Online Data Supplement

GENETIC ARCHITECTURE OF AMBULATORY BLOOD PRESSURE IN THE GENERAL POPULATION – INSIGHTS FROM CARDIOVASCULAR GENE-CENTRIC ARRAY

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Illumina HumanCVD BeadChip

A detailed list of the loci on the 50K IBC array (1) is available at http://bmic.upenn.edu/cvdsnp/. Genes and loci on this array were prioritized in three groups: [1] genes and loci with a high likelihood of functional significance (well-established contributors to vascular disease, genes implicated in genome-wide association studies and those with previous evidence of association) were captured with an $r^2 \geq 0.8$ for HapMap SNPs with minor allele frequency (MAF)>0.02 (Tier 1), [2] loci of potential relevance to cardiovascular disease (or large loci that required a high number of tags) were covered with $r^2 \geq 0.5$ for MAF>0.05 (Tier 2), [3] genes of lower interest to the IBC consortium investigators were represented only by non-synonymous and known functional variants with MAF>0.01 (Tier 3).

Extended information on CoLaus Study and Silesian Cardiovascular Study (SCS)

Both studies recruited subjects of white European ancestry (CoLaus – 6188 unrelated subjects, SCS - 703 subjects from 213 families and 435 unrelated individuals). The CoLaus cohort was recruited from the general population of Lausanne, Switzerland (2). The Silesian Cardiovascular Study was conducted in 3 reference centres in the south of Poland and is enriched in patients with high cardiovascular risk (defined as coronary artery disease, hypertension, multiple cardiovascular risk factors or combinations of the above) (3). In both cohorts subjects were phenotyped for basic demographic and anthropometric parameters and clinic BP was measured three times in a sitting position using appropriately sized cuff and an oscillometric method according to the guidelines of European Society of Hypertension (4). For the purpose of this analysis, only biologically unrelated subjects (all Colaus participants and 807 individuals from SCS) were selected. Full genetic and phenotypic information was available for 5356 and 753 biologically unrelated subjects from CoLaus and SCS, respectively.

Extended description of the statistical analysis

Heritability of BP was estimated using an algorithm implemented in Sequential Oligogenic Linkage Analysis Routines (SOLAR v2.0) software (5). Total phenotypic variance of BP was partitioned into genetic and environmental components. The genetic component was further divided into polygenic additive effect - narrow sense heritability (explained by the regression slope) and the non-additive effect of alleles (the source of deviation from the slope – dominant effect). The model also estimated effects of known demographic covariates (age, age^2 and sex). The estimates were then modelled to best fit the observed data using maximum likelihood approach and genetic component tested against null hypothesis assuming heritable component equal to 0.

The χ^2 test was used to examine whether distributions of genotypes for each SNP on the 50K IBC array were concordant with Hardy-Weinberg equilibrium in the parental generation of the GRAPHIC Study. SNPs which passed this and additional filters (see Online Data Supplement) were examined for their associations with BP under an additive model of inheritance using generalized estimating equations (GEE) with exchangeable correlation structure to account for familial correlations and with adjustment for age, age^2 and sex. Association analysis of top SNPs in the GRAPHIC Study with clinic BP in the CoLaus and SCS cohorts was based on age, age^2 and sex adjusted linear regression models fitted in PLINK http://pngu.mgh.harvard.edu/purcell/plink/ (6) under additive model of inheritance. BP values of subjects on antihypertensive treatment in all cohorts were adjusted for BP-lowering effect of therapy using a semi-parametric algorithm in GRAPHIC and SCS (7-8) or adding a constant of 15 mmHg (to SBP) and 10 mmHg (to DBP) in CoLaus (9). Genetic effects are shown as β-coefficients (β) per each extra minor allele copy of a SNP with the respective standard error (SE).

The semi-parametric algorithm used to adjust for effects of antihypertensive medication in the GRPAHIC and SCS is essentially based on the approach described earlier by Levy (7) and Tobin (8). In brief, this method is based on estimation of the residuals for treatment adjusted BPs from regression models conditioned on the distribution of untreated residuals of at least equal (or greater) magnitude (7). Based on this non-parametric algorithm treatment adjusted BP is the sum

of measured BP plus the difference between the adjusted residual and the initial residual (7). No adjustments are made to BP values from subjects who are not treated with antihypertensive medication. Genetic effects are shown as β-coefficients per each extra minor allele copy of a SNP with the respective standard error (SE).

Two sensitivity analyses [(i) no treatment correction, (ii) exclusion of subjects on antihypertensive treatment] were undertaken to examine if antihypertensive therapy had any impact on the observed associations.

To correct for multiple testing in the GRAPHIC Study for each association with the two principal phenotypes (mean 24-hour SBP and DBP) we calculated q-values based on the method proposed by Storey and Tibshirani (10) and available in the QVALUE software (http://genomine.org/qvalue/). The q-value of a test measures the proportion of false positives (false positive discovery rate) when this test is considered as significant. Q-value thresholds of 0.05 and 0.25 were selected to identify the findings that represent significant and suggestive associations, respectively.

Fixed-effect meta-analysis of SNPs on clinic BP in the GRAPHIC, CoLaus and SCS studies was conducted under additive model of inheritance using inverse variance weighted averages of β-coefficients and SEs in METAL (http://www.sph.umich.edu/csg/abecasis/metal/index.html). The inverse variance weighted fixed effects meta-analysis combines effects size estimates (β-coefficients) along with respective standard errors across studies by assigning each study a weight inversely proportional to the variance of its estimate (http://www.sph.umich.edu/csg/abecasis/metal/). The between-study heterogeneity was evaluated using χ^2 test.

Two-tail Fisher's exact test was used to analyse the distributions of rare, exonic and non-synonymous variants across different strata of associations with mean 24-hour mean SBP and DBP in the GRAPHIC Study.

The power estimates of the GRAPHIC Study to detect both nominal ($P<0.05$) and Bonferroni adjusted (33577 tests) effects of various sizes for mean 24-hour SBP and DBP across a range of different minor allele frequencies were derived using the average SE observed at each allele frequency and constructed in STATA.

Extended description of the bioinformatic analysis

All SNPs on the 50K IBC array were annotated to specific loci using resources available in public domain including information on design of the array at http://bmic.upenn.edu/cvdsnp/, NCBI (Built 36.3) and SNP Function Portal - web-based application for analysis of human genetic variants (11).

The most significant candidate region implicated in the association analysis (5' flanking sequence of 5,10-methylenetetrahydrofolate reductase (NADPH) gene /MTHFR/ and chloride channel 6 gene /CLCN6/) was aligned against syntenic rodent BP quantitative trait loci (QTLs) in UCSC Genome Browser (http://genome.ucsc.edu/) based on the information extracted from Rat Genome Database – RGD (http://rgd.mcw.edu/) and Mouse Genome Informatics (MGI) resource (http://www.informatics.jax.org/). CpG islands (definition based on Gardiner-Garden and Frommer criteria) (12) were identified and visualised using the UCSC Genome Browser. Conservation score statistics (PhastCons estimate) in the placental mammals (17 species plus human) was calculated based on a probabilistic two-state phylogenetic hidden Markov model (13). Functional potential of the candidate regions was examined using evolutionary and sequence pattern extraction through reduced representations (ESPERR) method for identification of non-neutral *cis*-regulatory DNA elements (14).

To examine in more detail associations between mean 24-hour BP and genes previously linked to SBP and DBP we identified such genes using the Gene Ontology Annotation Database (GOA) (http://www.ebi.ac.uk/GOA/downloads.html). The details of this GOA-based strategy are shown in **Table S1**.

Identification of processes associated with mean 24-hour SBP and DBP was conducted using Gene set-based analysis of polymorphisms (GeSBAP) web interface (15). GeSBAP implements a

pathwayomic paradigm (examination of blocks of functionally related genes rather than single molecules) in relation to data from large scale SNP-based genotyping experiments. The major computational algorithm of this programme screens for gene sets enrichments associated with the ranked value of statistical measure (–log P-value from the association analysis) and corrects for multiple testing by integrated false discovery rate computation (15). Gene ontology (GO) database was used as a reference repository of functionally annotated biological processes.

50K IBC array genotyping in GRAPHIC: Reasons for exclusion of SNPs prior to analysis

Of 49094 genotyped SNPs, 1775 were excluded because they belonged to admixture and ancestry informative control markers. Duplicate SNPs and those identified as copy number variants (n=106) were also removed. Additional filters resulted in exclusion of 13636 further SNPs because of low (<0.01) MAF (n=12443), location on sex chromosomes (n=638), low (<90%) genotyping call rate (n=424), >10 Mendelian errors suggestive of poor genotyping quality (which was possible to check because of the family-based structure of GRAPHIC) (n=20), Hardy-Weinberg equilibrium violation (p<0.0001) (n=107), and lack of unambiguous rs identification number (n=4).

rs13306560, rs17367504 and rs11801879 – conditional analysis

To examine inter-relationships between the associations with mean 24-hour DBP for rs13306560 and rs17367504 we undertook a conditional analysis. When genotypes at rs13306560 were added as a covariate to the regression analysis the association between mean 24-hour DBP and rs17367504 was largely abolished (P=0.0519). On the other hand, the association of rs13306560 remained highly significant when conditioned on rs17367504 ($P=9.9x10^{-5}$). This indicated an inter-relationship despite the low r^2 between the two SNPs. Further examination showed that D' coefficient between the two SNPs was high (0.99) and that there was almost entire concordance of genotypes between carriers of the minor allele for rs13306560 and a proportion of individuals carrying the minor allele for rs17367504. This suggests that the association with mean 24-hour DBP at rs17367504 is largely driven by its relationship with rs13306560. Simulation studies assuming a causal SNP with a MAF of 5% and a marker SNP with a MAF of 16% and an r^2 of 0.25, with similar P-values to those seen for rs13306560 and rs17367504 (for their individual associations), confirmed that in conditional analyses the P-values for each changed exactly in the way that was observed in our study. Further conditional analysis indicated that, similar to rs17367504, the association of rs11801879 with mean 24-hour DBP was driven by its linkage disequilibrium with rs13306560 (P=0.0482 when adjusted for 13306560).

