Supplementary Material

Supplementary Methods 1: Inclusion Criteria and protocol Seven hundred and twenty two essential hypertensive (EH) patients never treated before (n=616), or out of treatment for at least 6 months (n=106), who presented systolic blood pressure (BP) from 140 to 179 mmHg and diastolic BP from 90 to 109 mmHg, were enrolled [1].

We excluded patients with: severe/malignant hypertension or secondary hypertension; kidney or liver disease as evidenced by a serum creatinine level of more than 2 mg/dl or alanine/aspartate aminotransferase activity more than two times the upper limit compared to normal values; kidney transplantation or unilateral functioning kidney; cardiovascular risk ≥ 20% (Framingham risk score); history of cardiac arrhythmias, heart failure, ischemic heart disease; clinically overt endocrine, respiratory, immune disease, or metabolic disturbances; pregnancy or lactation, or use of contraceptive medications; history of angioedema; electrolyte disturbances; substance abuse or mental disorder.

At the end of 8 weeks of run-in period, inclusion and exclusion criteria were confirmed in n=539 patients who were included in the study. Fifty mg/day of losartan were administered orally for 4 weeks in open label. Venus blood samples were collected under fasting conditions for DNA extraction and genotyping, PRA and aldosterone, insulin and glucose, brain natriuretic peptide (BNP) measurements. Serum sodium and potassium were also measured. 24h urine samples were collected at week -8 and 0 to determine urinary sodium, potassium and microalbuminuria.

Laboratory parameters for the diagnosis of essential hypertension were measured [2,3].

Supplementary Methods 2: HCTZ protocol

HCTZ sample

The HCTZ study is formally identical to the SOPHIA study, except for the use of hydrochlorothiazide. EH were recruited in Sassari and Milano Units from 1997, according to the protocol of the Study Group of Pharmacogenomics of the Italian Society of Hypertension [4,5]. The HCTZ protocol received approval from the Ethics Committees of both Units. As for SOPHIA study, mild-to-moderate, never-treated EH patients were used after written informed consent was obtained. HCTZ sample (n=558) was composed of 399 individuals from Sassari and 159 from Milano. Figure S1 shows the entire patients' flow from week -8 (start screening) to week +4 (end of the study) and pre-analysis quality control steps.

Supplementary Methods 3: Replication cohorts

GERA2 cohort

Data and samples were collected in the Genetic Epidemiology of Responses to Antihypertensives (GERA2) study between 1997 and 2007. The ARB, candesartan, was administered in 300 whites with uncomplicated primary hypertension, stage 1-2, 30-59.9 years of age (Rochester MN). Patients were instructed to discontinue previous antihypertensive medications for 4 weeks. Once stable elevation of the BP was achieved (diastolic BP≥90 mmHg), candesartan was administered 16 mg daily for two weeks followed by 32 mg daily for four additional weeks. The delta BP (BP recorded at the end of the active treatment minus BP recorded at the end of the run-in treatment) is the phenotype considered [6].

GENRES cohort

Data and samples were collected between 1999 and 2004 in GENRES study, which is a double-

blind, placebo controlled, randomized, and cross-over study with 4 weeks monotherapies using four antihypertensive drugs (losartan, bisoprolol, amlodipine and hydrochlorothiazide). Three hundred and thirteen moderately hypertensive Finnish men, aged 35–60 years were included in the study. The inclusion criteria were DBP≥95 mmHg in repeated measurements or use of antihypertensive medication. Each patient received losartan 50 mg, bisoprolol 5 mg, hydrochlorothiazide 25 mg and amlodipine 5 mg daily, each as monotherapy, in a randomized order for 4 weeks. Office and 24-hour ambulatory BP measurements were performed at the end of each treatment period. Office BP measurements were carried out three times with 1-min intervals, after a 30-min rest in the sitting position, using a semi-automated oscillometric device.

The mean of the last two measurements was used in the analyses. Office BP measurements were available for 216 subjects during losartan therapy [7]. In this replication study, office BP data were used, because office BP data was used in SOPHIA study.

Supplementary Methods 4: Genotyping and Imputing

SOPHIA and HCTZ samples were genotyped using the Illumina Human1M-Duo array (Illumina Inc, San Diego, CA, USA) within HYPERGENES project [8] or the Illumina HumanOmniExpress array within InterOmics project (http://www.interomics.eu/).

