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1 **Large scale trans-ethnic meta-analyses identify novel rare and common variants**

2 **associated with blood pressure and hypertension**

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Abstract

High blood pressure is a major risk factor for cardiovascular disease and premature death. However, there is limited knowledge on specific causal genes and pathways. To better understand the genetics of blood pressure we genotyped 242,296 rare, low-frequency and common genetic variants in up to ~192,000 individuals, and used ~155,063 samples for independent replication. We identified 31 novel blood pressure or hypertension associated genetic regions in the general population, including three rare missense variants in *RBM47*, *COL21A1* and *RRAS* with larger effects (>1.5mmHg/allele) than common variants. Multiple rare, nonsense and missense variant associations were found in *A2ML1* and a low-frequency nonsense variant in *ENPEP* was identified. Our data extend the spectrum of allelic variation underlying blood pressure traits and hypertension, provide new insights into the pathophysiology of hypertension, and indicate new targets for clinical intervention.

High blood pressure (BP) or hypertension is a highly prevalent chronic disorder. It is estimated to be responsible for a larger proportion of global disease burden and premature mortality than any other disease risk factor[1]. Elevated systolic and/or diastolic BP increases the risk of several cardiovascular disorders including stroke, coronary heart disease (CHD), heart failure, peripheral arterial disease and abdominal aortic aneurysms[2]. BP is a complex, heritable, polygenic phenotype for which genome-wide association studies (GWAS) have identified over 67 genetic regions associated with BP and/or hypertension to date[3-11]. These variants are common (minor allele frequency, MAF > 0.05), mostly map to intronic or intergenic regions, with the causal alleles and genes not readily identified due to linkage disequilibrium (LD) [4,5], and explain only ~2% of trait variance [12]. Low-frequency (0.01 < MAF < 0.05) and rare (MAF ≤ 0.01) single nucleotide variants (SNVs), predominantly unexplored by GWAS may have larger phenotypic effects than common SNVs[13], and may help to explain the missing heritability, and identify causative genes as demonstrated previously[14].

To identify novel coding variants and loci influencing BP traits and hypertension we performed the largest meta-analysis to date that included a total of ~350,000 individuals, directly genotyped with the Exome chip. The Exome chip contains ~240,000 mostly rare and low-frequency variants (Methods). A single-variant discovery analysis was performed, and candidate SNVs were taken forward for validation using independent replication samples. Gene-based tests were used to identify BP associated genes harboring multiple rare variant associations. We next assessed whether the newly identified BP associated SNVs were associated with expression levels of nearby genes, and tested these variants in aggregate for a causal association of BP with other cardiovascular traits and risk factors. Our findings highlight the contribution of rare variants in the aetiology of blood pressure in the general population, and provide new insights into the pathophysiology of hypertension.

362

363 **Results**

364 **Discovery of single variant BP associations**

365 We genotyped 192,763 individuals from 51 studies, and assessed association of 242,296
366 SNVs with diastolic BP (DBP), systolic BP (SBP), pulse pressure (PP) and hypertension
367 (HTN; Supplementary Tables 1, 2 and 3; Methods; Supplementary Information). An
368 overview of the SNV discovery study design is given in Figure 1. A fixed effects meta-
369 analysis for each trait was performed using study-level association summary statistics from i)
370 samples of European (EUR) ancestry (up to 165,276 individuals), and ii) a trans-ethnic meta-
371 analysis of the EUR and additional South Asian (SAS) ancestry samples (EUR_SAS; up to
372 192,763 individuals). Two analyses of DBP, SBP and PP were performed, one in which the
373 trait was inverse normal transformed and a second in which the raw phenotype was analysed.
374 Both sets of results were consistent (Methods), therefore to minimise sensitivity to deviations
375 from normality in the analysis of rare variants, the results from the analyses of the
376 transformed traits were used for discovery. Strong correlations between the BP traits were
377 observed across studies (Methods), hence no adjustment of significance thresholds for
378 independent trait testing was applied.

379 The discovery meta-analyses identified 50 genomic regions with genome-wide significant
380 (GWS) evidence of association with at least one of the four BP traits tested ($P<5\times10^{-8}$;
381 Supplementary Table 4). There were 45 regions associated in the EUR_SAS samples, of
382 which 13 were novel (Figure 2). An additional five regions were GWS in the EUR only meta-
383 analyses of which two were novel (Supplementary Figure 1). In total, 16 genomic regions
384 were identified that were GWS for at least one BP trait that have not been previously
385 reported.

386 **Replication of single variant BP associations**

387 Next we sought support for our findings, in an independent replication dataset comprising of
388 18 studies, 16 of which were from the Cohorts for Heart and Aging Research in Genomic
389 Epidemiology+ (CHARGE+) consortium (Figure 1; Supplementary Information; Liu *et al.*
390 Nature Genetics, *submitted*). Variants were selected for replication first using the larger
391 (transformed) EUR_SAS data, with additional variants from the (transformed) EUR data also
392 selected. SNVs were selected if they mapped outside of known BP genomic regions and had
393 MAF ≥ 0.05 and $P < 1x10^{-5}$ or MAF < 0.05 and $P < 1x10^{-4}$ with at least one BP trait, *i.e.*
394 choosing a lower significance threshold for the selection of rare variants (full details of the
395 selection criteria are provided in the Methods). In total 81 candidate SNVs were selected for
396 replication (Supplementary Table 5). Eighty variants were selected from EUR_SAS
397 (transformed) results and one SNV at the *ZNF101* locus from the EUR (transformed)
398 analyses. The results for EUR_SAS and EUR were consistent (association statistics were
399 correlated, ρ=0.9 across ancestries for each of the traits). Of the 81 variants, 30 SNVs were
400 selected for association with DBP as the primary trait, 26 for SBP, 19 for PP and 6 for HTN,
401 with the primary trait defined as the BP trait with the smallest association *P*-value in the
402 EUR-SAS discovery analyses.

403 Meta-analyses were performed on results from analyses of untransformed DBP, SBP, PP and
404 HTN (as only results of untransformed traits were available from CHARGE+) in (i) up to
405 125,713 individuals of EUR descent, and (ii) up to 155,063 individuals of multiple ethnicities
406 (4,632 of Hispanic descent, 22,077 of African American descent, 2,641 SAS samples with the
407 remainder EUR; Figure 1; Supplementary Information). Given that a large proportion of the
408 ancestries in the trans-ethnic meta-analyses were not included in our discovery samples, we

409 used the EUR meta-analyses as the main data set for replication, but we also report any
410 additional associations identified within the larger trans-ethnic dataset.

411 Novel BP-SNV associations were identified based on two criteria (Figure 1; Methods).
412 Firstly, replication of the primary BP trait-SNV association was sought at a Bonferroni
413 adjusted *P*-value threshold in the replication data ($P \leq 6.17 \times 10^{-4}$, assuming $\alpha = 0.05$ for 81
414 SNVs tested and same direction of effect; Methods) without the need for GWS. Secondly,
415 meta-analyses of discovery and replication results across all four (untransformed) BP traits
416 were performed to assess the overall level of support across all samples for the 81 candidate
417 SNVs; those BP-SNV associations that were GWS (with statistical support in the replication
418 studies; $P<0.05$ and the same direction of effect) were also declared as novel.

419

420 Seventeen SNV-BP associations formally replicated with concordant direction of effect at a
421 Bonferroni adjusted significance level for the primary trait. Fourteen were in the EUR meta-
422 analyses, and amongst these was a rare non-synonymous (ns) SNV mapping to *COL21A1*
423 (Table 1a, Supplementary Table 6). Three associations were in the trans-ethnic meta-
424 analyses, these included two rare nsSNVs in *RBM47* and *RRAS* (Table 1a, Supplementary
425 Table 7; Methods).

426

427 In addition to the 17 SNV-BP trait associations that formally replicated, we identified 13
428 further SNV-associations that were GWS in the combined (discovery and replication) meta-
429 analyses. Ten of these were GWS in the combined EUR analyses, (Table 1b; Supplementary
430 Tables 6 and 8a), and three were GWS in the combined trans-ethnic meta-analyses (Table 1b;
431 Supplementary Tables 7 and 8b).

432

433 This gives a total of 30 novel SNV-BP associations (15 SNV-DBP, 9 SNV-SBP and 6 SNV-
434 PP; Tables 1a and 1b; Supplementary Figures 2 and 3). Five of the SNVs were GWS with
435 more than one BP trait (Figure 3: Tables 1a and 1b; Supplementary Table 8). Four loci
436 (*CERS5*, *TBX2*, *RGL3* and *OBFC1*) had GWS associations with HTN in addition to GWS
437 associations with DBP and SBP. The *PRKAG1* locus had GWS associations with both SBP
438 and PP.
439
440 Conditional analyses were performed to identify secondary signals of association within the
441 novel BP loci. The RAREMETALWORKER (RMW) package (Methods) [15] allows
442 conditional analyses to be performed using summary level data. Hence, analyses of the
443 transformed primary traits and HTN were re-run in RMW across the discovery studies
444 (Figure 4). The results of the RMW single variant tests were consistent with the initial
445 discovery analyses (Methods). Given that the RMW analyses are based on our discovery
446 samples, the larger EUR-SAS data was used as the main analysis to increase power, but we
447 also report any additional associations with evidence in EUR.
448
449 We identified secondary independent signals of association in four loci, *PREX1, PRKAG1*
450 and *RRP1B* within the EUR_SAS analyses and *COL21A1* in the EUR analyses
451 ($P_{conditional}$<1x10^{-4}; Methods; Supplementary Tables 9 and 10). Three independent association
452 signals were identified in the MYH6 locus in the EUR_SAS analyses (Supplementary Table
453 11).
454 **Gene-based BP associations**
455 To improve statistical power to detect associations in genes harbouring rare variants,
456 analytical methods that combine effects of variants across a gene into a single test have been
457 devised and are implemented in the RMW package [15]. We applied the gene-based sequence

458 kernel association test (SKAT) [16] and Burden tests [17] to the RMW dataset (MAF < 0.05 or

459 MAF < 0.01; Figure 4; Methods). One previously unidentified BP gene (*A2ML1*) was

460 associated with HTN (P= 7.73x10^{-7}) in the EUR_SAS studies and also in EUR studies (Table

461 2; Bonferroni-corrected threshold of significance P <2.8x10^{-6}, after adjusting for 17,996

462 genes tested, Methods). The gene showed residual association with the primary BP trait after

463 conditioning on the most associated SNV in the gene ($P_{conditional}$ =5.00x10^{-4}; Table 2),

464 suggesting that the association is due to multiple rare variants in the gene. One nonsense

465 variant (rs199651558, R893X, MAF=3.5x10^{-4}) was observed, and there were multiple

466 missense variants (Figure 5). *A2ML1* encodes alpha-2-macroglobulin-like 1 protein, and is a

467 member of the alpha macroglobulin superfamily, which comprises protease inhibitors

468 targeting a wide range of substrates. Mutations in this gene are associated with a disorder

469 clinically related to Noonan syndrome, a developmental disorder which involves cardiac

470 abnormalities[18]. We sought replication in the CHARGE+ studies for this gene, however there

471 was no evidence of association with HTN (P= 0.45). Given the very low frequencies of the

472 variants involved, however, studies in which the variants are polymorphic will be required to

473 replicate the association with HTN. The *DBH* gene was found to be associated with DBP

474 using the SKAT test (P=2.88x10^{-6}). However, this was not due to multiple rare variants as the

475 association was driven by rs77273740 (Supplementary Table 5) and the SNV was not

476 validated in the replication samples.