References:

1. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Glessner JT, Galver L, Barrett JC, Grant SF, Farlow DN, Chandrupatla HR, Hansen M, Ajmal S, Papanicolaou GJ, Guo Y, Li M, Derohannessian S, de Bakker PI, Bailey SD, Montpetit A, Edmondson AC, Taylor K, Gai X, Wang SS, Fornage M, Shaikh T, Groop L, Boehnke M, Hall AS, Hattersley AT, Frackelton E, Patterson N, Chiang CW, Kim CE, Fabsitz RR, Ouwehand W, Price AL, Munroe P, Caulfield M, Drake T, Boerwinkle E, Reich D, Whitehead AS, Cappola TP, Samani NJ, Lusis AJ, Schadt E, Wilson JG, Koenig W, McCarthy MI, Kathiresan S, Gabriel SB, Hakonarson H, Anand SS, Reilly M, Engert JC, Nickerson DA, Rader DJ, Hirschhorn JN, Fitzgerald GA. Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. *PLoS One*. 2008;3:e3583.
2. Firmann M, Mayor V, Vidal PM, Bochud M, Pecoud A, Hayoz D, Paccaud F, Preisig M, Song KS, Yuan X, Danoff TM, Stirnadel HA, Waterworth D, Mooser V, Waeber G, Vollenweider P. The CoLaus study: A population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. *BMC Cardiovasc Disord*. 2008;8:6.

3. Tomaszewski M, Charchar FJ, Barnes T, Gawron-Kiszka M, Sedkowska A, Podolecka E, Kowalczyk J, Rathbone W, Kalarus Z, Grzeszczak W, Goodall AH, Samani NJ, Zukowska-Szczechowska E. A common variant in low-density lipoprotein receptor-related protein 6 gene (LRP6) is associated with LDL-cholesterol. *Arterioscler Thromb Vasc Biol*. 2009;29:1316-1321.
4. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Boudier HA, Zanchetti A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Erdine S, Kiowski W, Agabiti-Rosei E, Ambrosioni E, Lindholm LH, Viigimaa M, Adamopoulos S, Bertomeu V, Clement D, Farsang C, Gaita D, Lip G, Mallion JM, Manolis AJ, Nilsson PM, O'Brien E, Ponikowski P, Redon J, Ruschitzka F, Tamargo J, van Zwieten P, Waeber B, Williams B. 2007 guidelines for the management of arterial hypertension: The task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens*. 2007;25:1105-1187.
5. Almasy L, Blangero J. Multipoint quantitative trait linkage analysis in general pedigrees. *Am J Hum Genet.* 1998;62:1198-1211.
6. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559-575.
7. Levy D, DeStefano AL, Larson MG, O'Donnell CJ, Lifton RP, Gavras H, Cupples LA, Myers RH. Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the Framingham Heart Study. *Hypertension*. 2000;36:477-483.
8. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: Antihypertensive therapy and systolic blood pressure. *Stat Med*. 2005;24:2911-35.
9. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, Najjar SS, Zhao JH, Heath SC, Eyheramendy S, Papadakis K, Voight BF, Scott LJ, Zhang F, Farrall M, Tanaka T, Wallace C, Chambers JC, Khaw KT, Nilsson P, van der Harst P, Polidoro S, Grobbee DE, Onland-Moret NC, Bots ML, Wain LV, Elliott KS, Teumer A, Luan J, Lucas G, Kuusisto J, Burton PR, Hadley D, McArdle WL, Wellcome Trust Case Control Consortium, Brown M, Dominiczak A, Newhouse SJ, Samani NJ, Webster J, Zeggini E, Beckmann JS, Bergmann S, Lim N, Song K, Vollenweider P, Waeber G, Waterworth DM, Yuan X, Groop L, Orho-Melander M, Allione A, Di Gregorio A, Guarrera S, Panico S, Ricceri F, Romanazzi V, Sacerdote C, Vineis P, Barroso I, Sandhu MS, Luben RN, Crawford GJ, Jousilahti P, Perola M, Boehnke M, Bonnycastle LL, Collins FS, Jackson AU, Mohlke KL, Stringham HM, Valle TT, Willer CJ, Bergman RN, Morken MA, Doring A, Gieger C, Illig T, Meitinger T, Org E, Pfeufer A, Wichmann HE, Kathiresan S, Marrugat J, O'Donnell CJ, Schwartz SM, Siscovick DS, Subirana I, Freimer NB, Hartikainen AL, McCarthy MI, O'Reilly PF, Peltonen L, Pouta A, de Jong PE, Snieder H, van Gilst WH, Clarke R, Goel A, Hamsten A, Peden JF, Seedorf U, Syvanen AC, Tognoni G, Lakatta EG, Sanna S, Scheet P, Schlessinger D, Scuteri A, Dorr M, Ernst F, Felix SB, Homuth G, Lorbeer R, Reffelmann T, Rettig R, Volker U, Galan P, Gut IG, Hercberg S, Lathrop GM, Zelenika D, Deloukas P, Soranzo N, Williams FM, Zhai G, Salomaa V, Laakso M, Elosua R, Forouhi NG, Volzke H, Uiterwaal CS, van der Schouw YT, Numans ME, Matullo G, Navis G, Berglund G, Bingham SA, Kooner JS, Connell JM, Bandinelli S, Ferrucci L, Watkins H, Spector TD, Tuomilehto J, Altshuler D, Strachan DP, Laan M, Meneton P, Wareham NJ, Uda M, Jarvelin MR, Mooser V, Melander O, Loos RJ, Elliott P, Abecasis GR, Caulfield M, Munroe PB. Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet*. 2009;44:666-676.
10. Storey JD, Tibshirani R. Statistical significance for genomewide studies. *Proc Natl Acad Sci U S A*. 2003;100:9440-9445.

11. Wang P, Dai M, Xuan W, McEachin RC, Jackson AU, Scott LJ, Athey B, Watson SJ, Meng F. SNP function portal: A web database for exploring the function implication of SNP alleles. *Bioinformatics*. 2006;22:e523-529.
12. Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. *J Mol Biol*. 1987;196:261-282.
13. Siepel A, Bejerano G, Pedersen JS, Hinrichs AS, Hou M, Rosenbloom K, Clawson H, Spieth J, Hillier LW, Richards S, Weinstock GM, Wilson RK, Gibbs RA, Kent WJ, Miller W, Haussler D. Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res*. 2005;15:1034-1050.
14. Taylor J, Tyekucheva S, King DC, Hardison RC, Miller W, Chiaromonte F. ESPERR: Learning strong and weak signals in genomic sequence alignments to identify functional elements. *Genome Res*. 2006;16:1596-1604.
15. Medina I, Montaner D, Bonifaci N, Pujana MA, Carbonell J, Tarraga J, Al-Shahrour F, Dopazo J. Gene set-based analysis of polymorphisms: Finding pathways or biological processes associated to traits in genome-wide association studies. *Nucleic Acids Res*. 2009;37:W340-344.

Table S1. Bioinformatics strategy to identify most relevant blood pressure genes on Illumina HumanCVD BeadChip.

Stage	Outcome
Identification of GO categories linked to “blood” and/or “pressure” terms in GOA database	336 GO processes
Identification of GO categories directly related to blood pressure and/or hypertension (based on contribution to blood pressure regulation and/or relevance to antihypertensive treatment) - independent assessment of 2 investigators	113 GO processes
Identification of genes with annotation to at least one GO category with the highest relevance to blood pressure regulation/hypertension	129 genes
Identification of genes annotated to at least one GO category with the highest relevance to blood pressure regulation/hypertension and present on Illumina HumanCVD BeadChip (after genotyping and quality control filters)	110 genes
Removal of genes with insufficient tagging – (Tier 3 of prioritizing)	105 genes

GO – Gene ontology; GOA - Gene Ontology Annotation Database, Tier 3 of prioritizing - genes of lower interest to the IBC consortium investigators represented only by non-synonymous and known functional variants with minor allele frequency >0.01

Table S2. Characteristics of the CoLaus cohort and Silesian Cardiovascular Study (SCS).

Variable	CoLaus	SCS
n	6188	807
M/F (%)	2937/3251 (47.5/52.5)	465/342 (57.6/42.4)
Age (years)	53.1±10.8	55.3±11.6
Body mass index (kg/m^2)	25.8±4.6	27.5±4.2
Clinic SBP (mmHg)	128.0±18.0	131.5±19.1
Clinic DBP (mmHg)	79.0±11.0	75.1±11.1
Antihypertensive treatment (%)	1131 (18.3)	478 (59.2)

Data are means and standard deviations or counts and percentages, M-men, F-women

Table S3. SNPs showing suggestive associations with mean 24-hour SBP in the GRAPHIC Study.

Ch	Locus	SNP	Location (bp)	Minor (coded)/ major allele	Minor allele frequency - parents	HWE - parents	β-coefficient (SE)	P-value	q-value
17	SPHK1	rs9892909	71884670	T/C	0.0977	1	-2.28 (0.50)	$5.3x10^{-6}$	0.0815
11	KCNQ1	rs2283210	2704481	G/C	0.0225	0.4039	-4.22 (0.97)	$1.5x10^{-5}$	0.1095
2	PDE1A	rs2623410	182880609	A/G	0.0122	1	-4.63 (1.13)	$4.3x10^{-5}$	0.2309
5	THBS4	rs443095	79398306	T/C	0.0176	0.2688	-4.08 (1.00)	$5.0x10^{-5}$	0.2309

SNP – single nucleotide polymorphism; Ch – chromosome; bp – base pair; HWE – statistical significance of Hardy-Weinberg equilibrium test; β-coefficient – estimated quantitative effect of each SNP minor allele copy on mean 24-hour SBP (adjusted for age, age^2, sex and antihypertensive medication); a negative β indicates lower BP in carriers of the minor allele; SE – standard error; P-value – statistical significance of association; q-value – false positive discovery rate

Table S4. SNPs showing significant or suggestive associations with mean 24-hour BP - analysis of association with clinic BP in the GRAPHIC Study.

