in Supplementary Table 7.

‖ P values were not mentioned or did not reach the specified significance level.

Table 3. CHARGE+ Exome Chip BP Consortium: significant genes in burden and sequence kernel association tests

Gene	Chr	Test*	T1/T5†	Phenotype	Beta (SE) /Qmeta‡	*P* value§	N Variants¦	CAF
PTPMT1	11	Burden	T5	HTN	0.05(0.01)	$3.5x10^{-7}$	4	0.053
NPR1	1	SKAT	T5	MAP	270678.8	$4.4x10^{-8}$	14	0.025
DBH	9	SKAT	T1	MAP	145331.4	$9.2x10^{-7}$	27	0.028

CAF, cumulative coded allele frequency for variants used in an analysis. The experiment wide significance level for gene-based tests is $P<1x10^{-6}$.
* The standard burden test collapses the rare variants into a single variable and tests the association between this variable with a phenotype; the sequence kernel association test (SKAT) was designed to detect effects of alleles that collectively contribute to higher and lower BP effects.
† Meta-analysis was conducted at the gene level to evaluate aggregate effects from multiple non-synonymous or splicing variants with MAFs<0.01 (T1) and <0.05 (T5).
‡ The burden test yields beta/SE and the SKAT test provides Qmeta.
§ In pooled samples of all ancestries.
¦ Number of variants used in analysis.

Online Methods

Study Participants

A total of 146,562 individuals of European American (EA) (n=120,473), African American (AA) (n=21,503), and Hispanic American (HA) (n=4,586) contributed from 16 studies (Supplementary Table 20 and Supplementary Note) were included in the discovery stage association analyses. The entire discovery sample was also included in the meta-analyses of discovery and follow-up stage results (Figure 1). All study participants provided written informed consent for genetic research, with the exception of the BioVU biorepository, in which DNA was extracted from discarded blood collected during routine clinical testing and was linked to de-identified medical records. All studies received approval to conduct this research from their respective Institutional Review Boards. Studies contributing to the discovery analyses included a wide range of mean measured BP values (110 to 142 mm Hg for SBP and 69 to 84 mmHg for DBP), hypertension prevalence (2% to 77%), and proportion of individuals taking anti-hypertensive medications (0.6 to 63%) (Supplementary Table 20).

Genotyping and Quality Control

All samples were genotyped on the Illumina Infinium Human Exome Array v1.0 or v1.1 (Supplementary Table 21). Ten studies (51,106 individuals) were jointly called at the Human Genetics Center of the University of Texas Health Science Center in Houston[76]. Six additional studies followed genotyping calling protocols from Illumina or from the CHARGE consortium, and strand assignment for allele encoding specified by the CHARGE consortium[76]. All studies followed quality control guidelines recommended by the CHARGE analysis committee. Quality control procedures were further applied at the cohort level as described in Supplementary Table 21. Variants were removed for genotype call rate less than 95%, HWE p-value less than $1x10^{-6}$,

24 and concordance rate (between overlapping variants from previous GWAS and the Exome Chip)
25 less than 95%; individual samples were removed for call rate less than 95%, discordance rate less
26 than 95% with GWAS data, or in the event of a suspected sample swap, sex mismatch, or
27 heterozygosity F-value greater than 10.

28 **BP Phenotypes**

29 In the discovery stage, the BP phenotypes included were SBP, DBP, PP (SBP minus DBP), and
30 MAP (1/3 SBP + 2/3 DBP). A participant was classified as having HTN if she/he had SBP ≥140
31 mm Hg, or DBP ≥90 mm Hg, or was taking anti-hypertensive medication. SBP and DBP values
32 were obtained from the first examination attended for longitudinal studies; when available, the
33 average of two single occasion measurements was used for SBP and DBP. To account for the
34 reduction in BP due to medication use, all individuals taking BP lowering medication had15 mm
35 Hg added to the measured SBP, and 10 mm Hg to the measured DBP[15]. The four continuous BP
36 traits are moderately or highly correlated such that among the larger contributing cohorts, the
37 ranges of correlations were: 0.70-0.82 (SBP-DBP), 0.92-0.95 (SBP-MAP), 0.73-0.89 (SBP-PP),
38 0.92-0.99 (DBP-MAP), 0.20-0.45 (DBP-PP), and 0.43-0.68 (MAP-PP). Such correlations
39 appeared to be consistent across different ethnic populations within these same studies.

