Original Article

Transcriptome-Wide Analysis Identifies Novel Associations With Blood Pressure

Tanja Zeller,* Claudia Schurmann,* Katharina Schramm,* Christian Müller,* Soonil Kwon,* Philipp S. Wild, Alexander Teumer, David Herrington, Arne Schillert, Licia Iacoviello, Adelheid Kratzer, Annika Jagodzinski, Mahir Karakas, Jingzhong Ding, Johannes T. Neumann, Kari Kuulasmaa, Christian Gieger, Tim Kacprowski, Renate B. Schnabel, Michael Roden, Simone Wahl, Jerome I. Rotter, Francisco Ojeda, Maren Carstensen-Kirberg, David-Alexandre Tregouet, Marcus Dörr, Thomas Meitinger, Karl J. Lackner, Petra Wolf, Stephan B. Felix, Ulf Landmesser, Simona Costanzo, Andreas Ziegler, Yongmei Liu, Uwe Völker, Walter Palmas, Holger Prokisch, Xiuqing Guo,* Christian Herder,* Stefan Blankenberg,* Georg Homuth*

See Editorial Commentary, pp xxx–xxx

Abstract—Hypertension represents a major cardiovascular risk factor. The pathophysiology of increased blood pressure (BP) is not yet completely understood. Transcriptome profiling offers possibilities to uncover genetics effects on BP. Based on 2 populations including 2549 individuals, a meta-analyses of monocytic transcriptome-wide profiles were performed to identify transcripts associated with BP. Replication was performed in 2 independent studies of whole-blood transcriptome data including 1990 individuals. For identified candidate genes, a direct link between long-term changes in BP and gene expression over time and by treatment with BP-lowering therapy was assessed. The predictive value of protein levels encoded by candidate genes for subsequent cardiovascular disease was investigated. Eight transcripts (*CRIP1*, *MYADM*, *TIPARP*, *TSC22D3*, *CEBPA*, *F12, LMNA*, and *TPPP3*) were identified jointly accounting for up to 13% (95% confidence interval, 8.7–16.2) of BP variability. Changes in *CRIP1*, *MYADM*, *TIPARP*, *LMNA*, *TSC22D3*, *CEBPA*, and *TPPP3* expression associated with BP changes—among these, *CRIP1* gene expression was additionally correlated to measures of cardiac hypertrophy. Assessment of circulating CRIP1 (cystein-rich protein 1) levels as biomarkers showed a strong association with increased risk for incident stroke (hazard ratio, 1.06; 95% confidence interval, 1.03–1.09; P=5.0×10^{-5}). Our comprehensive analysis of global gene expression highlights 8 novel transcripts significantly associated with BP, providing a link between gene expression and BP. Translational approaches further established evidence for the potential use of CRIP1 as emerging disease-related biomarker. (***Hypertension*****. 2017;70:00-00. DOI: 10.1161/HYPERTENSIONAHA.117.09458.)** • **Online Data Supplement**

Key Words: blood pressure ■ gene expression ■ genome-wide association study ■ hypertension ■ transcriptome

Hypertension as a major cardiovascular risk factor continues to be a significant health challenge[1] and imparts an increased risk of cardiovascular and kidney diseases.[2,3] Hypertension is determined by multiple factors, and during the past years, the immune system (mainly T cells) and inflammatory processes have emerged as key contributors to elevated blood pressure (BP) in several experimental animal and human models.[4,5] A shared pathophysiology with manifest cardiac

Received April 6, 2017; first decision April 22, 2017; revision accepted June 1, 2017.

From the General and Interventional Cardiology, University Heart Center Hamburg, Germany (T.Z., C.M., A.J., M.K., J.T.N., R.B.S., F.O., S.B.); DZHK (German Centre for Cardiovascular Research), Germany (T.Z., C.S., C.M., P.S.W., A.T., A.S., A.K., A.J., M.K., J.T.N., T.K., R.B.S., M.D., T.M., K.J.L., S.B.F., U.L., A.Z., U.V., S.B.); Institute of Human Genetics (K.S., T.M., H.P.), Molecular Epidemiology (C.G., S.W.), and Institute of Epidemiology II (C.G., S.W.), Helmholtz Zentrum München, Germany; Institut für Medizinische Biometrie und Statistik, Universitätsklinikum Schleswig-Holstein, Germany (C.M., A.S., A.Z.); Institute for Translational Genomics and Population Sciences, UCLA Medical Center (S.K., J.I.R., X.G.); Preventive Cardiology and Preventive Medicine (P.S.W.), Institute of Clinical Chemistry and Laboratory Medicine (K.J.L.), and Center for Thrombosis and Hemostasis (P.S.W.), University Medical Center of the Johannes Gutenberg-University Mainz, Germany; Institute for Community Medicine (A.T.) and Department of Internal Medicine B (M.D., S.B.F.), University Medicine Greifswald, Germany; Interfaculty Institute for Genetics and Functional Genomics, University Greifswald, Germany (C.S., T.K., U.V., G.H.); Department of Internal Medicine (D.H., J.D.) and Department of Epidemiology and Prevention (Y.L.), Wake Forest School of Medicine, Winston-Salem, NC; Department of Epidemiology and Prevention, IRCCS Istituto Neurologico Mediterraneo Neuromed, Pozzilli IS, Italy (L.I., S.C.); Department of Cardiology and Pneumology, Charite, Universitätsmedizin Berlin, Germany (A.K., U.L.); National Institute for Health and Welfare, Helsinki, Finland (K.K.); Institute for Clinical Diabetology, German Diabetes Center, Duesseldorf, Germany (M.R., M.C.-K., C.H.); Department of Endocrinology and Diabetology, Medical Faculty Düsseldorf, Germany (M.R.); German Center for Diabetes Research (DZD), Munich, Germany (M.R., S.W., M.C.-K., C.H.); Sorbonne Universités, UPMC, INSERM, UMR_S 1166, ICAN Institute for Cardiometabolism and Nutrition, Paris, France (D.-A.T.); Institute of Human Genetics, Technische Universität München, Germany (T.M., P.W., H.P.); ZIK_FunGene, Universität Greifswald, Germany (U.V., G.H.); and Columbia University Medical Center, New York, NY (W.P.).

*These authors contributed equally to this work.

The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.117.09458/-/DC1.

Correspondence to Tanja Zeller, Universitäres Herzzentrum Hamburg, Germany. E-mail t.zeller@uke.de

disease is suggested.[3,6] Nevertheless, there is also a substantial genetic heritability of 30% to 60% for hypertension.[7]

Large-scale genome-wide association studies on hypertension or BP traits have been published.[8–10] Some of the identified genetic variants primarily associated with higher BP also confer an increased risk for coronary artery disease, consistent with a causal relationship of increased BP and coronary artery disease risk.[11,12] However, the distinct genetic variants identified by genome-wide association studies to date exhibit small effect sizes and explain 3.5% of the BP variance.[10,11,13] Moreover, most genes near the identified genetic variants are not known to be functionally related to BP.[14]

Global gene expression profiling offers novel possibilities for functional genomics, and possible effects of genetic variants on clinical phenotypes may be uncovered. Recent gene expression analyses for hypertension and BP traits identified gene expression signatures involved in multiple biological processes that contribute to BP regulation.[15–19]

Here, we investigated (1) the global gene expression based on transcripts levels in relation to BP traits in a 2-stage meta-analysis of 4 population-based studies, (2) the relationship of changes in BP and transcript levels over time, and (3) the clinical application of candidate genes as BP-related biomarkers.

Material and Methods

A detailed description of the methods and the study samples is given in the online-only Data Supplement. All studies followed the recommendations of the Declaration of Helsinki, and study protocols were approved by the local ethics committees. Written informed consent was obtained from all study participants.

Study Workflow

The study workflow is outlined in Figure S1 in the online-only Data Supplement.

1. A discovery meta-analysis of global monocyte gene expression and BP traits (systolic BP [SBP], diastolic BP [DBP], and pulse pressure) was performed combining data from the population-based studies GHS (Gutenberg Health Study)[22] (n=1285) and MESA (Multi-Ethnic Study of Atherosclerosis)[23] (n=1264). Multiple testing was controlled by a false discovery rate approach implementing the Benjamin–Hochberg method. At this step, a false discovery rate threshold of 0.01 was used.
2. Transcripts displaying suggestive evidence for BP-associated changes were taken forward to external validation in whole-blood transcriptomic data sets of 1990 individuals from 2 independent population-based studies (SHIP-TREND [Study of Health in Pomerania-TREND][24] [n=997] and KORA [Cooperative Heath Research in the Augsburg Region][25] F4 [n=993]). Criteria were (1) evidence for statistical significance ($P<0.05$) in each study for at least 1 BP trait, and (2) consistent direction of effect in discovery and replication.

To relate expression of candidate transcripts to BP changes, transcript levels were assessed in different settings: (1) a setting of long-term changes in BP for 5 years in GHS (n=1092), and (2) in the setting of a clinical trial to test the influence of BP-lowering medication by routinely used telmisartan/amlodipine and olmesartan on candidate transcript levels for 6 months (n=406).

To assess additional clinical implications of the findings, candidate transcript levels were linked to measures of subclinical cardiovascular disease (CVD), and the relation of protein levels encoded by BP-related candidate transcripts was determined for incident cardiovascular events in serum samples of the population-based Moli-Sani Study[26] (n=379) to test the applicability as circulating biomarkers.

Gene Expression Profiling Using Microarray Technology

In GHS, KORA F4, and SHIP-TREND, RNA was processed using the Illumina TotalPrep-96 RNA Amp Kit (Ambion, Darmstadt, Germany), and labeled cRNA was hybridized to the Illumina HumanHT-12 v3 Expression BeadChip as described previously.[27] GHS 5-year follow-up samples were processed using the Illumina TotalPrep-96 RNA Amp Kit (Ambion), and labeled cRNA was hybridized to the IlluminaHT-12 v4 Expression BeadChips following manufactures recommendations. In MESA, the Illumina TotalPrep-96 RNA Amplification Kit (Ambion) and the Illumina HumanHT-12 v4 Expression BeadChip were used for gene expression profiling.[28]

Statistical Analyses

Identification of BP-Related Candidate Transcripts

The microarray data were pre-processed, normalized, log2-transformed, and quality controlled as described previously for GHS, KORA, SHIP-TREND,[27] and MESA.[28] Associations between mRNA levels and BP traits were estimated using linear regression models and adjusted for sex, age, body mass index, and the technical covariates plate layout, RNA Integrity Number, and storage time.[27] In MESA, the models were additionally adjusted for ethnicity. Association statistics from GHS and MESA were pooled by inverse-variance weighting of SEs using METAL (Meta Analyses Helper).[29] In the primary analyses, individuals receiving antihypertensive treatment were not excluded. In a sensitivity analysis, individuals receiving antihypertensive drug treatment were excluded, leading to 941 eligible individuals in GHS, 815 in MESA, 570 in KORA F4, and 602 in SHIP-TREND.

Analyses of Changes in Candidate Transcript Expression Over Time

A detailed description is given in the online-only Data Supplement. Briefly, association analysis between changes of BP traits and changes in candidate transcript expression after 5 years in the GHS was performed using linear regression. Adjustments were performed for sex, age, BP trait at baseline, and body mass index change between baseline and follow-up. Controlling for multiple testing was performed using the Benjamini–Hochberg method,[30] and the significance level was set to 0.05. For changes in gene expression for 5 years in the GHS, results are given as mRNA change per 10-mm Hg increase in BP trait (delta mRNA)±SE.

In the BP-lowering clinical trial, differential expression of candidate transcripts before and after BP-lowering therapy was calculated by linear mixed models adjusted for sex, age, and body mass index. Controlling for multiple testing was performed using the Benjamini–Hochberg method,[30] and the significance level was set to 0.05. For differential gene expression in the clinical trial, results are given as percent mRNA change (%mRNA change) after 6 months±SE.

Expression Quantitative Trait Loci (eQTL) Analysis in the GHS

eQTL analyses were performed in 1333 individuals from the GHS with available gene expression and available genome-wide genotyping data.[20] Two approaches were used to identify eQTLs related to BP or CVD: (1) *cis*-eQTLs were calculated for single nucleotide polymorphisms (SNPs) within ±250 kb around the transcription start site and a minor allele frequency ≥1%, and (2) eQTLs calculated based on published genome-wide association study results of BP-related traits retrieved from the genome-wide association studies catalogue (March 20, 2017).[31]

Results

BP-Related Gene Expression: Identification and Replication

The study characteristics are outlined in Table S1. Differential gene expression in relation to BP traits was assessed by a meta-analysis of GHS and MESA monocyte transcriptome

data. At a false discovery rate <0.01, 91, 35, and 51, unique transcripts differentially expressed in relation to BP traits were identified (Table S2).

Validation of monocyte transcript expression findings was performed using whole-blood transcriptome data of 2 independent cohort studies: SHIP-TREND and KORA F4 (Table S3). Eight unique transcripts fulfilled criteria for an independent validation at a $P<0.05$, including 5 for SBP, 6 for DBP, and 3 for pulse pressure (Table 1), encompassing *CRIP1*, *MYADM*, *TIPARP*, *TSC22D3*, *CEBPA*, *F12*, *LMNA*, and *TPPP3*. Of these, *CEBPA* showed decreased transcripts levels associated with increased BP, whereas the remaining transcript levels were positively associated with BP. Associations between BP traits and expression remained significant after excluding individuals receiving antihypertensive treatment (Table S4) and when including only white subjects in MESA (Table S5). The BP-related transcripts were expressed at comparable levels in monocytes and whole-blood cells (Figure S2).

Variation of BP Traits Attributable to Candidate Transcripts

To assess the variance in BP levels attributable to gene expression, the R^2 (percentage of phenotypic variance) was calculated (Table S6). In aggregate, the genes identified in the transcriptome analyses accounted in total for 2.82% to 11.33% (SBP), 2.11% to 8.31% (DBP), and 1.36% to 4.74% (pulse pressure) of the phenotypic variance of the respective BP traits. These data indicate that a larger proportion of BP variance is attributable to changes in gene expression levels as compared with an explained variance of only 3.5% by common genetic variants.[13]

Changes in BP and Corresponding Changes in Gene Expression

A direct link between changes in BP and candidate transcript expression in monocytes was assessed in different settings: (1) long-term BP changes for 5 years in the GHS population, and (2) by initiation of BP-lowering therapy for 6 months in a clinical trial.

Long-Term Changes in Transcript Expression in Relation to BP for 5 Years in GHS

In individuals with monocyte transcriptome data available at baseline and 5-year follow-up, a strong association between changes of BP traits and expression levels of *CRIP1*, *MYADM*, *TIPARP*, *TSC22D3*, *CEBPA*, *LMNA*, and *TPPP3* was observed (Table 2). Consistent with the data from the discovery/replication step, a negative association of *CEBPA* to BP changes was found, whereas the remaining transcripts were positively associated. These associations were independent of antihypertensive drug therapy. *CRIP1* transcript levels showed the strongest association with changes in BP traits after 5-year follow-up (% mRNA change per 10 mm Hg SBP: 2.93±0.45; $P=2.15\times10^{-10}$; delta mRNA DBP: 5.19±0.77; $P=8.46\times10^{-11}$; delta mRNA pulse pressure: 2.2±0.62; $P=5.0\times10^{-4}$). Figure S3a–S3g shows the association between BP changes and respective mRNA level according to categories of BP changes.

Changes in Transcript Expression in Relation to BP by Antihypertensive Medication

BP-lowering medication resulted in a reduction of BP after 6 months (Figure S4). It was expected that this BP reduction lead to a decrease of the expression of transcripts that positively correlated with BP in the discovery phase and vice versa. Accordingly, the reduction of BP associated with a decrease in the expression of 7 of the 8 candidate transcripts (Figure). The strongest differential expression was found for *CRIP1* (% mRNA change: −34.14%±3.55; $P=5.6\times10^{-14}$). Of note, *CEBPA* (% mRNA change: −51.84%±5.5; $P=7.5\times10^{-16}$) was the only transcript with a divergent expression association pattern, opposite what was expected.

In addition, we assessed the association between candidate transcripts and measures of cardiac hypertrophy. Of all candidate transcripts, *CRIP1* was most strongly associated with septal thickness end diastolic (log2-fold mRNA change [log2 change] per mm: 0.0198; $P=1.9\times10^{-3}$), left ventricular posterior wall thickness end diastolic (log2 change per cm: 0.003; $P=1.0\times10^{-4}$), left ventricular mass (log2 change per gram: 0.0006; $P=5.0\times10^{-4}$), relative wall thickness (log2 change per cm: 0.3697; $P=3.5\times10^{-2}$), and left ventricular hypertrophy (log2 difference between subjects with and without left ventricular hypertrophy: 0.1276; $P=5.2\times10^{-3}$; Table S7).

All BP modulation strategies confirmed the uniform response of candidate transcripts to BP changes either over time or by BP-lowering medication. The strongest response in all approaches was observed for *CRIP1*.

Protein Levels of CRIP1 and Incident Cardiovascular Events

For the protein encoded by the most strongly associated transcripts, *CRIP1*, we investigated the potential to serve as biomarker for future cardiovascular events. We assessed the predictive value of circulating CRIP1 (cystein-rich protein 1) serum levels for the incidence of stroke, heart failure, and coronary artery disease in a population-based sample from the Moli-Sani Study. A significant association was found for incident stroke events (Table 3), indicating a predictive value of CRIP1 as biomarker for stroke.

Genetic Interplay on BP-Related Transcripts

Because transcript levels might be influenced by genetic variants, eQTL analyses were performed in the GHS monocyte transcriptome data set for SNPs related to BP or CVD traits following 2 approaches (Figure S5). First, regulatory SNPs around the candidate transcripts were examined for CVD-related SNP-trait associations using the Genome-Wide Repository of Associations Between SNPs and Phenotypes (GRASP) database. For *CRIP1*, *TPPP3*, and *LMNA*, significant *cis*-eQTLs (n=25, n=191, and n=30, respectively) were identified. Of these, 2 SNPs around *CRIP1* were related to mitral annular calcium (eg, rs10151805; $P=4.55\times10^{-5}$)[32] and 5 SNPs to body mass index (eg, rs1475766; $P=6.36\times10^{-5}$)[29] with $P\leq10^{-4}$ in the GRASP database (Table S8A).