Ch	Locus	SNP	Mean 24-hour SBP		Mean 24-hour DBP		Clinic SBP		Clinic DBP	
			β-coefficient (SE)	P-value	β-coefficient (SE)	P-value	β-coefficient (SE)	P-value	β-coefficient (SE)	P-value
1	USH2A	rs2797221	**-3.92 (0.79)**	**$8.6x10^{-7}$**	**-2.44 (0.98)**	**0.0131**	-4.20 (1.63)	0.0100	-4.13 (1.39)	0.0031
17	SPHK1	rs9892909	*-2.28 (0.50)*	*$5.3x10^{-6}$*	-1.25 (0.38)	0.0009	-3.07 (0.83)	0.0002	-1.91 (0.53)	0.0004
11	KCNQ1	rs2283210	*-4.22 (0.97)*	*$1.5x10^{-5}$*	-2.55 (0.73)	0.0005	-5.63 (1.42)	$7.2x10^{-5}$	-2.24 (1.20)	0.0616
2	PDE1A	rs2623410	*-4.63 (1.13)*	*$4.3x10^{-5}$*	-2.10 (0.98)	0.0329	-8.02 (1.74)	$4.1x10^{-6}$	-2.00 (1.00)	0.0459
5	THBS4	rs443095	*-4.08 (1.00)*	*$5.0x10^{-5}$*	-1.37 (0.64)	0.0333	-3.89 (1.64)	0.0178	-1.21 (1.09)	0.2663
1	PTGS2	rs4648310	-2.41 (0.84)	0.0039	*-2.26 (0.50)*	*$5.9x10^{-6}$*	-2.90 (1.44)	0.0433	-2.64 (1.04)	0.0108
1	NPPB	rs11801879	-2.06 (0.58)	0.0004	*-1.80 (0.40)*	*$7.7x10^{-6}$*	-1.93 (0.94)	0.0398	-1.11 (0.59)	0.0582
1	MTHFR	rs17037388	-1.62 (0.45)	0.0004	*-1.26 (0.28)*	*$9.1x10^{-6}$*	-2.11 (0.70)	0.0027	-2.03 (0.43)	$3.0x10^{-6}$
1	MTHFR	rs17037390	-1.56 (0.46)	0.0006	*-1.25 (0.28)*	*$1.2x10^{-5}$*	-2.11 (0.71)	0.0028	-2.03 (0.44)	$3.1x10^{-6}$
1	MTHFR	rs17367504	-1.67 (0.46)	0.0003	*-1.25 (0.29)*	*$1.8x10^{-5}$*	-2.24 (0.72)	0.0019	-2.03 (0.44)	$3.6x10^{-6}$
1	MTHFR	rs13306561	-1.57 (0.46)	0.0006	*-1.20 (0.29)*	*$3.0x10^{-5}$*	-2.13 (0.72)	0.0030	-1.94 (0.43)	$7.9x10^{-6}$
17	NLRP1	rs925596	1.75 (0.55)	0.0014	*1.44 (0.36)*	*$5.2x10^{-5}$*	2.34 (0.86)	0.0067	1.56 (0.50)	0.0020
8	CPA6	rs6993502	-2.83 (1.12)	0.0110	*-2.61 (0.64)*	*$5.2x10^{-5}$*	-5.11 (1.77)	0.0039	-3.84 (1.18)	0.0012
2	STAT1	rs2280232	0.92 (0.40)	0.0204	*1.11 (0.28)*	*$5.7x10^{-5}$*	-0.06 (0.65)	0.9216	0.40 (0.40)	0.3289
6	AGER	rs1035798	1.27 (0.39)	0.0013	*1.10 (0.28)*	*$6.6x10^{-5}$*	1.88 (0.63)	0.0030	0.66 (0.39)	0.0851
3	CISH	rs873985	-1.36 (0.48)	0.0041	*-1.27 (0.32)*	*$6.7x10^{-5}$*	-2.11 (0.73)	0.0038	-1.21 (0.46)	0.0084
17	NOG	rs12603626	-1.04 (0.36)	0.0037	*-0.95 (0.24)*	*$6.8x10^{-5}$*	-0.71 (0.55)	0.1949	-0.45 (0.32)	0.1648

SNP – single nucleotide polymorphism, Ch – chromosome, β-coefficient – estimated quantitative effect of each SNP minor allele copy on BP (adjusted for age, age^2, sex and antihypertensive medication); a negative β indicates lower BP in carriers of the minor allele, SE – standard error, P-value – statistical significance of association, bold - data presented in Table 2, italic - data presented in Supplementary Tables 3 and 5

Table S5. SNPs showing suggestive associations with mean 24-hour DBP in the GRAPHIC Study.

Ch	Locus	SNP	Location (bp)	Minor (coded) /major allele	Minor allele frequency – parents	HWE - parents	β-coefficient (SE)	P-value	q-value
1	PTGS2	rs4648310	184907148	G/A	0.0273	0.0363	-2.26 (0.50)	$5.9x10^{-6}$	0.0815
1	NPPB	rs11801879	11851406	C/T	0.0898	0.5639	-1.80 (0.40)	$7.7x10^{-6}$	0.0856
1	MTHFR	rs17037388	11780623	G/A	0.1680	0.5770	-1.26 (0.28)	$9.1x10^{-6}$	0.0864
1	MTHFR	rs17037390	11783430	A/G	0.1685	0.5769	-1.25 (0.28)	$1.2x10^{-5}$	0.0959
1	MTHFR	rs17367504	11785365	G/A	0.1650	0.7344	-1.25 (0.29)	$1.8x10^{-5}$	0.1166
1	MTHFR	rs13306561	11788391	C/T	0.1689	0.9113	-1.20 (0.29)	$3.0x10^{-5}$	0.1819
17	NLRP1	rs925596	5428823	G/A	0.1129	0.2117	1.44 (0.36)	$5.2x10^{-5}$	0.2309
8	CPA6	rs6993502	68740520	G/A	0.0220	0.3899	-2.61 (0.64)	$5.2x10^{-5}$	0.2309
2	STAT1	rs2280232	191559011	G/T	0.2261	1	1.11 (0.28)	$5.7x10^{-5}$	0.2368
6	AGER	rs1035798	32259200	T/C	0.2314	1	1.10 (0.28)	$6.6x10^{-5}$	0.2382
3	CISH	rs873985	50626755	G/A	0.1514	1	-1.27 (0.32)	$6.7x10^{-5}$	0.2382
17	NOG	rs12603626	52037330	A/C	0.3701	0.4609	-0.95 (0.24)	$6.8x10^{-5}$	0.2382

SNP – single nucleotide polymorphism, Ch – chromosome, bp – base pair, HWE – statistical significance of Hardy-Weinberg equilibrium test, β-coefficient – estimated quantitative effect of each SNP minor allele copy on mean 24-hour DBP (adjusted for age, age^2, sex and antihypertensive medication); a negative β indicates lower BP in carriers of the minor allele; SE – standard error, P-value – statistical significance of association, q-value – false positive discovery rate

Table S6. SNPs showing significant and suggestive associations with mean 24-hour SBP in the GRAPHIC Study – sensitivity analyses.

Chr	Locus	SNP	Location (bp)	Minor (coded)/ major allele	MAF	HWE	Reference analysis	Sensitivity analysis 1	Sensitivity analysis 2
1	USH2A	rs2797221	213979265	T/C	0.0112	1	β=-3.92, SE=0.79, $P=8.6x10^{-7}$	β=3.25, SE=0.76, $P=1.9x10^{-5}$	β=2.93, SE=0.75, $P=8.3x10^{-5}$
17	SPHK1	rs9892909	71884670	T/C	0.0977	1	β=-2.28, SE=0.50, $P=5.3x10^{-6}$	β=-1.82, SE=0.48, $P=1.5x10^{-4}$	β=-1.39, SE=0.48 $P=3.9x10^{-3}$
11	KCNQ1	rs2283210	2704481	G/C	0.0225	0.4039	β=-4.22, SE=0.97, $P=1.5x10^{-5}$	β=-4.23, SE=0.91, $3.1x10^{-6}$	β=-3.78, SE=0.96, $P=7.9x10^{-5}$
2	PDE1A	rs2623410	182880609	A/G	0.0122	1	β=-4.63, SE=1.13, $4.3x10^{-5}$	β=-4.04, SE=1.05, $P=1.2x10^{-4}$	β=-3.66, SE=1.05, $P=7.3x10^{-4}$
5	THBS4	rs443095	79398306	T/C	0.0176	0.2688	β=-4.08 SE=1.00, $P=5.0x10^{-5}$	β=-3.66, SE=1.05 $P=2.5x10^{-4}$	β=-3.46, SE=1.02, $P=6.8x10^{-4}$

Ch – chromosome; SNP – single nucleotide polymorphism; bp – base pair; MAF – minor allele frequency in parents; HWE – statistical significance of Hardy-Weinberg equilibrium test (in parents), Reference analysis - the primary association analysis, Sensitivity analysis 1 - analysis based on using antihypertensive treatment unadjusted values, Sensitivity analysis 2 - analysis based on exclusion of subjects on antihypertensive treatment, β – regression coefficient, SE – standard error

Table S7. SNPs showing significant and suggestive associations with mean 24-hour DBP in the GRAPHIC Study – sensitivity analysis.

Chr	Locus	SNP	Location (bp)	Minor (coded)/ major allele	MAF	HWE	Reference analysis	Sensitivity analysis 1	Sensitivity analysis 2
1	MTHFR/ CLCN6	rs13306560	11788770	A/G	0.0527	0.1963	β=-2.63, SE=0.46, $P=1.2x10^{-8}$	β=-2.38, SE=0.42, $P=1.6x10^{-8}$	β=-2.29, SE=0.42, $P=5.4x10^{-8}$
1	PTGS2	rs4648310	184907148	G/A	0.0273	0.0363	β=-2.26, SE=0.50, $P=5.9x10^{-6}$	β=-2.14, SE=0.48, $P=7.3x10^{-6}$	β=-1.77, SE=0.48, $P=5.9x10^{-6}$
1	NPPB	rs11801879	11851406	C/T	0.0898	0.5639	β=-1.80, SE=0.40, $P=7.7x10^{-6}$	β=-1.70, SE=0.38, $P=6.0x10^{-6}$	β=-1.62, SE=0.38, $P=1.8x10^{-5}$
1	MTHFR	rs17037388	11780623	G/A	0.168	0.577	β=-1.26, SE=0.28, $P=9.1x10^{-6}$	β=-1.19, SE=0.27, $P=8.8x10^{-6}$	β=-1.10, SE=0.28, $P=6.2x10^{-5}$
1	MTHFR	rs17037390	11783430	A/G	0.1685	0.5769	β=-1.25, SE=0.28, $1.2x10^{-5}$	β=-1.20, SE=0.27, $P=9.6x10^{-6}$	β=-1.11, SE=0.28, $P=6.7x10^{-5}$
1	MTHFR	rs17367504	11785365	G/A	0.165	0.7344	β=-1.25, SE= 0.29, $P=1.8x10^{-5}$	β=-1.16, SE=0.27, $P=2.1x10^{-5}$	β=-1.09, SE=0.28, $P=1.1x10^{-4}$
1	MTHFR	rs13306561	11788391	C/T	0.1689	0.9113	β=-1.20, SE=0.29, $P=3.0x10^{-5}$	β=-1.12, SE=0.27, $P=3.6x10^{-5}$	β=-1.06, SE=0.28, $P=1.7x10^{-4}$
17	NLRP1	rs925596	5428823	G/A	0.1129	0.2117	β=1.44, SE=0.36, $5.2x10^{-5}$	β=1.25, SE=0.36, $P=4.5x10^{-4}$	β=1.39, SE=0.36, $P=1.0x10^{-4}$
8	CPA6	rs6993502	68740520	G/A	0.022	0.3899	β=-2.61 SE=0.64, $P=5.2x10^{-5}$	β=-2.58, SE=0.60, $P=1.6x10^{-5}$	β=-2.53, SE=0.62, $P=5.2x10^{-5}$
2	STAT1	rs2280232	191559011	G/T	0.2261	1	β=1.11, SE=0.28, $P=5.7x10^{-5}$	β=1.03, SE=0.26 $7.1x10^{-5}$	β=1.04, SE=0.27, $P=1.0x10^{-4}$

