A Meta-analysis of Gene Expression Signatures of Blood Pressure and Hypertension

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RESEARCH ARTICLE

A Meta-analysis of Gene Expression Signatures of Blood Pressure and Hypertension

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Abstract

Genome-wide association studies (GWAS) have uncovered numerous genetic variants (SNPs) that are associated with blood pressure (BP). Genetic variants may lead to BP changes by acting on intermediate molecular phenotypes such as coded protein sequence or gene expression, which in turn affect BP variability. Therefore, characterizing genes whose expression is associated with BP may reveal cellular processes involved in BP regulation and uncover how transcripts mediate genetic and environmental effects on BP variability. A meta-analysis of results from six studies of global gene expression profiles of BP and hypertension in whole blood was performed in 7017 individuals who were not receiving antihypertensive drug treatment. We identified 34 genes that were differentially expressed in relation to BP (Bonferroni-corrected $p<0.05$). Among these genes, *FOS* and *PTGS2* have been previously reported to be involved in BP-related processes; the others are novel. The top BP signature genes in aggregate explain 5%–9% of inter-individual variance in BP. Of note, rs3184504 in *SH2B3*, which was also reported in GWAS to be associated with BP, was found to be a trans regulator of the expression of 6 of the transcripts we found to be associated with BP (*FOS*, *MYADM*, *PP1R15A*, *TAGAP*, *S100A10*, and *FGBP2*). Gene set enrichment analysis suggested that the BP-related global gene expression changes include genes involved in inflammatory response and apoptosis pathways. Our study provides new insights into molecular mechanisms underlying BP regulation, and suggests novel transcriptomic markers for the treatment and prevention of hypertension.

Author Summary

The focus of blood pressure (BP) GWAS has been the identification of common DNA sequence variants associated with the phenotype; this approach provides only one dimension of molecular information about BP. While it is a critical dimension, analyzing DNA variation alone is not sufficient for achieving an understanding of the multidimensional complexity of BP physiology. The top loci identified by GWAS explain only about 1 percent of inter-individual BP variability. In this study, we performed a meta-analysis of gene expression profiles in relation to BP and hypertension in 7017 individuals from six studies. We identified 34 differentially expressed genes for BP, and discovered that the top BP signature genes explain 5%–9% of BP variability. We further linked BP gene expression signature genes with BP GWAS results by integrating expression associated SNPs (eSNPs) and discovered that one of the top BP loci from GWAS, rs3184504 in *SH2B3*, is a *trans* regulator of expression of 6 of the top 34 BP signature genes. Our study, in conjunction

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with prior GWAS, provides a deeper understanding of the molecular and genetic basis of BP regulation, and identifies several potential targets and pathways for the treatment and prevention of hypertension and its sequelae.

Introduction

Systolic and diastolic blood pressure (SBP and DBP) are complex physiological traits that are affected by the interplay of multiple genetic and environmental factors. Hypertension (HTN) is a critical risk factor for stroke, renal failure, heart failure, and coronary heart disease [1]. Genome-wide association studies (GWAS) have identified numerous loci associated with BP traits [2,3]. These loci, however, only explain a small proportion of inter-individual BP variability. In aggregate the 29 loci reported by the International Consortium of Blood Pressure (ICBP) consortium GWAS account for about one percent of BP variation in the general population [3]. Most genes near BP GWAS loci are not known to be mechanistically associated with BP regulation [3]. Therefore, further studies are needed to determine whether the genes implicated in GWAS demonstrate functional relations to BP physiology and to uncover the molecular actions and interactions of genetic and environmental factors involved in BP regulation.

Alterations in gene expression may mediate the effects of genetic variants on phenotype variability. We hypothesized that characterizing gene expression signatures of BP would reveal cellular processes involved in BP regulation and uncover how transcripts mediate genetic and environmental effects on BP variability. We additionally hypothesized that by integrating gene expression profiling with genetic variants associated with altered gene expression (eSNPs or eQTLs) and with BP GWAS results, we would be able to characterize the genetic architecture of gene expression effects on BP regulation.

Several previous studies have examined the association of global gene expression with BP [4,5] or HTN [6,7]. Most of these studies, however, were based on small sample sizes and lacked replication [4,5,6,7]. To address this challenge, we conducted an association study of global gene expression levels in whole blood with BP traits (SBP, DBP, and HTN) in six independent studies. In order to avoid the possibility that the differentially expressed genes we identified reflect drug treatment effects, we excluded individuals receiving anti-hypertensive treatment. The eligible study sample included 7017 individuals: 3679 from the Framingham Heart Study (FHS), 972 from the Estonian Biobank (EGCUT), 604 from the Rotterdam Study (RS) [8], 597 from the InCHIANTI Study, 565 from the Cooperative Health Research in the Region of Augsburg [KORA F4] Study [9], and 600 from the Study of Health in Pomerania [SHIP-TREND] [10]. We first identified differentially expressed BP genes in the FHS (n = 3679) followed by external replication in the other five studies (n = 3338). Subsequently, we performed a meta-analysis of all 7017 individuals from the six studies, and identified 34 differentially expressed genes associated with BP traits using a stringent statistical threshold based on Bonferroni correction for multiple testing of 7717 unique genes. The differentially expressed genes for BP (BP signature genes) were further integrated with eQTLs and with BP GWAS results in an effort to differentiate downstream transcriptomic changes due to BP from putatively causal pathways involved in BP regulation.