477

478 **Rare and common variant associations in established BP loci**

479 Of the 67 established BP loci, 35 loci were on the Exome chip (N=43 SNVs or close proxies

480 r^2>0.7). All 43 SNVs had at least nominal evidence of association with BP in our discovery

481 samples (P< 0.01; Supplementary Table 13). We also assessed if any of the established BP

482 loci contained coding variants that are associated with BP traits and in LD (r^2>0.2) with the

483 known BP variants on the Exome chip (Supplementary Table 13), using the 1000G phase 3
484 release for LD annotation. Focusing on SNVs that were GWS for any BP trait from our
485 transformed discovery data for either ancestry, there were 25 coding variants, of which 6
486 were predicted to be damaging at loci labelled *CDC25A, SLC39A8, HFE, ULK4, ST7L-*
487 *CAPZA1-MOV10* and *CYP1A1-ULK3*. Three of these are published variants at loci labelled
488 *SLC39A8, HFE* and *ST7-CAPZA1-MOV10.* At *CYP1A1-ULK3*, the coding variant was in
489 moderate LD with the reported variant, but was less significantly associated with DBP in our
490 EUR_SAS dataset (P=2.24x10^{-8} compared to P=1.68x10^{-15} for the published variant). At the
491 ULK4 locus the predicted damaging coding variant had similar association as the published
492 coding variant (predicted to be benign), and prior work has already indicated several
493 associated nsSNVs in strong LD in *ULK4* [19]. The nsSNV within the CDC25A locus
494 (rs11718350 in *SPINK8*) had similar association with DBP as the intergenic published SNV
495 in our EUR_SAS dataset (P=2.00x10^{-8} compared to P=2.27x10^{-8} for the published variant).
496 Overall at least 5 of the known loci are consistent with having a coding causal variant.

497 Gene-based SKAT tests of all genes that map within 1 Mb of a previously reported SNV
498 association (Supplementary Table 14), indicated no genes with multiple rare or low-
499 frequency variant associations. Single variant conditional analyses showed that rs33966350, a
500 rare nonsense variant in *ENPEP* (MAF=0.01) was associated with SBP ($P_{conditional}$ = 1.61x10^{-5})
501 in the EUR_SAS samples (Supplementary Tables 14 and 15; Methods) independently of
502 the known SNV (rs6825911). *ENPEP* encodes aminopeptidase A (APA) an enzyme of the
503 renin-angiotensin-aldosterone system (RAAS) that converts angiotensin II (AngII) to AngIII.

504 There were no other established loci with convincing low-frequency or rare SNV associations
505 in the EUR_SAS samples. However, *HOXC4*, had evidence of a second independent signal
506 with a rare missense SNV in EUR samples (rs78731604; MAF = 0.005, $P_{conditional}$= 5.76x10^{-5};
507 Supplementary Table 15). The secondary signal in the *HOXC4* region, mapped to

508 *CALCOCO1*, ~300kb from the known SNV. The gene association (MAF≤0.01, P=2.37x10^{-5})
509 was below the required significance threshold and attributable to rs78731604, which is not
510 predicted to have detrimental effects on protein structure. Therefore replication of this
511 association is required. Three loci (*ST7L-CAPZA1-MOV10, FIGN-GRB14,* and *TBX5-TBX3*)
512 had evidence of a second independent signal in the region in EUR_SAS samples with a
513 common variant ($P_{conditional}$<1x10^{-4}; Supplementary Table 15) that has not been previously
514 reported.

515 Having identified 30 novel loci associated with BP traits, as well as additional new
516 independent SNVs at four novel loci and five known loci, we calculated the percent of the
517 trait variance explained (Methods). This was 2.08%/2.11%/1.15% for SBP/DBP/PP for the
518 43 previously reported BP-SNVs covered in our dataset, increasing to 3.38%/3.41%/2.08%
519 respectively with the inclusion of the 30 lead SNVs from novel loci, plus new independent
520 SNV-BP associations identified from novel and known loci.

521

522 **Effect of BP SNVs on cardiovascular traits & risk factors**

523 Amongst our novel BP-SNV associations, some have previously been reported to be
524 associated with other cardiovascular traits and risk factors (Supplementary Table 16); these
525 include coronary heart disease (CHD: *PHACTR1*, *ABO*) [20,21], QT interval (*RNF207*) [22], heart
526 rate (*MYH6*) [23], and cholesterol levels (2q36.3, *ABO*, *ZNF101*) [24].

527 To test the impact of BP variants on cardiovascular endpoints and risk factors we created
528 three weighted genetic risk scores (GRS) according to DBP/SBP/PP based on the newly
529 identified and previously published BP variants (up to N=125; Methods). The GRS models
530 were used to test the causal effect of BP on the following traits: ischemic stroke (including

531 the subtypes, cardiometabolic, large and small vessel [25]), CHD, heart failure, [26] left ventricular
532 mass [27], left ventricular wall thickness [27], high-density lipoprotein cholesterol (HDL-c), low-
533 density lipoprotein (LDL-c), triglycerides, total cholesterol, body mass index (BMI), waist-
534 hip ratio adjusted BMI, height and estimated glomerular filtration rate (eGFR) (Methods). As
535 expected, BP was positively associated with increased CHD risk (OR [95% CI]=1.62 [1.28-
536 2.05] per 10mmHg increase in DBP, P=5.99x10^{-5}; 1.39 [1.22-1.59] per 10mmHg increase in
537 SBP, P=6.07×10^{-7}; 1.70 [1.34-2.16], per 10mmHg increase in PP, P=1.20x10^{-5}; Table 3), and
538 increased risk of ischemic stroke (OR [95% CI]=1.93 [1.47-2.55] per 10mmHg increase in
539 DBP, P=2.81×10^{-6}; 1.57 [1.35-1.84] per 10mmHg increase in SBP, P=1.16×10^{-8}; 2.12 [1.58-
540 2.84], per 10mmHg increase in PP, P=5.35x10^{-7}). The positive association with ischemic
541 stroke was primarily due to large vessel stroke (Table 3). DBP and SBP were also positively
542 associated with left ventricular mass (9.57 [3.98-15.17] gram increase per 10mmHg increase
543 in DBP, P=8.02x10^{-4} and 5.13 [1.77-8.48] gram increase per 10mmHg increase in SBP,
544 P=0.0027) and left ventricular wall thickness (0.10cm [0.06-0.13] increase per 10mmHg
545 increase in DBP, P=1.88x10^{-8} and 0.05cm [0.03-0.07] increase per 10mmHg increase in SBP,
546 P=5.52x10^{-6}, Table 3). There was no convincing evidence to support the BP associated
547 variants having an effect on lipid levels (P>0.1), BMI (P>0.005), waist hip ratio adjusted
548 BMI (P>0.1), height (P>0.06), eGFR (P>0.02) or heart failure (P>0.04). The causal
549 associations with CHD, stroke, and left ventricular measures augment the results from a
550 previous association analysis using 29 BP variants [28]. Our data strongly support the previous
551 observations of no causal relationship between BP and eGFR. Lack of evidence of a BP
552 effect with heart failure may only be due to lack of power, as the association was in the
553 expected direction.
554
555 **Potential functional variants at BP loci and candidate genes**

556 Twenty-six of our newly discovered BP associated SNVs had MAF > 0.05 and therefore due
557 to extensive LD with other SNVs not genotyped on the Exome array, identifying the causal
558 genes requires additional information. If a SNV is associated with increased or decreased
559 expression of a particular gene, *i.e.* it is an expression quantitative trait locus (eQTL) this
560 suggests the gene on which the SNV acts could be in the causal pathway. To help identify
561 potential candidate causal genes in the novel BP loci (Supplementary Table 9), expression
562 quantitative trait loci (eQTL) information from publicly available databases were investigated
563 across a range of tissues and cells (MuTHER for LCL, adipose and skin and GTEx for nine
564 tissues including the heart and tibial artery; Methods).

565 The DBP increasing allele of the nsSNV, rs7302981-A, was associated with increased
566 expression of *CERS5* in four tissues: LCLs (P_{MuTHER}=3.13x10^{-72}) skin (P_{MuTHER}=2.40x10^{-58})
567 adipose (P_{MuTHER}=2.87x10^{-54}) and nerve (P_{GTEx}=4.5x10^{-12}) (Supplementary Figure 4).
568 Additional testing (Methods) provided no evidence against colocalisation of the eQTL and
569 DBP association signals, implicating *CERS5* as a candidate causal gene for this DBP locus.
570 *CERS5* (LAG1 homolog, ceramide synthase 5) is involved in the synthesis of ceramide, a
571 lipid molecule that is involved in a several cellular signaling pathways. CERS5 knockdown
572 has been shown to reduce cardiomyocyte hypertrophy in mouse models[29]. However, it is
573 unclear whether the blood pressure raising effects at this locus are the cause or result of any
574 potential effects on cardiac hypertrophy. Future studies investigating this locus in relation to
575 parameters of cardiac hypertrophy and function (*e.g.* ventricular wall thickness) should help
576 address this question.

577 The DBP raising allele of the nsSNV (rs867186-A) was associated with increased expression
578 of *PROCR* in adipose tissue (P_{MuTHER}=3.24x10^{-15}) and skin (P_{MuTHER}=1.01x10^{-11})
579 (Supplementary Figure 4). There was no evidence against colocalisation of the eQTL and
580 DBP association thus supporting *PROCR* as a candidate causal gene. *PROCR* encodes the

581 Endothelial Protein C receptor, a serine protease involved in the blood coagulation pathway,
582 and rs867186 has previously been associated with coagulation and haematological factors.[30,31]
583 The PP decreasing allele of the nsSNV, rs10407022-T, which is predicted to have detrimental
584 effects on protein structure (Methods) was associated with increased expression of *AMH* in
585 muscle (P_{GTEx}=9.95x10^{-15}), thyroid (P_{GTEx}=8.54x10^{-7}), nerve (P_{GTEx}=7.15x10^{-8}), tibial artery
586 (P_{GTEx}=6.46x10^{-9}), adipose (P_{GTEx}=4.69x10^{-7}), and skin (P_{GTEx}=5.88x10^{-8}) (Supplementary
587 Figure 4). There was no evidence against colocalisation of the eQTL and PP association,
588 which supports *AMH* as a candidate causal gene for PP. Low AMH protein levels have been
589 previously associated with hypertensive status in women with the protein acting as a marker
590 of ovarian reserve [32]. The intergenic SBP raising allele of rs4728142-A was associated with
591 reduced expression of *IRF5* in skin (P_{MuTHER}=5.24x10^{-31}) and LCLs (P_{MuTHER}=1.39x10^{-34}),
592 whole blood (P_{GTEx}=3.12x10^{-7}) and tibial artery (P_{GTEx}=1.71x10^{-7}).

593

594 Three novel rare nsSNVs were identified that map to *RBM47*, *RRAS* (both associated with
595 SBP) and *COL21A1* (associated with PP). They had larger effect sizes than common variant
596 associations (>1.5mmHg per allele; Supplementary Figure 5) and were predicted to have
597 detrimental effects on protein structure (Supplementary Table 16; Methods). In *RBM47*,
598 rs35529250 (G538R) is located in a highly conserved region of the gene and was most
599 strongly associated with SBP (MAF=0.008; +1.59 mmHg per T allele; *P*=5.90x10^{-9}). *RBM47*
600 encodes the RNA binding motif protein 47 and is responsible for post-transcriptional
601 regulation of RNA, through its direct and selective binding with the molecule.[33] In *RRAS*,
602 rs61760904 (D133N) was most strongly associated with SBP (MAF=0.007; +1.51 mmHg per
603 T allele; *P*=8.45x10^{-8}). *RRAS* encodes a small GTPase belonging to the Ras subfamily of
604 proteins H-RAS, N-RAS, and K-RAS and has been implicated in actin cytoskeleton
605 remodelling, and controlling cell proliferation, migration and cycle processes [34]. The nsSNV

606 in *COL21A1* (rs200999181, G655A) was most strongly associated with PP (MAF=0.001;
607 +3.14 mmHg per A allele; P=1.93x10^{-9}). *COL21A1* encodes the collagen alpha-1 chain
608 precursor of type XXI collagen, a member of the FACIT (fibril-associated collagens with an
609 interrupted triple helix) family of proteins [35]. The gene is detected in many tissues, including
610 the heart and aorta. Based on our results, these three genes represent good candidates for
611 functional follow-up. However, due to the incomplete coverage of all SNVs across the region
612 on the Exome chip, it is possible that other non-genotyped SNVs may better explain some of
613 these associations. We therefore checked for variants in LD (r^2>0.3) with these three rare
614 nsSNVs in the UK10K + 1000G dataset [36] to ascertain if there are other candidate SNVs at
615 these loci (Supplementary Table 17). There were no SNVs within 1Mb of the *RBM47* locus
616 in LD with the BP associated SNV. At the *COL21A*1 locus there were only SNVs in
617 moderate LD, and these were annotated as intronic, intergenic or in the 5'UTR. At the *RRAS*
618 locus, there were two SNVs in strong LD with the BP associated SNV, which both mapped to
619 introns of the *SCAF1* gene and are not predicted to be damaging. All SNVs in LD at both loci
620 were rare as expected (Supplementary Table 17) supporting a role for rare variants. Hence,
621 the rare BP associated nsSNVs at *RBM47, COL21A1* an*d RRAS* remain the best causal
622 candidates.
623

624 **Pathway and network analyses**

625 To identify connected gene sets and pathways implicated by the BP associated genes we used
626 Meta-Analysis Gene-set Enrichment of variant Associations (MAGENTA)[37] and GeneGo
627 MetaCore (Thomson Reuters, UK). MAGENTA tests for over-representation of BP
628 associated genes in pre-annotated pathways (gene sets) (Methods and Supplementary Table
629 18a). GeneGo Metacore identifies potential gene networks. The MAGENTA analysis was

630 used for hypothesis generation and results were compared with the GeneGo Metacore outputs
631 to cross-validate findings.