The aim of the second approach was to investigate whether known BP-associated variants have a regulatory effect on candidate transcript expression. A total of 191 previously published BP-related SNPs were tested

Table 1. Differentially Expressed Transcripts Associated With BP Traits

Gene	Gene Description	Discovery	Independent Replication		Combined Analysis	
		Meta GHS/MESA	KORA F4	SHIP-TREND	Meta KORA/SHIP	Discovery+ Replication
		P Value (mRNA Difference [%])				
Systolic blood pressure						
CRIP1	Cysteine-rich protein 1	7.36×10^{-26} (3.3)	2.2×10^{-2} (1.1)	4.8×10^{-4} (2.1)	6.66×10^{-5} (1.5)	3.34×10^{-26} (2.5)
MYADM	Myeloid-associated differentiation marker	1.71×10^{-14} (1.9)	8.8×10^{-4} (1.5)	8.1×10^{-4} (2.1)	2.90×10^{-6} (1.7)	2.77×10^{-19} (1.8)
TIPARP	TCDD-inducible poly(ADP-ribose) polymerase	1.62×10^{-12} (1.5)	1.9×10^{-2} (1.3)	5.6×10^{-4} (1.1)	6.60×10^{-5} (0.8)	7.90×10^{-15} (1.1)
TSC22D3	TSC22 domain family member 3	1.08×10^{-13} (2.3)	2.0×10^{-2} (1.4)	2.0×10^{-4} (1.9)	1.35×10^{-5} (1.7)	1.35×10^{-17} (2.0)
CEBPA	CCAAT/enhancer binding protein alpha	5.62×10^{-5} (–0.8)	4.1×10^{-3} (–1.1)	1.1×10^{-3} (–1.5)	1.65×10^{-5} (–1.3)	8.29×10^{-9} (–0.96)
Diastolic blood pressure						
CRIP1	Cysteine-rich protein 1	2.17×10^{-16} (4.7)	2.1×10^{-2} (2.2)	1.2×10^{-2} (2.4)	6.41×10^{-4} (2.3)	2.29×10^{-17} (3.7)
MYADM	Myeloid-associated differentiation marker	2.21×10^{-6} (2.1)	7.0×10^{-4} (3.1)	9.3×10^{-3} (2.6)	1.99×10^{-5} (2.9)	3.08×10^{-10} (2.3)
TSC22D3	TSC22 domain family member 3	9.18×10^{-5} (2.1)	3.0×10^{-3} (3.6)	3.7×10^{-2} (1.7)	6.60×10^{-4} (2.3)	2.20×10^{-7} (2.1)
CEBPA	CCAAT/enhancer binding protein alpha	2.11×10^{-6} (–1.7)	4.2×10^{-4} (–2.7)	8.8×10^{-4} (–2.4)	1.21×10^{-6} (–2.6)	2.90×10^{-11} (–2.0)
LMNA	Lamin A/C	9.11×10^{-5} (2.8)	1.0×10^{-2} (1.9)	2.5×10^{-3} (1.9)	7.06×10^{-5} (1.9)	5.02×10^{-8} (2.2)
TPPP3	Tubulin polymerization-promoting protein family member 3	4.85×10^{-16} (5.4)	1.9×10^{-2} (1.5)	4.0×10^{-2} (1.2)	1.87×10^{-3} (1.4)	1.54×10^{-12} (2.6)
Pulse pressure						
MYADM	Myeloid-associated differentiation marker	6.30×10^{-12} (2.3)	2.3×10^{-2} (1.4)	2.1×10^{-2} (1.9)	1.31×10^{-3} (1.6)	6.78×10^{-14} (2.1)
TIPARP	TCDD-inducible poly(ADP-ribose) polymerase	4.81×10^{-8} (1.6)	1.3×10^{-2} (0.9)	2.6×10^{-3} (1.3)	1.22×10^{-4} (1.0)	6.23×10^{-11} (1.3)
F12	Coagulation factor XII	4.69×10^{-7} (1.6)	1.4×10^{-2} (1.1)	1.0×10^{-2} (1.0)	3.91×10^{-4} (1.1)	1.64×10^{-9} (1.3)

P values and effect estimates were calculated by linear regression models and by pooled analyses using inverse-variance weighting. Differences of mRNA (%) are given per 10 mm Hg BP difference. BP indicates blood pressure; GHS, Gutenberg Health Study; KORA, Cooperative Heath Research in the Augsburg Region; MESA, Multi-Ethnic Study of Atherosclerosis; meta, meta-analysis; and SHIP-TREND, Study of Health in Pomerania-TREND.

for associations with the 8 BP candidate transcripts (Table S8B). Two *trans*-eQTLs were identified (rs653178-T and rs3184504-C) that increased expression of 4 of the candidate genes (*CRIP1*, *MYADM*, *TPPP2*, and *TIPARP*; Figure S5). These SNPs were located on chromosome 12q24.12 and were in high linkage disequilibrium (pairwise disequilibrium coefficient=0.99; Table S9). Both SNPs, rs653178 (intronic to *ATXN2*) and rs3184504 (nonsynonymous SNP in *SH2B3*), have already been described as *trans*-eQTLs in monocytes and whole blood,[15,19] indicating the importance of these candidate genes for coregulatory mechanisms underlying BP regulation. In our data, *CRIP1* expression was most strongly associated with both SNPs (rs653178: mRNA change per T allele±SE: 5.49%±1.05; $P=3.59\times10^{-7}$; rs3184504: mRNA change per C allele±SE: 5.42%±1.06; $P=6.53\times10^{-7}$). The T-allele of rs3184504 was significantly associated with increased DBP (0.8 mm Hg per T-allele; $P=0.043$) but not to SBP ($P=0.342$) in the GHS.

Discussion

We demonstrated a direct link between the levels of 8 candidate transcripts and BP at a large scale. Our data show that transcript expression changes account for a large proportion of BP variance, and for the most relevant transcript—*CRIP1*—we showed a potential clinical application as circulating biomarker.

This study is one of the largest to investigate global gene expression of BP traits at the population level including >4500 individuals, harmonized data sets on monocytic and whole-blood gene expression and BP phenotypes, as well as data on long-term gene expression changes for 5 years from the same individuals.

Several findings from our study contribute to a more detailed understanding to BP genetics. First, compared with genetic variants, gene expression changes are associated with a considerably larger proportion of phenotypic BP variance. The genetic variants identified to date explain 3.5% of the BP variance,[10] whereas the expression of the 8 candidate transcripts in aggregate accounted for up to 11% in our data. Changes in transcript expression, therefore, seem to reflect the biological changes of BP and hypertension in a better way compared with genetic variants.

Second, 8 transcripts were identified that associated with BP changes. These transcripts are not only expressed in blood cells but also in various human cells and tissues as shown by RNA sequencing in the Genotype-Tissue Expression project[32] (Figure S6). Among these genes, *CRIP1*, *MYADM*, *TIPARP*, *F12*, and *TSC22D3* have been previously implicated in hypertension.[15,19,33–35] For the other transcripts, a connection to diseases related to BP, such as obesity and CVD, as well as important roles in the immune system have been described,[36] but the association with BP traits is novel. These findings

Table 2. Association of Long-Term Changes of Transcript Expression to Changes in BP During 5 Years of Follow-Up in the GHS

	All Individuals (n=1092)		Individuals Without Antihypertensive Treatment (n=703)		All Individuals (n=1092)		Individuals Without Antihypertensive Treatment (n=703)		All Individuals (n=1092)		Individuals Without Antihypertensive Treatment (n=703)	
	SBP (*)				DBP (†)				PP (‡)			
Gene	mRNA Change (%)*	*P* Value	mRNA Change (%)*	*P* Value	mRNA Change (%)†	*P* Value	mRNA Change (%)†	*P* Value	mRNA Change (%)‡	*P* Value	mRNA Change (%)‡	*P* Value
CRIP1	2.93	2.15×10^{-10}§	4.09	9.51×10^{-10}§	5.19	8.46×10^{-11}§	6.65	3.51×10^{-9}§	2.2	5.02×10^{-4}§	2.92	1.24×10^{-3}§
MYADM	1.56	1.81×10^{-4}§	2.39	9.09×10^{-5}§	2.38	8.93×10^{-4}§	3.65	3.53×10^{-4}§	1.4	1.39×10^{-2}§	1.88	2.15×10^{-2}
TIPARP	1.3	4.09×10^{-4}§	1.57	3.17×10^{-3}§	1.81	4.14×10^{-3}§	1.98	2.61×10^{-2}	1.27	1.12×10^{-2}§	1.58	2.68×10^{-2}
TSC22D3	1.15	1.82×10^{-2}§	1.11	1.18×10^{-1}	1.56	6.08×10^{-2}	1.88	1.12×10^{-1}	1.15	8.37×10^{-2}	0.69	4.65×10^{-1}
CEBPA	−0.82	3.97×10^{-2}§	−1.01	8.62×10^{-2}	−1.74	1.14×10^{-2}§	−1.04	2.90×10^{-1}	−0.43	4.27×10^{-1}	−1.18	1.39×10^{-1}
F12	0.07	8.29×10^{-1}	−0.49	2.99×10^{-1}	−0.05	9.29×10^{-1}	−0.42	5.92×10^{-1}	0.16	7.14×10^{-1}	−0.63	3.23×10^{-1}
LMNA	1.23	3.96×10^{-3}§	1.48	1.85×10^{-2}§	1.85	1.16×10^{-2}§	2.26	3.13×10^{-2}	1.12	5.46×10^{-2}	1.16	1.67×10^{-1}
TPPP3	2.28	7.36×10^{-6}§	2.32	1.16×10^{-3}§	5.29	1.87×10^{-9}§	5.16	1.62×10^{-5}§	0.93	1.78×10^{-1}	0.73	4.44×10^{-1}

Linear regression models were adjusted for sex, age, and BP trait at baseline and body mass index change between baseline and follow-up. Changes in mRNA expression level (%) are given per 10-mm Hg SBP increase (*), 10-mm Hg DBP increase (†), and per 10-mm Hg PP increase (‡). Significant associations with a false discovery rate ≤0.05 are indicated by §. BP indicates blood pressure; CEBPA, CCAAT/enhancer-binding protein alpha; CRIP1, cysteine-rich protein 1; DBP, diastolic blood pressure; F12, coagulation factor XII; GHS, Gutenberg Health Study; LMNA, lamin A/C; MYADM, myeloid-associated differentiation marker; PP, pulse pressure; SBP, systolic blood pressure; TIPARP, TCDD-inducible poly(ADP-ribose) polymerase; TPPP3, tubulin polymerization-promoting protein family member 3; and TSC22D3, TSC22 domain family member 3.

seem plausible because numerous studies suggest that hypertension represents an inflammatory state and an involvement of the immune system and, in particular, monocytes in the development of hypertension.[3,37] For instance, selective ablation of lysozyme M-positive myelomonocytic cells attenuated angiotensin II–induced hypertension.[5] Likewise, Itani et al[4] showed that human T cells become activated by hypertensive stimuli, such as angiotensin II.

Previous studies identified BP-related signature genes, including *MYADM* and *TIPARP*.[15] By using monocytes, an

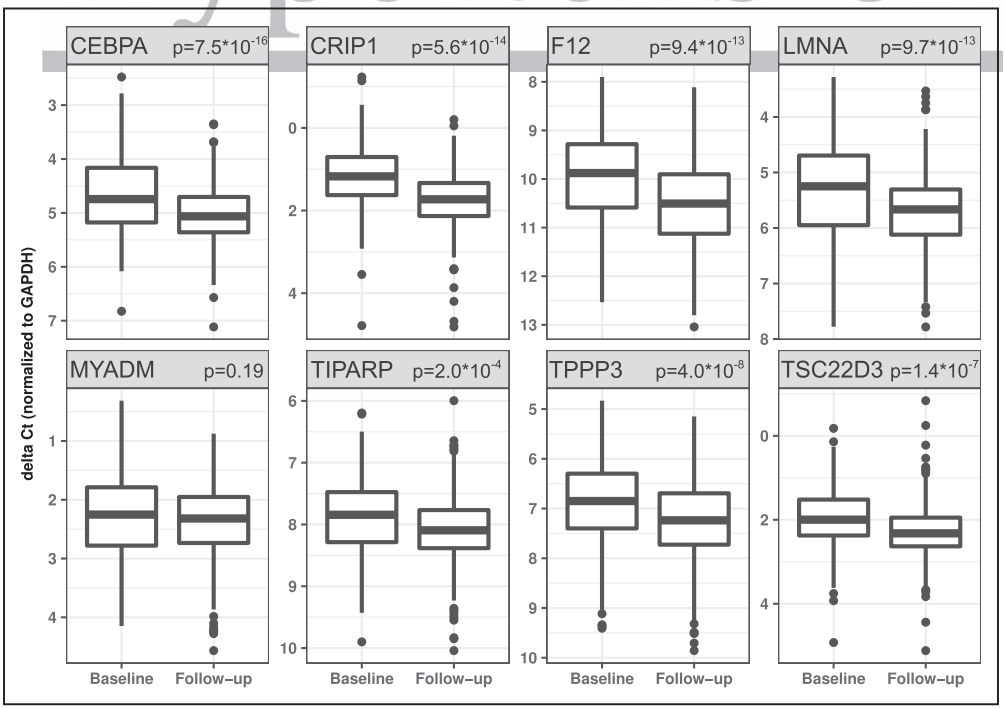


Figure. Relationship of blood pressure (BP) reduction and transcript expression by BP-lowering medication for 6 mo (n=406). Transcript expression was measured by quantitative polymerase chain reaction. Linear mixed models adjusted for age, sex, and body mass index were used to calculate differential gene expression. CEBPA indicates CCAAT/enhancer-binding protein alpha; CRIP1, cysteine-rich protein 1; LMNA, lamin A/C; MYADM, myeloid-associated differentiation marker; TIPARP, TCDD-inducible poly(ADP-ribose) polymerase; TPPP3, tubulin polymerization-promoting protein family member 3; and TSC22D3, TSC22 domain family member 3.

Table 3. Association of Serum CRIP1, CRP, NT-proBNP, and hsTnI Protein Levels to Cardiovascular End Points in the Moli-Sani Project

End Point	Marker	Hazard Ratio (95% CI)*	*P* Value
Stroke (n=50)	CRIP1	1.0588 (1.03–1.0884)	4.97×10^{-5}
	CRP	1.0606 (0.9965–1.1289)	6.41×10^{-2}
	NT-proBNP	1.0001 (0.9988–1.0015)	8.52×10^{-1}
	hsTnI	1.0677 (0.9207, 1.2382)	3.86×10^{-1}
Heart failure (n=139)	CRIP1	1.0138 (0.9913–1.0368)	2.30×10^{-1}
	CRP	1.0677 (1.0131–1.1253)	1.45×10^{-2}
	NT-proBNP	1.0005 (0.9996–1.0015)	2.67×10^{-1}
	hsTnI	1.1538 (1.0586–1.2577)	1.13×10^{-3}
Coronary heart disease (n=107)	CRIP1	1.0158 (0.991–1.0413)	2.14×10^{-1}
	CRP	1.0859 (1.0237–1.1519)	6.16×10^{-3}
	NT-proBNP	1.0008 (0.9997–1.0019)	1.64×10^{-1}
	hsTnI	1.1283 (1.0118–1.2582)	2.99×10^{-2}

Cases included subjects with incident stroke, heart failure, and coronary heart disease. Associations between incident events and protein levels were tested by Cox regression adjusted for age, sex, and systolic blood pressure at baseline. CI indicates confidence interval; CRIP1, cystein-rich protein 1 (ng/mL); CRP, C-reactive protein (mg/L); hsTnI, high sensitive troponin I (pg/mL); and NT-proBNP, N-terminal pro-B-type natriuretic peptide (pg/mL).

*The hazard ratio refers to a 1-unit change of a given biomarker.

important cell type of the innate immune system and effector in inflammation, we identified additional transcripts in relation to BP (*CRIP1*, *TSC22D3*, *CEBPA*, *LMNA*, *TPPP3*, and *F12*). We hypothesize that these genes might contribute to BP regulation and development via their role in the immune system and provide a starting point for further experimental work.

Finally, and clinically most relevant, circulating levels of the protein encoded by the most relevant transcript found—*CRIP1*—are associated with incident stroke, a sequela of hypertension, implying a potential role of CRIP1 as biomarker.

CRIP1 is a particular interesting candidate transcript for further investigation. In our data, *CRIP1* consistently showed the strongest association (1) to BP at the population level, (2) to BP reduction mediated by antihypertensive medication, and (3) to longitudinal changes in BP during a 5-year time frame. The investigation of the genetic interplay by eQTL analyses revealed that the expression of *CRIP1* (along with *MYADM, TIPARP*, and *TPPP3*) was highly associated with variants in the *SH2B3/LNK* locus. SH2B3 is a negative regulator of growth factors and cytokine signaling, and previous data have already implicated this locus as a master regulator involved in BP regulation.[14,19,38] A *Sh2b3*$^{-/-}$ knockout leads to markedly elevated BP in response to low dose of angiotensin II.[16]

We speculate that the *SH2B3* effect on BP is mediated—at least partly—by *CRIP1*. CRIP1 belongs to the LIM/double-zinc finger protein family, and a relationship of CRIP1 to hypertension and renal disease has recently been shown. In the renin-expressing juxtaglomerular cells, crucial for BP control, CRIP1 expression was highly increased.[39,40] CRIP1 is also strongly expressed in immune cells, again indicating a link between CRIP1 and BP regulation via the immune system. Along with the results presented here, these data highlight *CRIP1* a promising BP-related candidate transcript for further examinations.

The main strength of this study is the large size of global and harmonized gene expression data that were associated with BP traits at a population level and the analysis of different cell types, including monocytes and whole blood providing a broader view. However, by using these different cell types, we might have missed additional transcripts that would have been discovered when using monocytes only. As we took care to include transcriptomic data derived using the same methodology (Illumina HT-12 Array) and standardized procedures,[27] no additional, independent population-based monocyte transcriptome data set is, to the best of our knowledge, currently available for replication.

As an additional strength, we were able to include data of longitudinal nature (for 5 years and after BP-lowering medication) to provide information on gene expression over time.

A limitation of our work is that, to date, no independent population-based cohort with follow-up expression data is available for replication of our longitudinal data analyses. Furthermore, the highest proportion of explained BP variance by transcripts was observed in the discovery cohorts, and an independent validation in cohorts with available monocytic RNA would be valuable. Moreover, data on the predictive value of circulating CRIP1 levels need to be confirmed in further studies including a broad range of cardiovascular end points. It needs to be considered that CRIP1 levels were determined in a sample of moderate size (n=400), and the coefficient of variation of the ELISA immunoassay was >10%. The lower sample size and the moderate precision of the ELISA assay can cause a bias into the results. We speculate that by increasing the sample size or using a more valid ELISA assay (which is currently not available), the coefficient of variation might improve and increase the precision of the results, also for additional cardiovascular end points. Furthermore, the precise molecular mechanisms underlying the observed associations still require additional experimental follow-up projects.