6	AGER	rs1035798	32259200	T/C	0.2314	1	β=1.10, SE=0.28, P=6.6x10^{-5}	β=1.07, SE=0.26, 3.8x10^{-5}	β=1.01, SE=0.27, P=2.1x10^{-4}
3	CISH	rs873985	50626755	G/A	0.1514	1	β=-1.27, SE= 0.32, P=6.7x10^{-5}	β=-1.12, SE=0.30, P=1.0x10^{-4}	β=-1.29, SE=0.30 P=1.8x10^{-5}
17	NOG	rs12603626	52037330	A/C	0.3701	0.4609	β=-0.95 SE=0.24 P=6.8x10^{-5}	β=-0.94, SE=0.23, P=3.4x10^{-5}	β=-0.77, SE=0.23, P=8.8x10^{-4}

Ch – chromosome; SNP – single nucleotide polymorphism; bp – base pair; MAF – minor allele frequency in parents; HWE – statistical significance of Hardy-Weinberg equilibrium test (in parents); Reference analysis - the primary association analysis, Sensitivity analysis 1 - analysis based on using antihypertensive treatment unadjusted values, Sensitivity analysis 2 - analysis based on exclusion of subjects on antihypertensive treatment, β – regression coefficient, SE – standard error

Table S8. SNPs showing significant or suggestive associations with mean 24-hour BP in the GRAPHIC Study - analysis of association with clinic BP in CoLaus cohort.

Ch	Locus	SNP	Minor (coded)/ major allele	MAF	HWE	r^2hat	Clinic SBP		Clinic DBP	
							β-coefficient (SE)	P-value	β-coefficient (SE)	P-value
1	USH2A	rs2797221	T/C	0.0162	0.1101	0.96	1.76 (1.38)	0.2020	1.44 (0.89)	0.1064
17	SPHK1	rs9892909	T/C	0.1182	0.0452	0.83	-0.14 (0.54)	0.7923	0.08 (0.35)	0.8086
11	KCNQ1	rs2283210	G/C	0.0087	1.0000	0.58	-2.06 (2.30)	0.3717	-0.88 (1.48)	0.5502
2	PDE1A	rs2623410	T/C	0.0107	0.0922	0.61	2.41 (2.22)	0.2783	0.88 (1.43)	0.5357
5	THBS4	rs443095	T/C	0.0257	0.4005	1.00	-1.93 (1.10)	0.0787	-0.65 (0.71)	0.3564
1	NPPB	rs11801879	C/T	0.0842	0.6143	0.73	-0.11 (0.72)	0.8771	-0.09 (0.46)	0.8532
1	MTHFR	rs17037390	A/G	0.1422	0.1606	1.00	-0.67 (0.50)	0.1870	-0.59 (0.32)	0.0702
1	MTHFR	rs17367504	G/A	0.1419	0.1774	1.00	-0.66 (0.50)	0.1938	-0.57 (0.32)	0.0778
1	MTHFR	rs13306561	C/T	0.1603	0.0958	0.88	-0.61 (0.50)	0.2284	-0.55 (0.32)	0.0909
17	NLRP1	rs925596	G/A	0.1191	0.5246	0.70	-0.13 (0.58)	0.8159	-0.19 (0.37)	0.6016
8	CPA6	rs6993502	G/A	0.0254	1.0000	1.00	0.96 (1.10)	0.3860	0.44 (0.71)	0.5349
2	STAT1	rs2280232	G/T	0.3339	0.8354	0.69	0.27 (0.41)	0.5123	0.26 (0.26)	0.3121
6	AGER	rs1035798	T/C	0.2184	0.3473	0.96	0.47 (0.43)	0.2730	0.40 (0.28)	0.1433
3	CISH	rs873985	G/A	0.1458	0.4414	1.00	-0.22 (0.49)	0.6607	0.07 (0.32)	0.8340
17	NOG	rs12603626	A/C	0.3544	0.6267	0.99	0.31 (0.37)	0.3967	0.04 (0.24)	0.8578

SNP – single nucleotide polymorphism, Ch – chromosome, MAF – minor allele frequency, HWE – level of statistical significance of Hardy-Weinberg equilibrium test, r^2hat – imputation accuracy coefficient, β-coefficient – estimated quantitative effect of each SNP minor allele copy on BP (adjusted for age, age^2, sex and antihypertensive medication); a negative β indicates lower BP in carriers of the minor allele, SE – standard error, P-value – level of statistical significance, information on PTGS2 rs4648310 and MTHFR rs17037388 SNPs was not available in CoLaus Study

Table S9. Association between rs13306560 and mean 24-hour DBP in the GRAPHIC Study – body mass index-related sensitivity analysis.

SNP	Minor (coded) allele	Phenotype	Covariates in the model	β (SE)	P-value
rs13306560	A	mean 24-hour DBP	age,age^2, sex	-2.63 (0.46)	1.2×10^{-8}
			age,age^2, sex, body mass index	-2.59 (0.47)	3.3×10^{-8}

SNP – single nucleotide polymorphism; β – estimated quantitative effect of each minor allele copy of rs13306560 on adjusted (for antihypertensive treatment effect and other covariates) mean 24-hour DBP, SE – standard error; P-value – level of statistical significance

Table S10. The most significant associations between mean 24-hour SBP and SNPs in 105 most relevant BP candidate genes.

No	Ch	Gene symbol	Total number of SNPs	Top SNP	Location (bp)	Minor (coded) allele	Minor allele frequency - parents	HWE - parents	β-coefficient	Standard error	P-value	q-value
1	17	ACE	24	rs4295	58910030	G	0.3855	1	-0.52	0.34	0.1309	0.9436
2	16	ACSM3	8	rs2301770	20709673	T	0.0742	0.6477	-0.84	0.66	0.2074	0.9515
3	10	ACTA2	6	rs17114302	90699305	C	0.0645	1	1.21	0.81	0.1355	0.9436
4	12	ACVRL1	6	rs11169953	50590666	T	0.3096	0.3071	0.63	0.38	0.103	0.9374
5	11	ADM	5	rs11042727	10289959	G	0.0752	0.8232	-1.11	0.65	0.0862	0.9368
6	1	ADORA3	35	rs2800889	111844255	T	0.022	1	1.33	0.94	0.159	0.9438
7	8	ADRA1A	82	rs2644628	26770589	T	0.0518	0.7494	2.89	0.89	0.0011	0.5473
8	5	ADRA1B	13	rs2030373	159280059	T	0.2439	0.9325	-0.66	0.39	0.0945	0.9374
9	20	ADRA1D	14	rs8183794	4158448	T	0.1772	0.5918	-1.13	0.42	0.0073	0.7503
10	10	ADRA2A	14	rs17186196	112821777	G	0.0815	0.0186	-1.2	0.59	0.0406	0.9066
11	2	ADRA2B	5	rs29000579	96140615	G	0.0249	0.4722	1.43	1.03	0.1652	0.9462
12	4	ADRA2C	17	rs4916612	3719953	A	0.0903	0.4505	2.14	0.69	0.002	0.5731
13	10	ADRB1	21	rs7076938	115779365	C	0.2778	0.9379	-0.62	0.4	0.1274	0.9436
14	5	ADRB2	12	rs33942282	148189011	A	0.0352	1	-1.73	1.1	0.1161	0.9429
15	8	ADRB3	6	rs28434339	37944313	T	0.0289	0.578	2.09	1	0.0356	0.8928
16	11	ADRBK1	4	rs12274774	66803077	A	0.0122	1	1.53	1.18	0.1937	0.9512
17	1	AGT	44	rs11568030	228913466	A	0.0513	0.105	-1.28	0.69	0.0641	0.9327
18	3	AGTR1	51	rs12695903	149932302	A	0.0537	0.5292	-1.47	0.58	0.0119	0.7764
19	1	AGTRAP	9	rs4073395	11723290	T	0.3203	0.9428	-0.77	0.36	0.033	0.8802
20	11	AGTRL1	3	rs11544374	56761153	A	0.2678	0.5239	0.45	0.39	0.2454	0.9561
21	19	APOE	7	rs8106922	50093506	G	0.3925	0.3586	-0.17	0.36	0.6349	0.9814
22	12	ATP2A2	5	rs1860561	109267624	A	0.2227	0.2058	0.55	0.42	0.1929	0.9509
23	20	AVP	11	rs2422840	3024384	G	0.2177	0.0099	-0.69	0.41	0.0937	0.9374
24	12	AVPR1A	4	rs1042615	61830476	T	0.4329	1	0.74	0.36	0.0384	0.8979
25	14	BDKRB2	29	rs4905459	95741884	A	0.0816	0.6741	-1.33	0.61	0.0281	0.8779
26	2	BMPR2	10	rs17199235	203128104	G	0.1235	0.3852	0.81	0.52	0.1185	0.9436
27	5	CARTPT	7	rs10515114	71049590	C	0.0991	0.162	-0.44	0.61	0.4734	0.9750
28	3	CAV3	24	rs2268484	8748950	C	0.2769	0.5851	-0.75	0.38	0.0505	0.9156
29	14	CMA1	6	rs1956917	24050332	T	0.3569	0.276	-0.49	0.37	0.1849	0.9494