632 Using MAGENTA there was an enrichment (P<0.01 and FDR<5% in either the EUR_SAS
633 or the EUR participants) of six gene sets with DBP, three gene sets with HTN and two gene
634 sets for SBP (Supplementary Table 18b). The RNA polymerase I promoter clearance
635 (chromatin modification) pathway showed the most evidence of enrichment with genes
636 associated with DBP ($P_{Reactome}$=8.4×10^{-5}, FDR=2.48%). NOTCH signalling was the most
637 associated pathway with SBP ($P_{Reactome}$ = 3.00×10^{-4}, FDR = 5%) driven by associations at the
638 *FURIN* gene. The inorganic cation anion solute carrier (SLC) transporter pathway had the
639 most evidence of enrichment by HTN associated genes ($P_{Reactome}$=8.00×10^{-6}, FDR=2.13%).

640 Using GeneGo MetaCore, five network processes were enriched (FDR<5%; Methods;
641 Supplementary Tables 19 and 20). These included several networks with genes known to
642 influence vascular tone and BP (inflammation signalling, P=1.14×10^{-4}) and (blood vessel
643 development P=2.34×10^{-4}). The transcription and chromatin modification network
644 (P=2.85×10^{-4}) was also enriched, a pathway that was also highlighted in the MAGENTA
645 analysis, with overlap of the same histone genes (*HIST1H4C*, *HIST12AC*, *HIST12BC*,
646 *HISTH1T*) and has also been recently reported in an integrative network analysis of published
647 BP loci and whole blood expression profiling [38]. Two cardiac development pathways were
648 enriched: the oxidative stress-driven (ROS/NADPH) (P=4.12×10^{-4}) and the Wnt/β-
649 catenin/integrin-driven (P=0.0010). Both these cardiac development pathways include the
650 *MYH6*, *MYH7*, and *TBX2* genes, revealing a potential overlap with cardiomyopathies and
651 hypertension, and suggesting some similarity in the underlying biological mechanisms.

652

653 **Discussion**

654 By conducting the largest ever genetic study of BP, we identified further novel common
655 variants with small effects on BP traits, similar to what has been observed for obesity and
656 height[39,40]. More importantly, our study identified some of the first rare coding variants of
657 strong effect (>1.5mmHg) that are robustly associated with BP traits in the general
658 population, complementing and extending the previous discovery and characterisation of
659 variants underlying rare Mendelian disorders of blood pressure regulation [41]. Using SNV
660 associations in 17 genes reported to be associated with monogenic disorders of blood
661 pressure (Methods) we found no convincing evidence of enrichment ($P_{\mathrm{enrichment}} = 0.044$). This
662 suggests that BP control in the general population may occur through different pathways to
663 monogenic disorders of BP re-enforcing the importance of our study findings. The
664 identification of 30 novel BP loci plus further new independent secondary signals within four
665 novel and five known loci (Methods) has augmented the trait variance explained by 1.3%,
666 1.2% and 0.93% for SBP, DBP and PP respectively within our data-set. This suggests that
667 with substantially larger sample sizes, for example through UK BioBank [42], we expect to
668 identify 1000s more loci associated with BP traits, and replicate more of our discovery SNV
669 associations that are not yet validated in the current report.

670 The discovery of rare missense variants has implicated several interesting candidate genes,
671 which are often difficult to identify from common variant GWAS, and should therefore lead
672 to more rapidly actionable biology. *A2ML1*, *COL21A1*, *RRAS* and *RBM47* all warrant further
673 follow-up studies to define the role of these genes in regulation of BP traits, as well as
674 functional studies to understand their mechanisms of action. *COL21A1* and *RRAS* warrant
675 particular interest since both are involved in blood vessel remodelling, a pathway of known
676 aetiological relevance to hypertension.

677 We observed a rare nonsense SBP associated variant in *ENPEP* (rs33966350; W317): this
678 overlaps a highly conserved region of both the gene and protein and is predicted to result in

679 either a truncated protein with reduced catalytic function or is subject to nonsense mediated
680 RNA decay. ENPEP converts angiotensin II (AngII) to Ang-III. AngII activates the
681 angiotensin 1 (AT1) receptor resulting in vasoconstriction, while AngIII activates the
682 angiotensin 2 (AT2) receptor that promotes vasodilation and protects against hypertension.[43]
683 The predicted truncated protein may lead to predominant AngII signaling in the body, and
684 increases in BP. This new observation could potentially inform therapeutic strategies. Of
685 note, angiotensin-converting-enzyme (ACE) inhibitors are commonly used in the treatment of
686 hypertension. However, patients who suffer from adverse reactions to ACE inhibitors, such
687 as dry cough and skin rash, would benefit from alternative drugs that target RAAS. Murine
688 studies have shown that in the brain, AngIII is the preferred AT1 agonist that promotes
689 vasoconstriction and increases blood pressure, as opposed to AngII in the peripheral system.
690 These results have motivated the development of brain specific APA inhibitors to treat
691 hypertension[44]. Our results confirm APAs, such as ENPEP, as a valid target to modify blood
692 pressure, but suggest that long-term systemic reduction in APA activity may lead to an
693 increase in blood pressure. Future studies are needed to examine the effects of the W317X
694 variant on the RAAS system, specifically in the brain and peripheral vasculature, in order to
695 test the benefits of the proposed therapeutic strategy in humans.

696 In addition to highlighting new genes in pathways of established relevance to BP and
697 hypertension, and identifying new pathways, we have also identified multiple signals at new
698 loci. For example there are three distinct signals at the locus containing the *MYH6*/*MYH7*
699 genes, and we note that *TBX2* maps to one of the novel regions. These genes are related to
700 cardiac development and/or cardiomyopathies, and provide an insight into the shared
701 inheritance of multiple complex traits. Unravelling the causal networks within these
702 polygenic pathways may provide opportunities for novel therapies to treat or prevent both
703 hypertension and cardiomyopathies.

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810

811 **Methods**

812 **Overview of discovery studies**

813 The cohorts contributing to the discovery meta-analyses comprise studies from three
814 consortia (CHD Exome+, ExomeBP, and GoT2D/T2D-GENES). The total number of unique
815 samples was 192,763. All participants provided written informed consent and the studies
816 were approved by their local Research Ethics Committees and/or Institutional Review
817 Boards.

818

819 The CHD Exome+ consortium included ten different collections (77,385 samples): eight
820 studies (49,898 samples) are of European (EUR) ancestry (EPIC-CVD, EPIC-InterAct,
821 CCHS, CGPS, CIHDS, PROSPER, MORGAM, WOSCOPS) and two studies (27,487
822 samples) of South Asian ancestry (BRAVE, PROMIS). The ExomeBP consortium included
823 25 studies (75,620 samples) of EUR ancestry (Airwave, ASCOT, 1958BC, BRIGHT,
824 CROATIA_Korcula, DIABNORD, EGCUT, FENLAND, FINRISK97/02, GS:SFHS,
825 GLACIER, GoDARTS, GRAPHIC, HELIC-MANOLIS, HUNT, LBC1921, LBC1936,
826 LIFELINES, MDC, NFBC1986, OBB, PIVUS, TwinsUK, ULSAM, UKHLS). The GoT2D
827 consortium comprised 14 studies (39,758 samples) of Northern EUR ancestry from Denmark
828 (ADDITION, HEALTH2006/2008, INTER99, SDC and Vejle Biobank), Finland (DPS, DR's
829 EXTRA, FIN-D2D 2007, FINRISK 2007, FUSION, METSIM and PPP-Botania), and
830 Sweden (ANDIS and SDR). The participating studies and their characteristics, including
831 study design, BP ascertainment, measurement and exclusion criteria are detailed in
832 Supplementary Tables 1 and 2. Note, any studies contributing to multiple consortia were only
833 included once in all meta-analyses.

834 **Phenotypes**

835 Four blood pressure (BP) traits were analysed, these included three quantitative traits:
836 systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP); and one
837 binary outcome: hypertension (HTN). For individuals known to be taking BP lowering
838 medication, 15 / 10 mmHg was added to the raw SBP / DBP values, respectively, to obtain
839 medication-adjusted SBP / DBP values [45]. PP was defined as the difference between SBP
840 and DBP, post-adjustment. For the HTN phenotype, individuals were classified as
841 hypertensive cases if they satisfied at least one of the following criteria: (i) SBP ≥ 140
842 mmHg, (ii) DBP ≥ 90 mmHg, (iii) taking anti-hypertensive or BP lowering medication. All
843 other individuals were included within the hypertension analysis as controls. The four BP
844 traits are correlated (SBP:DBP correlations were between 0.6 and 0.8, and SBP:PP
845 correlations were approximately 0.8). However, they measure partly distinct physiological
846 features including, cardiac output, vascular resistance, and arterial stiffness, all measures for
847 determining a cardiovascular risk profile. Therefore the genetic architecture of the individual
848 phenotypes are of interest and a multi-phenotype mapping approach was not adopted. Details
849 of the BP phenotypes for each participating study are provided in Supplementary Table 1.

850

851 **Genotyping**

852 All samples were genotyped using one of the Illumina HumanExome Beadchip arrays
853 (http://genome.sph.umich.edu/wiki/Exome_Chip_Design; Supplementary Table 3).
854 Genotyping was performed across the different studies using different arrays that contained
855 Exome chip SNVs and across several different sites (Supplementary Table 3). Most studies
856 followed comparable protocols for genotyping and quality control of resultant data. Full
857 details are given in Supplementary Information. All genotypes were aligned to the plus strand
858 of the human genome reference build 37 prior to any analyses and any unresolved mappings

859 were removed. As an additional check, genotype cluster plots were reviewed for all the novel
860 rare variants (both lead and secondary signals) and for rare variants that contributed to the
861 gene-based testing to ensure good quality genotype calls.

862

863 **Discovery SNV analyses**

864 Two analyses of the continuous traits SBP, DBP, PP, were conducted within each
865 contributing study: inverse normal transformed and untransformed. The analyses of the
866 transformed traits were performed in order to minimise sensitivity to deviations from
867 normality in the analysis of rare variants, and hence were used for discovery of new SNV-BP
868 associations. The residuals from the null model obtained after regression of the medication-
869 adjusted trait on the covariates (age, age^2, sex, BMI, and disease status for CHD) within a
870 linear regression model, were ranked and inverse normalised. These normalised residuals
871 were used to test trait-SNV associations. All SNVs that passed QC were analysed for
872 association, without any further filtering by MAF, assuming an additive allelic effects model.
873 Full details of the analyses conducted within each study contributing to each of the consortia
874 are given in the Supplementary Information.