Raw intensity data were analysed with the Illumina Software Genome Studio for genotype calling, using the Illumina reference cluster file. A DNA call rate threshold was set at 0.95 and DNAs with call rate ≤ 0.95 were excluded from the final data set. For each DNA, data from X chromosome were used to check for discordance with ascertained sex. Genome-wide imputation was performed with MACH [9] using as reference the HapMap CEU haplotypes (release 22). Measured SNPs with >99% call rate, minor allele frequency (MAF) >1% were included in the data set.

Imputed SNPs with low imputation quality (Rsq<0.3 or MAF<3%) were not used in the association analysis.

Supplementary Methods 5: Quality Control and Principal component Analysis

All QC steps were performed in accordance with the protocol written by C.A. Anderson *et al.* [10]

Four hundred and ninety four genotyped individuals of the SOPHIA sample underwent quality control. Thirty 9 samples having call rate <0.95 were excluded. Sixteen subjects with genotypic sex mismatch (difference between the gender reported in clinical data and the one estimated with sex SNPs genotyped) were identified and removed from the analysis.

Using genome-wide IBD estimation (PLINK version 1.7 [11]) we identified and removed from the analysis 6 duplicated and 18 related subjects (10 family components, 5 siblings, 3 second degree).

In the SOPHIA~~,~~ we performed PCA using 1M SNPs and the EIGENSOFT package (version 3.0) [12,13]. We removed 19 outliers, defined as individuals that exceed 6 standard deviations from the whole sample along any of the principal components.

Results for the first 2 PCs are described in **Supplementary Figure 1**. The plot clearly shows subjects clustering according to their geographical origin. For each treatment cohort the samples are represented as macro-groups (Continental Italy and Sardinia).

We selected the first 10 PCs to include them as covariates in the linear regression model.

One individual was filtered out for a reduced proportion of heterozygous genotypes and 23 for incomplete phenotypic data.

After quality control the final sample comprised of 372 subjects (280 males, 92 females).

SNPs with a MAF <3% and with call rate <99% were removed leaving 1,705,664 SNPs for analysis.

After association tests, the cluster plots of all genotyped SNPs, associated with $P \leq 10^{-5}$, were manually inspected to check the fidelity of genotype assignment.

Author Contributors

Nicola Glorioso designed the study.

Giuseppe Argiolas, Michele Bardini, Gianpaolo Bernini, Emanuela Bulla, Patrizia Bulla, Ezio Degli Esposti, Giovanbattista Desideri, Francesco Fallo, Claudio Ferri, Ferruccio Galletti, Nicola Glorioso, Chiara Lanzani, Lorenzo Malatino, Paolo Manunta, Paolo Mulatero, Franco Perticone, Giuseppe Regolisti, Angela Sciaqua, Giuseppe Antonio Scioli, Andrea Semplicini, Benedetta Stancanelli, Alessandra Sturani, Chiara Troffa, Franco Veglio and Tracy A Williams , collected the samples and provided the clinical data.

Francesca Frau, Giovanni Fresu, M. Francesca Ortu, Daniela Antonella Piras, Silvia Pitzoi, Roberta Zaninello, managed DNA samples.

Cristina Barlassina, Daniele Braga, Dinesh Velayutham, performed genotyping.

Martina Chittani, Valeria Glorioso and Francesca Frau performed statistical analysis.

Cristina Barlassina, Daniele Cusi, Francesca Frau, Nicola Glorioso, and Erika Salvi contributed experimental design and interpretation.

Eric Boerwinkle, Kimmo Kontula and Timo P. Hiltunen and Stephen T. Turner, provided *in-silico* replication samples.

Cristina Barlassina, Daniele Cusi, Francesca Frau, Nicola Glorioso and Erika Salvi wrote the manuscript.