30	7	COL1A2	28	rs369982	93886418	A	0.4404	0.2047	-0.75	0.33	0.0237	0.8558
31	4	CORIN	59	rs16860440	47298612	G	0.0879	0.4321	1.16	0.62	0.0614	0.9310
32	11	CSRP3	11	rs1346118	19176075	C	0.166	0.2608	0.7	0.48	0.1463	0.9436
33	20	CST3	6	rs911122	23573746	G	0.4058	0.4357	-0.63	0.37	0.0859	0.9368
34	14	CTSG	8	rs1885597	24112908	G	0.457	0.285	-0.62	0.35	0.0786	0.9336
35	8	CYP11B1	2	rs5297	143952659	C	0.083	0.6798	0.39	0.61	0.5231	0.9786
36	8	CYP11B2	8	rs11781082	143996903	A	0.2837	0.2186	0.41	0.38	0.2835	0.9589
37	1	CYP2J2	6	rs10493270	60161262	A	0.0864	1	1.11	0.66	0.0917	0.9374
38	2	DES	7	rs907684	220007516	C	0.0366	0.6422	-1.44	0.86	0.0915	0.9374
39	19	DMPK	5	rs2854300	50966232	G	0.041	0.4094	-1.07	0.89	0.2294	0.9545
40	11	DRD2	19	rs11214611	112835429	G	0.1479	0.4578	0.96	0.53	0.0666	0.9327
41	3	DRD3	18	rs3732783	115373479	G	0.0503	0.7385	-1.32	0.79	0.0944	0.9374
42	1	ECE1	17	rs213051	21494123	G	0.3125	1	-0.96	0.39	0.0129	0.7796
43	6	EDN1	27	rs4714384	12405839	C	0.306	0.1622	0.66	0.39	0.0886	0.9374
44	1	EDN2	27	rs4660541	41727031	T	0.2669	0.3798	-0.93	0.38	0.016	0.8174
45	20	EDN3	10	rs11570248	57307333	G	0.0855	0.6907	1.22	0.59	0.0401	0.9066
46	4	EDNRA	17	rs6822565	148656962	C	0.2319	0.3343	1.29	0.38	0.0007	0.5238
47	13	EDNRB	14	rs3027129	77384402	T	0.0625	0.7916	-1.01	0.69	0.1451	0.9436
48	2	EPAS1	38	rs7579899	46391108	A	0.4062	0.6978	-0.94	0.36	0.0083	0.7545
49	8	EPHX2	12	rs11780471	27400636	A	0.0674	0.4541	0.8	0.73	0.2738	0.9581
50	1	ACOT11	47	rs11589310	54785469	T	0.21	0.3473	0.45	0.42	0.279	0.9581
51	6	FOXC1	12	rs2745593	1548401	G	0.4922	0.7546	-0.42	0.34	0.2128	0.9515
52	16	FOXC2	8	rs4843165	85162542	T	0.2696	0.9367	-0.91	0.36	0.0119	0.7764
53	17	GAA	5	rs12452616	75704676	A	0.2778	0.2425	-0.21	0.39	0.5873	0.9803
54	14	GCH1	6	rs4411417	54390313	C	0.2021	0.5608	-0.47	0.43	0.2727	0.9581
55	1	GCLM	5	rs7533596	94120717	A	0.373	0.7386	-0.25	0.36	0.498	0.9756
56	12	GNB3	9	rs5439	6819143	C	0.0911	0.4502	-0.43	0.7	0.5383	0.9790
57	3	GPX1	2	rs1800668	49370761	T	0.3047	0.4171	0.34	0.39	0.3841	0.9692
58	11	HBB	6	rs11036351	5202576	T	0.4604	0.2849	0.76	0.35	0.0329	0.8802
59	5	HBEGF	8	rs1042184	139706145	T	0.0745	0.258	-0.47	0.62	0.4494	0.9722
60	15	HCN4	16	rs7172808	71450685	G	0.1611	0.729	1.12	0.52	0.0316	0.8802
61	22	HMOX1	15	rs17883419	34121971	T	0.044	1	-1.13	0.73	0.1207	0.9436
62	1	ADORA3	35	rs2789535	111884087	G	0.4854	1	0.18	0.35	0.6143	0.9811
63	16	HSD11B2	2	rs6499129	66015752	C	0.0991	0.0344	1.3	0.62	0.0359	0.8939
64	1	HSPB7	6	rs1056207	16217414	T	0.0883	0.6928	1.15	0.63	0.0671	0.9327

65	12	IGF1	34	rs17882264	101395486	G	0.0894	0.0524	-1.24	0.54	0.022	0.8521
66	21	KCNE1	28	rs1892593	34748230	A	0.3213	0.1516	-0.61	0.37	0.0984	0.9374
67	21	KCNE2	11	rs12626687	34655160	A	0.3047	0.5557	-0.97	0.37	0.008	0.7545
68	7	KCNH2	11	rs3807373	150299654	T	0.2988	0.3709	0.94	0.39	0.0174	0.8265
69	12	KCNJ8	2	rs829064	21831775	G	0.167	0.7363	0.31	0.48	0.5144	0.9780
70	11	KCNQ1	195	rs2283210	2704481	G	0.0225	0.4039	-4.22	0.97	$1.5^{x}10^{-5}$	0.1095
71	3	KNG1	23	rs1392952	187913716	T	0.1245	1	-0.84	0.54	0.1214	0.9436
72	3	MYL3	6	rs6768627	46870380	T	0.0669	1	1.04	0.71	0.1428	0.9436
73	3	NISCH	10	rs17263770	52494894	C	0.1294	0.4063	0.64	0.51	0.2094	0.9515
74	17	NOS2A	67	rs8081248	23106091	A	0.4455	0.4471	-0.62	0.38	0.1024	0.9374
75	7	NOS3	26	rs2853796	150334848	G	0.4692	0.802	-1.02	0.35	0.0038	0.6720
76	1	NPPA	10	rs198372	11832101	A	0.1309	0.4093	-1.48	0.49	0.0026	0.5955
77	1	NPPB	11	rs11801879	11851406	C	0.0898	0.5639	-2.06	0.58	0.0004	0.5028
78	2	NPPC	14	rs11679292	232507906	T	0.0195	1	-2.77	0.84	0.0009	0.5241
79	1	NPR1	6	rs7543790	151936937	T	0.0567	0.3694	1.12	0.75	0.1317	0.9436
80	9	NPR2	6	rs1407295	35795161	A	0.0836	0.8378	1	0.71	0.1585	0.9437
81	5	NPR3	22	rs1421811	32750027	C	0.4007	0.1033	-0.64	0.34	0.0587	0.9279
82	7	NPY	8	rs16135	24294445	T	0.0742	0.2563	-0.82	0.72	0.2598	0.9578
83	4	NPY1R	6	rs12510421	164469886	C	0.104	0.0021	1.07	0.6	0.0759	0.9327
84	4	NPY2R	7	rs10212868	156346717	C	0.3926	0.359	-1	0.35	0.0042	0.6886
85	12	P2RX4	5	rs7961979	120155644	A	0.1338	0.6851	-0.67	0.5	0.1849	0.9494
86	9	PCSK5	156	rs1538843	77791088	G	0.0758	0.508	-1.5	0.58	0.0096	0.7607
87	6	PLN	3	rs9481825	118982785	A	0.1672	0.3129	1.17	0.46	0.0109	0.7755
88	2	POMC	9	rs6545975	25238989	C	0.4053	0.3993	-0.59	0.34	0.0842	0.9368
89	3	PPARG	59	rs4684846	12313849	G	0.249	0.6763	-1.33	0.41	0.0011	0.5473
90	9	PTGS1	20	rs10306108	124171301	C	0.0654	1	1.29	0.71	0.0677	0.9327
91	1	PTGS2	24	rs4648310	184907148	G	0.0273	0.0363	-2.41	0.84	0.0039	0.6786
92	1	REN	28	rs11571084	202395455	A	0.0411	1	1.58	0.91	0.0819	0.9368
93	3	SCN5A	43	rs6797133	38631037	A	0.3916	1	0.59	0.34	0.0861	0.9368
94	7	SEMA3C	2	rs17154557	80323912	A	0.0562	0.1273	0.57	0.88	0.5149	0.9780
95	21	SOD1	4	rs9974610	31940240	G	0.1763	0.1313	0.6	0.5	0.2305	0.9550
96	6	SOD2	21	rs2758352	160042911	A	0.2312	0.6609	1.05	0.41	0.0108	0.7755
97	7	SRI	8	rs4728737	87677464	G	0.0508	1	1.66	0.95	0.0812	0.9368
98	19	TBXA2R	8	rs8105780	3543171	T	0.3495	0.4095	0.24	0.38	0.5214	0.9786
99	1	TGFB2	25	rs17047682	216589648	G	0.0674	1	1.42	0.67	0.0342	0.8855

100	11	TH	6	rs6356	2147527	A	0.3594	0.3775	0.39	0.39	0.3151	0.9659
101	19	TNNI3	9	rs3729838	60360122	C	0.1799	0.9156	0.5	0.48	0.2967	0.9602
102	1	TNNT2	13	rs2799691	199614966	C	0.2544	0.0699	0.53	0.39	0.173	0.9466
103	15	TPM1	10	rs4075583	61127280	G	0.3465	0.0532	0.75	0.35	0.0308	0.8802
104	1	UTS2	12	rs707476	7840693	T	0.3105	0.1257	-0.76	0.38	0.044	0.9069
105	7	WNT2	11	rs39310	116739977	C	0.2681	0.233	-0.67	0.38	0.0768	0.9327

Ch – chromosome, top SNP – single nucleotide polymorphism in a given gene with the most significant association with mean 24-hour SBP, bp – base pair, HWE - statistical significance of Hardy-Weinberg equilibrium test, β-coefficient - estimated quantitative effect of each SNP minor (coded) allele copy on mean 24-hour SBP (adjusted for age, age^2, sex and BP-lowering medication), P-value –statistical significance, q-value – false positive discovery rate

Table S11. The most significant associations between mean 24-hour DBP and SNPs in 105 most relevant BP candidate genes**.**