In conclusion, using large-scale transcriptome data, our analyses highlight 8 transcripts significantly associated with BP. In particular, *CRIP1* emerged as an attractive candidate to further elucidate the pathomechanisms of hypertension and to envisage in the long-term therapeutic intervention with respect to BP control.

Perspectives

The results from the present study show that several blood-based gene transcripts are associated with BP and long-term

changes of BP, directly linking gene expression with BP. In addition, circulating levels of the protein encoded by the identified CRIP1 gene strongly associated with incident stroke events. These findings suggest that BP-related transcripts could serve as marker for diagnosis, monitoring, or treatment of hypertension in clinical practice. In particular, CRIP1 might additionally serve as circulating marker for future risk of development of CVD and stroke.

Sources of Funding

The Gutenberg Health Study is funded through the government of Rhineland-Palatinate (Stiftung Rheinland-Pfalz für Innovation, contract AZ 961-386261/733), the research programs Wissenschafft Zukunft and Center for Translational Vascular Biology of the Johannes Gutenberg-University of Mainz, and its contract with Boehringer Ingelheim and PHILIPS Medical Systems, including an unrestricted grant for the Gutenberg Health Study. SHIP-TREND (Study of Health in Pomerania-TREND) is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs, as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania, and the network Greifswald Approach to Individualized Medicine (GANI_MED) funded by the Federal Ministry of Education and Research (grant 03IS2061A). Generation of whole-blood transcriptome data has been supported by the Federal Ministry of Education and Research (grant no. 03ZIK012). The University of Greifswald is a member of the Caché Campus program of the InterSystems GmbH. The KORA (Cooperative Heath Research in the Augsburg Region) research platform and the KORA Augsburg studies are financed by the Helmholtz Zentrum München, German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education, Science, Research, and Technology and by the State of Bavaria. We thank the field staff in Augsburg who were involved in the conduct of the studies. The German Diabetes Center is funded by the German Federal Ministry of Health and the Ministry of School, Science, and Research of the State of North-Rhine-Westphalia. The Diabetes Cohort Study was funded by a German Research Foundation project grant (DFG; RA 459/2-1). This study was supported, in part, by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.). This work was supported by the German Federal Ministry of Education and Research (BMBF) funded Systems Biology of Metabotypes grant (SysMBo #0315494A). Additional support was obtained from the German Federal Ministry of Science and Research (National Genome Research Network NGFNplus Atherogenomics, 01GS0834) and the Leibniz Association (WGL Pakt für Forschung und Innovation). The MESA (Multi-Ethnic Study of Atherosclerosis) is supported by National Institutes of Health contracts HHSN2682015000031, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168 and N01-HC-95169 and by grants UL1-TR-000040, UL1-TR-001079, and UL1-RR-025005. Analysis was supported, in part, by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center grant DK063491 to the Southern California Diabetes Endocrinology Research Center. The MESA Epigenomics and Transcriptomics Study was funded by National Heart, Lung, and Blood Institute grant R01HL101250 to Wake Forest University Health Sciences. The enrollment phase of the Moli-Sani Study was supported by research grants from Pfizer Foundation (Rome, Italy), the Italian Ministry of University and Research (MIUR, Rome, Italy)–Programma Triennale di Ricerca, Decreto no. 1588, and Instrumentation Laboratory, Milan, Italy.

Disclosures

S. Blankenberg has received research funding from Abbott, Abbott Diagnostics, Bayer, Boehringer Ingelheim, SIEMENS, and Thermo Fisher; he received honoraria for lectures from Abbott, Abbott Diagnostics, Astra Zeneca, Bayer, Boehringer Ingelheim, Medtronic, Pfizer, Roche, SIEMENS Diagnostics, SIEMENS, and Thermo Fisher; and he served as member of Advisory Boards and for consulting for Boehringer Ingelheim, Bayer, Novartis, Roche, and Thermo Fisher. D.-A. Tregouet is supported by the Institute for Cardiometabolism and Nutrition (ICAN, ANR-10-IAHU-05), and T. Zeller is supported by the German Center for Cardiovascular Research (DZHK, 81Z1710101). The other authors report no conflicts.

References

1. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R; Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 2002;360:1903–1913.
2. Nabel EG. Cardiovascular disease. *N Engl J Med*. 2003;349:60–72. doi: 10.1056/NEJMra035098.
3. Hay M. The good and the bad: immune cells and hypertension. *Circ Res*. 2015;117:830–831. doi: 10.1161/CIRCRESAHA.115.307506.
4. Itani HA, McMaster WG Jr, Saleh MA, et al. Activation of human T cells in hypertension: studies of humanized mice and hypertensive humans. *Hypertension*. 2016;68:123–132. doi: 10.1161/HYPERTENSIONAHA.116.07237.
5. Wenzel P, Knorr M, Kossmann S, et al. Lysozyme M-positive monocytes mediate angiotensin II-induced arterial hypertension and vascular dysfunction. *Circulation*. 2011;124:1370–1381. doi: 10.1161/CIRCULATIONAHA.111.034470.
6. Harrison DG, Guzik TJ, Lob HE, Madhur MS, Marvar PJ, Thabet SR, Vinh A, Weyand CM. Inflammation, immunity, and hypertension. *Hypertension*. 2011;57:132–140. doi: 10.1161/HYPERTENSIONAHA.110.163576.
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. Surendran P, Drenos F, Young R, et al; CHARGE-Heart Failure Consortium; EchoGen Consortium; METASTROKE Consortium; GIANT Consortium; EPIC-InterAct Consortium; Lifelines Cohort Study; Wellcome Trust Case Control Consortium; Understanding Society Scientific Group; EPIC-CVD Consortium; CHARGE+ Exome Chip Blood Pressure Consortium; T2D-GENES Consortium; GoT2DGenes Consortium; ExomeBP Consortium; CHD Exome+ Consortium. Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. *Nat Genet*. 2016;48:1151–1161. doi: 10.1038/ng.3654.
9. Ehret GB, Ferreira T, Chasman DI, et al; CHARGE-EchoGen Consortium; CHARGE-HF Consortium; Wellcome Trust Case Control Consortium. The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. *Nat Genet*. 2016;48:1171–1184. doi: 10.1038/ng.3667.
10. Warren HR, Evangelou E, Cabrera CP, et al; International Consortium of Blood Pressure (ICBP) 1000G Analyses; BIOS Consortium; Lifelines Cohort Study; Understanding Society Scientific group; CHD Exome+ Consortium; ExomeBP Consortium; T2D-GENES Consortium; GoT2DGenes Consortium; Cohorts for Heart and Ageing Research in Genome Epidemiology (CHARGE) BP Exome Consortium; International Genomics of Blood Pressure (iGEN-BP) Consortium; UK Biobank CardioMetabolic Consortium BP Working Group. Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. *Nat Genet*. 2017;49:403–415. doi: 10.1038/ng.3768.
11. Munroe PB, Barnes MR, Caulfield MJ. Advances in blood pressure genomics. *Circ Res*. 2013;112:1365–1379. doi: 10.1161/CIRCRESAHA.112.300387.
12. Lieb W, Jansen H, Loley C, et al; CARDIoGRAM. Genetic predisposition to higher blood pressure increases coronary artery disease risk. *Hypertension*. 2013;61:995–1001. doi: 10.1161/HYPERTENSIONAHA.111.00275.
13. Ehret GB, Caulfield MJ. Genes for blood pressure: an opportunity to understand hypertension. *Eur Heart J*. 2013;34:951–961. doi: 10.1093/eurheartj/ehs455.
14. Ehret GB, Munroe PB, Rice KM, et al; International Consortium for Blood Pressure Genome-Wide Association Studies; CARDIoGRAM Consortium; CKDGen Consortium; KidneyGen Consortium; EchoGen consortium; CHARGE-HF Consortium. Genetic variants in novel

pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103–109. doi: 10.1038/nature10405.
15. Huan T, Esko T, Peters MJ, et al; International Consortium for Blood Pressure GWAS (ICBP). A meta-analysis of gene expression signatures of blood pressure and hypertension. *PLoS Genet*. 2015;11:e1005035. doi: 10.1371/journal.pgen.1005035.
16. Huan T, Meng Q, Saleh MA, et al. Integrative network analysis reveals molecular mechanisms of blood pressure regulation. *Mol Syst Biol*. 2015;11:799.
17. Chon H, Gaillard CA, van der Meijden BB, Dijstelbloem HM, Kraaijenhagen RJ, van Leenen D, Holstege FC, Joles JA, Bluyssen HA, Koomans HA, Braam B. Broadly altered gene expression in blood leukocytes in essential hypertension is absent during treatment. *Hypertension*. 2004;43:947–951. doi: 10.1161/01.HYP.0000123071.35142.72.
18. Zeller T, Wild P, Szymczak S, et al. Genetics and beyond–the transcriptome of human monocytes and disease susceptibility. *PLoS One*. 2010;5:e10693. doi: 10.1371/journal.pone.0010693.
19. Rotival M, Zeller T, Wild PS, et al; Cardiogenics Consortium. Integrating genome-wide genetic variations and monocyte expression data reveals trans-regulated gene modules in humans. *PLoS Genet*. 2011;7:e1002367. doi: 10.1371/journal.pgen.1002367.
20. Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR; 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature*. 2015;526:68–74. doi: 10.1038/nature15393.
21. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
22. Wild PS, Sinning CR, Roth A, Wilde S, Schnabel RB, Lubos E, Zeller T, Keller T, Lackner KJ, Blettner M, Vasan RS, Münzel T, Blankenberg S. Distribution and categorization of left ventricular measurements in the general population: results from the population-based Gutenberg Heart Study. *Circ Cardiovasc Imaging*. 2010;3:604–613. doi: 10.1161/CIRCIMAGING.109.911933.
23. Bild DE, Bluemke DA, Burke GL, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. *Am J Epidemiol*. 2002;156:871–881.
24. Völzke H, Alte D, Schmidt CO, et al. Cohort profile: the study of health in Pomerania. *Int J Epidemiol*. 2011;40:294–307. doi: 10.1093/ije/dyp394.
25. Holle R, Happich M, Löwel H, Wichmann HE; MONICA/KORA Study Group. KORA–a research platform for population based health research. *Gesundheitswesen*. 2005;67(suppl 1):S19–S25. doi: 10.1055/s-2005-858235.
26. Di Castelnuovo A, de Curtis A, Costanzo S, Persichillo M, Olivieri M, Zito F, Donati MB, de Gaetano G, Iacoviello L; MOLI-SANI Project Investigators. Association of D-dimer levels with all-cause mortality in a healthy adult population: findings from the MOLI-SANI study. *Haematologica*. 2013;98:1476–1480. doi: 10.3324/haematol.2012.083410.
27. Schurmann C, Heim K, Schillert A, et al. Analyzing illumina gene expression microarray data from different tissues: methodological aspects of data analysis in the metaxpress consortium. *PLoS One*. 2012;7:e50938. doi: 10.1371/journal.pone.0050938.
28. Ding J, Reynolds LM, Zeller T, et al. Alterations of a cellular cholesterol metabolism network are a molecular feature of obesity-related type 2 diabetes and cardiovascular disease. *Diabetes*. 2015;64:3464–3474. doi: 10.2337/db14-1314.
29. Speliotes EK, Willer CJ, Berndt SI, et al; MAGIC; Procardis Consortium. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet*. 2010;42:937–948. doi: 10.1038/ng.686.
30. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc*. 1995;57:289–300.
31. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, Klemm A, Flicek P, Manolio T, Hindorff L, Parkinson H. The NHGRI GWAS catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res*. 2014;42(database issue):D1001–D1006. doi: 10.1093/nar/gkt1229.
32. Thanassoulis G, Campbell CY, Owens DS, et al; CHARGE Extracoronary Calcium Working Group. Genetic associations with valvular calcification and aortic stenosis. *N Engl J Med*. 2013;368:503–512. doi: 10.1056/NEJMoa1109034.
33. Yagil C, Hubner N, Monti J, Schulz H, Sapojnikov M, Luft FC, Ganten D, Yagil Y. Identification of hypertension-related genes through an integrated genomic-transcriptomic approach. *Circ Res*. 2005;96:617–625. doi: 10.1161/01.RES.0000160556.52369.61.
34. Ivy JR, Oosthuyzen W, Peltz TS, Howarth AR, Hunter RW, Dhaun N, Al-Dujaili EA, Webb DJ, Dear JW, Flatman PW, Bailey MA. Glucocorticoids induce nondipping blood pressure by activating the thiazide-sensitive cotransporter. *Hypertension*. 2016;67:1029–1037. doi: 10.1161/HYPERTENSIONAHA.115.06977.
35. Papageorgiou PC, Chan CT, Yeo EL, Backx PH, Floras JS. Coagulation factor XIIa-kinin-mediated contribution to hypertension of chronic kidney disease. *J Hypertens*. 2014;32:1523–1533; discussion 1533. doi: 10.1097/HJH.0000000000000192.
36. Bennett CE, Nsengimana J, Bostock JA, Cymbalista C, Futers TS, Knight BL, McCormack LJ, Prasad UK, Riches K, Rolton D, Scarrott T, Barrett JH, Carter AM. CCAAT/enhancer binding protein alpha, beta and delta gene variants: associations with obesity related phenotypes in the Leeds Family Study. *Diab Vasc Dis Res*. 2010;7:195–203. doi: 10.1177/1479164110366274.
37. Wenzel U, Turner JE, Krebs C, Kurts C, Harrison DG, Ehmke H. Immune mechanisms in arterial hypertension. *J Am Soc Nephrol*. 2016;27:677–686. doi: 10.1681/ASN.2015050562.
38. Rudemiller NP, Lund H, Priestley JR, Endres BT, Prokop JW, Jacob HJ, Geurts AM, Cohen EP, Mattson DL. Mutation of SH2B3 (LNK), a genome-wide association study candidate for hypertension, attenuates Dahl salt-sensitive hypertension via inflammatory modulation. *Hypertension*. 2015;65:1111–1117. doi: 10.1161/HYPERTENSIONAHA.114.04736.
39. Brunskill EW, Sequeira-Lopez ML, Pentz ES, Lin E, Yu J, Aronow BJ, Potter SS, Gomez RA. Genes that confer the identity of the renin cell. *J Am Soc Nephrol*. 2011;22:2213–2225. doi: 10.1681/ASN.2011040401.
40. Castellanos-Rivera RM, Pentz ES, Lin E, Gross KW, Medrano S, Yu J, Sequeira-Lopez ML, Gomez RA. Recombination signal binding protein for Ig-κJ region regulates juxtaglomerular cell phenotype by activating the myo-endocrine program and suppressing ectopic gene expression. *J Am Soc Nephrol*. 2015;26:67–80. doi: 10.1681/ASN.2013101045.

Novelty and Significance

What Is New?

- Transcriptomics data from populations were analyzed providing novel insights into the genetics of blood pressure (BP). Eight transcripts, measured in monocytes and whole blood, were found to be related to BP changes.

What Is Relevant?

- Changes in transcript levels are related to BP changes. Levels of CRIP1, cysteine-rich protein 1, associated with future BP-related disease, such as incident stroke.

Summary

Using transcriptome data, this study highlights 8 transcripts significantly associated with BP. *CRIP1* emerged as candidate to further elucidate the pathogenesis and mechanisms of hypertension.

Hypertension
Journal of the American Heart Association

Transcriptome-Wide Analysis Identifies Novel Associations With Blood Pressure

Tanja Zeller, Claudia Schurmann, Katharina Schramm, Christian Müller, Soonil Kwon, Philipp S. Wild, Alexander Teumer, David Herrington, Arne Schillert, Licia Iacoviello, Adelheid Kratzer, Annika Jagodzinski, Mahir Karakas, Jingzhong Ding, Johannes T. Neumann, Kari Kuulasmaa, Christian Gieger, Tim Kacprowski, Renate B. Schnabel, Michael Roden, Simone Wahl, Jerome I. Rotter, Francisco Ojeda, Maren Carstensen-Kirberg, David-Alexandre Tregouet, Marcus Dörr, Thomas Meitinger, Karl J. Lackner, Petra Wolf, Stephan B. Felix, Ulf Landmesser, Simona Costanzo, Andreas Ziegler, Yongmei Liu, Uwe Völker, Walter Palmas, Holger Prokisch, Xiuqing Guo, Christian Herder, Stefan Blankenberg and Georg Homuth

Hypertension. published online August 7, 2017;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0194-911X. Online ISSN: 1524-4563

ONLINE SUPPLEMENT

Transcriptome-wide analysis identifies novel associations with blood pressure

Running title: Blood pressure related gene expression

Zeller T[1,2*], Schurmann C[2,3*#], Schramm K[4*], Müller C[1,2,5*], Kwon S[6*], Wild PS[2,7,8], Teumer A[2,9], Herrington D[10], Schillert A[2,5], Iacoviello L[11], Kratzer A[2,12], Jagodzinski A [1,2], Karakas M[1,2], Ding J[10], Neumann JT[1,2], Kuulasmaa K[13], Gieger C[14,15], Kacprowski T[2,3] Schnabel RB[1,2], Roden M[16,17,18], Wahl S [14,15,18], Rotter JI[6], Ojeda F[1], Carstensen-Kirberg M[16,18], Tregouet DA[19], Dörr M[2,20], Meitinger T[2,4,21] Lackner KJ[2,22], Wolf P[5,21], Felix SB[2,20], Landmesser U[2,12] Costanzo S[11], Ziegler A[2,5], Liu Y[23], Völker U[2,3,24], Palmas W[25], Prokisch H[4,21], Guo X[6*], Herder C[16,18*], Blankenberg S[1,2*], Homuth G[*3,24]

1. Department of General and Interventional Cardiology, University Heart Center Hamburg, Germany
2. DZHK (German Centre for Cardiovascular Research), Germany
3. Interfaculty Institute for Genetics and Functional Genomics, University Greifswald, Germany
4. Institute of Human Genetics, Helmholtz Zentrum München, Germany
5. Institut für Medizinische Biometrie und Statistik, Universitätsklinikum Schleswig-Holstein, Germany
6. Institute for Translational Genomics and Population Sciences, UCLA Medical Center, USA.
7. Preventive Cardiology and Preventive Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, Germany
8. Center for Thrombosis and Hemostasis, Mainz, Germany
9. Institute for Community Medicine, University Medicine Greifswald, Germany
10. Department of Internal Medicine, Wake Forest School of Medicine, USA
11. IRCCS Istituto Neurologico Mediterraneo Neuromed, Department of Epidemiology and Prevention, Italy
12. Department of Cardiology and Pneumology, Charite, Universitätsmedizin Berlin, Germany
13. National Institute for Health and Welfare, Helsinki, Finland
14. Molecular Epidemiology, Helmholtz Zentrum München, Germany
15. Institute of Epidemiology II, Helmholtz Zentrum München, Germany
16. Institute for Clinical Diabetology, German Diabetes Center, Germany
17. Department of Endocrinology and Diabetology, Medical Faculty Düsseldorf, Germany
18. German Center for Diabetes Research (DZD), Germany
19. Sorbonne Universités, UPMC, INSERM, UMR_S 1166, ICAN Institute for Cardiometabolism and Nutrition, Paris, France.
20. Department of Internal Medicine B, University Medicine Greifswald, Germany
21. Institute of Human Genetics, Technische Universität München, Germany

22. Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, Germany
23. Department of Epidemiology & Prevention, Wake Forest School of Medicine, North Carolina, USA
24. ZIK_FunGene, Universität Greifswald, Germany
25. Columbia University Medical Center, New York, USA

* equal contribution

Supplementary Material

1. Description of study cohorts
2. Definition and measurement of subclinical phenotypes and biomarkers
3. Preparation and quality control of RNA
4. Genotyping and imputation in the Gutenberg Health Study
5. Measurement of Human Cystein Rich Protein 1 (CRIP1) protein levels in the Moli-Sani Study
6. Statistical analyses

Supplementary Tables

Table S1: Study characteristics

Table S2: Gene-specific transcripts associated with blood pressure traits in the discovery analysis (Excel file).