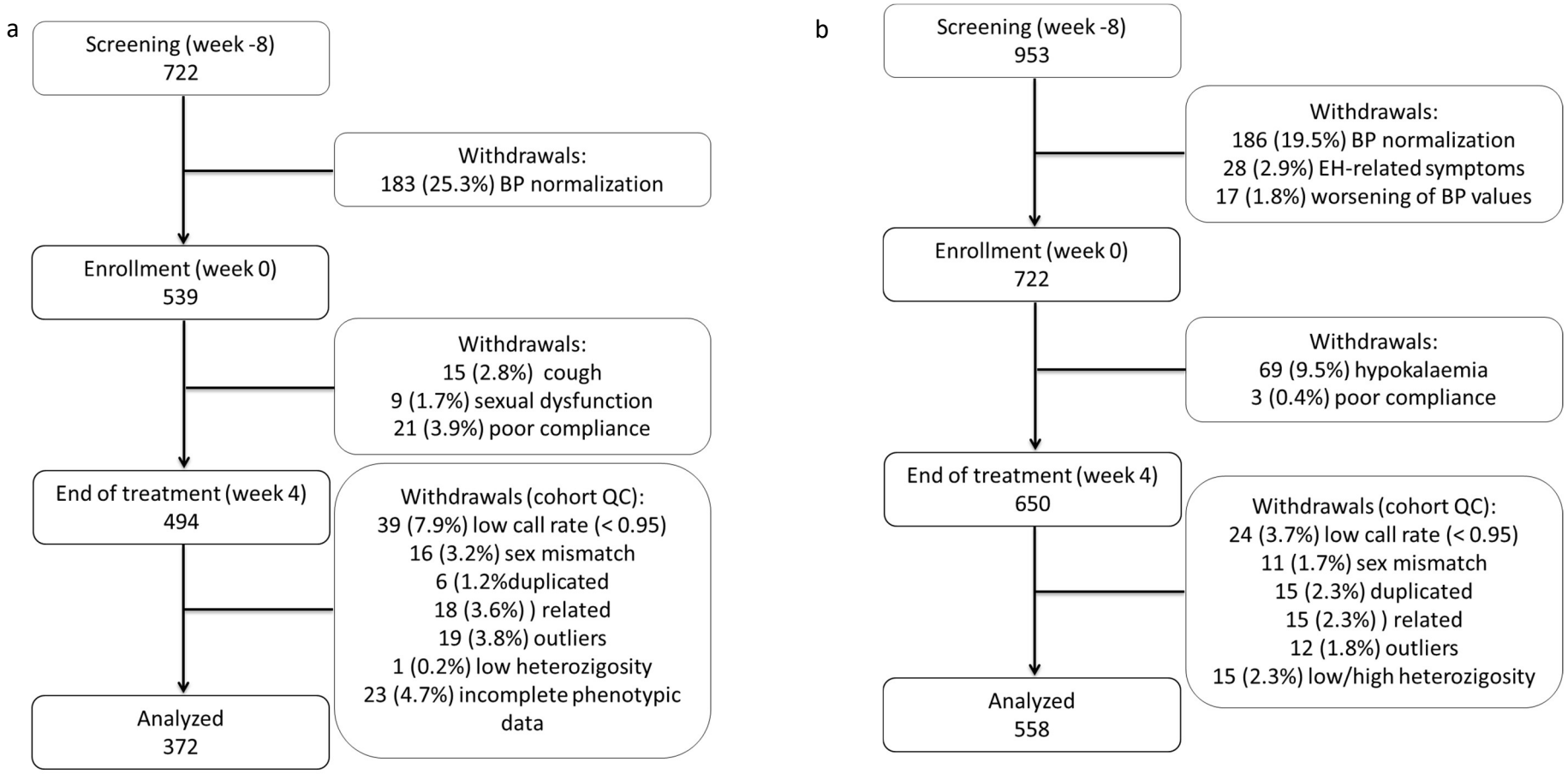
All authors interpreted the results and approved the final version of the manuscript.