No	Ch	Gene symbol	Total number of SNPs	Top SNP	Location (bp)	Minor (coded) allele	Minor allele frequency - parents	HWE - parents	β-coefficient	Standard error	P-value	q-value
1	17	ACE	24	rs4611524	58945384	T	0.4243	0.0474	-0.48	0.23	0.0398	0.9061
2	16	ACSM3	8	rs2301770	20709673	T	0.0742	0.6477	-0.47	0.46	0.3138	0.9654
3	10	ACTA2	6	rs17114302	90699305	C	0.0645	1	0.53	0.49	0.2848	0.9597
4	12	ACVRL1	6	rs11169953	50590666	T	0.3096	0.3071	0.5	0.26	0.0542	0.9240
5	11	ADM	5	rs11042727	10289959	G	0.0752	0.8232	-0.89	0.45	0.0494	0.9150
6	1	ADORA3	35	rs2800889	111844255	T	0.022	1	0.44	0.79	0.5742	0.9798
7	8	ADRA1A	82	rs17296809	26762260	C	0.061	1	1.59	0.49	0.0011	0.5473
8	5	ADRA1B	13	rs2030373	159280059	T	0.2439	0.9325	-0.49	0.26	0.0557	0.9240
9	20	ADRA1D	14	rs8183794	4158448	T	0.1772	0.5918	-0.35	0.3	0.244	0.9560
10	10	ADRA2A	14	rs17186196	112821777	G	0.0815	0.0186	-0.5	0.43	0.2421	0.9558
11	2	ADRA2B	5	rs29000579	96140615	G	0.0249	0.4722	1.05	0.66	0.1092	0.9386
12	4	ADRA2C	17	rs4916613	3723384	T	0.04	0.4027	2.01	0.68	0.003	0.6368
13	10	ADRB1	21	rs7076938	115779365	C	0.2778	0.9379	-0.49	0.28	0.0803	0.9358
14	5	ADRB2	12	rs33942282	148189011	A	0.0352	1	-0.88	0.72	0.2181	0.9519
15	8	ADRB3	6	rs28434339	37944313	T	0.0289	0.578	0.62	0.67	0.3577	0.9677
16	11	ADRBK1	4	rs12274774	66803077	A	0.0122	1	0.28	0.91	0.7554	0.9846
17	1	AGT	44	rs11568030	228913466	A	0.0513	0.105	-0.36	0.49	0.4705	0.9750
18	3	AGTR1	51	rs12695903	149932302	A	0.0537	0.5292	-0.81	0.48	0.0933	0.9374
19	1	AGTRAP	9	rs11121816	11723065	G	0.3413	0.5316	0.68	0.36	0.0608	0.9466
20	11	AGTRL1	3	rs2282624	56758487	C	0.3242	0.6687	-0.4	0.4	0.3191	0.9706
21	19	APOE	7	rs8106922	50093506	G	0.3925	0.3586	-0.37	0.25	0.1369	0.9436
22	12	ATP2A2	5	rs1860561	109267624	A	0.2227	0.2058	0.3	0.27	0.2545	0.9561
23	20	AVP	11	rs2422840	3024384	G	0.2177	0.0099	-0.26	0.28	0.346	0.9669
24	12	AVPR1A	4	rs1042615	61830476	T	0.4329	1	0.34	0.24	0.1537	0.9436
25	14	BDKRB2	29	rs4905459	95741884	A	0.0816	0.6741	-0.62	0.43	0.147	0.9436
26	2	BMPR2	10	rs17199235	203128104	G	0.1235	0.3852	0.53	0.36	0.1425	0.9436
27	5	CARTPT	7	rs10515114	71049590	C	0.0991	0.162	-0.28	0.42	0.5044	0.9766
28	3	CAV3	24	rs2268484	8748950	C	0.2769	0.5851	-0.41	0.26	0.1164	0.9429
29	14	CMA1	6	rs10220622	24046810	T	0.0635	0.5962	-0.64	0.62	0.3063	0.9410

30	7	COL1A2	28	rs369982	93886418	A	0.4404	0.2047	-0.45	0.22	0.0443	0.9069
31	4	CORIN	59	rs16860440	47298612	G	0.0879	0.4321	0.61	0.4	0.128	0.9436
32	11	CSRP3	11	rs1346118	19176075	C	0.166	0.2608	0.52	0.33	0.1095	0.9389
33	20	CST3	6	rs911122	23573746	G	0.4058	0.4357	-0.06	0.25	0.794	0.9859
34	14	CTSG	8	rs12878759	24115524	G	0.0391	0.1976	-1.38	0.83	0.0971	0.9310
35	8	CYP11B1	2	rs5297	143952659	C	0.083	0.6798	0.07	0.4	0.8698	0.9874
36	8	CYP11B2	8	rs11781082	143996903	A	0.2837	0.2186	0.48	0.25	0.048	0.9069
37	1	CYP2J2	6	rs10493270	60161262	A	0.0864	1	0.73	0.44	0.0964	0.9374
38	2	DES	7	rs907684	220007516	C	0.0366	0.6422	0.04	0.51	0.9409	0.9879
39	19	DMPK	5	rs2854300	50966232	G	0.041	0.4094	-0.73	0.58	0.2076	0.9515
40	11	DRD2	19	rs11214611	112835429	G	0.1479	0.4578	0.23	0.34	0.4963	0.9756
41	3	DRD3	18	rs3732783	115373479	G	0.0503	0.7385	-1.53	0.54	0.0047	0.7067
42	1	ECE1	17	rs213051	21494123	G	0.3125	1	-0.2	0.27	0.4579	0.9723
43	6	EDN1	27	rs4714384	12405839	C	0.306	0.1622	0.28	0.27	0.2982	0.9605
44	1	EDN2	27	rs4660541	41727031	T	0.2669	0.3798	-0.59	0.28	0.0348	0.8875
45	20	EDN3	10	rs11570248	57307333	G	0.0855	0.6907	1.01	0.37	0.0067	0.7461
46	4	EDNRA	17	rs6822565	148656962	C	0.2319	0.3343	0.92	0.28	0.0012	0.5561
47	13	EDNRB	14	rs3027129	77384402	T	0.0625	0.7916	-0.78	0.45	0.0842	0.9368
48	2	EPAS1	38	rs7579899	46391108	A	0.4062	0.6978	-0.22	0.24	0.3619	0.9679
49	8	EPHX2	12	rs11780471	27400636	A	0.0674	0.4541	0.18	0.49	0.7144	0.9834
50	1	ACOT11	47	rs11589310	54785469	T	0.21	0.3473	0.29	0.29	0.3291	0.9664
51	6	FOXC1	12	rs2745593	1548401	G	0.4922	0.7546	-0.02	0.24	0.9489	0.9879
52	16	FOXC2	8	rs4843165	85162542	T	0.2696	0.9367	-0.39	0.26	0.1217	0.9436
53	17	GAA	5	rs12452616	75704676	A	0.2778	0.2425	-0.15	0.26	0.5712	0.9796
54	14	GCH1	6	rs4411417	54390313	C	0.2021	0.5608	-0.05	0.3	0.8749	0.9874
55	1	GCLM	5	rs7533596	94120717	A	0.373	0.7386	0.27	0.25	0.2822	0.9586
56	12	GNB3	9	rs5439	6819143	C	0.0911	0.4502	-0.1	0.44	0.814	0.9859
57	3	GPX1	2	rs1800668	49370761	T	0.3047	0.4171	0.26	0.25	0.3037	0.9634
58	11	HBB	6	rs11036351	5202576	T	0.4604	0.2849	0.67	0.24	0.0049	0.7067
59	5	HBEGF	8	rs1042184	139706145	T	0.0745	0.258	-0.43	0.41	0.2915	0.9602
60	15	HCN4	16	rs7172808	71450685	G	0.1611	0.729	0.52	0.34	0.1237	0.9436
61	22	HMOX1	15	rs17883419	34121971	T	0.044	1	-0.69	0.55	0.208	0.9515
62	1	ADORA3	35	rs2789535	111884087	G	0.4854	1	-0.08	0.25	0.7562	0.9846
63	16	HSD11B2	2	rs6499129	66015752	C	0.0991	0.0344	0.68	0.42	0.1066	0.9374
64	1	HSPB7	6	rs1056207	16217414	T	0.0883	0.6928	0.55	0.44	0.2066	0.9515