875 Two meta-analyses were performed for each trait, one with EUR only, and one with SAS and
876 EUR ancestries combined (EUR_SAS). We performed inverse variance weighted fixed effect
877 meta-analysis for continuous traits (SBP, DBP and PP) and sample size weighted meta-
878 analysis for the binary trait (HTN) as implemented in METAL [46]. Contributing studies used
879 PCs to adjust for population stratification, consequently minimal inflation in the association
880 test statistics, λ, was observed (λ =1.07 for SBP, 1.10 for DBP, 1.04 for PP and <1 for HTN
881 in the transformed discovery meta-analysis in EUR_SAS; λ = 1.06 for SBP, 1.09 for DBP,
882 1.05 for PP and <1 for HTN in the transformed discovery meta-analysis in EUR;

883 Supplementary Figure 6). We used I^2 to calculate heterogeneity across studies
884 (Supplementary Figure 2). The meta-analyses were performed independently in two different
885 centres and concordance of results between the two centres was achieved following thorough
886 quality control checks. Given the studies contributing to the discovery analyses include
887 studies ascertained on CHD or T2D, we explored potential systematic bias in calculated
888 effect estimates amongst these studies. No evidence of bias in the overall effect estimates was
889 obtained.

890 The results for the transformed traits were taken forward and used to select candidate SNVs
891 for replication. Comparison of the results from the transformed and untransformed analyses
892 were made, and the correlations of association *P*-values across all SNVs on the Exome-chip
893 between the results were strong ($r^2 > 0.9$).

894 **Replication SNV analyses**

895 We extracted SNVs associated with any of the transformed traits (SBP, DBP, PP) or HTN
896 and annotated them using the Illumina SNV annotation file - 'humanexome-
897 12v1_a_gene_annotation.txt' independently across two sites. Given the difference in power
898 to detect common versus low frequency and rare variants, two different significance
899 thresholds were chosen for SNV selection. For SNVs with MAF ≥ 0.05, $P \leq 1\text{x}10^{-5}$, while, P
900 $\leq 1\text{x}10^{-4}$ was used for SNVs with MAF < 0.05. By choosing a significance threshold of
901 $P<1\text{x}10^{-4}$ we maximized the opportunity to follow-up rare variants (making the assumption
902 that any true signals at this threshold could replicate at Bonferroni adjusted significance,
903 $P\leq6.17\text{x}10^{-4}$, assuming $\alpha=0.05$ for 81 SNVs). All previously published BP associated SNVs
904 and any variants in LD with them ($r^2 > 0.2$), were removed from the list of associated SNVs
905 as we aimed to replicate new findings only. SNVs for which only one study contributed to the
906 association result or showed evidence of heterogeneity ($P_{het} < 0.0001$) were removed from

907 the list as they were likely to be an artefact. Where SNVs were associated with multiple traits,
908 to minimise the number of tests performed, only the trait with the smallest *P*-value was
909 selected as the primary trait in which replication was sought. Where multiple SNVs fitted
910 these selection criteria for a single region, only the SNV with the smallest *P*-value was
911 selected. In total, 81 SNVs were selected for validation in independent samples. These 81
912 SNVs had concordant association results for both transformed and non-transformed traits.
913 Eighty SNVs were selected from EUR_SAS and had consistent support in the EUR results,
914 and one variant in *ZNF101* was selected based on support in EUR samples only. In the next
915 step, we looked up the 81 SNV-BP associations using data from a separate consortium, the
916 CHARGE+ consortium (who had analysed untransformed DBP, SBP, PP and HTN) and two
917 studies (UHP and Lolipop) from the ExomeBP consortium (Supplementary Information,
918 Supplementary Tables 2 and 3). The analysed residuals from CHARGE+ were approximately
919 normally distributed in their largest studies and plots of the untransformed distributions are
920 provided in Supplementary Figure 7.

921 Two meta-analyses of the replication datasets were performed: one in the EUR samples only,
922 and a second with EUR, African American, Hispanics and SAS ancestries (“ALL”). Both
923 used inverse variance weighted fixed effects meta-analysis (SBP/DBP/PP) and sample size
924 weighted meta-analysis (HTN) as implemented in METAL. Evidence of replication was only
925 confirmed if P (1-tailed) $< 0.05/81=6.17\times10^{-4}$ and the effect (beta) was in the direction
926 observed in our meta-analyses for the selected trait. A combined meta-analysis in METAL
927 was performed of discovery (untransformed results as only untransformed data was available
928 within the CHARGE+ consortium) and replication datasets across the four traits to assess the
929 overall support for each locus. For the combined meta-analyses, a GWS threshold of,
930 $P\leq5\times10^{-8}$, was used to declare a SNV as novel rather than a less stringent experiment wide
931 threshold, as GWS is used to declare significance in GWAS and we wish to minimise the

932 possibility of false positive associations. (Note that the GWS threshold of $P \leq 5x10^{-8}$ is also
933 equivalent to an exome-wide threshold of $P \leq 2x10^{-7}$ adjusted for four traits).

934

935 Note: all final BP-associated variants that validated were associated at $P<10^{-5}$ in the
936 discovery dataset (for the primary trait). Hence, we could have used the same inclusion
937 criteria for both common and rare SNVs. Therefore the optimal threshold to choose for future
938 experiments may need further consideration.

939 **Conditional analyses and gene-based tests**

940 The RAREMETALWORKER (RMW) tool [15] does not require individual level data to
941 perform conditional analyses and gene-based tests
942 (http://genome.sph.umich.edu/wiki/RAREMETALWORKER) and therefore was selected for
943 these analyses. All studies that contributed to the SNV discovery analyses were re-contacted
944 and asked to run the RMW tool. Within Exome BP consortium, only FENLAND,
945 GoDARTS, HELIC-MANOLIS, and UKHLS were unable to run RMW, while two new
946 studies were included, INCIPE and NFBC1966 (Supplementary Table 1 and 2). Only EPIC-
947 InterAct did not contribute to the RMW analyses from the CHD Exome+ consortium. All
948 studies were included from GoT2D. In total, 43 studies (147,402 samples) were included in
949 the EUR analyses and 45 studies (173,329 samples) in the EUR_SAS analyses. Further
950 details are provided in the Supplementary Information and Supplementary Tables 2 and 3.
951 Comparison of the single variant discovery results with the new single variant results from
952 RMW, revealed very good correlation across the continuous BP traits (>0.77 in the
953 EUR_SAS and > 0.74 in the EUR only analyses). The differences were attributable to having
954 fewer samples and slightly different studies in the RMW dataset compared with the discovery
955 samples.

956 For each novel locus, the genomic coordinates and size of the region were defined according
957 to recombination rates (Supplementary Table 9) around the lead variant. For known loci, a 1
958 Mb window was used (Supplementary Table 14). Conditional analysis was performed across
959 each region, in both the EUR and EUR_SAS samples, for the transformed phenotype
960 corresponding to the validated BP trait for novel loci and the published BP trait for known
961 loci.

962 Gene based tests were performed with both the EUR and EUR_SAS datasets using the
963 Sequence Kernel Association Test (SKAT) [16] within RMW as it allows for the SNVs to have
964 different directions and magnitudes of effect. Burden tests were also performed but are not
965 presented as only SKAT provided significant results. The variants in the gene-based tests
966 using SKAT were weighted using the default settings. Briefly, this used a beta distribution
967 density function to give more weight to rare variants, Beta(MAFj, 1, 25) where MAFj
968 represents the pooled MAF for variant j across all studies. Annotation of all variants with
969 information on genes and exon positions were derived from the UCSC reference file
970 "refFlat.txt.gz" (http://hgdownload.soe.ucsc.edu/goldenPath/hg19/database/) and analyses
971 restricted to coding SNVs with MAF < 5% and MAF <1%. Association of genes with the
972 transformed DBP, SBP, PP traits and HTN were deemed to be associated if $P < 2.8 \times 10^{-6}$
973 (Bonferroni correction for 17,996 genes). To confirm the gene associations were not
974 attributable to a solitary SNV within the gene, a gene-based test conditional on the most
975 associated SNV was performed ($P_{conditional} < 0.001$). The QC of all SNVs contributing to the
976 gene based tests including the number of samples and studies contributing to the associations
977 were checked prior to claiming association. We also sought replication in the CHARGE+
978 studies for any significant gene associations in the discovery analyses.

979

Pathway analyses

(1) Magenta

We tested seven databases in MAGENTA[37] (BioCarta[47], Kyoto Encyclopedia of Genes and Genomes (KEGG)[48], Ingenuity[49], Panther[50], Panther Biological Processes[50], Panther Molecular Functions [50] and Reactome [51,52]) for overrepresentation of the SNV discovery results from both EUR and EUR_SAS ancestries. Each of the four BP phenotypes, HTN and transformed DBP, SBP, and PP were tested. MAGENTA maps SNVs to a gene based on their chromosome and position. For SNVs not situated in a gene a ±20kb flanking region is used to capture variants that potentially regulate that gene and the SNV with the smallest *P*-value is subsequently used to represent the gene. To adjust gene associations for potential confounding, the model takes into account six possible predictors: (i) gene size (kb); (ii) number of genotyped variants per kb; (iii) the number of independent variants per kb; (iv) the number of recombination hotspots per kb; (v) the genetic distance (cM) per kb; and (vi) the LD units per kb. The proportion of genes that have an adjusted *P*-value less than the 95th percentile of the gene *P*-value meta-analysis distribution is the “leading edge fraction”. The enrichment *P*-value for a pathway is the proportion of random pathways of identical size, in 10,000 permutations, whose leading edge fraction is greater than or equal to the observed leading edge fraction. Pathways exhibiting $P<0.01$ and FDR<5% were considered statistically significant.

(2) GeneGo MetaCore Network analyses

A set of BP genes based on previously published studies and our current Exome chip results (locus defined as $r^2 > 0.4$ and 500kb on either side of tag SNV; Supplementary Table 19) was tested for enrichment using the THOMSON REUTERS MetaCore™ Single Experiment Analysis workflow tool. The manually uploaded data set was mapped onto selected

MetaCore ontology databases such as: pathway maps, process networks, GO processes and diseases / biomarkers, for which functional information is derived from experimental literature. Outputs were sorted based on the *P*- and FDR-values. Stringent thresholds were adopted for determining whether the given gene set was enriched for a particular process ($P<0.05$, FDR$<5\%$).

Genetic Risk Score

To assess the effect of BP on CHD, ischemic stroke (and three of its subtypes: large vessel, small vessel and cardioembolic stroke) left ventricular mass, left ventricular wall thickness, heart failure, HDL-c, LDL-c, total cholesterol, triglycerides and eGFR, we performed a weighted generalized linear regression of the genetic associations with each outcome variable on the genetic associations with BP accounting for the correlations between genetic variants. When genetic variants are uncorrelated, the estimates from such a weighted linear regression analysis using summarized data, and a genetic risk score analysis using individual-level data, are equal [53]. We refer to the analysis as a genetic risk score (also known as a polygenic risk score) analysis as this is likely to be more familiar to applied readers. As some of the genetic variants in our analysis are correlated, a generalized weighted linear regression model is fitted that accounts for the correlations between variants. This is undertaken as follows:

If β_X are the genetic associations (beta-coefficients) with the risk factor (here, BP) and β_Y are the genetic associations with the outcome, then the causal estimate from a weighted generalized linear regression is $({\beta_X}^T\Omega^{-1}\beta_X)^{-1}{\beta_X}^T\Omega^{-1}\beta_Y$, and the standard error is $\hat{\sigma}\sqrt{({\beta_X}^T\Omega^{-1}\beta_X)^{-1}}$, where ^{T} is a matrix transpose, $\hat{\sigma}$ is the estimate of the residual standard error from the regression model, and the weighting matrix Ω has terms $\Omega_{j_1 j_2} = \sigma_{Y j_1}\sigma_{Y j_2}\rho_{j_1 j_2}$, where σ_{Yj} is the standard error of the genetic association with the outcome for the *j*th SNV,