Supplemental References

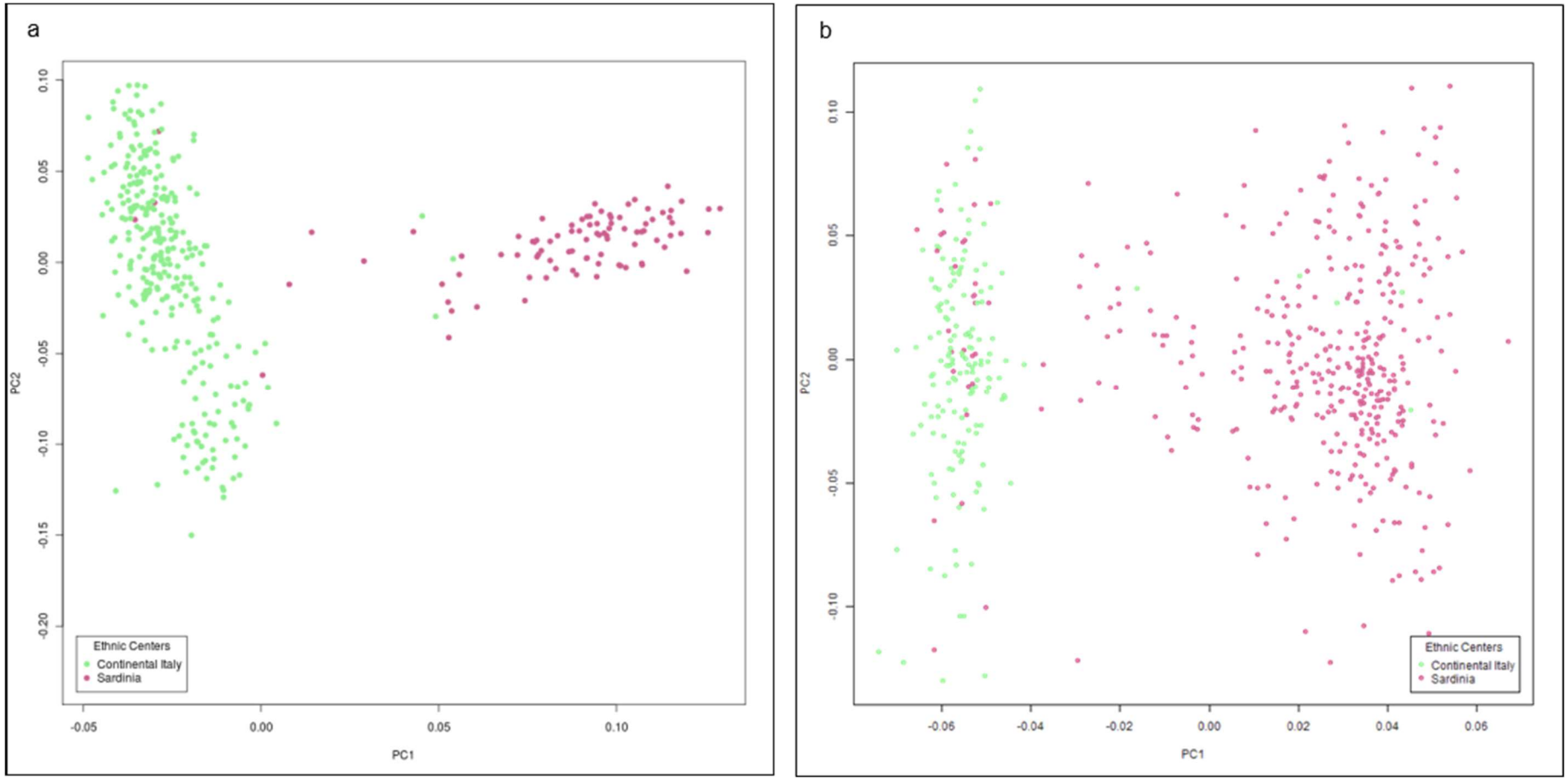
1. Glorioso N, Argiolas G, Filigheddu F, *et al*. Study Group on Cardiovascular Pharmacogenomics of Italian Society of Hypertension. Conceptual basis and methodology of the SOPHIA study. *Pharmacogenomics* 8(11), 1497-509 (2007).
2. Chobanian AV, Bakris GL, Black HR, *et al.* Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. National Heart, Lung, and Blood Institute; National High Blood Pressure Education Program Coordinating Committee. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 42(6), 1206-52 (2003).
3. European Society of Hypertension-European Society of Cardiology Guidelines Committee. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens* 21(6), 1011-53 (2003).
4. Williams TA, Mulatero P, Filigheddu F, *et al*. Role of HSD11B2 polymorphisms in essential hypertension and the diuretic response to thiazides. *Kidney Int* 67(2), 631-7 (2005).
5. Turner ST, Boerwinkle E, O'Connell JR, *et al*. Genomic Association Analysis of Common Variants Influencing Antihypertensive Response to hydrochlorothiazide. *Hypertension* Jun 10 (2013).
6. Turner ST, Bailey KR, Schwartz GL, Chapman AB, Chai HS, Boerwinkle E. Genomic association analysis identifies multiple loci influencing antihypertensive response to an angiotensin II receptor blocker. *Hypertension* 59(6), 1204-11 (2012).
7. Hiltunen TP, Suonsyrjä T, Hannila-Handelberg T, *et al*. Predictors of antihypertensive drug responses: initial data from a placebo-controlled, randomized, cross-over study with four antihypertensive drugs (The GENRES Study). *Am J Hypertens* 20(3), 311-8 (2007).