40 **Association Analyses and Meta-analyses**

41 *Power Estimation*: Nearly 90 percent of the markers on the Exome Chip are low-frequency
42 (MAF 0.01-0.05) or rare (MAF <0.01) variants. Power for association was evaluated for MAP
43 assuming a mean of 100 mm Hg with standard deviation of 10 mm Hg using QUANTO[77] for a
44 sample size n=150,000 at the significance level of 3.4×10^{-7} for a variant with MAF of 0.0005,
45 0.001, 0.005, or 0.01. To reach 80% power, an effect size of 5, 3.5, 1.6, or 1.1 mm Hg, is needed,
46 respectively, for a variant with MAF=0.0005, 0.001, 0.005, or 0.01.

47 *The Fraction of the Common Variants Tagged by the Exome Chip:* We downloaded the phase 3
48 genotype data for the European ancestry from HapMap project. The phase 3 file
49 "hapmap3_r2_b36_fwd.CEU.qc.poly" includes 1,416,121 variants (1,352,770 with MAF>0.01
50 and 1,223,919 with MAF> 0.05). We used the PLINK command "show-tags" to estimate the
51 number of common variants (MAF>0.05) that can be tagged by Exome Chip variants. We
52 estimated that 172,220 (linkage disequilibrium $r^2 \geq 0.5$) and 88,186 (linkage disequilibrium
53 $r^2 \geq 0.8$) common SNPs (MAF >0.05) can be tagged by the Exome Chip variants. Compared to
54 the number of variants tagged by a GWAS chip (e.g. Affymetrix 500K), the Exome Chip tags
55 much fewer common variants.
56 *Cohort-specific Analysis*: Gene-based (or region-based) testing was performed using the seqMeta
57 package[78]. Covariates included age, age-squared, sex, body mass index (BMI), and principle
58 components (if applicable) to account for population structure. All variants were recoded to
59 conform to the alleles specified in a "Recode" file distributed to each study. In all analyses,
60 variant effects were modeled additively. Conditional analysis was performed to identify
61 independent BP signals at previously reported BP loci[5-15] using the seqMeta package[78] by
62 adjusting at the cohort level for the previously reported GWAS SNP with the smallest p-value in
63 association analysis. Similarly, for any newly identified locus with multiple variants, conditional
64 analysis was performed by adjusting for the most significant variant in the region to identify non-
65 redundant signals.
66 *Meta-analysis at the Single Variant Level*: Meta-analysis of single variant associations from
67 discovery and follow-up stage results was performed using the inverse variance weighted fixed-
68 effects method[79] implemented in the seqMeta package[78]. In the discovery stage, the primary
69 meta-analysis was performed in all samples to identify variants showing consistent effects with

70 BP traits across multiple ancestry groups. Secondary analysis was performed in each of the three
71 ancestries separately to identify novel variants with different ancestral origin. Meta-analysis was
72 also performed on results from conditional analysis and compared with the original meta-
73 analysis to identify non-redundant signals. Although we performed association and meta-analysis
74 on all genotyped variants that passed quality control, we only reported results from about
75 147,000 variants that had minor allele counts (MACs) ≥30 in meta-analyses of all samples. Since
76 the BP traits are highly correlated, we used an array-wide Bonferroni-corrected significance
77 threshold of 3.4×10^{-7} (=0.05/147,000). The Exome Chip array contains numerous previously
78 published variants or their LD proxies, mostly from GWAS using imputed genotype information
79 for a variety of human traits. Using exome chip experimental genotypes, associations from
80 previous BP GWAS[5-15] were considered significant with *P* values ≤ 0.05/n, where n is the
81 number of previously identified SNPs or SNPs that showed at least moderate LD ($r^2 \geq 0.3$) on the
82 Exome Chip.
83 *Meta-analysis at the Gene Level*: Meta-analysis was also conducted at the gene level to evaluate
84 aggregate effects from multiple non-synonymous and splicing variants with MAFs ≤0.01 (T1)
85 and ≤ 0.05 (T5) in a gene using both the sequence kernel association test (SKAT)[43]and the
86 standard burden test[41,42] implemented in the seqMeta package[78]. The standard burden test
87 collapses the rare variants and has optimal properties when these variants all have the same
88 directionality and magnitude of effect on phenotype. In contrast, SKAT aggregates individual
89 variant score test statistics and offers better power compared to the burden test when there are a
90 variety of effect sizes and directions, e.g. both protective and deleterious effects in a gene[43].
91 Approximately 17,000 genes were included two or more non-synonymous variants in the
92 primary meta-analysis of all study samples. An association was deemed to be signficant at *P*<1

93 $\times 10^{-6}$ for gene-based tests. Among up to 154,543 individuals of European ancestry from CHD
94 Exome+ Consortium, ExomeBP Consortium, GoT2DGenes Consortium, T2D-GENES
95 consortium (Supplementary Note), gene-based SKAT was applied to HTN and inverse normal
96 transformed DBP, SBP, PP using the RAREMETAL software package[80]. We performed lookup
97 in their SKAT results for the genes that displayed $P<1 \times 10^{-6}$ in Stage 1 analysis of this study.