1029 and $\rho_{j_1 j_2}$ is the correlation between the j_1th and j_2th SNPs. The presence of the estimated
1030 residual standard error allows for heterogeneity between the causal estimates from the
1031 individual SNVs as overdispersion in the regression model (in the case of underdispersion,
1032 the residual standard error estimate is set to unity). This is equivalent to combining the causal
1033 estimates from each SNV using a multiplicative random-effects model [54].
1034
1035 For each of DBP, SBP and PP, the score was created using both the novel and known BP
1036 SNVs or a close proxy (r^2>0.8). Both the sentinel SNV association and any secondary SNV
1037 associations that remained after adjusting for the sentinel SNV were included in the genetic
1038 risk score. For the 30 validated novel SNV-BP associations, βs were taken from the
1039 independent replication analyses (Table 1a and 1b) to weight the SNV in the genetic risk
1040 score. For the secondary SNVs from the novel loci (N=7) and known loci (N=5), βs were
1041 taken from the discovery analyses (Supplementary Tables 10 and 15). For the 82 published
1042 known SNVs, 43 SNVs were either genotyped or had proxies on the Exome chip and the βs
1043 were taken from discovery results (Supplementary Table 13), the remaining βs were taken
1044 from published effect estimates. This strategy for selecting betas for use in the GRS was
1045 taken to minimize the influence of winners curse. The associations between the BP variants
1046 with CHD, HDL-c, LDL-c, total cholesterol, log(triglycerides) and log(eGFR) were obtained
1047 using the CHD Exome+ Consortium studies (N=82,056, N=80,395, N=77,021, N=80,455,
1048 N= 77,779 and N=51,039 respectively) the associations with BMI, waist-hip ratio adjusted
1049 BMI and height were obtained from the GIANT consortium (unpublished Exome chip
1050 results, N=526,508, 344,369 and 458,927 respectively) and for ischemic stroke, left
1051 ventricular mass, left ventricular wall thickness and heart failure (N= 25,799, N= 11,273,
1052 N=11,311, and N= 23,821) from METASTROKE [25], EchoGen [27] and CHARGE-HF [26]. A
1053 causal interpretation of the association of GRS with the outcome as the effect of BP on the

1054 outcome assumes that the effects of genetic variants on the outcome are mediated via blood
1055 pressure and not via alternate causal pathways, for example via LV thickness. There are also
1056 limitations of the Mendelian randomization approach in distinguishing between the causal
1057 effects of different measures of blood pressure, due to the paucity of genetic variants
1058 associated with only one measure of blood pressure (for example, SBP, DBP or PP).

1059

1060 **eQTL analyses**

1061 The MuTHER dataset contains gene expression data from 850 UK twins for 23,596 probes
1062 and 2,029,988 (HapMap 2 imputed) SNVs. All cis–associated SNVs with FDR<1%, within
1063 each of the 30 novel regions (IMPUTE info score >0.8) were extracted from the MuTHER
1064 project dataset for each of the tissues, LCL (n=777), adipose (n=776) and skin (n=667) [54].
1065 The pilot phase of the GTEx Project (dbGaP Accession phs000424.v3.p1) provides
1066 expression data from up to 156 individuals for 52,576 genes annotated in Gencode v12
1067 (including pseudo genes) and 6,820,472 genotyped SNVs (using the Human Omni5-Quad
1068 array) and imputed SNVS (to the 1000 Genomes project reference post-QC, info > 0.4, MAF
1069 > 5%) [55]. The eQTL analysis was focused on nine tissues having more than 80 samples and
1070 genes expressed at least 0.1 RPKM in 10 or more individuals in a given tissue. All transcripts
1071 with a transcription start site (TSS) within one of the 30 new BP loci and for which there was
1072 a cis-associated SNV (IMPUTE info score > 0.4) within 1Mb of the TSS at FDR<5%, were
1073 identified. Data were extracted for: subcutaneous adipose tissue (n=94), tibial artery (n=112),
1074 heart (left ventricle) (n=83), lung (n=119), skeletal muscle (n=138), tibial nerve (n=88), skin
1075 (sun exposed, lower leg) (n=96), thyroid (n=105) and whole blood (n=156). We were not able
1076 to evaluate presence of expression signals in kidney tissue, since the currently available
1077 sample size in GTEx (n=8) is too small to enable calculation of eQTLs. Only data for SNVs

1078 with MAF > 0.05 were considered. From each resource, we report eQTL signals, which reach
1079 the resource-specific thresholds for significance described above, for SNVs that are in LD
1080 (r^2>0.8) with our sentinel SNV.

1081 For identified eQTLs, we tested whether they colocalised with the BP associated SNV using
1082 a Bayesian approach based on summary statistics [56]. Colocalisation analyses were considered
1083 to be significant if the posterior probability of colocalisation was greater than 0.95.

1084 **Annotation of variants and literature review**

1085 *In silico* prediction of the functional effect of detected variants was based on the annotation
1086 from the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/), the Ensembl Variant Effect
1087 Predictor (VEP) tool (http://www.ensembl.org/info/docs/tools/index.html) and from the
1088 Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA
1089 (http://evs.gs.washington.edu/EVS/) on March 2015 and February 2016. Annotation was
1090 performed for each known Ensembl transcript or regulatory feature regardless of the biotype.
1091 Information about the effect on transcript and protein level, including various effect
1092 prediction scores (PolyPhen, SIFT, Grantham), evolutionary conservations scores
1093 (phastCons, GERP) and variant frequency in various populations (AFR, AMR, ASN, EAS,
1094 EUR, SAS), was collected. Details of the literature review are provided in the Supplementary
1095 Information.

1096 **Trait variance explained**

1097 The percentage trait variance explained for SBP, DBP, PP was assessed with 5,861
1098 individuals from a population-based cohort (1958BC) with complete information for all
1099 phenotypes and covariates.

1100 Two different genetic models were investigated: one containing the 43 previously known BP
1101 associated SNVs covered on the Exome chip, as a baseline reference; and a second model
1102 additionally including the 30 novel lead SNVs and 9 of the new independent additional SNVs
1103 from both novel and known loci with multiple signals, giving a total of 82 known and novel
1104 SNV predictors. These 9 additional new independent SNVs were taken as the secondary
1105 signals identified within the novel and known loci from the conditional analyses of the EUR
1106 dataset, as 1958BC contains European subjects only. Hence the 9 independent variants
1107 included: 4 secondary variants from novel loci for EUR (*PREX1, COL21A1, PRKAG1* and
1108 *MYH6* (there was only 1 in EUR) loci; Supplementary Table 10) and 5 secondary variants
1109 from known loci for EUR (*ST7L-CAPZA1-MOV10, FIGN-GRB14, ENPEP, TBX5-TBX3 and*
1110 *HOXC4*; Supplementary Table 15).

1111 The residual trait was obtained by adjusting each of the medication-adjusted BP traits with
1112 sex and BMI variables, the same covariates used within the discovery SNV analyses (not age
1113 or age^2 as all 1958BC individuals were aged 44 years). A linear regression was fitted for the
1114 residual trait regressed on all SNVs within the corresponding model and adjusted for the first
1115 ten principal components (PCs). The r^2 calculated from this regression model was used as the
1116 percentage trait variance explained. The comparison of r^2 measures between the 43-SNV
1117 model and the 82-SNV model was used to indicate the incremental increase in percentage
1118 trait variance explained due to all the novel SNVs identified.

1119

1120 **Monogenic Enrichment analyses**

1121 To determine if sub-significant signals of association were present in a set of genes associated
1122 with monogenic forms of disease, we performed an enrichment analysis of the discovery

single variant meta-analysis association results for all four traits for both for individuals of EUR ancestry and EUR_SAS ancestry datasets.

The monogenic gene set included: *WNK1*, *WNK4*, *KLHL3*, *CUL3*, *PPARG*, *NR3C2*, *CYP11B1*, *CYP11B2*, *CYP17A1*, *HSD11B2*, *SCNN1A*, *SCNN1B*, *SCNN1G*, *CLCNKB*, *KCNJ1*, *SLC12A1*, *SLC12A3* [3]. The association results of coding SNVs in these genes were extracted and the number of tests with $P<0.001$ observed. In order to determine how often such an observation would be observed by chance, we constructed 1,000 matched gene sets. The matching criteria for each monogenic gene was the intersection of all genes in the same exon length quintile and all genes in the same coding variant count decile. Within the matched sets, the number of variants with $P<0.001$ was observed. The empirical *P*-value was calculated as the fraction of matched sets with an equal or larger number of variants less than 0.001.

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1162

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Table 1a. Novel blood pressure trait associated loci. Variants with formal replication

Variant information				Discovery		Replication			Combined		
Locus	rsID	Chr:Pos (EA:EAF)	Trait	P_t	P_U	N	β	*P*	N	β	*P*
EUR											
RNF207	rs709209	1:6.28 (A:0.655)	PP	4.57x10^{-6}	1.60x10^{-6}	122780	0.17	5.83x10^{-4}	284683	0.20	9.62x10^{-9}
c5orf56	rs12521868	5:131.78 (T:0.373)	DBP	1.59x10^{-6}	3.03x10^{-7}	122795	-0.18	2.29x10^{-5}	282023	-0.19	6.12x10^{-11}
PHACTR1	rs9349379	6:12.90 (A:0.566)	SBP	2.11x10^{-8}	1.78x10^{-7}	122809	0.24	4.06x10^{-4}	284673	0.29	8.84x10^{-10}
COL21A1	rs200999181†	6:55.94 (A:0.002)	PP	3.08x10^{-8}	2.46x10^{-7}	121487	2.70	1.90x10^{-4}	242486	3.25	6.27x10^{-10}
ABO	rs687621	9:136.14 (A:0.615)	DBP	8.80x10^{-8}	2.55x10^{-7}	122798	0.16	1.96x10^{-4}	276014	0.19	5.45x10^{-10}
ADO	rs10995311	10:64.56 (C:0.567)	DBP	1.86x10^{-6}	1.14x10^{-6}	122798	0.23	8.47x10^{-8}	266456	0.21	1.12x10^{-12}
LMO1	rs110419	11:8.25 (A:0.48)	DBP	9.41x10^{-6}	2.22x10^{-5}	122798	0.16	1.81x10^{-4}	279935	0.16	3.04x10^{-8}
OR5B12	rs11229457	11:58.21 (T:0.236)	SBP	1.58x10^{-6}	4.62x10^{-5}	122809	-0.32	7.53x10^{-5}	284680	-0.31	2.70x10^{-8}
CERS5	rs7302981	12:50.54 (A:0.361)	DBP	1.35x10^{-13}	4.60x10^{-11}	122798	0.24	2.64x10^{-8}	284718	0.25	1.38x10^{-17}
MYH6	rs452036	14:23.87 (A:0.327)	PP	4.59x10^{-11}	2.80x10^{-13}	122780	-0.21	1.81x10^{-5}	284672	-0.28	2.96x10^{-16}
DPEP1	rs1126464	16:89.70 (C:0.256)	DBP	1.19x10^{-9}	4.35x10^{-11}	118677	0.24	1.68x10^{-6}	261564	0.28	1.02x10^{-15}
TBX2	rs8068318†	17:59.48 (T:0.698)	DBP	7.46x10^{-13}	5.71x10^{-10}	122798	0.26	3.23x10^{-8}	281978	0.26	1.95x10^{-16}
RGL3	rs167479	19:11.53 (T:0.486)	DBP	2.22x10^{-23}	1.97x10^{-22}	122797	-0.29	3.01x10^{-11}	283332	-0.33	1.99x10^{-31}
PREX1	rs6095241	20:47.31 (A:0.452)	DBP	5.65x10^{-6}	2.29x10^{-5}	122798	-0.18	2.56x10^{-5}	281322	-0.17	4.75x10^{-9}
ALL ancestry											
RBM47	rs35529250†	4:40.43 (T:0.01)	SBP	6.56x10^{-7}	6.15x10^{-6}	148878	-1.43	5.02x10^{-4}	306352	-1.55	2.42x10^{-8}
OBFC1	rs4387287	10:105.68 (A:0.157)	SBP	2.23x10^{-8}	1.32x10^{-7}	147791	0.28	3.37x10^{-4}	320494	0.36	9.12x10^{-10}
RRAS	rs61760904†	19:50.14 (T:0.008)	SBP	1.96x10^{-6}	1.90x10^{-5}	148878	1.38	5.70x10^{-4}	322664	1.50	8.45x10^{-8}

SNV-BP associations are reported for the newly identified BP loci that replicated at $P<6.2x10^{-4}$ (Bonferroni correction for the 81 variants selected for replication for a primary blood pressure trait; Methods). Loci are categorised into EUR and ALL ancestry based on the meta-analysis used to replicate the variants for the primary BP trait shown in column labelled 'Trait'. In the columns that contains the discovery meta-analyses results, P_t represents the *P*-value for association of the variant with the transformed primary BP trait in the EUR_SAS discovery meta-analyses (which was also used to select the variant for replication) and P_u represents the *P*-value for association with the untransformed primary BP trait in the ancestry in which the variant replicated. N, **β** and *P*, denotes the number of samples, estimated allelic effect and *P*-value for association with the primary BP trait, are provided for the untransformed primary BP trait in the replication data and also from the combined (discovery and replication) meta-analyses. NB: ALL ancestry corresponds to all ancestries in the combined (discovery + replication) meta-analyses

Locus – Gene or region containing the SNV, rsID - dbSNP rsID. Chr:Pos (EA:EAF) – Chromosome:NCBI Build 37 position in Mb (effect allele:effect allele frequency), Trait – primary blood pressure trait for which the variant was and also replicated, β - effect estimate, N:sample size, EUR - European.