8. Lanzani C, Citterio L, Glorioso N, *et al*. Adducin- and ouabain-related gene variants predict the antihypertensive activity of rostafuroxin, part 2: clinical studies. *Sci Transl Med* 2(59), 59ra87 (2010).
9. Salvi E, Kutalik Z, Glorioso N, *et al*. Genomewide association study using a high-density single nucleotide polymorphis array and case-control design identifies a novel essential hypertension susceptibility locus in the promoter region of endothelial NO synthase. *Hypertension* 59(2), 248-55 (2012).
10. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol* 34(8), 816-3 (2010).
11. Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. *Nat Protoc* 5(9), 1564-73 (2010).
12. 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* 81(3), 559-75 (2007).
13. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 38(8), 904-9 (2006).
14. Patterson N, Price AL, Reich D: Population structure and eigenanalysis. *PLoS Genet* 2, 2074-2093 (2006).

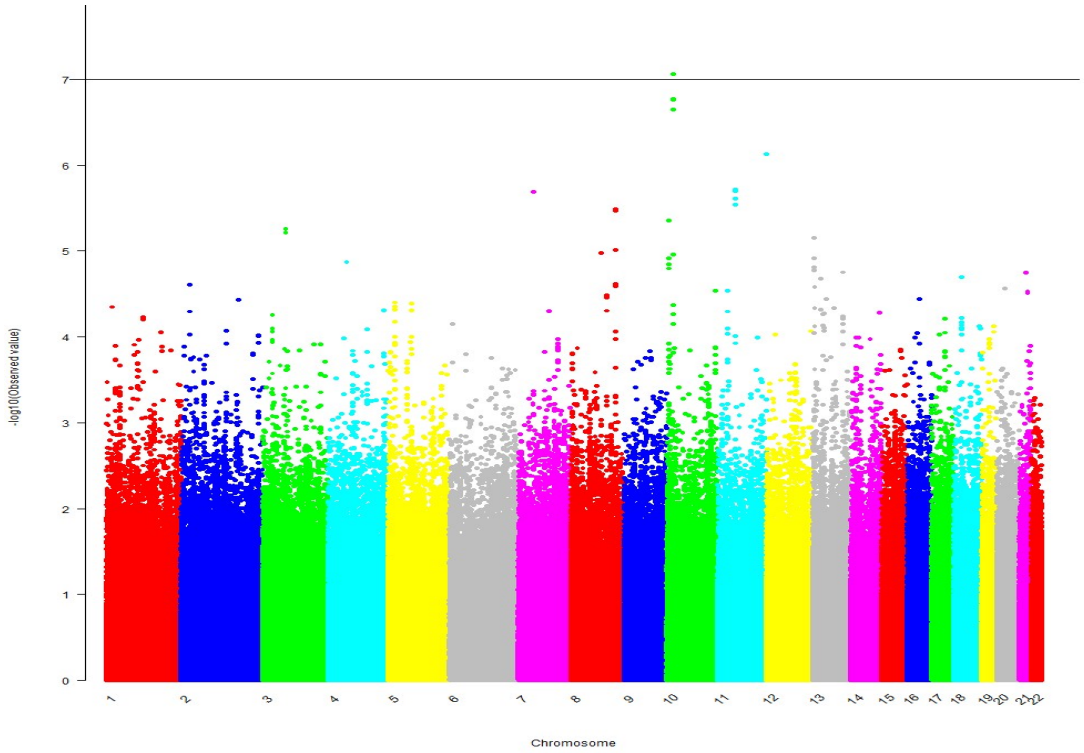
Supplementary Figures



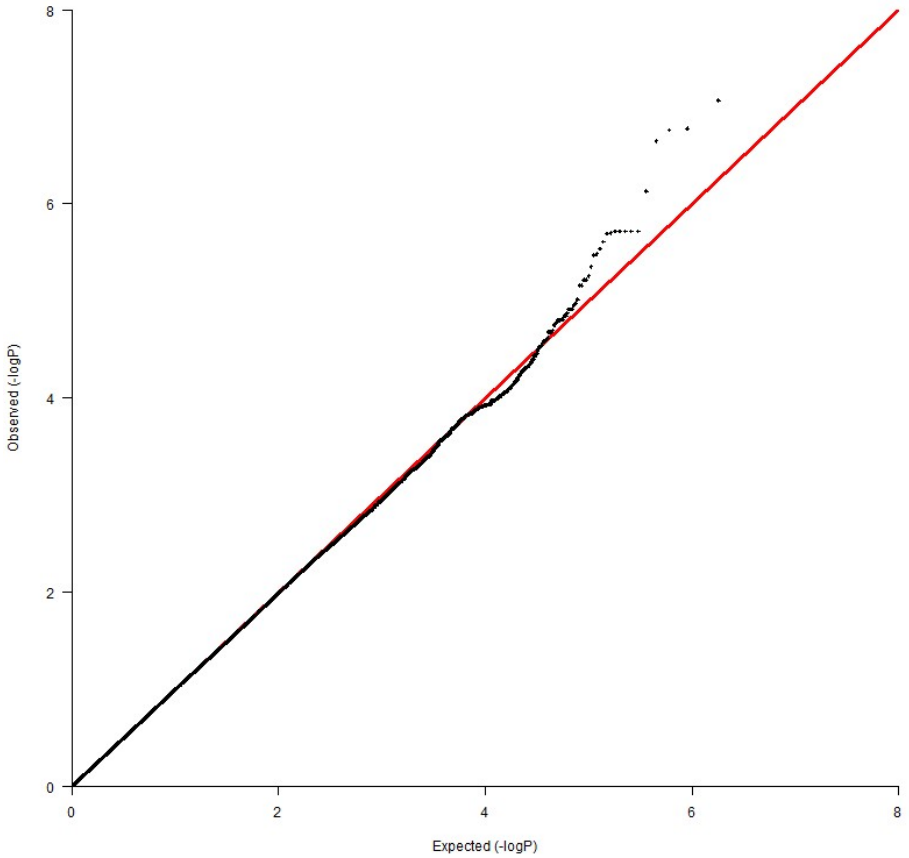
Supplementary Figure 1: Flow diagram of the participants of a) Sophia and b) HCTZ studies. EH, essential hypertension; BP blood pressure; QC quality control.



Supplementary Figure 2. Principal component plot of a) Sophia and b) HCTZ samples.
Samples are represented as macro-groups: Continental Italy in green and Sardinia in purple.