65	12	IGF1	34	rs17882264	101395486	G	0.0894	0.0524	-0.46	0.37	0.2196	0.9520
66	21	KCNE1	28	rs1892593	34748230	A	0.3213	0.1516	-0.1	0.24	0.6963	0.9826
67	21	KCNE2	11	rs12626687	34655160	A	0.3047	0.5557	-0.59	0.25	0.0211	0.8460
68	7	KCNH2	11	rs3807373	150299654	T	0.2988	0.3709	0.29	0.26	0.2751	0.9581
69	12	KCNJ8	2	rs829064	21831775	G	0.167	0.7363	0.11	0.32	0.7343	0.9836
70	11	KCNQ1	195	rs2283210	2704481	G	0.0225	0.4039	-2.55	0.73	0.0005	0.5210
71	3	KNG1	23	rs1392952	187913716	T	0.1245	1	-0.22	0.37	0.5551	0.9790
72	3	MYL3	6	rs6768627	46870380	T	0.0669	1	0.73	0.48	0.1251	0.9436
73	3	NISCH	10	rs17263770	52494894	C	0.1294	0.4063	0.59	0.36	0.1071	0.9378
74	17	NOS2A	67	rs8081248	23106091	A	0.4455	0.4471	-0.26	0.25	0.2965	0.9602
75	7	NOS3	26	rs2853796	150334848	G	0.4692	0.802	-0.32	0.23	0.1611	0.9461
76	1	NPPA	10	rs198372	11832101	A	0.1309	0.4093	-1.19	0.33	0.0003	0.4853
77	1	NPPB	11	rs11801879	11851406	C	0.0898	0.5639	-1.8	0.4	$7.7^{x}10^{-6}$	0.0856
78	2	NPPC	14	rs11679292	232507906	T	0.0195	1	-1.78	0.64	0.0053	0.7067
79	1	NPR1	6	rs7543790	151936937	T	0.0567	0.3694	0.93	0.55	0.0883	0.9374
80	9	NPR2	6	rs1407295	35795161	A	0.0836	0.8378	0.88	0.46	0.055	0.9240
81	5	NPR3	22	rs1421811	32750027	C	0.4007	0.1033	-0.37	0.24	0.1144	0.9421
82	7	NPY	8	rs16135	24294445	T	0.0742	0.2563	0.1	0.47	0.8279	0.9861
83	4	NPY1R	6	rs12510421	164469886	C	0.104	0.0021	0.39	0.39	0.3188	0.9664
84	4	NPY2R	7	rs10213571	156346015	C	0.391	0.2645	-0.53	0.24	0.024	0.8558
85	12	P2RX4	5	rs7961979	120155644	A	0.1338	0.6851	-0.21	0.35	0.551	0.9790
86	9	PCSK5	156	rs1538843	77791088	G	0.0758	0.508	-0.79	0.39	0.042	0.9066
87	6	PLN	3	rs9481825	118982785	A	0.1672	0.3129	0.8	0.31	0.0099	0.7607
88	2	POMC	9	rs6545975	25238989	C	0.4053	0.3993	-0.26	0.23	0.2577	0.9574
89	3	PPARG	59	rs4684846	12313849	G	0.249	0.6763	-0.72	0.28	0.009	0.7557
90	9	PTGS1	20	rs10306108	124171301	C	0.0654	1	1.04	0.44	0.019	0.8330
91	1	PTGS2	24	rs4648310	184907148	G	0.0273	0.0363	-2.26	0.5	$5.9^{x}10^{-6}$	0.0815
92	1	REN	28	rs11571084	202395455	A	0.0411	1	0.71	0.58	0.2253	0.9540
93	3	SCN5A	43	rs6797133	38631037	A	0.3916	1	0.34	0.23	0.1352	0.9436
94	7	SEMA3C	2	rs17154557	80323912	A	0.0562	0.1273	0.27	0.58	0.6382	0.9814
95	21	SOD1	4	rs9974610	31940240	G	0.1763	0.1313	0	0.33	0.9943	0.9880
96	6	SOD2	21	rs2758352	160042911	A	0.2312	0.6609	0.66	0.28	0.018	0.8265
97	7	SRI	8	rs4728737	87677464	G	0.0508	1	0.81	0.57	0.1613	0.9461
98	19	TBXA2R	8	rs8105780	3543171	T	0.3495	0.4095	0.29	0.26	0.2696	0.9579
99	1	TGFB2	25	rs17047682	216589648	G	0.0674	1	0.85	0.47	0.0704	0.9327

100	11	TH	6	rs6356	2147527	A	0.3594	0.3775	0.18	0.25	0.4677	0.9742
101	19	TNNI3	9	rs3729838	60360122	C	0.1799	0.9156	0.29	0.32	0.3626	0.9679
102	1	TNNT2	13	rs2799691	199614966	C	0.2544	0.0699	0.17	0.26	0.525	0.9786
103	15	TPM1	10	rs4075583	61127280	G	0.3465	0.0532	0.34	0.23	0.1463	0.9436
104	1	UTS2	12	rs707476	7840693	T	0.3105	0.1257	-0.55	0.25	0.0296	0.8802
105	7	WNT2	11	rs39310	116739977	C	0.2681	0.233	-0.23	0.27	0.3822	0.9692

Ch – chromosome, top SNP – single nucleotide polymorphism in a given gene with the most significant association with mean 24-hour SBP, bp – base pair, HWE - statistical significance of Hardy-Weinberg equilibrium test, β-coefficient - estimated quantitative effect of each SNP minor (coded) allele copy on mean 24-hour DBP (adjusted for age, age^2, sex and BP-lowering medication), P-value –statistical significance, q-value – false positive discovery rate

Table S12. Analysis of SNPs identified in meta-analysis of genome-wide association studies and present on the 50K IBC array in relation to mean 24-hour BP in the GRAPHIC Study.

Chr	locus	GWAS leading SNP	Ref	GRAPHIC SNP	r^2	mean 24-hour SBP		mean 24-hour DBP	
						β-coefficient (SE)	P-value	β-coefficient (SE)	P-value
1	MTHFR	rs17367504	1	rs17367504	1	-1.67 (0.46)	0.0003	-1.25 (0.29)	$1.8x10^{-5}$
12	ATP2B1	rs11105354	2	rs11105354	1	-0.26 (0.48)	0.5797	-0.18 (0.34)	0.6012
12	SH2B3	rs3184504	2	rs3184504	1	0.88 (0.36)	0.0149	0.78 (0.24)	0.0013
12	C12orf30	rs17696736	2	rs17696736	1	0.48 (0.37)	0.1931	0.48 (0.24)	0.0471
15	CSK	rs1378942	1	rs2472304	0.96	0.33 (0.37)	0.3758	0.12 (0.25)	0.6292

Ch – chromosome, GWAS – genome-wide association scan, SNP – single nucleotide polymorphism, r^2 – linkage disequilibrium measure between a variant identified in GWAS and the SNP genotyped in the GRAPHIC, SBP – systolic blood pressure, DBP – diastolic blood pressure, β-coefficient – estimated quantitative effect of each SNP minor allele copy on mean 24-hour BP (adjusted for age, age^2, sex and BP-lowering medication), SE – standard error, P-value – statistical significance, Ref 1 – Nat Genet. 2009;44:666-676, Ref 2 – Nat Genet. 2009;41:677-687

Table S13. Gene ontology biological processes associated with mean 24-hour BP in the GRAPHIC Study - Gene set-based analysis of polymorphisms (GeSBAP).

GO symbol	GO biological process	mean 24-hour SBP – FDR	mean 24-hour DBP – FDR
GO:0007243	protein kinase cascade	0.0467	0.0405
GO:0042127	regulation of cell proliferation	0.0467	0.0423
GO:0006915	apoptosis	NS	0.0072
GO:0042981	regulation of apoptosis	NS	0.0072
GO:0008219	cell death	NS	0.0312

GO – gene ontology; FDR – statistical significance after correction for multiple testing, NS – non-significant

Table S14. Distribution of SNPs nominally associated with mean 24-hour BP at P<0.01 according to their characteristic and frequency - sensitivity analysis.

Descriptor	Illumina gene-centric array	SNPs associated with mean 24-hour SBP at p<0.01	p-value	SNPs associated with mean 24-hour DBP at p<0.01	P-value
All SNPs	33,577	443	-	424	-
Exonic SNPs	2,298 (6.8%)	31 (7%)	0.8490	40 (9.4%)	0.0420
Non-synonymous SNPs	1,634 (4.9%)	23 (5.2%)	0.7383	28 (6.6%)	0.1110
All SNPs with MAF<0.05	4,355 (13.0%)	79 (17.8%)	0.0035	84 (19.8%)	8.0×10^{-5}
Exonic SNPs with MAF<0.05 *	516 (11.8%)	7 (8.9%)	0.4857	10 (11.9%)	1.0
Non-synonymous SNPs with MAF<0.05*	406 (9.3%)	6 (7.6%)	0.8440	9 (10.7%)	0.5745
All SNPs with MAF<0.02	1,188 (3.5%)	32 (7.2%)	0.0002	39 (9.2%)	1.1×10^{-7}
Exonic SNPs with MAF<0.02†	162 (13.6%)	0	-	6 (15.4%)	0.8117
Non-synonymous SNPs with MAF<0.02†	128 (10.8%)	0	-	5 (12.8%)	0.6040

MAF – minor allele frequency, * percentage calculated in relation to total number of SNPs with MAF<0.05, † percentage calculated in relation to total number of SNPs with MAF<0.02

Figure S1. SNPs and mean 24-hour SBP and DBP in the GRAPHIC Study – distribution of nominal P-values from generalised estimating equations-based analysis across autosomes.

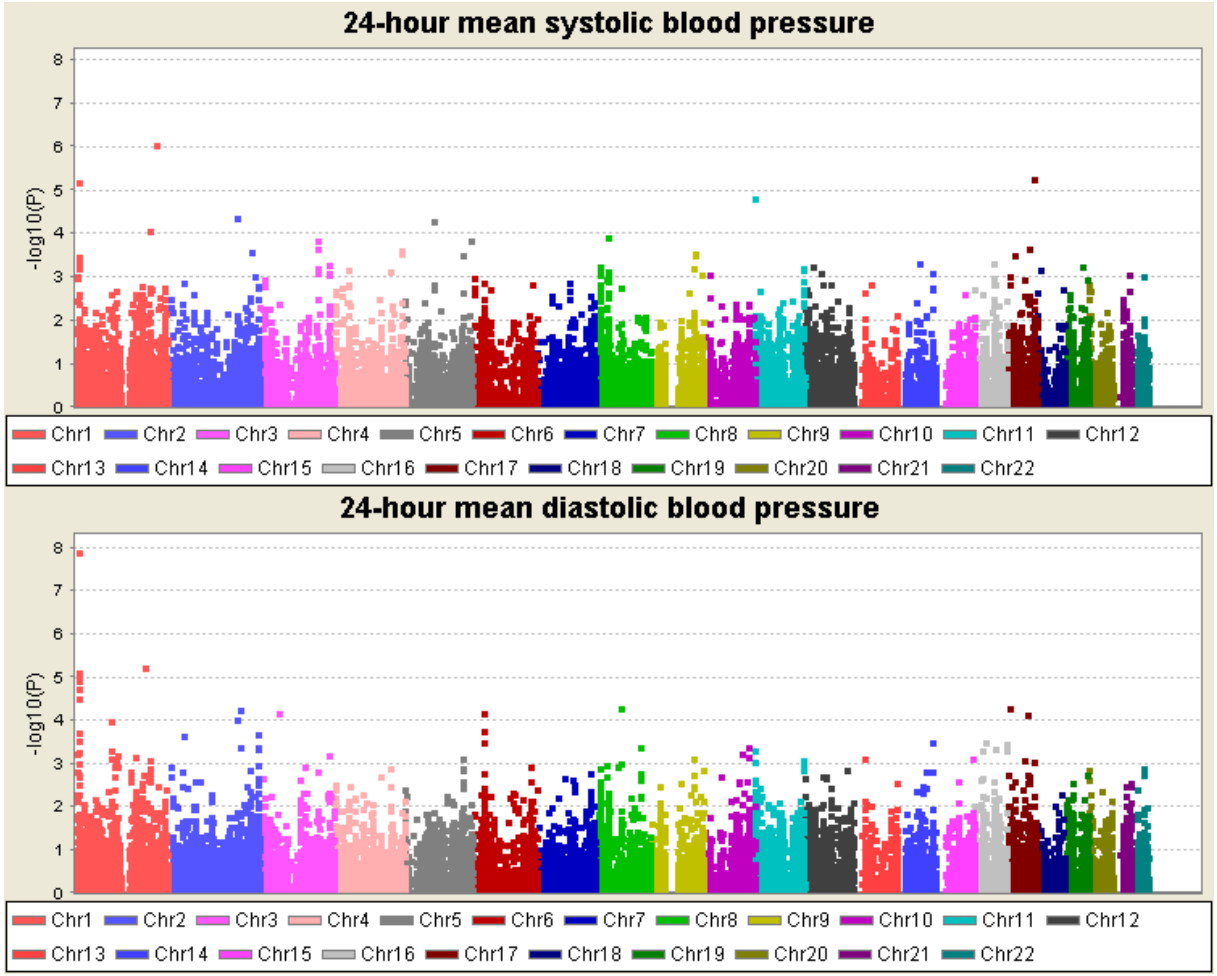

Figure S2. Power to detect associations with mean 24-hour SBP and DBP in the GRAPHIC study.
The upper panel shows the power estimates for mean 24-hour SBP to detect nominal associations (left side) and after Bonferroni correction (right side). The lower panel shows the same estimates for mean 24-hour DBP. In each case, lines represent power to detect a per allele effect of 0.5mm (orange), 1mm (green), 1.5mm (red), 2mm (purple) and 3mm (blue).