† indicates it is a non-synonymous SNV (nsSNV) or is linkage disequilibrium with a nsSNV (r^2>0.8) that is predicted to be damaging

Table 1b. Novel blood pressure trait associated loci. Variants with GWS evidence of association in combined meta-analyses

Variant information				Discovery		Replication			Combined		
Locus	rsID	Chr:Pos (EA:EAF)	Trait	P_t	P_U	N	β	*P*	N	β	*P*
EUR											
2q36.3	rs2972146	2:227.10 (T:0.652)	DBP§ (HTN)	1.51x10^{-9}	2.47x10^{-7}	122798	0.13	2.20x10^{-3}	275610	0.17	8.40x10^{-9}
ZBTB38	rs16851397	3:141.13 (A:0.953)	DBP§ (SBP)	6.87x10^{-6}	3.20x10^{-5}	122798	-0.38	1.20x10^{-4}	284717	-0.38	3.01x10^{-8}
PRDM6	rs1008058	5:122.44 (A:0.135)	SBP	5.09X10^{-7}	1.01x10^{-8}	43109	0.46	3.61x10^{-3}	176362	0.55	2.99x10^{-10}
GPR20	rs34591516	8:142.37 (T:0.055)	SBP§ (DBP)	1.54x10^{-6}	1.01x10^{-7}	122807	0.51	4.20x10^{-4}	282009	0.64	6.10x10^{-10}
HOXB7	rs7406910	17:46.69 (T:0.118)	SBP	6.07X10^{-10}	2.74x10^{-9}	122809	-0.20	4.89x10^{-2}	284690	-0.46	3.80x10^{-8}
AMH	rs10407022†	19:2.25 (T:0.82)	PP	1.63X10^{-7}	1.73x10^{-7}	118656	-0.19	1.62x10^{-3}	252525	-0.26	5.94x10^{-9}
ZNF101	rs2304130	19:19.79 (A:0.914)	DBP	1.66X10^{-8}	1.92x10^{-8}	122798	-0.17	1.71x10^{-2}	284705	-0.29	1.53x10^{-8}
PROCR	rs867186	20:33.76 (A:0.873)	DBP	1.44X10^{-6}	4.15x10^{-7}	122798	0.21	2.48x10^{-3}	284722	0.26	1.19x10^{-8}
RRP1B	rs9306160	21:45.11 (T:0.374)	DBP§ (SBP)	1.04x10^{-8}	1.90x10^{-6}	100489	-0.16	4.30x10^{-4}	249817	-0.18	6.80x10^{-9}
TNRC6B	rs470113	22:40.73 (A:0.804)	PP	1.48X10^{-10}	1.31x10^{-9}	122780	-0.14	1.37x10^{-2}	284683	-0.25	1.67x10^{-9}
ALL ancestry											
7q32.1	rs4728142	7:128.57 (A:0.433)	SBP	8.10x10^{-6}	4.21x10^{-6}	150542	-0.21	8.62x10^{-4}	338338	-0.24	3.45x10^{-8}
PRKAG1	rs1126930†	12:49.40 (C:0.036)	PP	2.12x10^{-6}	4.62x10^{-7}	151481	0.36	3.74x10^{-3}	314894	0.50	3.34x10^{-8}
SBNO1	rs1060105	12:123.81 (T:0.209)	DBP	6.66x10^{-7}	1.09x10^{-6}	150532	-0.15	2.67x10^{-3}	336413	-0.18	3.07x10^{-8}

SNV-BP associations are reported for the newly identified BP loci that showed genome-wide significant association (P<5x10^{-8}) in the combined discovery and replication meta-analyses. In the columns that contain results from the discovery meta-analyses, P_t represents the *P*-value for association of the variant with the transformed *primary* BP trait in the EUR_SAS discovery meta-analyses (used to select the variant for replication) and P_u represents the *P*-value for association with the untransformed BP trait in the ancestry in which the variant was validated. For four loci (2q36.3, *ZBTB38*, *GPR20* and *RRP1B*) P_t denotes the association *P*-value with the primary trait, which is given in parentheses, however, the locus was validated for a secondary trait, which is listed. Loci are categorised into EUR and ALL ancestry based on the ancestry in which the variant showed association with a blood pressure trait at P<5x10^{-8}. N, **β** and *P*, denotes the number of samples, estimated allelic effect and *P*-value for association with the validated BP trait, are provided for the untransformed BP trait in the replication data and also from the combined (discovery and replication) meta-analyses. NB: ALL ancestry corresponds to all ancestries in the combined (discovery + replication) meta-analyses.

Locus – Gene or region containing the SNV, rsID - dbSNP rsID. Chr:Pos (EA:EAF) – Chromosome:NCBI Build 37 position in Mb (effect allele:effect allele frequency), Trait - blood pressure trait for which association is reported, EUR - European.
§ indicates the trait with which the variant showed the strongest association in the combined meta-analyses. For variants where this trait is different from the primary phenotype, the primary BP trait used to select the variants is shown in the parantheses
† indicates it is a non-synonymous SNV (nsSNV) or is linkage disequilibrium with a nsSNV (r^2>0.8) that is predicted to be damaging

Table 2 Gene-based associations with BP traits from the SKAT model

Gene	Chr:Start-End	n_var	Trait	MAF_c	MAF_{min}	MAF_{max}	*P*	P_{cond}
EUR (Novel)								
A2ML1	12:8.975-9.03	51	HTN	0.01	3.43×10^{-6}	0.0077	2.36×10^{-6}	9.17×10^{-4}
EUR_SAS (Novel)								
A2ML1	12:8.975-9.03	54	HTN	0.01	2.91×10^{-6}	0.0067	6.83×10^{-7}	5.00×10^{-4}

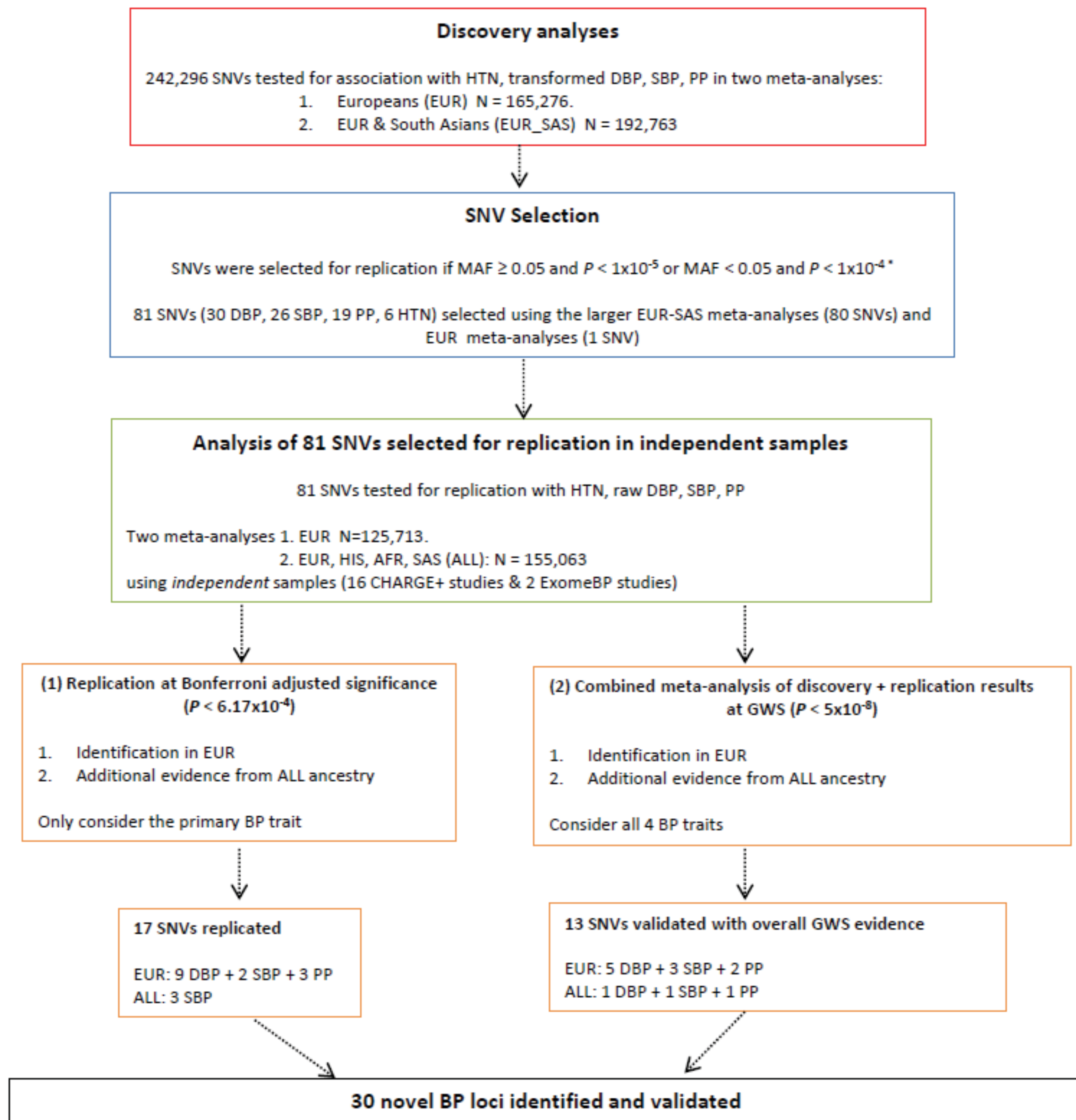
Novel blood pressure associated gene with $P<2.8 \times 10^{-6}$ (Bonferroni corrected *P*-value for the 17,996 genes tested) from the RareMetalWorker (RMW) SKAT test are given. Results are shown from both the EUR and EUR_SAS ancestry datasets. There was no evidence of association of this gene with HTN in the CHARGE+ studies used for replication ($P= 0.45$).
Chr:Start-End – Chromosome: GRCh37 start and end bp coordinates of the Gene in Mb, n_var - number of exonic variants tested., Trait – HTN (Hypertension) or transformed primary quantitative blood pressure trait, MAF_c - minor allele frequency filter applied to select variants for the test, MAF_{min} - minimum MAF of variants tested, MAF_{max} - maximum MAF of variants tested, *P* - *P*-value for association of the gene with the trait, P_{cond} - *P*-value for association of gene with the phenotype conditioned on the exonic variant that had the smallest association *P*-value with the phenotype in the single variant association RMW meta-analysis.

Table 3 Results of the genetic risk score analyses across CVD traits and risk factors.