Tables S3: Gene-specific transcripts associated with blood pressure traits in the validation analysis (Excel file).

Tables S4: Gene-specific transcripts associated with blood pressure traits in the discovery and validation analysis after exclusion of individuals receiving antihypertensive treatment (Excel file).

Tables S5: Gene-specific transcripts associated with blood pressure traits in the discovery analysis including only subjects of Caucasian ethnicity in MESA (Excel file)

Table S6: Explained variance (%) in BP levels attributable to the expression of genes in the participating studies.

Table S7: Association of monocyte candidate gene expression with blood pressure-related, subclinical cardiovascular phenotypes in the Gutenberg Health Study.

Tables S8: Single nucleotide polymorphisms from the GRASP database
(A) and published BP related variants (B) used for analyses of expression quantitative trait loci (Excel file).

Table S9: Blood pressure-related eQTLs and SNPs related to blood pressure

Table S10: Baseline characteristics of subjects selected for CRIP1 measurements from the Moli-Sani Study.

Supplementary Figures

Figure S1: Study workflow

Figure S2: Candidate gene expression by study and cell types.

Figure S3: Associations between blood pressure changes and changes in mRNA levels of candidate genes after 5-years follow-up in the Gutenberg Health Study

Figure S4: Reduction of blood pressure between baseline and 6 month follow up in a clinical trial.

Figure S5:
A) Single nucleotide polymorphisms from the GRASP database used for analyses of expression quantitative trait loci. B). Single nucleotide polymorphisms from the GWAS catalogue used for analyses of expression quantitative trait loci.

Figure S6: Median candidate gene expression in various tissues measured by RNA-sequencing in the GTEx project

Supplementary Material

1. Description of study cohorts

Ethics statement

The study followed the recommendations of the Declaration of Helsinki. The study protocols of all studies were approved by the respective ethics committees. Written informed consent was obtained from all study participants.

GHS

The Gutenberg Health Study (GHS) is designed as a community-based, prospective, observational, single-center cohort study in the Rhine-Main area of Western Germany. [1] The sample was drawn randomly from the governmental local registry offices in the city of Mainz and the district of Mainz-Bingen. The sample was stratified 1:1 for sex and residence (urban and rural) and in equal strata for decades of age. Individuals between 35 and 74 years of age were enrolled. Exclusion criteria were insufficient knowledge of the German language and physical or psychological inability to participate in the examinations at the study center. Baseline examination of 15,010 study participants was performed between 2007 and 2012. Starting in April 2012, a second clinical follow-up visit was started after 5 years. At baseline and after 5 years, detailed medical, biochemical, and molecular information and biomaterial have been acquired.

MESA

The Multi-Ethnic Study of Atherosclerosis (MESA) was designed to investigate the characteristics of subclinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms) and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease. The cohort is a diverse, population-based sample of 6,814 asymptomatic men and women aged 45-84. Approximately 38 percent of the recruited participants are white, 28 percent African American, 22 percent Hispanic, and 12 percent Asian (predominantly of Chinese descent). Participants were recruited during 2000-2002 from 6 field centers across the U.S. (at Wake Forest University; Columbia University; Johns Hopkins University; the University of Minnesota; Northwestern University, and the University of California – Los Angeles). Since its inception in 2000, five clinic visits (exams) collected extensive clinical, socio-demographic, lifestyle and behavior, laboratory, nutrition, and medication data. [2] The present analysis is primarily based on analyses of purified monocyte samples from the April 2010-February 2012 examination (exam 5) of 1,264 randomly selected MESA participants (55-94 years old, Caucasian (47%), African American (21%) and Hispanic (32%), female 51%) from four MESA field centers (Baltimore, MD; Forsyth County, NC; New York, NY; and St. Paul, MN).

KORA

KORA (Cooperative Heath Research in the Augsburg Region) exists since 1996 in the region of Augsburg in the southwest of Germany, and builds on the MONICA (Monitoring of trends and determinants in cardiovascular disease) project initiated in 1984 [3]. KORA is a regional research platform for population-based surveys and a cohort of more than 18,000 subjects are actively followed up to date. The KORA F4 survey (2006-2008) was

the 7-year follow-up survey of KORA S4 (1999-2001), which included a population-based sample aged 25 to 74 years from the city of Augsburg and two adjacent counties.

SHIP-TREND

The Study of Health in Pomerania (SHIP) is a longitudinal population-based cohort in West Pomerania, a region in the northeast of Germany. The study assesses the prevalence and incidence of common population-relevant diseases and their risk factors. Baseline examinations for SHIP-TREND were carried out between 2008 and 2012, comprising 4,420 participants. Study design and sampling methods were previously described [4]. The present project is based on a subset of 997 individuals aged 20 to 81 years of the SHIP-TREND study population.

Blood pressure lowering clinical trial

The influence of routinely used BP lowering medication over 6 month on gene expression was assessed in a clinical trial (EudraCT No.: 2009-017010-68). The study used the combination of telmisartan and amlodipine versus olmesartan and hydrochlorothiazide in hypertensive patients. Participants were selected at an age of 35 years or older, and being treated, but with uncontrolled hypertension (defined as 20/10 mmHg above target blood pressure of <140/90 mmHg [<130/80 mmHg for renal impaired and/ or diabetic patients]) or controlled hypertension and ≥ 3 cardiovascular risk factors and/or metabolic syndrome and/or diabetes mellitus and/or end organ damage. Exclusion criteria included pretreatment with amlodipine, Diuretics and AT1Blocker/ACEInhibitor within the last 3 months; pretreatment with telmisartan within the last 3 months, myocardial infarction within the last 6 months, previous stroke or hemodynamically relevant stenosis of carotic arteria and cardiac or peripheral bypass surgery within the last 6 month. Laboratory and clinical phenotyping includes various markers and collection of biomaterial available in all 600 individuals at 2 time points (baseline and 6 month follow up).

The Moli Sani Study

The cohort of the Moli-Sani Study was recruited in the Molise region, Italy, from city hall registries by a multistage sampling. First, townships were sampled in major areas by cluster sampling; then, within each township, participants aged 35 years or over were selected by simple random sampling. Exclusion criteria were pregnancy at the time of recruitment, lack of in understanding, current multiple trauma or coma, or refusal to sign the informed consent. A total of 24,325 men (47%) and women (53%) over the age of 35 were examined at baseline from 2005 to 2010. Participation was 70%. The cohort was followed-up for a median of 4.2 years (maximum 6.5 years) at December 2011 and will be followed-up every 5 years [5].(http://www.moli-sani.org). To determine of CRIP1 serum levels by ELISA, incident cases of cardiovascular endpoints (stroke, heart failure, coronary heart disease) as well as a random sample of the cohort were selected. In total, 107 cases of incident coronary heart disease, 50 cases of incident stroke, 139 cases of incident heart failure and a random sample of 133 subjects were selected. The baseline characteristics of the selected subjects is given in Table S 10.

2. Definition and measurement of clinical phenotypes and biomarkers

Definitions of SBP and DBP were standardized between all studies. PP was calculated as the difference between the systolic and diastolic pressure readings. Hypertension was defined as SBP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg at rest obtained as the mean of the second and third measurement, or by taking any antihypertensive drugs within the last 2 weeks. BP was measured with an Omron HEM-705CP device in GHS, SHIP-TREND and the clinical trial, using a Hawksley random-zero sphygmomanometer in KORA and a Dinamap PRO 100 automated oscillometric device in MESA.

Hyperlipidemia was defined as LDL/HDL ratio $>$ 3.5. Echocardiography was performed in every individual using a standardized protocol according to current American and European guidelines and as described in [1]. An iE33 echocardiography system with an S5-1 sector array transducer was used (Philips Electronics, Amsterdam, The Netherlands). Trained and certified medical technical assistants performed the examination. The following linear echocardiographic variables were studied in the present investigation: interventricular end-diastolic septum diameter (IVSD), LV internal end-diastolic diameter (LVIDD), LV end-diastolic posterior wall diameter (LVPWD), and LV end-systolic diameter (LVESD), LV hypertrophy (LVH). Derived from these variables, RWT as (IVSD_LVPWD)/LVEDD, and LV mass (LVM) according to the American Society of Echocardiography (ASE). The estimated glomerular filtration rate (eGFR) was calculated by the CKD-EPI formula. [6].

NT-proBNP levels were measured using a commercially available assay on the ELECSYS 2010 using the Elecsys proBNP II assay (ECLIA, Roche Diagnostics, Mannheim, Germany) with a LoD of 5 ng/L. The assay range of the assay was 5–35,000 ng/L. The inter- and intra-coefficient of variation were 5.4% and 2.3%, respectively. hsCRP levels were measured with the routine laboratory using an Abbott Architect c8000 system and the CRP Vario immunoassay. The inter- and intra-coefficient of variation were 5.6% and 3.7%, respectively. hsTnI levels were measured using a high-sensitivity cardiac troponin assay (ARCHITECT STAT highly sensitive Troponin I immunoassay, Abbott Diagnostics, USA, ARCHITECT i2000SR). The limit of detection for the assay was 1.9 ng/L (range 0-50000 ng/L). The assay had a ten percent coefficient of variation at a concentration of 5.2 ng/L. The inter- and intra-coefficient of variation were 4.5% and 5.8%, respectively.

3. Preparation and quality control of RNA

Preparation of RNA samples in GHS, KORA F4, and SHIP-TREND has been described previously. [7]. Briefly, total RNA was isolated from whole blood in KORA and SHIP-TREND, and from monocytes in GHS. For all three studies, purity and concentration of RNA were determined by NanoDrop measurement and RNA quality was measured on a 2100 Bioanalyzer and the RNA 6000 Nano Lab Chips (Agilent Techonology, Inc., Santa Clara, CA) by the RNA integrity number (RIN). Only samples with a RIN above 7 were used for gene expression profiling. In MESA, monocytic RNA was isolated using the AllPrep DNA/RNA Mini Kit (Qiagen, Inc., Hilden, Germany). RNA quality metrics included optical density measurements, using a NanoDrop spectrophotometer and evaluation of the RIN using the Agilent 2100 Bioanalyzer with RNA 6000 Nano Lab chips (Agilent Technology, Inc., Santa Clara, CA) following manufacturer's instructions. RNA with a RIN above 9 was used for gene expression profiling.

In the blood pressure lowering clinical trial, total RNA was isolated from peripheral blood mononuclear cells (PBMCs) using Trizol/Chloroform extraction.

Separation of PBMCs was conducted within 20 min after blood collection. Briefly, 8 mL blood was collected using the Vacutainer CPT Cell Preparation Tube System (BD, Heidelberg, Germany) and centrifuged. After separation, cells were washed and were resuspended in 1.5 mL Trizol Reagent (Invitrogen, Karlsruhe, Germany). 300 mL chloroform was added and phases were separated by centrifugation. For precipitation of RNA isopropanol was added. After a final washing step, total RNA was eluted in 40 µL RNase-free water. RNA quality metrics included optical density measurements, using a NanoDrop spectrophotometer. Integrity of total RNA was evaluated on an Agilent Bioanalyzer 2100 (Agilent Technologies, Boeblingen, Germany). Only samples with a RIN above 7 were used for subsequent gene expression analysis by semi-quantitative real-time PCR.

Semi-quantitative Polymerase Chain Reaction (PCR)

For semi-quantitative PCR, total RNA (100ng) was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit including RNase Inhibitor (Life Technologies, Darmstadt). The resulting cDNA was diluted 1:20 with RNase-/DNase-free water and 2.5µl were used for gene expression analysis by real-time PCR amplification. The amplification was carried out on a 7900HT Fast Real-Time PCR System (Life Technologies, Darmstadt) using the TaqMan Gene Expression Master Mix (Life Technologies, Darmstadt) and TaqMan Gene Expression assays for the following genes: CEBPA (Hs00269972_s1), CRIP1 (Hs00906229_g1), F12 (Hs01557543_g1), *LMNA* (Hs00153462_m1), *MYADM* (Hs01880197_s1), *TIPARP* (Hs00296054_m1), *TPPP3* (Hs00372228_g1), *TSC22D3* (Hs00929365_m1). For normalization, *GAPDH* endogenous control (Life Technologies, Darmstadt) were used.

4. Genotyping and imputation in the Gutenberg Health Study

Genomic DNA was extracted from buffy coats prepared from EDTA blood samples. Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0 as described by the Affymetrix user manual. Genotypes were called using the Affymetrix Birdseed v2 calling algorithm, and quality control was performed using GenABEL (http://mga.bionet.nsc.ru/nlru/GenABEL). Individuals with a call rate <97% or an autosomal heterozygosity >3 SDs around the mean were excluded. Standard quality criteria were applied to exclude SNPs (MAF<1%, genotype call rate <98%, and P value of deviation from HWE $<10^{-4}$). Imputation based on 1,000 genomes Phase I Integrated Release Version 2 (NCBI Build 37) was performed using MACH version 1.0.18.c. In total, 1,133 individuals with genotype and monocytic gene expression data were available for expression quantitative trait loci (eQTL) analysis.

5. Measurement of Human Cystein Rich Protein 1 (***CRIP1) protein levels in the Moli-Sani Study***

To investigate whether the protein encoded by *CRIP1* may be utilized as a circulating protein biomarker for clinically relevant endpoints, serum CRIP1 levels were determined in a population setting of 379 individuals from the cohort of the Moli-Sani Study [8]. This subsample was selected from the overall Moli-Sani cohort using a case cohort sampling design including incident cardiovascular cases of stroke (n=50), heart failure (n= 139) and coronary heart disease (CHD, a composite endpoint of myocardial infarction and coronary death, n=107) and an age-weighted random samples of the cohort (n=133).

Subjects with multiple events were included in each of the respective groups, resulting in a total overlap between groups of 50 subjects. CRIP1 protein levels were measured using an enzyme-linked immunosorbent assay (EKU03572, Biomatik, Wilmington, Delaware**,** USA). The lower limit of detection was 0.057 ng/mL. The inter-assay coefficient of variation (CV) was 15.5%; the intra-assay CV was 11.9%.

6. Statistical Methods

Proportion of BP variance attributable to candidate transcript expression

The proportion of SBP, DBP and PP explained by mRNA expression was calculated separately for each candidate transcript. Therefore, a generalized R^2 was computed based on the likelihoods from linear regressions, i) containing all covariates plus the candidate transcript expression and ii) with the covariates only as independent variables, as proposed by Magee [9]. Confidence intervals were estimated in 5,000 bootstrap iterations. The aggregated variance was calculated analogously, but with all candidate transcripts as independent variables.

Analysis of differential gene expression in the clinical trial

Expression values of candidate transcripts measured by qPCR were normalized for GAPDH Ct values prior to association analysis and are represented as deltaCt values (deltaCt_mRNA = Ct_mRNA – Ct_GAPDH). Differential gene expression in the clinical trial was assessed comparing gene expression at baseline and 6-month follow-up. Associations with Benjamini-Hochberg [10] based FDR ≤ 0.05 were considered significant.

Long term changes in gene expression analysis in the Gutenberg Health Study

Longitudinal gene expression data (Baseline and 5-year follow up) of 1,092 GHS individuals was pre-processed, log_2-transformed and batch effects were removed by quantile normalization followed by ComBat. [11] The difference between SBP and DBP and expression of each candidate transcript at baseline and 5-year follow-up was calculated for each individual. Association analyses between changes of each BP trait and candidate transcript expression were performed using linear regression and were adjusted for sex, age at baseline and BMI change between baseline and follow-up. Adjustments for multiple testing were performed using the Benjamini-Hochberg method[10], and the significance level was set to 0.05. Results are given as percent mRNA change (%mRNA change) after 6 months ± standard error.

Expression quantitative trait loci (eQTL) analysis in the GHS

eQTL analyses were performed in 1,333 individuals from the GHS with available gene expression and available genome-wide genotyping data based on Affymetrix SNP 6.0 microarrays with imputation based on the 1,000 genome reference[12]. Two approaches were used to identify eQTLs related to BP or cardiovascular disease:

A.) For each candidate transcript, cis-eQTLs were calculated for SNPs within ±250kb around the transcription start site and a minor allele frequency (MAF) ≥ 1%. The Genome-wide Repository of Associations between SNPs and Phenotypes (GRASP) database version 2.0.0.0 [13] was studied for significant cis- eQTLs that were at least moderately associated to a BP or a cardiovascular disease related trait (p-value≤10^{-4}).

B.) Published GWAS results of BP-related traits were retrieved from the GWAS catalogue (2017-03-20) [14]. Latest identified novel BP variants not present in the GWAS catalogue were retrieved from the relevant publications[15-18].