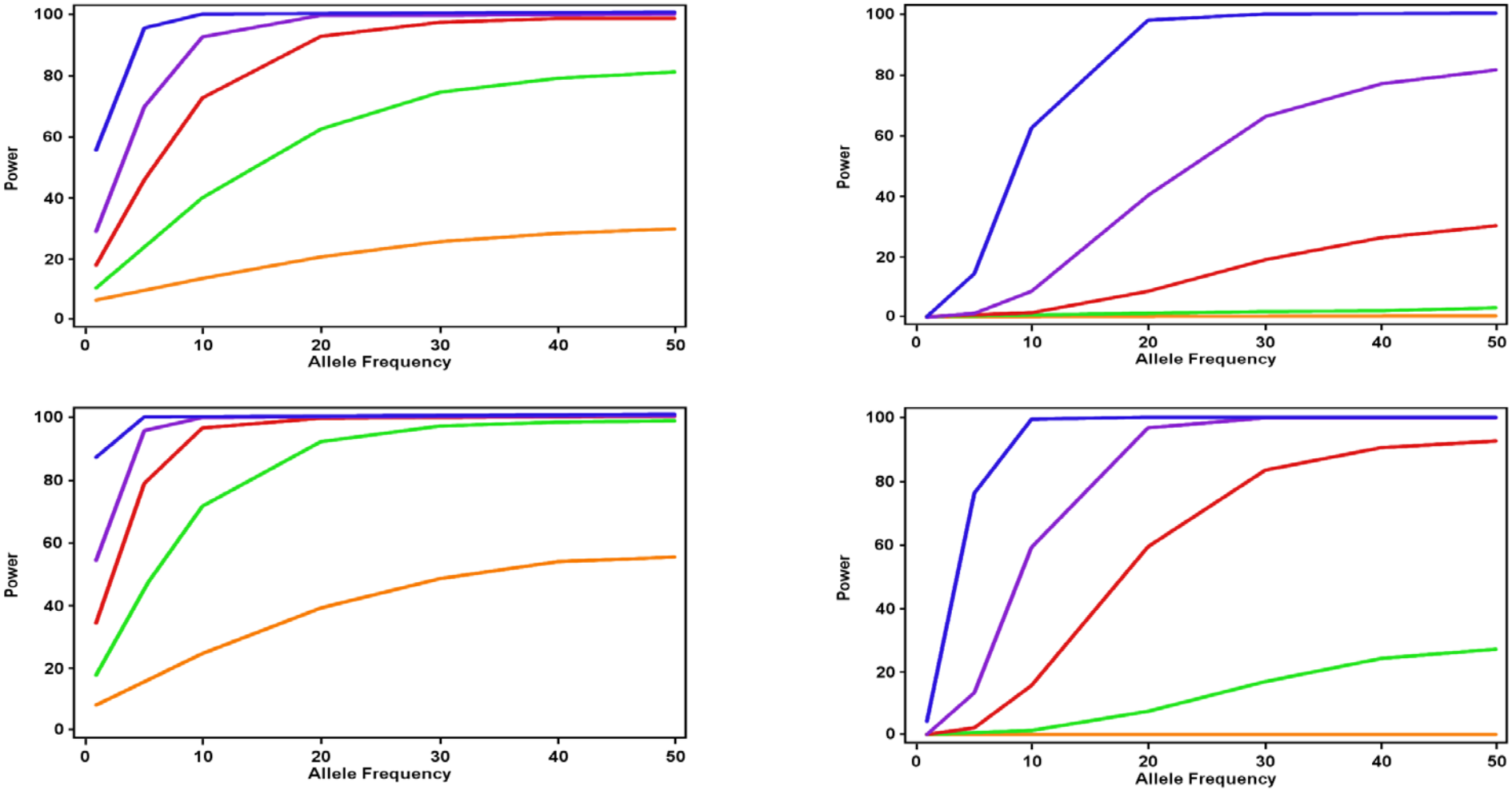

Figure S3. Linkage disequilibrium of all HapMap SNPs in proximity of 500 Kb to rs13306560.
rs13306560 is in significant LD with rs13306567 (r^2>0.8). Other SNPs are shown as pink diamonds. The locations of known genes in the region are shown in green and the recombination hotspots are represented as blue peaks.

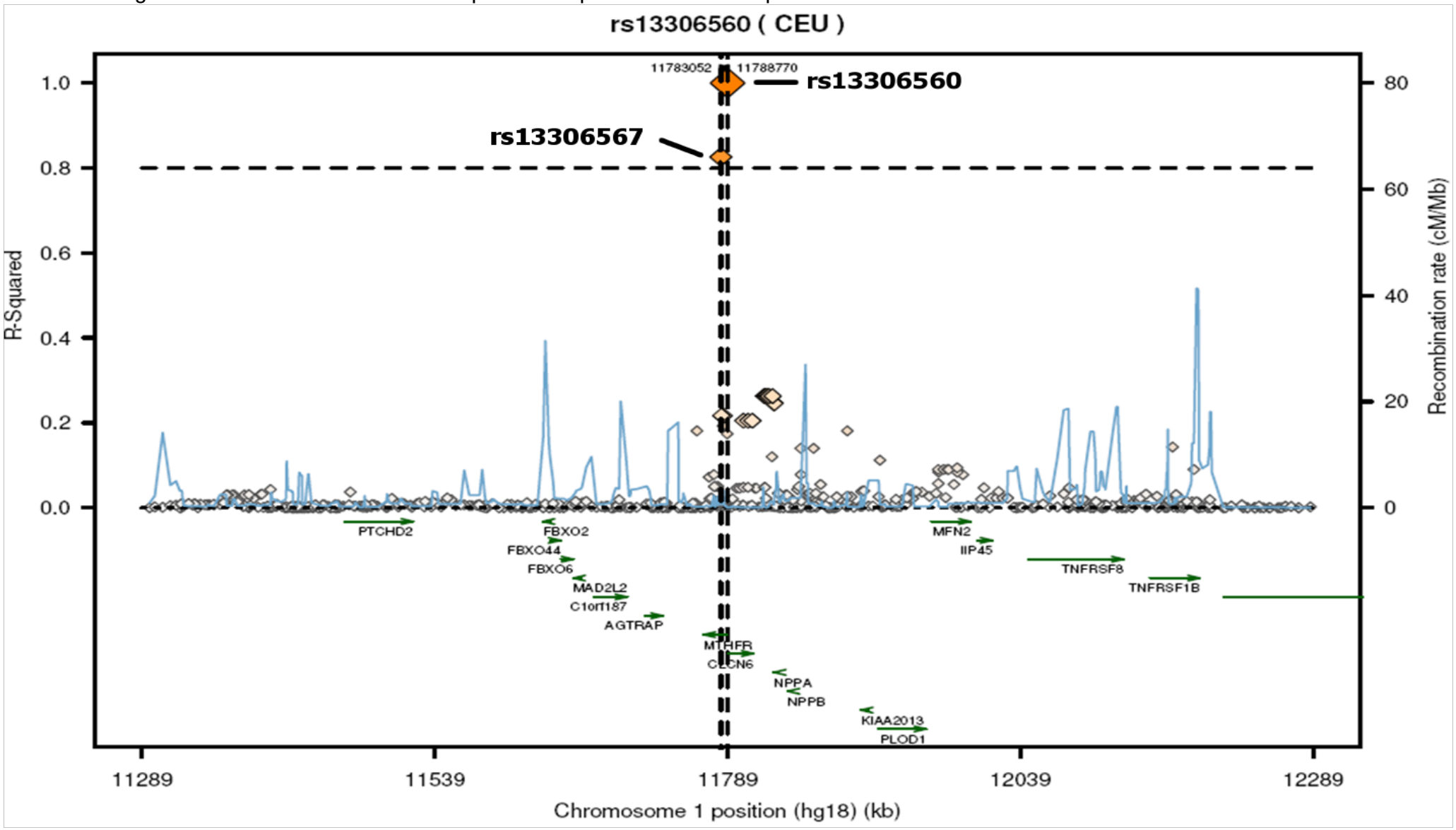

Figure S4. MTHFR/CLCN6 5'flanking region in UCSC Genome Browser
rs13306560 is the only confirmed polymorphism (arrow) within intergenic junction of MTHFR and CLCN6 (shown on reverse strand). This 5'flanking segment shows a high GC content (64.4%) and is a part of the larger 1104-bp CpG island (highlighted in light green) spanning the promoter regions of both genes. The region lies within 6 rat quantitative trait loci (QTLs) for BP (blood pressure QTLs 7,103,131,139,147 and 155) (highlighted in lapis) and 1 QTL (Athsq1) for atherosclerosis in mouse (highlighted in orange). It exhibits significant conservation across placental mammals (average PhastCons estimate of 0.51) and scores high in evolutionary and sequence pattern extraction through reduced representations (ESPERR) programme (shown in light blue as 7xReg Potential).