98 **The Follow-up Study at the Single Variant Level**

99 The follow-up study was performed in external samples (follow-up samples) including a total of
100 180,726 individuals from the CHD Exome+ Consortium, ExomeBP Consortium, GoT2DGenes
101 Consortium, T2D-GENES consortium (Supplementary Note). Summary information about
102 participants, genotyping and quality control in the follow-up samples are presented in
103 Supplementary Note. The follow-up samples provided SNP association statistics for DBP, PP,
104 SBP, and HTN but not MAP for a total of 180,726 individuals. Significant variants ($P \leq 3.4 \times 10^{-7}$
105) in the discovery samples were considered replicated in the follow-up samples with $P \leq 0.05/n$
106 with their pre-specified BP trait in the follow-up sample alone, where n was the number of
107 variants tested in the follow-up samples. Both the significant variants from discovery and
108 additional variants with $P \leq 1 \times 10^{-5}$ from the discovery samples were selected for joint meta-
109 analysis with the follow-up samples. The primary meta-analysis of the discovery and follow-up
110 results was performed in individuals of all ancestries. The secondary meta-analysis was
111 conducted in the EA only samples. The inverse variance weighted method was used in meta-
112 analysis of the discovery and follow-up stage results for DBP, PP and SBP. Because the follow-
113 up samples provided only z-scores and sample sizes for HTN, the optimally weighted z-score
114 method[81] was used in meta-analysis of HTN. The threshold of $P \leq 3.4 \times 10^{-7}$ was required for
115 significance in meta-analyses of the discovery and follow-up samples.

116 **Functional Inference**

117 We applied several computational strategies to infer biological functions associated with
118 candidate genes of the 31 novel loci reaching $P < 3.4 \times 10^{-7}$ (Table 1) and 39 validated loci
119 (Supplementary Table 3): 1) To test whether SNPs in Table 1 and Supplementary Table 3 were
120 significantly enriched among pre-specified gene sets defined in pathways, or by shared roles in
121 particular diseases or biological processes, we performed gene pathway, disease, and Gene
122 Ontology (GO) enrichment analysis using GeneGo software and Literature Lab[45] data mining of
123 literature (Supplementary Methods); 2) To investigate whether the coding and non-coding
124 variants listed in Table 1 may influence the transcriptional regulation, we compared BP
125 candidate SNPs with ENCODE and Roadmap Epigenomics regulome features summarized for
126 mainly *cis* regulatory function in HaploReg[47] and RegulomeDB[46]. The inclusion of coding
127 variants in this analysis was justified by previous research showing that transcriptional regulation
128 can be influenced by both non-coding and coding variations; a recent publication has shown that
129 ~15% of human codons simultaneously specify both amino acids and transcription factor
130 recognition sites[82]; and 3) To identify genes that encode proteins especially connected to other
131 proteins and therefore inferred to be important, we performed protein-protein interaction network
132 analysis (PPI) on SNPs in Table 1. The PPI network was constructed using the NCBI PPI
133 database information, which sources information from HPRD, BIND, BioGRID and EcoCys
134 databases. By design, 2% of the Exome Chip variants were identified from previous GWAS. To
135 investigate if these previous GWAS SNPs may artificially increase the extent of GeneGO
136 enrichment in known functional classes, we performed GeneGO enrichment analysis on 10
137 randomly selected sets of genes from the Exome Chip (with replacement) with the size of new

138 and previously BP candidates discovered. None of these random sets showed gene-set
139 enrichment with significance comparable to the enrichment for the BP SNPs.
140 To further assess putative functionality for the novel loci, we performed cis-eQTL
141 analysis between each of the newly identified variants with gene expression within 1 Mb
142 flanking that variant in peripheral whole blood samples of ~ 5000 individuals from the
143 Framingham Heart Study (FHS). Statistical significance in the FHS expression data was
144 evaluated at FDR<10% for newly identified variants[83]. We also searched for cis-associations
145 between novel variants and gene transcripts within 1 Mb flanking the lead SNP based on
146 databases of previously published expression quantitative trait locus (eQTL) analyses at the false
147 discovery rate (FDR) <10%[51,84].

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