In total, 270 unique SNPs with genome-wide association to at least one BP trait were found. Of those, 191 SNPs with a MAF ≥ 1% present in GHS were tested, leading to a significance level of 2.6×10^{-4}.
All eQTL analyses were performed using linear models assuming an additive genetic model, and were adjusted for age, sex and technical covariates [7].

Association of candidate transcript expression and sub-clinical phenotypes in the GHS

In 1,285 GHS subjects, associations between sub-clinical phenotypes and candidate transcript expression from microarrays were calculated using linear regression models adjusted for age, sex and technical covariates as described above. For each sub-clinical phenotype independently, associations with a Benjamini-Hochberg based FDR ≤ 0.05 were considered significant. Results are given as mRNA change per 10mmHg increase in blood pressure trait (delta mRNA) ± standard error.

Association of CRIP1 serum levels with clinically relevant endpoints

In the Moli-Sani Study, CRIP1 serum levels were tested for association with cardiovascular endpoints. For this, multivariate associations of stroke, heart failure, and coronary heart disease with CRIP1 serum levels were calculated using Cox regression adjusted for age, sex, systolic BP and plate layout. Since subjects were selected to the case-cohort sample with unequal probabilities, inverse of the sampling probabilities were used as weights in the Cox regression as proposed by Kulathinal et al. [19].

References

1. Wild PS, Sinning CR, Roth A, Wilde S, Schnabel RB, Lubos E, Zeller T, Keller T, Lackner KJ, Blettner M, Vasan RS, Munzel T, Blankenberg S. Distribution and categorization of left ventricular measurements in the general population: Results from the population-based gutenberg heart study. *Circulation. Cardiovascular imaging*. 2010;3:604-613
2. Bild DE, Bluemke DA, Burke GL, et al. Multi-ethnic study of atherosclerosis: Objectives and design. *American journal of epidemiology*. 2002;156:871-881
3. Holle R, Happich M, Lowel H, Wichmann HE. Kora--a research platform for population based health research. *Gesundheitswesen*. 2005;67 Suppl 1:S19-25
4. Volzke H, Alte D, Schmidt CO, et al. Cohort profile: The study of health in pomerania. *International journal of epidemiology*. 2011;40:294-307
5. Di Castelnuovo A, de Curtis A, Costanzo S, Persichillo M, Olivieri M, Zito F, Donati MB, de Gaetano G, Iacoviello L, Investigators M-SP. Association of d-dimer levels with all-cause mortality in a healthy adult population: Findings from the moli-sani study. *Haematologica*. 2013;98:1476-1480
6. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J. A new equation to estimate glomerular filtration rate. *Annals of internal medicine*. 2009;150:604-612
7. Schurmann C, Heim K, Schillert A, et al. Analyzing illumina gene expression microarray data from different tissues: Methodological aspects of data analysis in the metaxpress consortium. *PloS one*. 2012;7:e50938
8. Bonaccio M, Di Castelnuovo A, Rago L, de Curtis A, Assanelli D, Badilini F, Vaglio M, Costanzo S, Persichillo M, Cerletti C, Donati MB, de Gaetano G, Iacoviello L. T-wave axis deviation is associated with biomarkers of low-grade inflammation. Findings from the moli-sani study. *Thrombosis and haemostasis*. 2015;114:1199-1206
9. Magee L. R 2 measures based on wald and likelihood ratio joint significance tests. *The American Statistician*. 1990;44:250–253
10. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing *Journal of the Royal Statistical Society* 1995;57:289-300
11. Muller C, Schillert A, Rothemeier C, Tregouet DA, Proust C, Binder H, Pfeiffer N, Beutel M, Lackner KJ, Schnabel RB, Tiret L, Wild PS, Blankenberg S, Zeller T, Ziegler A. Removing batch effects from longitudinal gene expression - quantile normalization plus combat as best approach for microarray transcriptome data. *PloS one*. 2016;11:e0156594
12. The 1000 Genomes Project C, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR. A global reference for human genetic variation. *Nature*. 2015;526:68-74
13. Leslie R, O'Donnell CJ, Johnson AD. Grasp: Analysis of genotype-phenotype results from 1390 genome-wide association studies and corresponding open access database. *Bioinformatics*. 2014;30:i185-194
14. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, Klemm A, Flicek P, Manolio T, Hindorff L, Parkinson H. The nhgri gwas catalog, a curated resource of snp-trait associations. *Nucleic acids research*. 2014;42:D1001-1006
15. Liu C, Kraja AT, Smith JA, et al. Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. *Nature genetics*. 2016;48:1162-1170
16. Ehret GB, Ferreira T, Chasman DI, et al. The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. *Nature genetics*. 2016;48:1171-1184

17. Surendran P, Drenos F, Young R, et al. Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. *Nature genetics*. 2016;48:1151-1161
18. Warren HR, Evangelou E, Cabrera CP, et al. Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. *Nature genetics*. 2017;49:403-415
19. Kulathinal S, Karvanen J, Saarela O, Kuulasmaa K. Case-cohort design in practice - experiences from the morgam project. *Epidemiologic perspectives & innovations : EP+I*. 2007;4:15
20. Westra HJ, Peters MJ, Esko T, et al. Systematic identification of trans eqtls as putative drivers of known disease associations. *Nature genetics*. 2013;45:1238-1243
21. Newton-Cheh C, Johnson T, Gateva V, et al. Genome-wide association study identifies eight loci associated with blood pressure. *Nature genetics*. 2009;41:666-676
22. Wain LV, Verwoert GC, O'Reilly PF, et al. Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. *Nature genetics*. 2011;43:1005-1011
23. Dubois PC, Trynka G, Franke L, et al. Multiple common variants for celiac disease influencing immune gene expression. *Nature genetics*. 2010;42:295-302
24. Zhernakova A, Stahl EA, Trynka G, et al. Meta-analysis of genome-wide association studies in celiac disease and rheumatoid arthritis identifies fourteen non-hla shared loci. *PLoS genetics*. 2011;7:e1002004
25. Kottgen A, Pattaro C, Boger CA, et al. New loci associated with kidney function and chronic kidney disease. *Nature genetics*. 2010;42:376-384
26. Kottgen A, Albrecht E, Teumer A, et al. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nature genetics*. 2013;45:145-154
27. Levy D, Ehret GB, Rice K, et al. Genome-wide association study of blood pressure and hypertension. *Nature genetics*. 2009;41:677-687
28. Gieger C, Radhakrishnan A, Cvejic A, et al. New gene functions in megakaryopoiesis and platelet formation. *Nature*. 2011;480:201-208
29. Shameer K, Denny JC, Ding K, et al. A genome- and phenome-wide association study to identify genetic variants influencing platelet count and volume and their pleiotropic effects. *Human genetics*. 2014;133:95-109
30. Ehret GB, Munroe PB, Rice KM, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103-109
31. Shin SY, Fauman EB, Petersen AK, et al. An atlas of genetic influences on human blood metabolites. *Nature genetics*. 2014;46:543-550
32. Gudbjartsson DF, Bjornsdottir US, Halapi E, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nature genetics*. 2009;41:342-347
33. Eriksson N, Tung JY, Kiefer AK, Hinds DA, Francke U, Mountain JL, Do CB. Novel associations for hypothyroidism include known autoimmune risk loci. *PloS one*. 2012;7:e34442

Supplementary Tables

Table S1. Study characteristics. Data presented as mean ± standard deviation or number and percent. *Prevalent hypertension defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg or antihypertensive drug treatment during the last two weeks. **P-values were calculated by Student's t-test for differences of continuous traits and by chi-squared test for differences of dichotomous traits.

Study Characteristics	Discovery			Replication		
	GHS n = 1,285	MESA n = 1,264	P-value**	SHIP-TREND n = 993	KORA F4 n= 997	P-value**
Mean age [years]	54.7±11.0	60.2 ± 9.5	<0.001	50.1±13.7	70.38±5.37	<0.001
Females (%)	663 (51.6)	650 (51.4)	0.963	554 (56.0)	493 (49.6)	0.005
Current smoker, n (%)	239 (18.6)	170 (13.5)	<0.001	213 (21.5)	66 (6.7)	<0.001
Body mass index (BMI) [kg/m^2]	27.0±4.6	29.3 ± 5.2	<0.001	27.3±4.6	28.9±4.5	<0.001
Hyperlipidemia, n (%)	351 (27.3)	206 (16.3)	<0.001	141 (14.2)	155 (15.7)	0.435
Diabetes Mellitus, n (%)	79 (6.2)	104 (8.2)	0.050	28 (2.8)	211 (21.2)	<0.001
CAD, n (%)	58 (4.6)	55(4.4)	0.918	72 (7.3)	117 (11.8)	<0.001
Systolic blood pressure (SBP) [mmHg]	132.2 ± 17.8	122.9 ± 18.9	<0.001	124.4 ± 16.9	128.7 ± 20.0	<0.001
Diastolic blood pressure (DBP) [mmHg]	83.5 ± 9.7	71.4 ± 9.6	<0.001	76.6 ± 9.8	74.0 ± 10.1	<0.001
Pulse pressure (PP) [mmHg]	48.7 ± 13.0	51.5 ± 15.0	<0.001	47.8 ± 12.2	54.7 ± 14.7	<0.001
Heart rate, bpm	68.7 ± 10.9	62.9 ± 9.3	<0.001	71.0 ± 10.7	71.6 ± 11.3	0.169
Prevalent Hypertension, n (%)*	618 (48.1)	557 (44.1)	0.046	460 (46.5)	682 (68.7)	<0.001
Antihypertensive drug treatment	344 (26.8)	449 (35.5)	<0.001	388 (39.2)	421 (42.4)	0.166
Caucasian, n (%)	1,285	590 (46.7)	<0.001	993	997	1

Tables S2: Gene-specific transcripts associated with blood pressure traits in the discovery analysis (see Excel file Supplementary Table S2).

Tables S3: Gene-specific transcripts associated with blood pressure traits in the validation analysis (see Excel file Supplementary Table S3).

Tables S4: Gene-specific transcripts associated with blood pressure traits in the discovery and validation analysis after exclusion of individuals receiving antihypertensive treatment (see Excel file Supplementary Table S4).

Tables S5: Gene-specific transcripts associated with blood pressure traits in the discovery analysis including only subjects of Caucasian ethnicity in MESA (see Excel file Supplementary Table S5)

Table S6: Variance (%) in BP levels attributable to transcript expression. Values refer to the generalized R^2 which was computed based on the likelihoods from linear regressions for each BP trait / candidate gene combination. The total attributable variance refers to the aggregated proportion of variance by the transcripts, which were significantly associated with the given trait across all studies (bold).

Gene	Monocytes						Whole blood					
	GHS			MESA			KORA			SHIP		
	SBP	DBP	PP	SBP	DBP	PP	SBP	DBP	PP	SBP	DBP	PP
	Attributable variance (AV) % (95% confidence interval)											
CEBPA	**1.63** (0.19, 2.95)	**1.42** (0.03, 2.67)	0.66 0.33, 1.49)	**0.12** (0.00, 0.46)	**0.39** (0.00, 0.66)	0.00 (0.00, 0.21)	**0.35** (0.00, 1.14)	**0.47** (0.00, 1.27)	0.14 (0.00, 0.66)	**1.02** (0.00, 2.20)	**1.04** (0.00, 2.15)	0.26 (0.00, 0.84)
CRIP1	**5.52** (2.93, 8.03)	**3.68** (1.47, 5.72)	2.96 (0.94, 4.81)	**2.98** (0.61, 4.12)	**1.86** (0.23, 3.11)	1.94 (0.00, 2.69)	**0.36** (0.00, 1.09)	**0.32** (0.00, 1.02)	0.22 (0.00, 0.79)	**1.18** (0.00, 2.41)	**0.61** (0.00, 1.51)	0.68 (0.00, 1.66)
F12	1.46 (0.02, 2.74)	0.42 (0.40, 1.09)	**1.36** (0.12, 2.65)	0.20 (0.00, 0.67)	0.02 (0.00, 0.35)	**0.27** (0.00, 0.65)	0.58 (0.00, 1.56)	0.17 (0.00, 0.67)	**0.68** (0.00, 1.73)	0.02 (0.00, 0.25)	0.46 (0.00, 1.26)	**0.59** (0.00, 1.45)
LMNA	1.52 (0.00, 2.90)	**1.25** (0.07, 2.43)	0.66 (0.41, 1.56)	0.74 (0.00, 1.07)	**0.33** (0.00, 1.05)	0.59 (0.00, 0.63)	0.17 (0.00, 0.69)	**0.65** (0.00, 1.62)	0.00 (0.00, 0.21)	0.20 (0.00, 0.76)	**0.58** (0.00, 1.53)	0.00 (0.00, 0.19)
MYADM	**3.96** (1.65, 6.15)	**2.17** (0.51, 3.70)	**2.50** 0.54, 4.30)	**0.77** (0.00, 1.26)	**0.03** (0.00, 0.20)	**1.13** (0.00, 1.67)	**1.65%** (0.00, 3.26)	**1.89** (0.10, 3.53)	**0.77** (0.00, 1.83)	**1.02** (0.00, 2.14)	**0.61** (0.00, 1.53)	**0.52** (0.00, 1.31)
TIPARP	**3.64** (1.60, 5.51)	**1.56** 0.12, 2.86)	**2.72** (0.88, 4.43)	**0.55** (0.00, 0.91)	0.49 (0.00, 1.18)	**0.27** (0.00, 0.39)	**0.63** (0.00, 1.65)	0.23 (0.00, 0.84)	**0.68** (0.00, 1.70)	**1.16** (0.00, 2.44)	0.36 (0.00, 1.07)	**0.93** (0.00, 2.09)
TPPP3	5.12 (2.54, 7.54)	**4.71** (2.34, 6.89)	1.94 (0.25, 3.44)	2.12 (0.05, 3.27)	**0.95** (0.00, 1.82)	1.69 (0.00, 2.61)	1.05 (0.00, 2.41)	**0.83** (0.00, 1.99)	0.70 (0.00, 1.87)	0.10 (0.00, 0.49)	**0.42** (0.00, 1.19)	0.01 (0.00, 0.25)
TSC22D3	**4.05** (1.75, 6.22)	**1.03** (0.21, 2.13)	3.96 (1.56, 6.18)	**0.89** (0.00, 1.48)	**0.35** (0.00, 0.75)	0.75 (0.00, 1.30)	**0.79** (0.00, 1.77)	**1.25** (0.00, 2.57)	0.23% (0.00, 0.77)	**1.24** (0.00, 2.49)	**0.37** (0.00, 1.04)	1.01 (0.00, 2.17)
Total AV by candidate genes	11.33 (7.57, 14.48)	8.31 (4.95, 10.79)	4.74 (1.92, 7.10)	3.43 (0.44, 4.39)	2.11 (0.00, 2.97)	2.67 (0.16, 3.83)	2.82 (0.09, 4.44)	4.05 (0.92, 6.00)	1.36 (0.00, 2.73)	4.21 (1.25, 6.17)	3.03 (0.15, 4.70)	1.37 (0.00, 2.60)

Table S7: Association of candidate transcript expression with blood pressure-related, subclinical cardiovascular phenotypes in the Gutenberg Health Study. Each trait was tested for association to mRNA by a linear regression adjusted for age, sex and technical covariates, and significant associations (FDR < 0.05) are shown in bold. IVSD = Interventricular septal thickness end-diastolic (mm) LVIDD = left ventricular internal diameter end-diastolic (cm); LVPWD = left ventricular posterior wall thickness end-diastolic (cm); LVM= left ventricular mass (g); RWT = relative wall thickness (cm); LVH = left ventricular hypertrophy (0: LVH; 1: no LVH), NTproBNP = N-terminal pro B-type natriuretic peptide (pg/mL).

Gene	IVSD	LVIDD	LVPWD	LVM	RWT	LVH	Stiffness index	NT-pro BNP
	P-value (log_2-fold mRNA change per unit trait)							
CRIP1	**0.0019** (0.0198)	0.3877 (0.0021)	**0.0001** (0.03)	**0.0005** (0.0006)	**0.0088** (0.3697)	**0.0052** (0.1276)	0.651 (-0.0017)	0.4461 (0.0069)
MYADM	0.4948 (-0.0034)	0.4896 (0.0013)	0.4607 (0.0044)	0.7331 (0)	0.6963 (-0.0425)	0.1333 (-0.0515)	0.4338 (-0.0023)	0.259 (0.0079)
TIPARP	0.2419 (-0.0054)	0.2605 (-0.0019)	0.4515 (-0.0042)	0.1338 (-0.0002)	0.8738 (-0.0162)	0.1151 (-0.0507)	0.1534 (-0.004)	0.0468 (0.013)
TSC22D3	**0.0048** (0.0164)	0.7672 (0.0006)	0.1148 (0.0111)	0.0189 (0.0004)	0.0502 (0.2517)	0.0191 (0.0963)	0.0145 (-0.0085)	0.796 (-0.0021)
CEBPA	0.1006 (0.007)	0.588 (0.0009)	0.0955 (0.0086)	0.0706 (0.0002)	0.2875 (0.1002)	0.1369 (0.0419)	0.8103 (0.0006)	0.4772 (0.0043)
F12	0.3003 (0.0044)	0.7302 (-0.0005)	0.8679 (0.0009)	0.6823 (0)	0.4496 (0.0708)	1 (0)	0.645 (0.0012)	0.4218 (0.0048)
LMNA	**0.0002** (0.031)	0.7639 (0.0009)	0.0119 (0.0252)	**0.0026** (0.0007)	**0.0041** (0.5258)	**0.0015** (0.1788)	0.595 (0.0026)	0.1599 (-0.0166)
TPPP3	0.1193 (0.0112)	0.7369 (-0.0009)	**0.0037** (0.0251)	0.1082 (0.0003)	0.0267 (0.3513)	0.4471 (0.0387)	0.4822 (0.003)	0.8027 0.0025)

Tables S8: Single nucleotide polymorphisms from the GRASP database (A) and published BP related variants (B) used for analyses of expression quantitative trait loci (see Excel file Supplementary Table S8).

Table S9: Blood pressure-related eQTLs and SNPs related to blood pressure. Genome-wide significant associations for blood pressure and other disease traits were taken from the GWAS Catalog (www.ebi.ac.uk/gwas/) and novel BP GWAS publications which were not present in the GWAS Catalog in March 2017 [15], [17], [16], [18]. Associations between the SNP and gene expression were assessed in 1,133 samples from the GHS (with available gene expression and genotyping data) by linear regression under the assumption of an additive genetic model and in publically available data of 5,311 whole blood samples from Westra et al [20]; genenetwork.nl/bloodeqtlbrowser/). Associations with a p-value < 2.6×10^{-4} (=0.05/191 SNPs tested) were considered significant. The linkage disequilibrium between rs3184504 and rs653178 is r^2 0.99. n.s: not significant.