Outcome	Units	N	DBP (per 10mmHg increase)		SBP (per 10mmHg increase)		PP (per 10mmHg increase)	
			Effect [95% CI]	*P*	Effect [95% CI]	*P*	Effect [95% CI]	*P*
CHD	OR	82,056	1.62 [1.28, 2.05]	5.99×10^{-5}	1.39 [1.22, 1.59]	6.07×10^{-7}	1.70 [1.34, 2.16]	1.20×10^{-5}
Ischemic stroke	OR	25,799	1.93 [1.47, 2.55]	2.81×10^{-6}	1.57 [1.35, 1.84]	1.16×10^{-8}	2.12 [1.58, 2.84]	5.35×10^{-7}
Cardioembolic stroke	OR	16,113	1.43 [0.86, 2.39]	0.1683	1.33 [0.99, 1.80]	0.0584	1.73 [1.00, 3.02]	0.0518
Large vessel stroke	OR	13,903	2.26 [1.25, 4.08]	0.0068	1.85 [1.32, 2.59]	3.61×10^{-4}	3.05 [1.64, 5.68]	4.37×10^{-4}
Small vessel stroke	OR	15,617	1.96 [1.13, 3.41]	0.0168	1.56 [1.13, 2.16]	0.0064	1.98 [1.09, 3.61]	0.0248
Heart failure	OR	13,282	1.48 [1.02, 2.17]	0.0409	1.25 [1.00, 1.57]	0.0512	1.33 [0.88, 2.02]	0.1757
Left ventricular mass	g	11,273	9.57 [3.98,15.17]	8.02×10^{-4}	5.13 [1.77, 8.48]	0.0027	5.97 [-0.38,12.31]	0.0653
Left ventricular wall thickness	cm	11,311	0.10 [0.06, 0.13]	1.88×10^{-8}	0.05 [0.03, 0.07]	5.52×10^{-6}	0.05 [0.01, 0.09]	0.0187
HDL	mg/dl	80,395	0.25 [-1.00, 1.51]	0.6930	0.21 [-0.50, 0.92]	0.5622	0.47 [-0.79, 1.73]	0.4668
LDL	mg/dl	77,021	-1.57 [-5.20, 2.06]	0.3972	0.07 [-2.03, 2.16]	0.9498	1.87 [-1.86, 5.59]	0.3255
Total cholesterol	mg/dl	80,455	-1.34 [-5.90, 3.22]	0.5639	0.70 [-1.93, 3.32]	0.6029	3.68 [-0.97, 8.33]	0.1209
Triglycerides	mg/dl	77,779	0.02 [-0.03, 0.08]	0.3859	0.02 [-0.01, 0.05]	0.2697	0.03 [-0.03, 0.08]	0.3025
BMI	INVT	526,508	-0.10 [-0.18,-0.01]	0.0342	-0.07 [-0.13,-0.02]	0.0058	-0.12 [-0.23,-0.02]	0.0165
WHRadjBMI	INVT	344,369	0.03 [-0.04, 0.11]	0.4025	0.03 [-0.02, 0.08]	0.2170	0.06 [-0.03, 0.15]	0.1885
Height	INVT	458,927	0.02 [-0.15, 0.18]	0.8592	-0.04 [-0.15, 0.06]	0.4170	-0.18 [-0.37, 0.01]	0.0683
eGFR	INVT	51,039	-0.02 [-0.15, 0.11]	0.7810	-0.03 [-0.10, 0.04]	0.4080	-0.07 [-0.20, 0.06]	0.2741

CHD, coronary heart disease; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated glomerular filtration rate; DBP, diastolic blood pressure; SBP systolic blood pressure; PP, pulse pressure; OR, odds ratio; g, grams; INVT, inverse norma transformed (hence no units); N, sample size; *P*, *P*-value of association of BP with the trait listed; CI, confidence interval. Results are considered significant if $P < 0.0038$, which corresponds to a Bonferroni correction for 13 phenotypes tested.

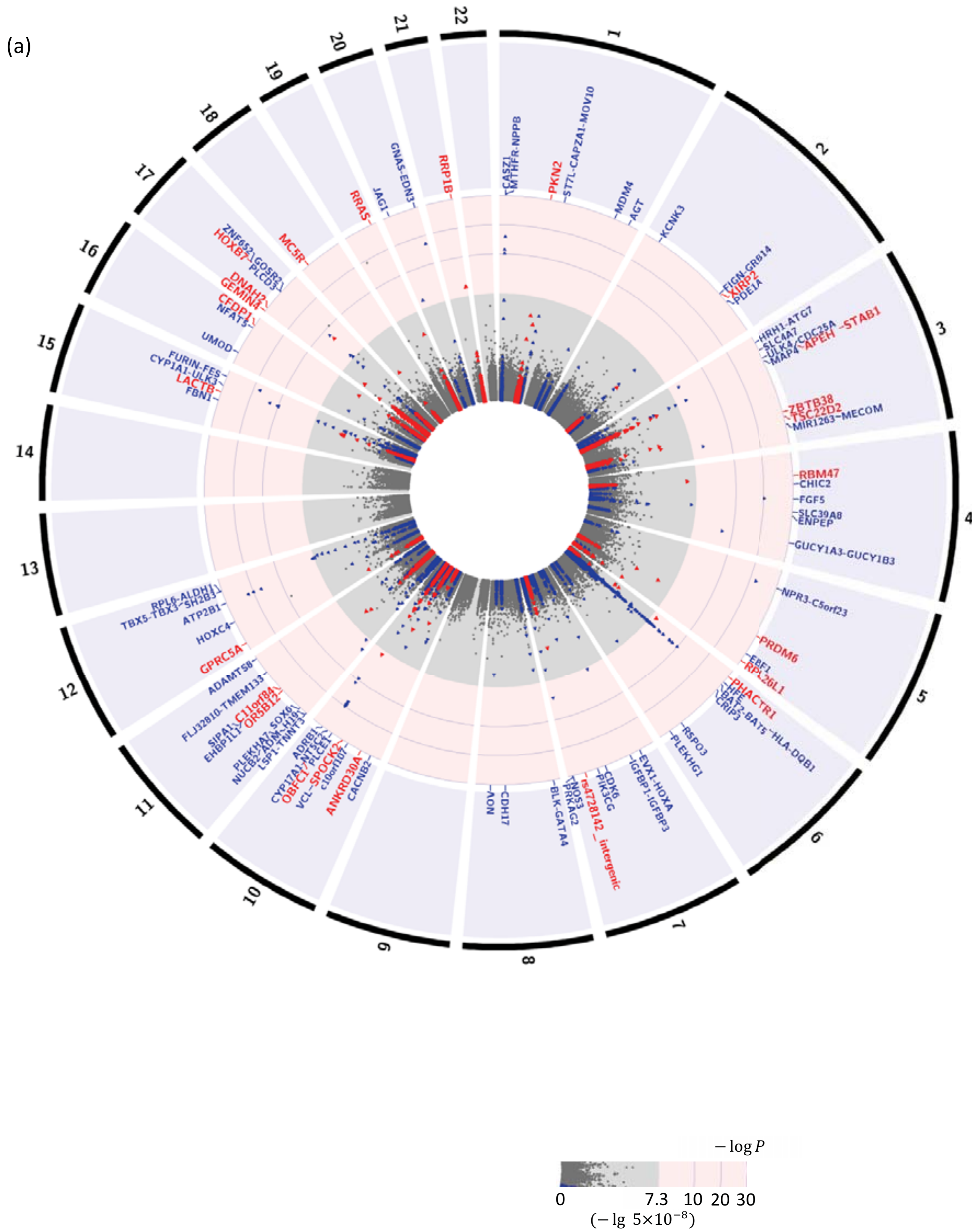
Figure 1 Study design and work flow diagram of single variant discovery analyses.



EUR=European, SAS=South Asian, HIS=Hispanic, AA=African American, HTN=hypertension, BP=blood pressure, SBP=systolic blood pressure, DBP= diastolic blood pressure, PP=pulse pressure, N=sample size, MAF=minor allele frequency, *P*=*P*-value significance threshold, SNV=single-nucleotide variant, GWS=genome-wide significance *Further details of the selection criteria are provided in the methods.

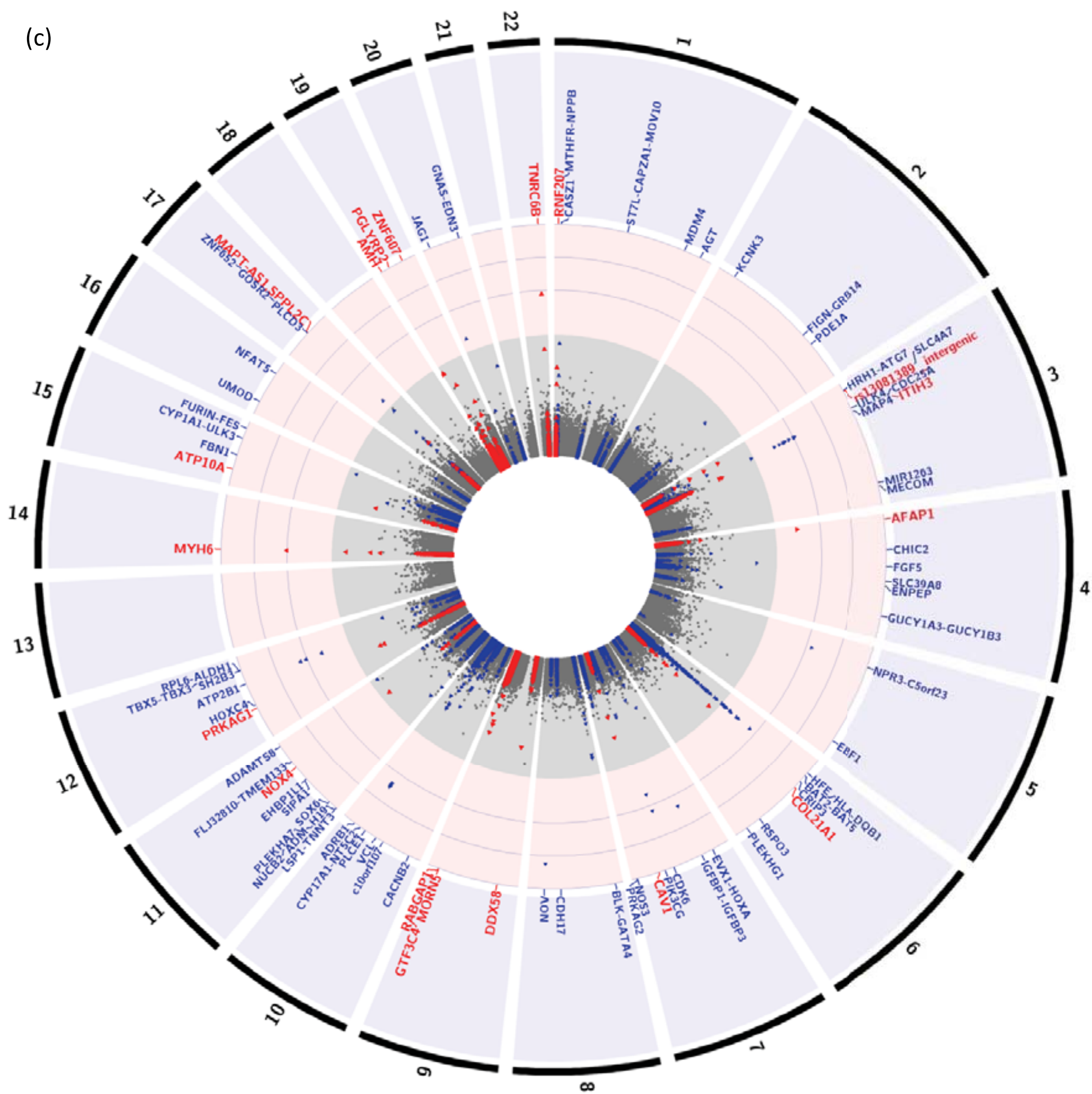
Figure 2 Discovery SNV-BP associations. Results are provided for (a) transformed SBP (b) transformed DBP (c) transformed PP and (d) HTN in the European and South Asian (EUR_SAS) discovery samples. The y-axis represents $-\log_{10}P$ for association. Red triangles represent variants that map to one of the 81 regions selected for replication, blue triangles represent SNVs that map to previously published BP regions, and grey triangles represent all remaining SNVs. SNVs are ordered according to chromosome (black lines on the outside of the plot) and physical position. Genes that SNVs map to are given in the outer lilac blocks.