Supplementary Figure 3. Manhattan plot of single SNP linear regression test statistics for Δ SBP4. Regression analysis adjusted for gender, age, basal systolic blood pressure and principal components. Results are reported as –log10 (P value) by genomic position.



Supplementary Figure 4. Quantile-quantile plot of single nucleotide polymorphism p-values from genome wide association analysis of systolic blood pressure response (Δ SBP4) to losartan.

Supplementary Tables

Supplementary Table 1. Genome-wide association results for SBP response to losartan associated SNPs with P value <10^{-4}. All imputed SNPs have an imputation quality (Rsq) >0.8. To retrieve information about SNPs and their genomic context (the nearest gene) we used the hg18 (NCBI 36) assembly. P indicates p values of association; SE, standard error; Chr, chromosome; SNP, single nucleotide polymorphism; bp, base pairs.

Supplementary Table 2. Association results for SBP and DBP response to losartan. To retrieve information about single nucleotide polymorphisms and their genomic context (the nearest gene) we used the hg18 (National Center for Biotechnology Information 36) assembly. P indicates, p values of association; SE, standard error; Chr, chromosome; SNP, single nucleotide polymorphism.

Supplementary Table 3. Association results for losartan and HCTZ. To retrieve information about single nucleotide polymorphisms and their genomic context (the nearest gene) we used the hg18 (National Center for Biotechnology Information 36) assembly. P indicates p values of association; SE, standard error; Chr, chromosome; SNP, single nucleotide polymorphisms.