SNP / allele	SNP annotation			SNP-BP associations		SNP-trait associations	SNP-gene expression associations		
	Chr	Gene	MAF	SBP	DBP	Other traits	Gene	Monocytes	Whole blood [20]
rs653178 / T	12	ATXN2 (intron)	48.6%		3×10^{-18} [21]	Mean arterial pressure [22]	CRIP1	3.59×10^{-7}	1.02×10^{-6}
						Celiac disease [23, 24]	TPPP3	1.35×10^{-4}	n.s.
						Chronic kidney disease [25]	MYADM	7.86×10^{-5}	5.12×10^{-7}
						Urate levels [26]	TIPARP	2.6×10^{-7}	n.s.
rs3184504 / C	12	SH2B3 (exon, missense)	48.9%	5×10^{-9} [27]	3×10^{-14} [27]	Platelet count [28, 29]	CRIP1	6.53×10^{-7}	1.32×10^{-6}
					4×10^{-25} [30]	Kynurenine levels [31]	TPPP3	1.64×10^{-4}	n.s.
						Eosinophil counts [32]	MYADM	6.47×10^{-5}	8.06×10^{-7}
						Hypothyroidism [33]	TIPARP	3.83×10^{-7}	n.s.

Table S10: Baseline characteristics of subjects selected for CRIP1 measurements from the Moli-Sani Study.

Study Characteristics	Moli-Sani Study n = 379
Mean age [years]	54.5 ± 9.4
Females, %	47.2
Current smoker, %	17.5
Body mass index (BMI) [kg/m^2]	28.5 ± 4.6
Diabetes Mellitus, %	5.3
CAD, %	1.3
Systolic blood pressure (SBP) [mmHg]	140.7 ± 20.4
Diastolic blood pressure (DBP) [mmHg]	83.2 ± 9.6
Pulse pressure (PP) [mmHg]	57.5 ± 16.5
Heart rate, bpm	68.0 ± 11.5
Prevalent Hypertension, %	55.0
Antihypertensive drug treatment, %	30.5

Supplementary Figures

Figure S1. Study workflow.
I) A meta-analysis of transcriptomics data and BP traits was performed for discovery in GHS and MESA. A FDR < 0.01 was used to indicate statistical significance.
II) External validation in a whole blood data set of independent studies (SHIP-Trend and KORA F4). A statistical significance of p < 0.05 in each study for at least one BP trait and consistent direction of effects were used to selected candidate genes. SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; CVD: cardiovascular disease; RCT randomized clinical trial.

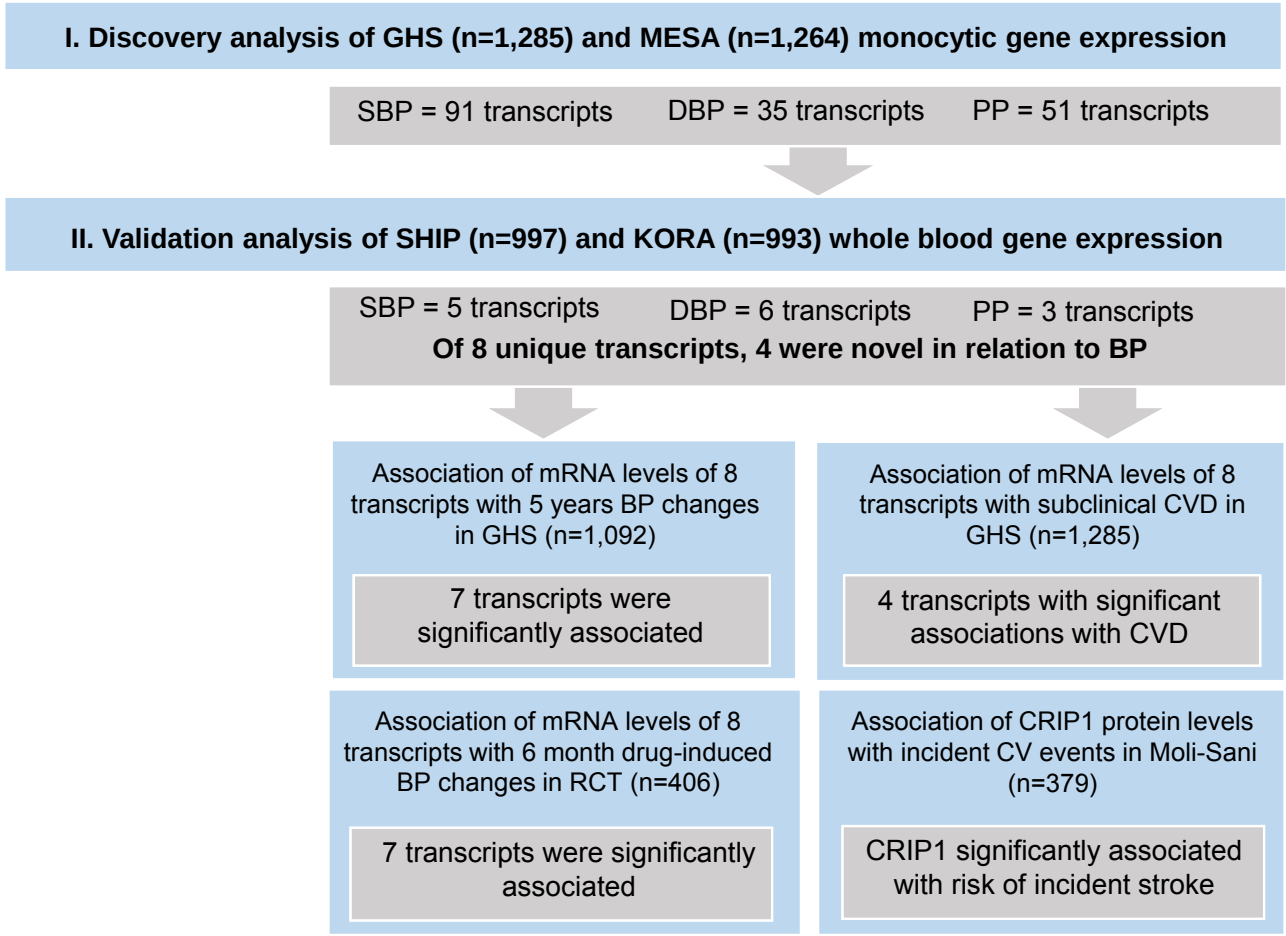


Figure S2: Candidate gene expression by study and cell types.
To consider study specific technical differences between studies, expression levels were normalized to the overall mean of all genes on the microarrays.

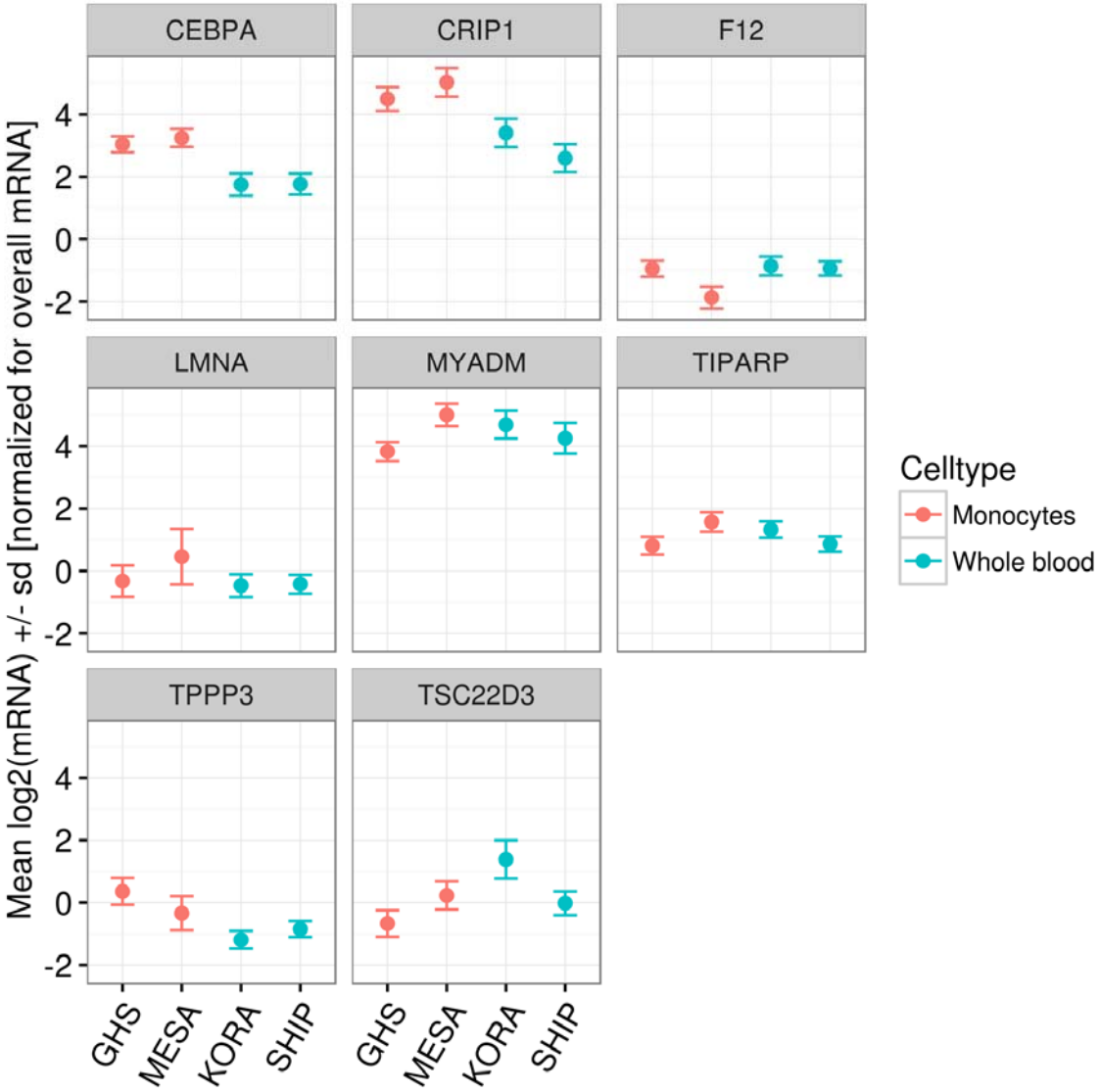


Figure S3a: Association between BP changes and *CRIP1* mRNA expression changes after 5-years follow-up in the GHS. A) Association to systolic BP; B) Association to diastolic BP. Numbers indicate the total number of individuals per group**.** BP: blood pressure

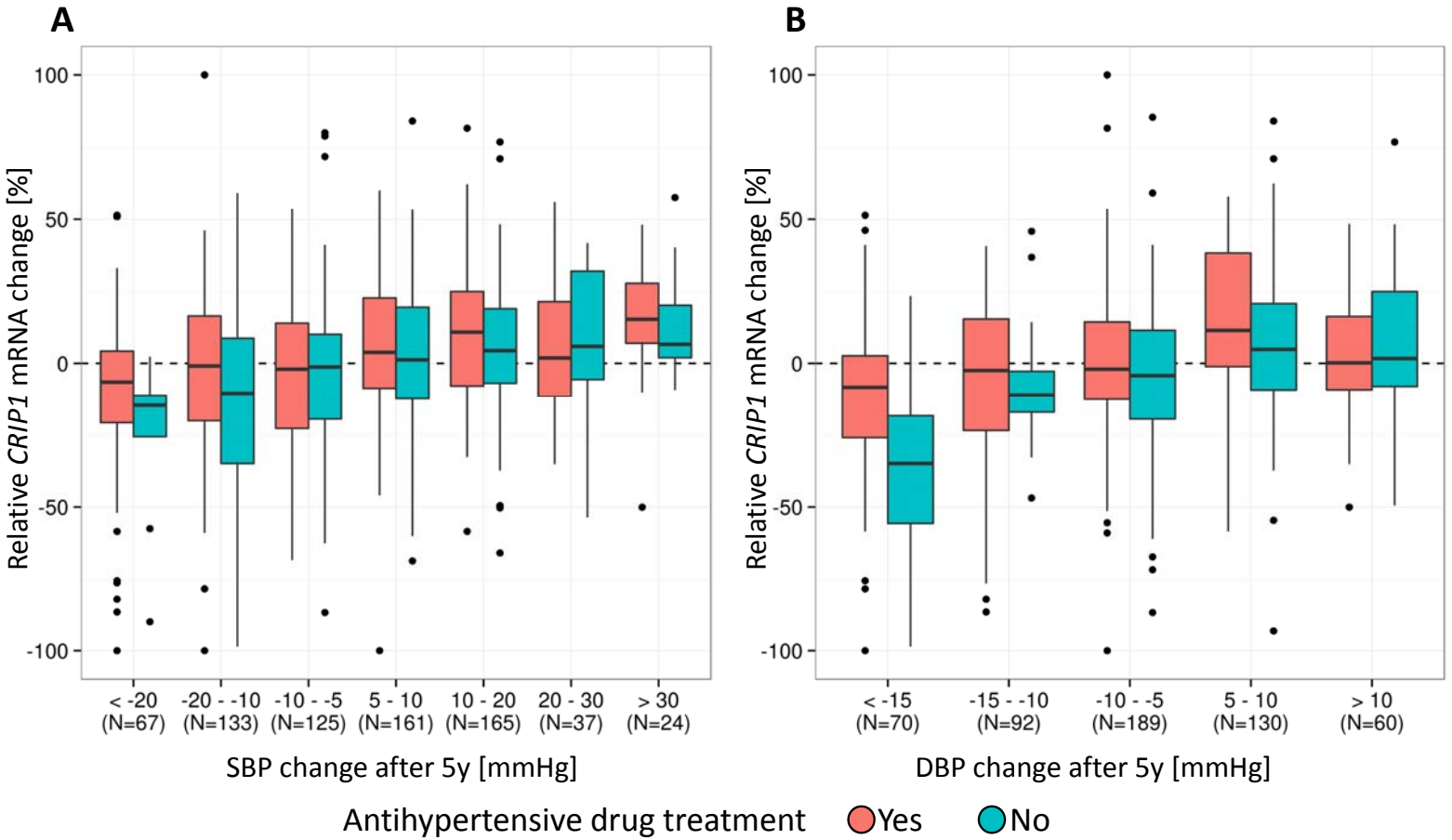


Figure S3b: Association between BP changes and *MYADM* mRNA expression changes after 5-years follow-up in the GHS. A) Association to systolic BP; B) Association to diastolic BP. Numbers indicate the total number of individuals per group**.** BP: blood pressure

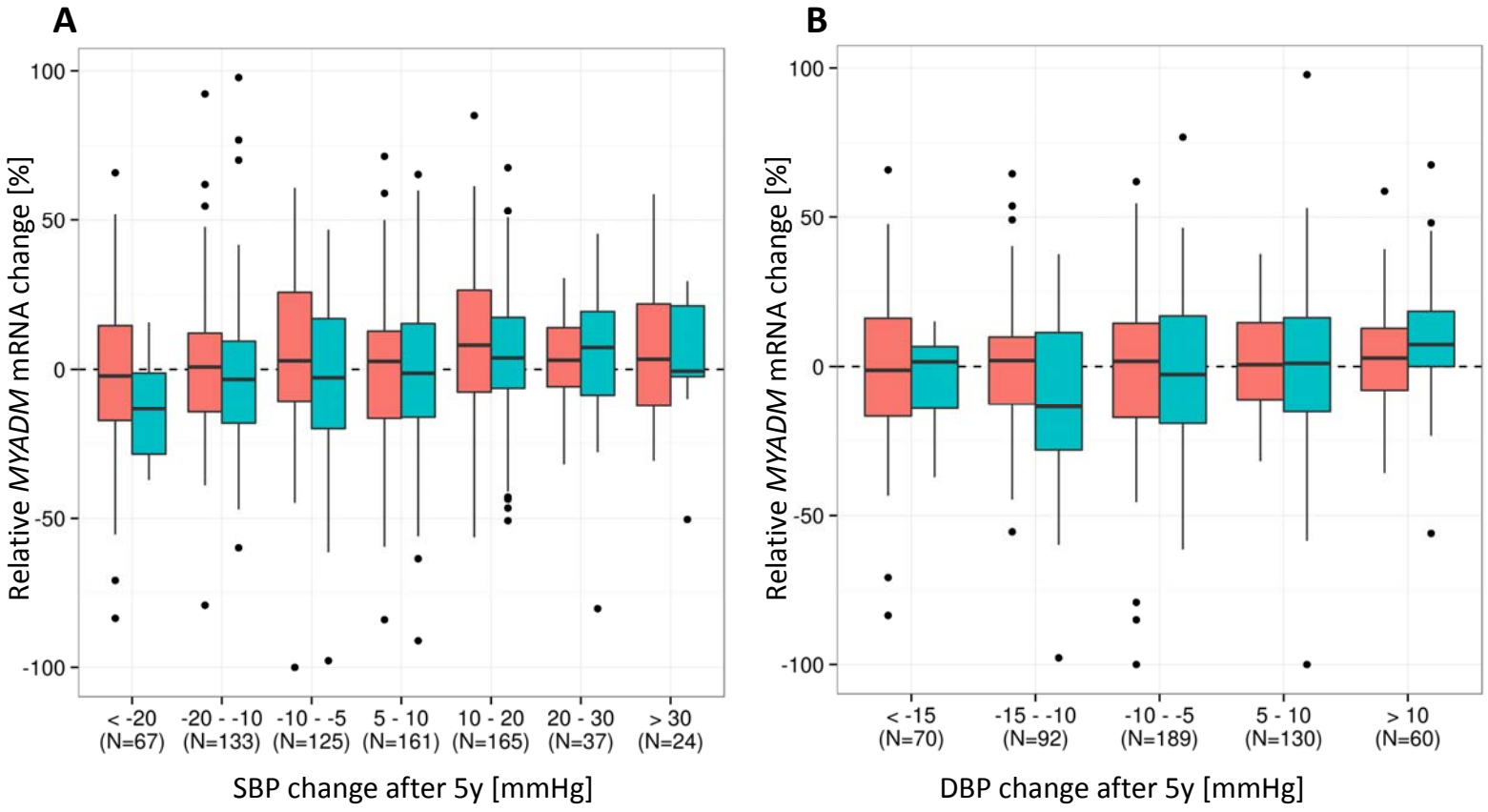


Figure S3c: Association between BP changes and *TIPARP* mRNA expression changes after 5-years follow-up in the GHS. A) Association to systolic BP; B) Association to diastolic BP. Numbers indicate the total number of individuals per group**.** BP: blood pressure

