Published in final edited form as:

Hypertension. 2017 October ; 70(4): 743–750. doi:10.1161/HYPERTENSIONAHA.117.09458.

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**[*]

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,

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

[*]These authors contributed equally to this work.

See Editorial Commentary, pp 695-697

Disclosures

The other authors report no conflicts.

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.)

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; $P=5.0\times10^{-5}$). 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.

Keywords

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 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=2.15×10^{-10}; delta mRNA DBP: 5.19±0.77; P=8.46×10^{-11}; delta mRNA pulse pressure: 2.2±0.62; P=5.0×10^{-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=5.6×10^{-14}). Of note, *CEBPA* (% mRNA change: −51.84%±5.5; P=7.5×10^{-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=1.9×10^{-3}), left ventricular posterior wall thickness end diastolic (log2 change per cm: 0.003; P=1.0×10^{-4}), left ventricular mass (log2 change per gram: 0.0006; P=5.0×10^{-4}), relative wall thickness (log2 change per cm: 0.3697; P=3.5×10^{-2}), and left ventricular hypertrophy (log2 difference between subjects with and without left ventricular hypertrophy: 0.1276; P=5.2×10^{-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=4.55×10^{-5})[32] and 5 SNPs to body mass index (eg, rs1475766; P=6.36×10^{-5})[29] with $P \lesssim 10^{-4}$ 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 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=3.59×10^{-7}; rs3184504: mRNA change per C allele±SE: 5.42% ±1.06; P=6.53×10^{-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 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 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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.

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).

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. [PubMed: 12493255]
2. Nabel EG. Cardiovascular disease. N Engl J Med. 2003; 349:60–72. DOI: 10.1056/NEJMra035098 [PubMed: 12840094]
3. Hay M. The good and the bad: immune cells and hypertension. Circ Res. 2015; 117:830–831. DOI: 10.1161/CIRCRESAHA.115.307506 [PubMed: 26494923]
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 [PubMed: 27217403]
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 [PubMed: 21875910]
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 [PubMed: 21149826]
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. [PubMed: 11040222]
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. Transancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. Nat Genet. 2016; 48:1151–1161. DOI: 10.1038/ng.3654 [PubMed: 27618447]

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 [PubMed: 27618452]
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 [PubMed: 28135244]
11. Munroe PB, Barnes MR, Caulfield MJ. Advances in blood pressure genomics. Circ Res. 2013; 112:1365–1379. DOI: 10.1161/CIRCRESAHA.112.300387 [PubMed: 23661711]
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 [PubMed: 23478099]
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 [PubMed: 23303660]
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 [PubMed: 21909115]
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 [PubMed: 25785607]
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. [PubMed: 25882670]
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 [PubMed: 15007037]
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 [PubMed: 20502693]
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 [PubMed: 22144904]
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 [PubMed: 26432245]
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. [PubMed: 19414839]
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 [PubMed: 20643817]
23. Bild DE, Bluemke DA, Burke GL, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. Am J Epidemiol. 2002; 156:871–881. [PubMed: 12397006]
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 [PubMed: 20167617]

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 [PubMed: 16032513]
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 [PubMed: 23645692]
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 [PubMed: 23236413]
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 [PubMed: 26153245]
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 [PubMed: 20935630]
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:D1001–D1006. (database issue). DOI: 10.1093/nar/gkt1229 [PubMed: 24316577]
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 [PubMed: 23388002]
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 [PubMed: 15731461]
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 [PubMed: 26953322]
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 [PubMed: 24733030]
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 [PubMed: 20460359]
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 [PubMed: 26319245]
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 [PubMed: 25776069]
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 [PubMed: 22034642]
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 [PubMed: 24904090]

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.

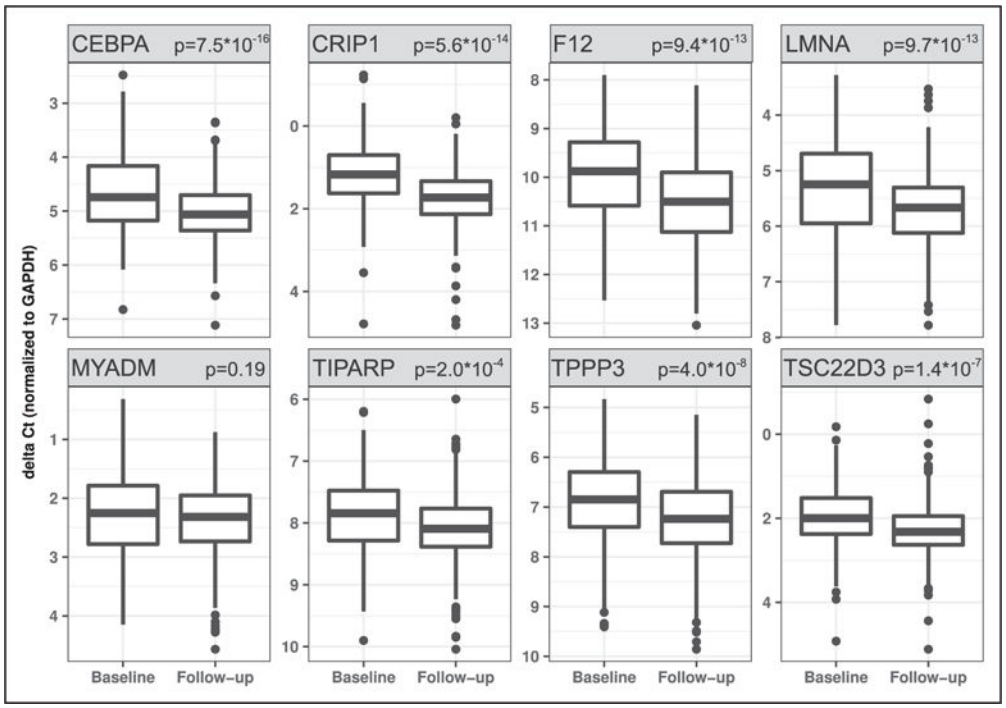

Figure.

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 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^{-1tl} (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.

Table 2

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

Gene	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 (‡)			
	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.

Table 3

Association of Serum CRIP1, CRP, NT-proBNP, and hsTnl 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.