(a)



(b)

(c)



d)

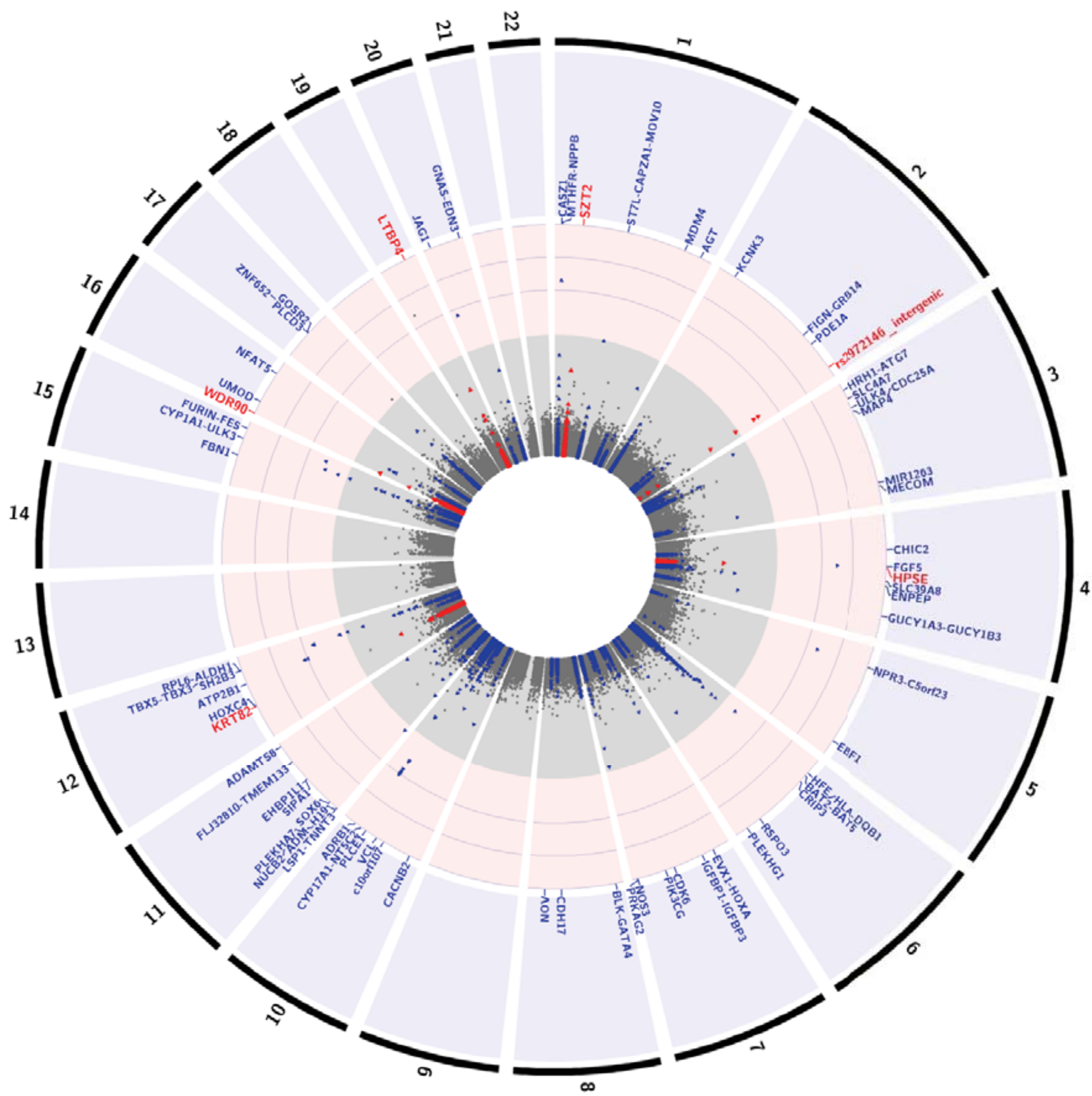
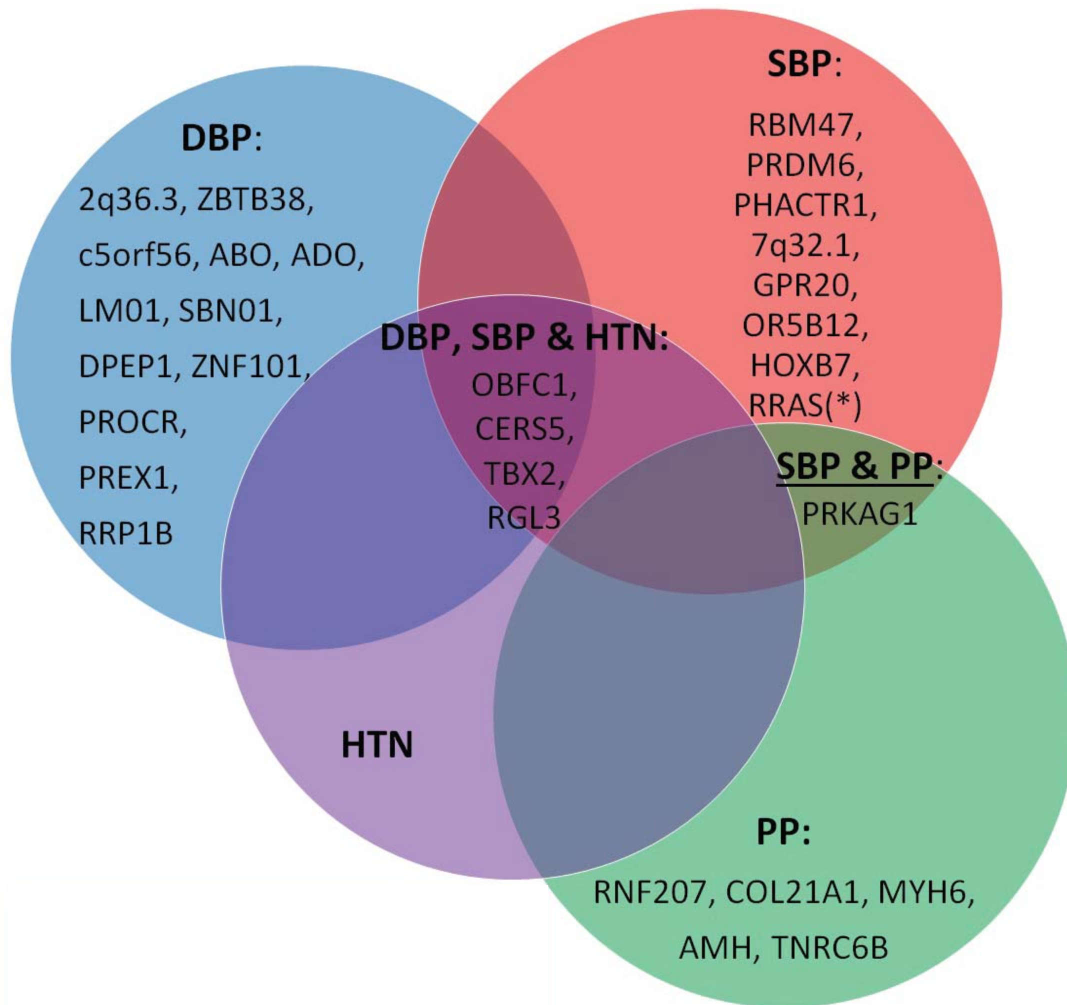
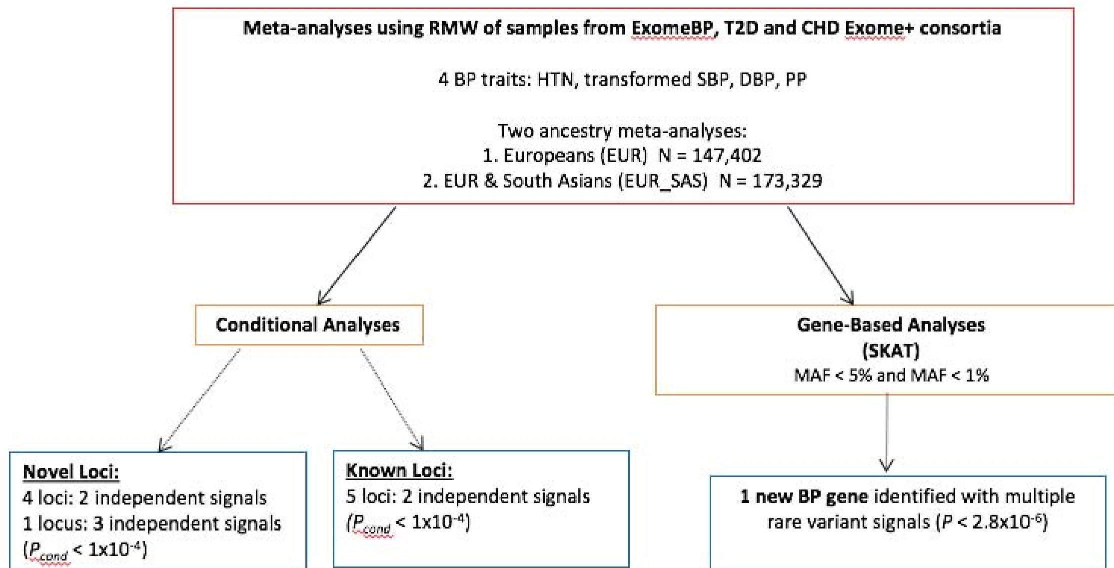


Figure 3 Overlap of the 30 novel loci associations across SBP, DBP, PP and HTN.



The Venn diagram shows which of the 30 newly identified BP loci are associated with multiple BP traits. Only SNV-BP trait associations that were genome-wide significant ($P < 5x10^{-8}$) in the combined discovery and replication meta-analyses are listed for any given BP trait, within the corresponding ancestry dataset that the given locus was validated for (see Tables 1a and 1b). The association of *RRAS* variant with SBP was replicated in the independent samples, but did not achieve GWS in the combined discovery and replication meta-analysis and is therefore only included for SBP. HTN=hypertension, SBP=systolic blood pressure, DBP= diastolic blood pressure, PP=pulse pressure.

Figure 4 Study design for conditional analyses and rare variant gene-based discovery analyses.



RMW=RareMetalWorker, EUR=European, SAS = South Asian, HTN=hypertension, BP=blood pressure, SBP=systolic blood pressure, DBP= diastolic blood pressure, PP=pulse pressure. N=sample size, MAF=minor allele frequency, *P*=*P*-value significance threshold, P_{cond}=conditional *P*-value significance threshold

Figure 5 Locus plot for *A2ML1* and secondary amino acid structure of the gene product.

(a) Locus plot for *A2ML1* associated with HTN identified through gene based tests. The variants' positions along the gene (x axis, based on human genome build 37) and the $-\log_{10}$(*P*-value of association) (y axis) are indicated. The variants are colour coded: nonsense (blue), missense, predicted damaging (green), and missense (red). The schematic above the x-axis represents the intron / exon (black vertical bars) structure, the untranslated regions are shown as grey vertical bars.

(b) The white box denotes the full-length amino acid sequence for each of the two gene products. Black numbers denote amino acid residue positions of note. Coloured boxes depict putative functional domains (see below). Coloured vertical lines indicate the amino acid substitutions corresponding to the variants depicted in the locus plots above using the same colour coding. Bold, italic indicates the SNV association with smallest *P*-value.

Dark grey – signal peptide sequence. Brown – regions of intramolecular disulfide bonds. For simplicity only those regions coinciding with variants described were indicated. Black – bait region described to interact with proteases. Purple – thiol ester sequence region aiding in interaction with proteases. Light grey – alpha helical regions thought to mediate A2ML1 interaction with LRP1, facilitating receptor-mediated endocytosis.