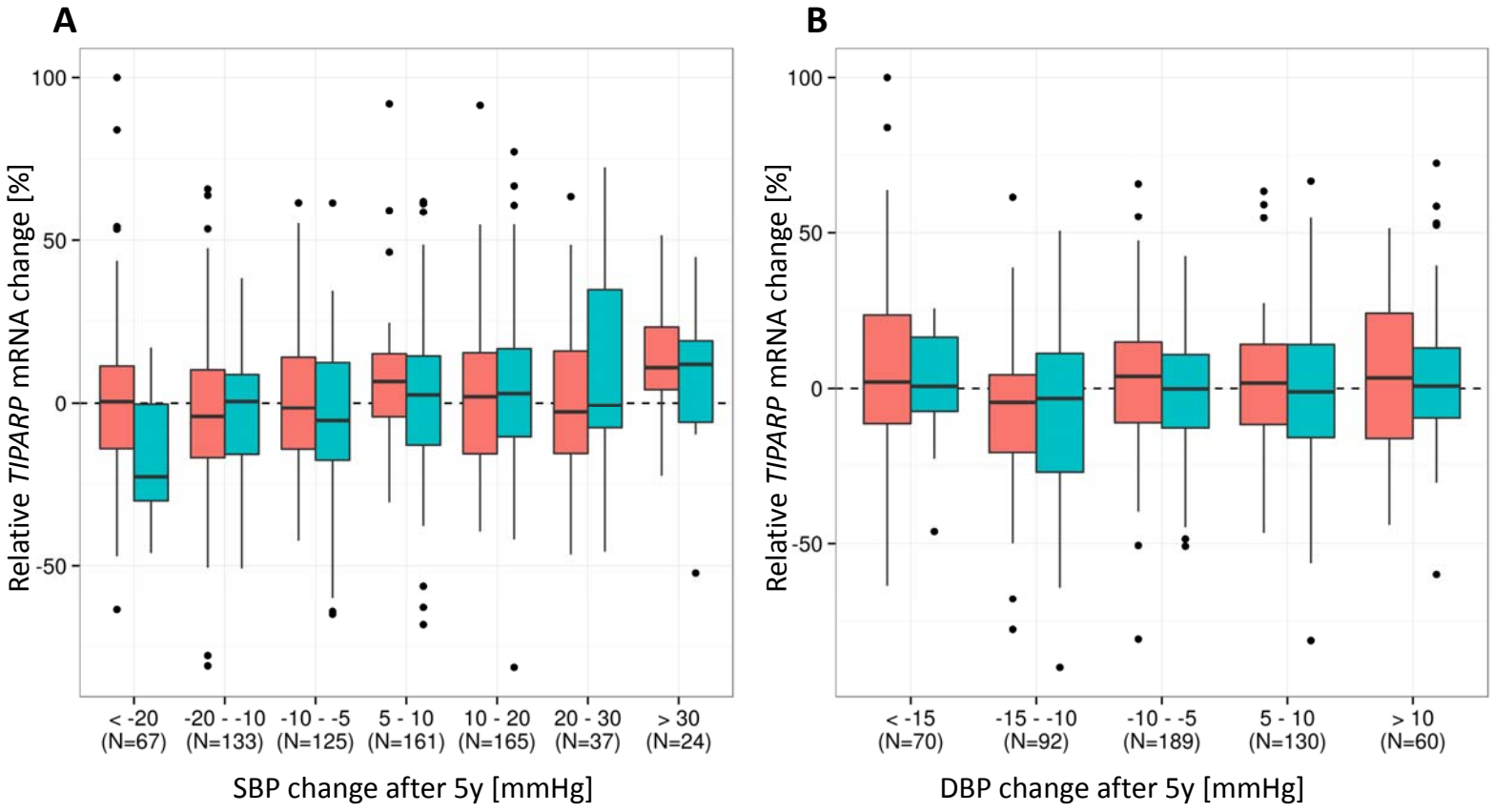


Figure S3d: Association between BP changes and *TSC22D3* mRNA expression changes after 5-years follow-up in the GHS. A) Association to systolic BP; B) Association to diastolic BP. Numbers indicate the total number of individuals per group**.** BP: blood pressure

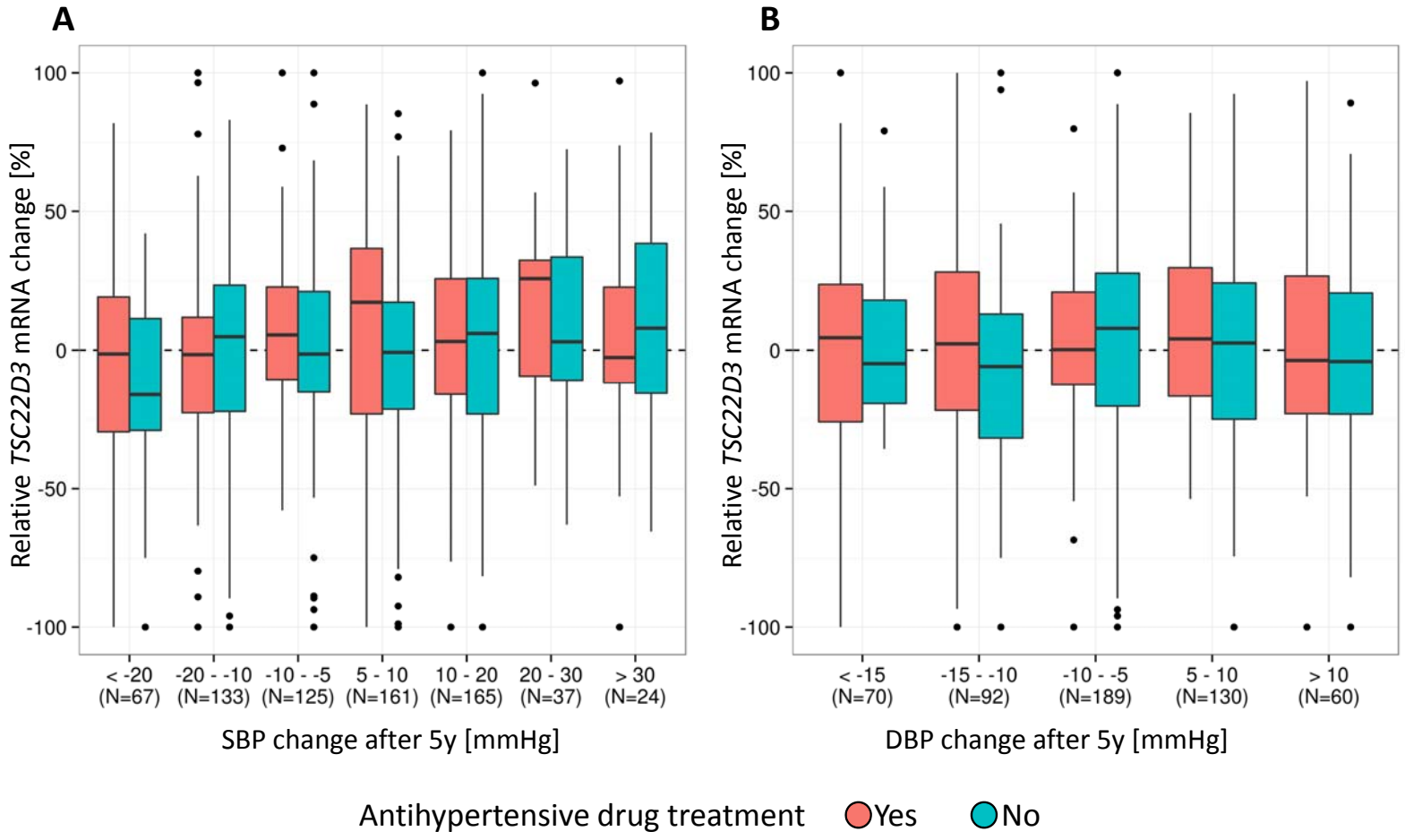


Figure S3e: Association between BP changes and *CEBPA* mRNA expression changes after 5-years follow-up in the GHS. A) Association to systolic BP; B) Association to diastolic BP. Numbers indicate the total number of individuals per group**.** BP: blood pressure

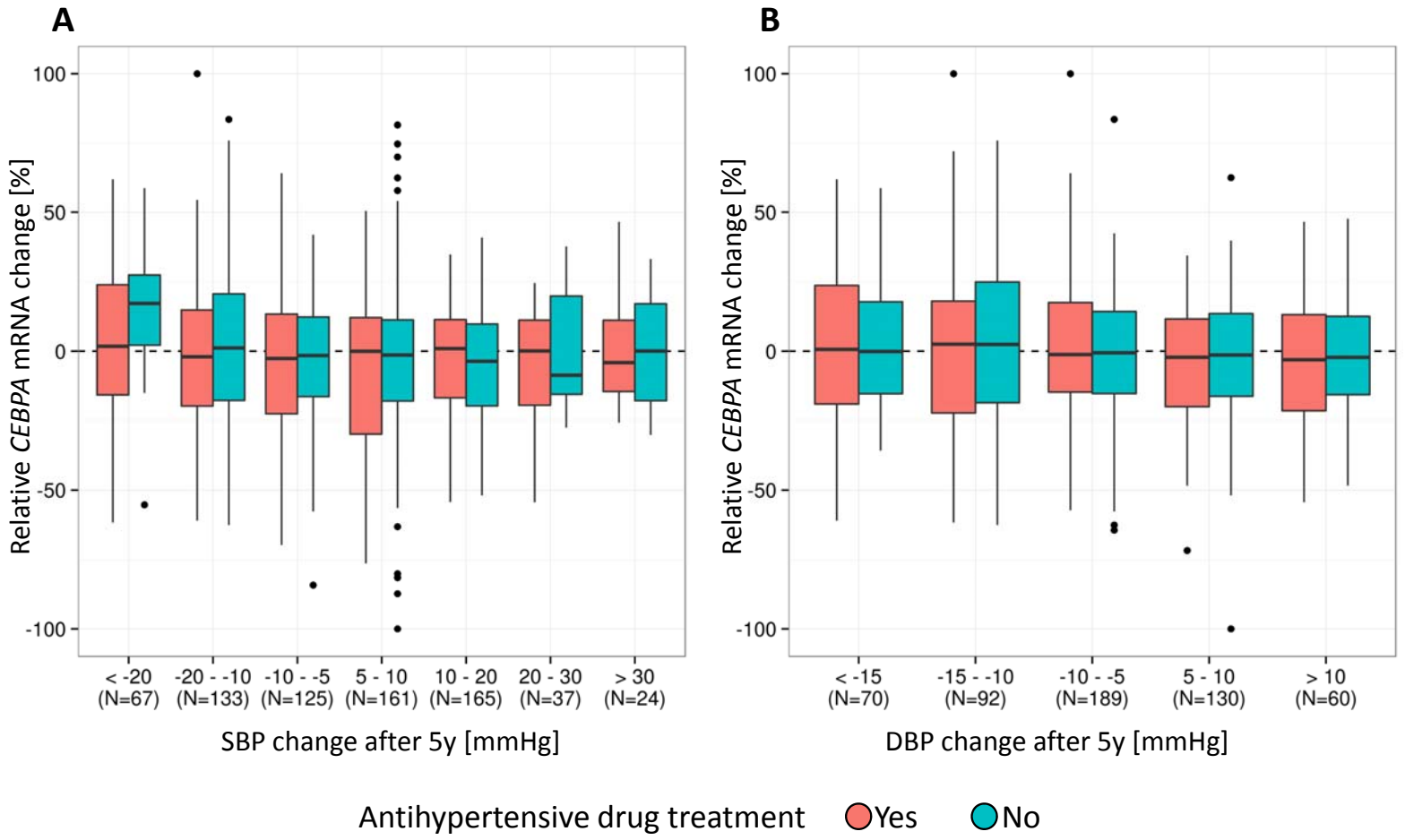


Figure S3f: Association between BP changes and *LMNA* mRNA expression changes after 5-years follow-up in the GHS. A) Association to systolic BP; B) Association to diastolic BP. Numbers indicate the total number of individuals per group**.** BP: blood pressure

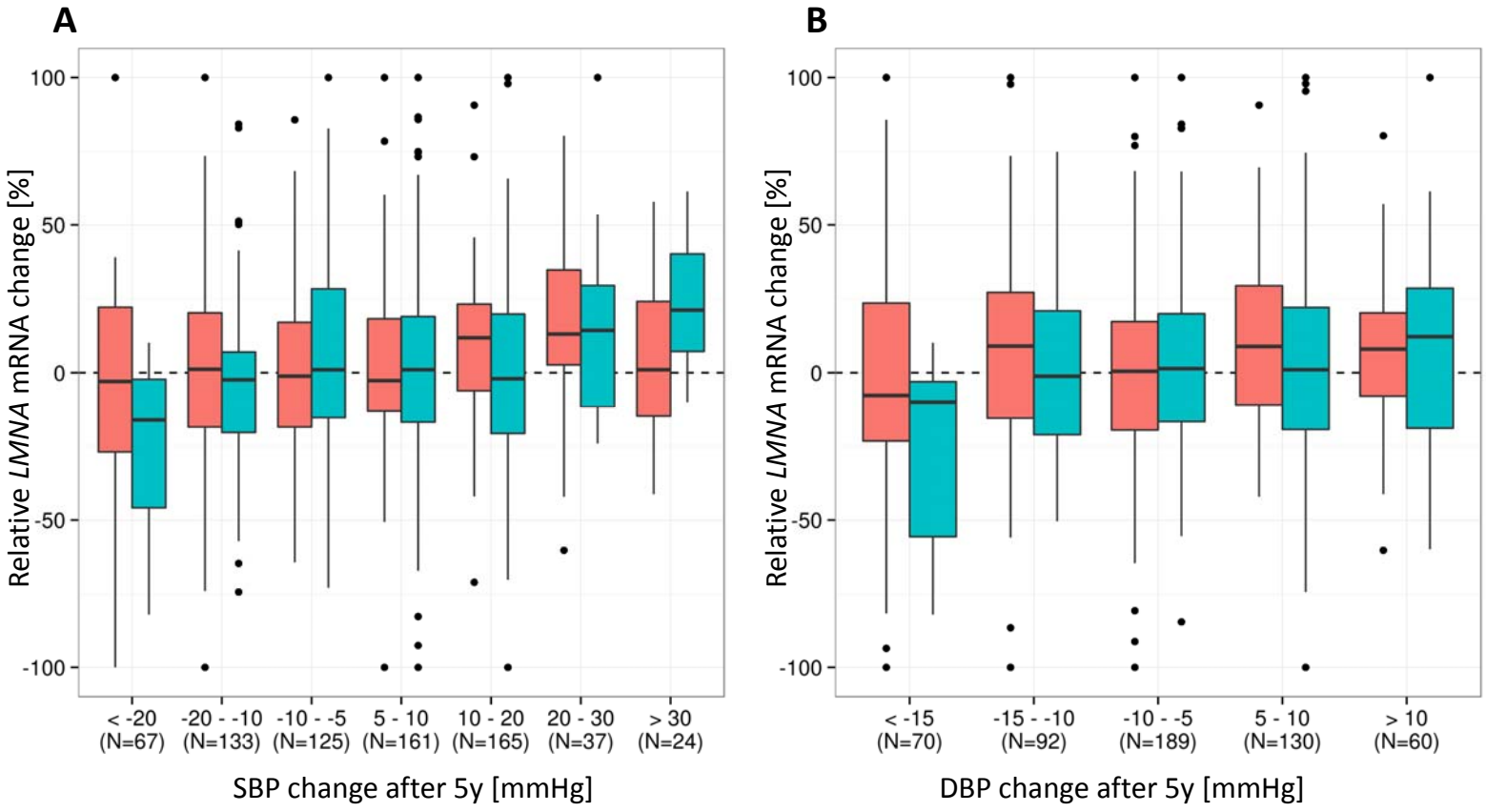


Figure S3g: Association between BP changes and *TPPP3* mRNA expression changes after 5-years follow-up in the GHS. A) Association to systolic BP; B) Association to diastolic BP. Numbers indicate the total number of individuals per group**.** BP: blood pressure

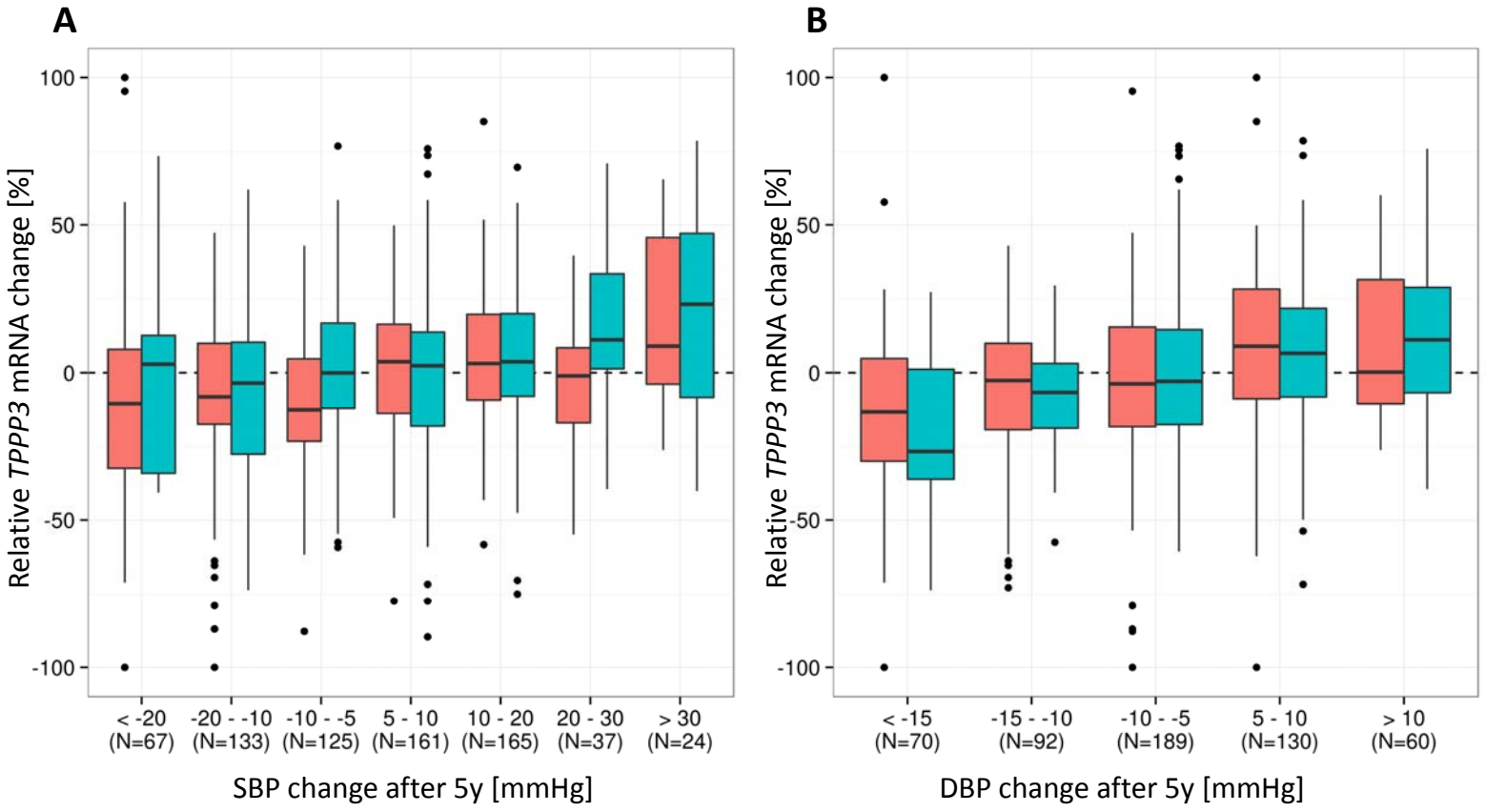


Figure S4: Reduction of blood pressure between baseline and 6 month follow up in a clinical trial.

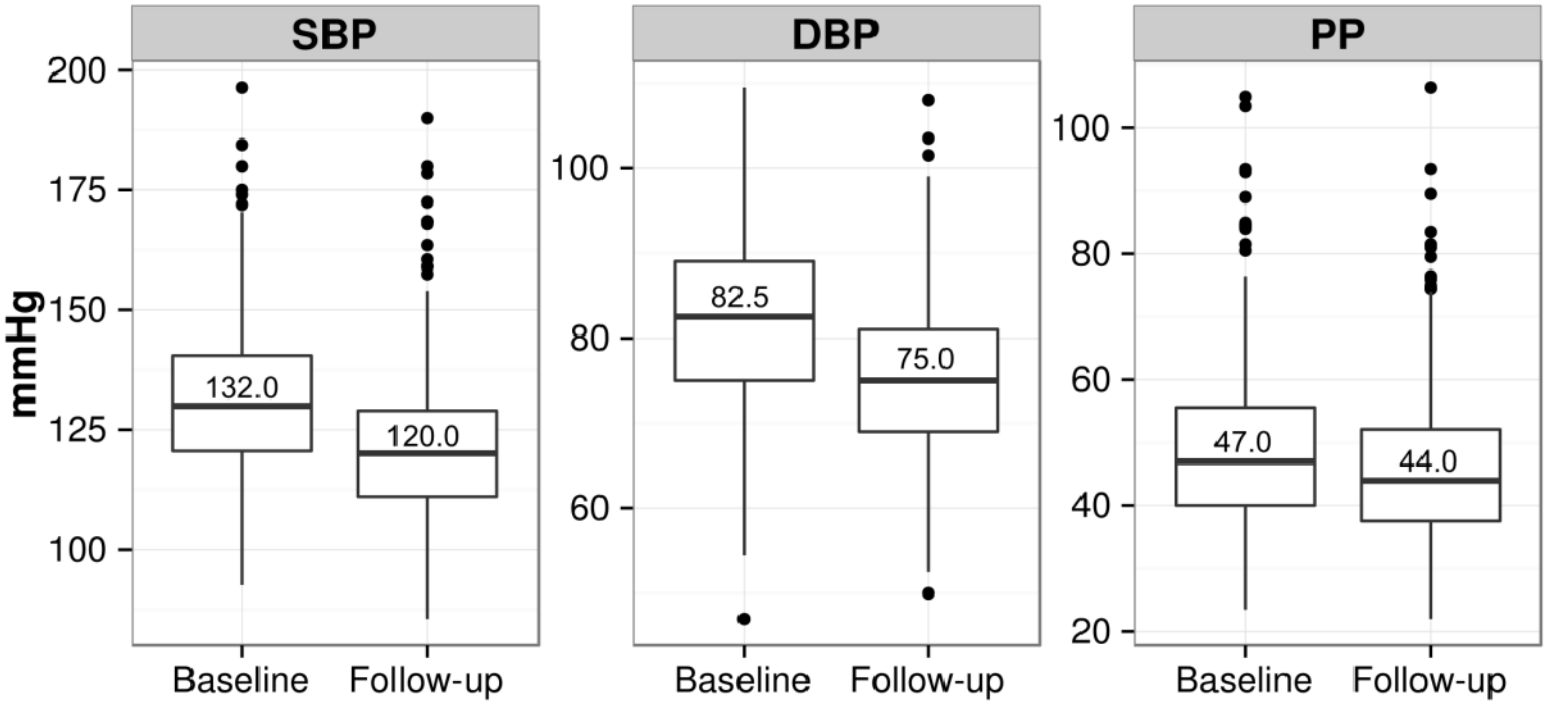


Figure S5A: Single nucleotide polymorphisms from the GRASP database used for analyses of expression quantitative trait loci. For each candidate transcript, cis-eQTLs were calculated for SNPs within ±250kb around the transcription start site and a minor allele frequency ≥ 1%. The Genome-wide Repository of Associations between SNPs and Phenotypes (GRASP) database version 2.0.0.0 (https://grasp.nhlbi.nih.gov) was studied for significant cis-eQTLs which were at least moderately associated to a BP or a cardiovascular disease related trait (p-value≤10^{-4}).

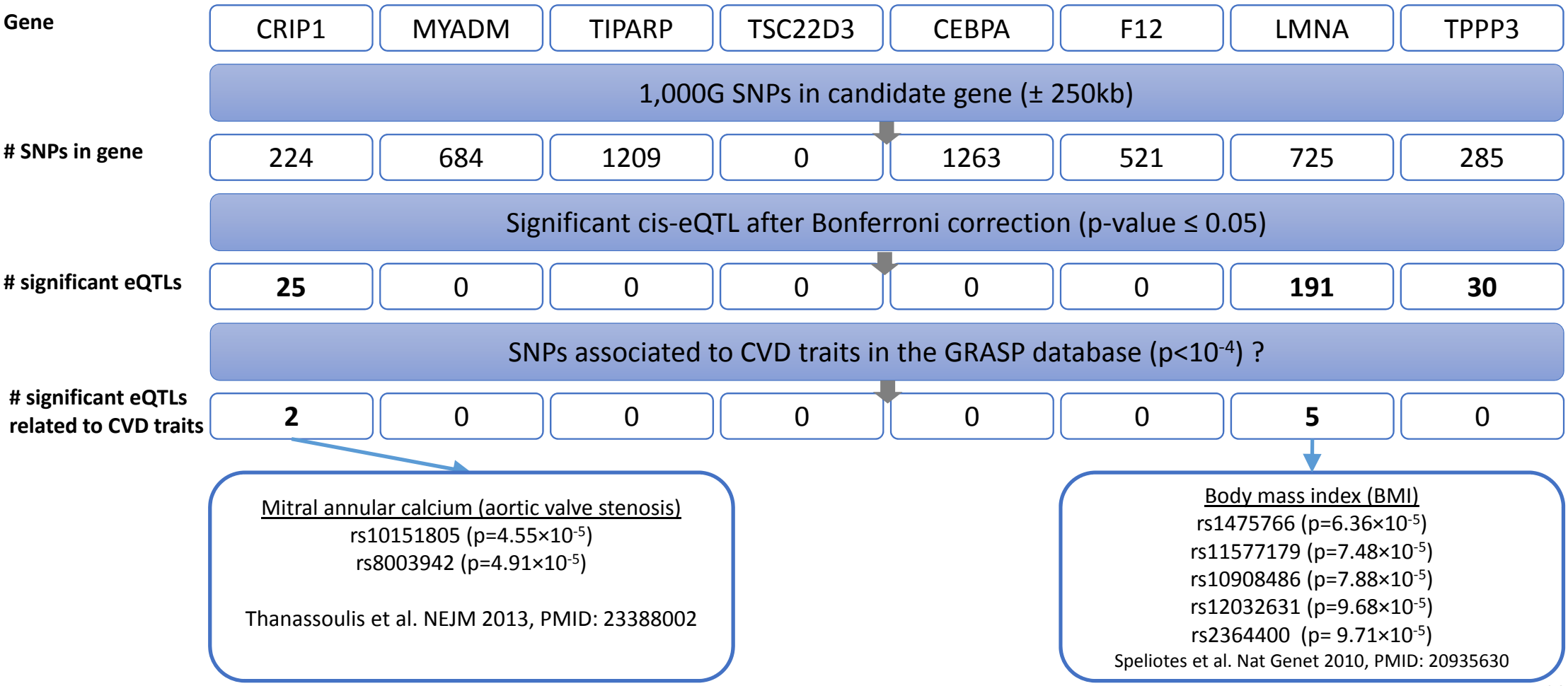
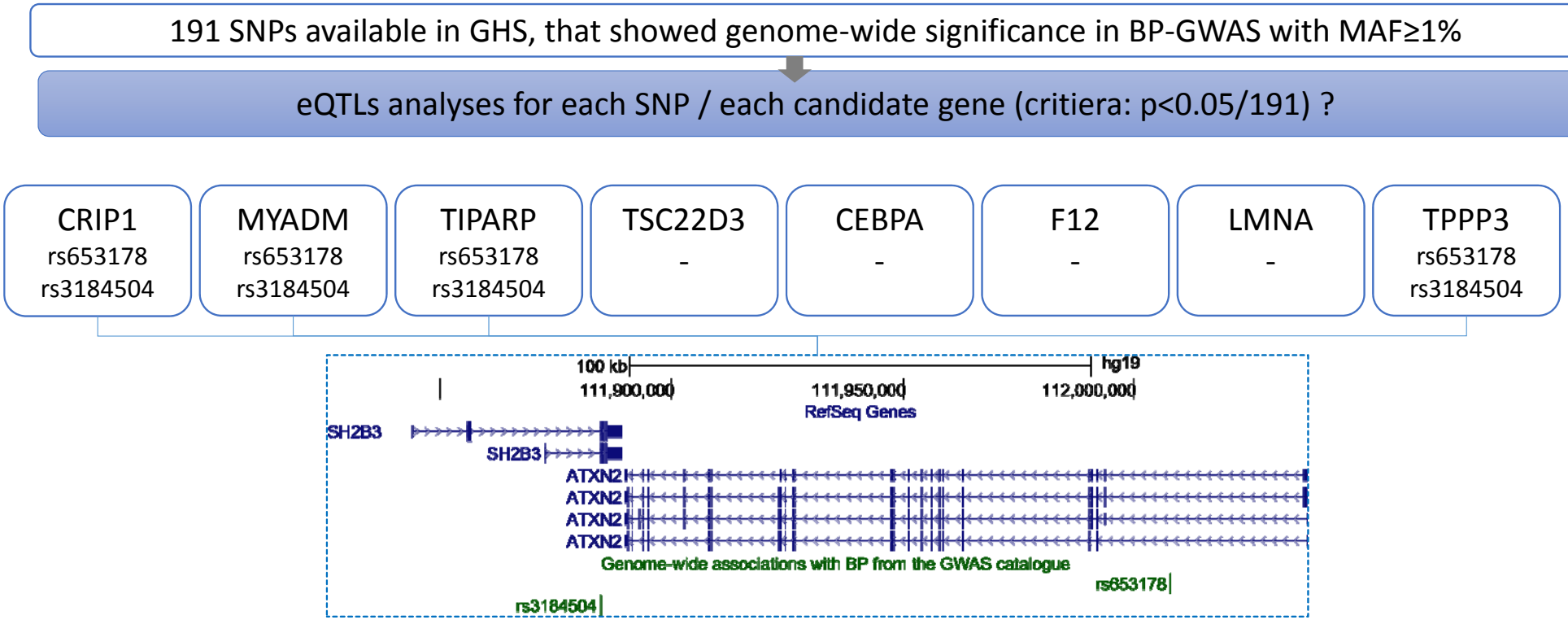
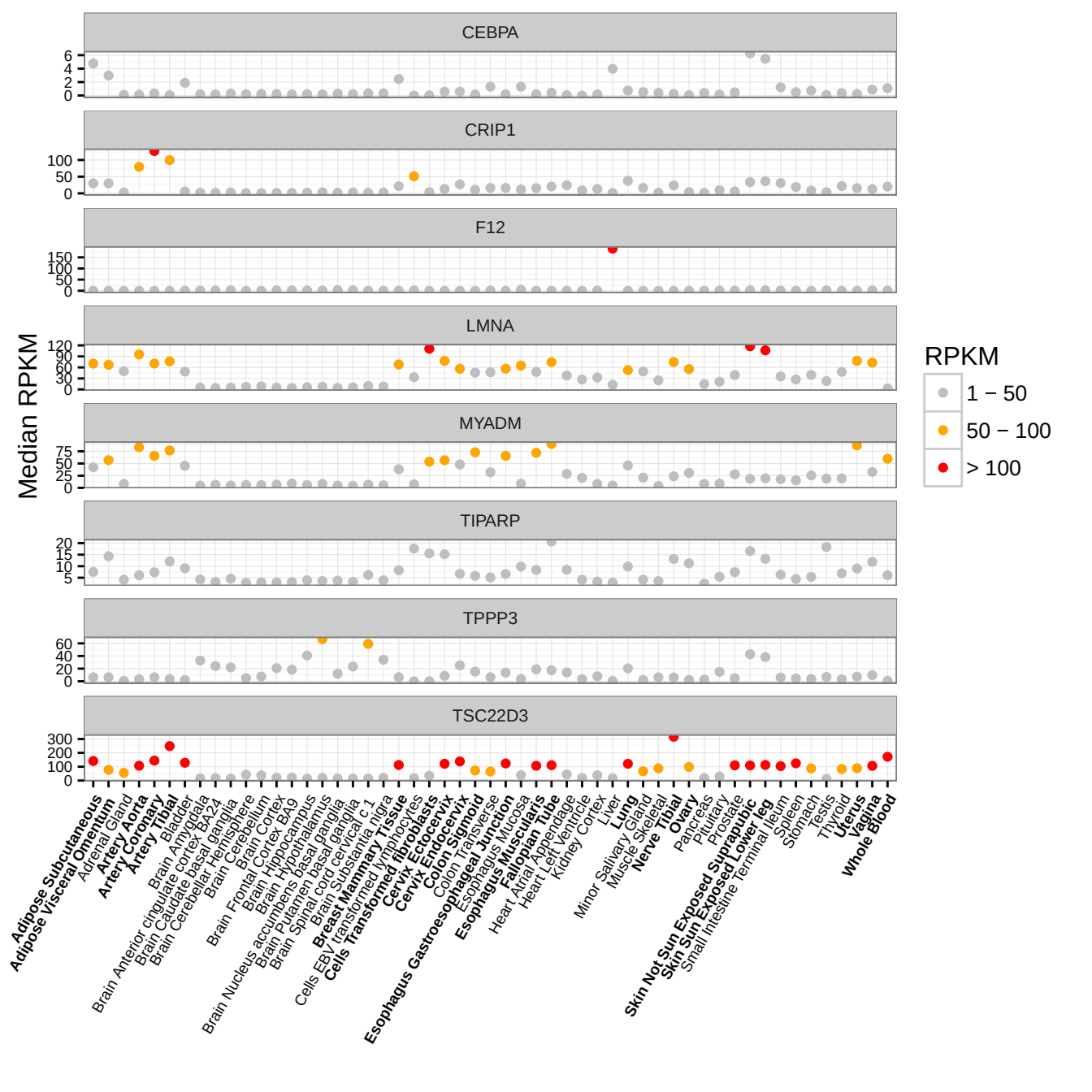


Figure S5B: Single nucleotide polymorphisms from the GWAS catalogue used for analyses of expression quantitative trait loci.
Published GWAS results of blood pressure (BP) related traits were retrieved from the GWAS catalogue (https://www.ebi.ac.uk/gwas/). In total, 191 unique SNPs with genome-wide association to at least one BP trait and a minor allele frequency (MAF) ≥ 1% in the Gutenberg Health Study (GHS) were found, leading to a significance level of 2.6 x 10^{-4}. Two SNPs within the SH2B adaptor protein 3 (SH2B3) genomic locus were identified as putative trans-regulators of *CRIP1*, *MYADM*, *TIPARP* and *TPPP3* mRNA expression in monocytes.



From UCSC Genome Browser (https://genome.ucsc.edu)

Figure S6: Median candidate gene expression in various tissues measured by RNA-sequencing in the GTEx project. RPKM: Reads Per Kilobase of transcript per Million mapped reads


CEBPA
CRIP1
F12
LMNA
MYADM
TIPARP
TPPP3
TSC22D3
Median RPKM
RPKM
1 − 50
50 − 100
> 100
Adipose Subcutaneous
Adipose Visceral Omentum
Adrenal Gland
Artery Aorta
Artery Coronary
Artery Tibial
Bladder
Brain Amygdala
Brain Anterior cingulate cortex BA24
Brain Caudate basal ganglia
Brain Cerebellar Hemisphere
Brain Cerebellum
Brain Cortex
Brain Frontal Cortex BA9
Brain Hippocampus
Brain Hypothalamus
Brain Nucleus accumbens basal ganglia
Brain Putamen basal ganglia
Brain Spinal cord cervical c.1
Brain Substantia nigra
Cells EBV transformed lymphocytes
Cells Transformed fibroblasts
Breast Mammary Tissue
Cervix Ectocervix
Cervix Endocervix
Colon Sigmoid
Colon Transverse
Esophagus Gastroesophageal Junction
Esophagus Mucosa
Esophagus Muscularis
Fallopian Tube
Heart Atrial Appendage
Heart Left Ventricle
Kidney Cortex
Liver
Lung
Minor Salivary Gland
Muscle Skeletal
Nerve Tibial
Ovary
Pancreas
Pituitary
Prostate
Skin Not Sun Exposed Suprapubic
Skin Sun Exposed Lower leg
Small Intestine Terminal Ileum
Spleen
Stomach
Testis
Thyroid
Uterus
Vagina
Whole Blood