Results

Clinical characteristics

After excluding individuals receiving anti-hypertensive treatment, the eligible sample size was 7017 (FHS, n = 3679; EGCUT, n = 972; RS, n = 604; InCHIANTI, n = 597; KORA F4, n = 565

Table 1. Clinical characteristics of the study cohorts.

	FHS N = 3,679	EGCUT N = 972	RS N = 604	InCHIANTI N = 597	KORA F4 N = 565	SHIP-TREND N = 600
Age (yr)	51 ± 12	36 ± 14	58 ± 8	71 ± 16	72 ± 5	46 ± 13
Sex, male (%)	42	49	46	46	51	43
Hypertension (%)	11	19	35	45	26	12
BMI (kg/m^2)	27.2 ± 5.3	24.8 ± 4.4	26.8 ± 4.1	27.0 ± 4.2	29.8± 4.6	26 ± 4.2
Systolic BP (mm Hg)	118 ± 15	122 ± 16	132 ± 20	132 ± 20	129± 21	120 ± 15
Diastolic BP (mm Hg)	74 ±9	76 ± 10	82 ± 11	78 ±10	73±11	75 ± 9

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and SHIP-TREND, n = 600). Clinical characteristics of participants from the four studies are presented in **Table 1**. The mean age varied across the cohorts (FHS = 51, EGCUT = 36, RS = 58, InCHIANTI = 71, KORA F4 = 72 and SHIP-TREND = 46 years) as did the proportion of individuals with hypertension (11% in FHS, 19% in EGCUT, 35% in RS, 45% in InCHIANTI, 26% in KORA, and 12% in SHIP).

Identification and replication of differentially expressed BP signature genes

At a Bonferroni corrected $p<0.05$, we identified 73, 31, and 8 genes that were differentially expressed in relation to SBP, DBP, and HTN, respectively in the FHS, which used an Affymetrix array for expression profiling, and 6, 1, and 1 genes in the meta-analysis of the 5 cohorts that used an Illumina array (Illumina cohorts): EGCUT, RS, InCHIANTI, KORA F4 and SHIP-TREND (**S1 Table**). For each differentially expressed BP gene in the FHS or in the Illumina cohorts, we attempted replication in the other group. At a replication $p<0.05$ (Bonferroni corrected), 13 unique genes that were identified in the FHS were replicated in the Illumina cohorts, including 10 for SBP (*CD97*, *TAGAP*, *DUSP1*, *FOS*, *MCL1*, *MYADM*, *PPP1R15A*, *SLC31A2*, *TAGLN2*, and *TIPARP*), 5 for DBP (*CD97*, *BHLHE40*, *PRF1*, *CLC*, and *MYADM*), and 2 for HTN (*GZMB* and *MYADM*) (**Table 2**). Each of the unique BP signature genes in the Illumina cohorts, 6 for SBP (*TAGLN2*, *BHLHE40*, *MYADM*, *SLC31A2*, *DUSP1*, and *MCL1*), 1 for DBP (*BHLHE40*) and 1 for HTN (*SLC31A2*), replicated in the FHS. All 6 Illumina cohorts BP signature genes that replicated in the FHS were among the 13 FHS BP signature genes that replicated in the Illumina cohorts. The BP signature genes identified in the FHS showed enrichment in the Illumina cohorts at *pi1* = 0.88, 0.75, and 0.99 for SBP, DBP, and HTN respectively (*pi1* value indicates the proportion of significant signals among the tested associations [11]; see details in the Methods section). **Fig. 1** shows that the mean gene expression levels of the top BP signature genes were consistent with the BP phenotypic changes observed in the FHS and the Illumina cohorts.

The 73 SBP signature genes in the FHS (55 of these 73 genes were measured in the Illumina cohorts) at a Bonferroni corrected $p<0.05$ in aggregate explained 9.4% of SBP phenotypic variance in the Illumina cohorts, and the 31 DBP signature genes from the FHS (22 of these 31 genes were measured in the Illumina cohorts) in aggregate explained 5.3% of DBP phenotypic variance in the Illumina cohorts. These results suggest that in contrast to common genetic variants identified by BP GWAS, which explain in aggregate only about 1% of inter-individual BP variation [3], changes in gene expression levels explains a considerably larger proportion of phenotypic variance in BP.

Table 2. Differentially expressed genes associated with BP and hypertension at Bonferroni correction $p<0.05$ in meta-analysis of the six cohorts.

Gene	Chr.	Gene Description	FHS Beta	FHS s.e.	FHS pvalue	Illumina Beta	Illumina s.e.	Illumina pvalue	Meta *	Meta s.e.	Meta pvalue
—SBP Signature genes											
SLC31A2	9	solute carrier family 31 (copper transporters), member 2	2.4E-03	3.3E-04	1.2E-13	2.1E-03	3.3E-04	9.9E-11	2.3E-03	2.3E-04	<1E-16
MYADM	19	myeloid-associated differentiation marker	2.5E-03	3.2E-04	2.2E-14	2.7E-03	3.9E-04	2.2E-12	2.6E-03	2.5E-04	<1E-16
DUSP1	5	dual specificity phosphatase 1	2.2E-03	3.9E-04	1.1E-08	2.1E-03	4.2E-04	3.7E-07	2.2E-03	2.9E-04	2.0E-14
TAGLN2	1	transgelin 2	2.0E-03	4.1E-04	1.0E-06	2.0E-03	4.0E-04	1.3E-06	2.0E-03	2.9E-04	5.8E-12
CD97	19	CD97 molecule	1.7E-03	3.2E-04	1.4E-07	1.5E-03	3.5E-04	1.6E-05	1.6E-03	2.4E-04	1.0E-11
BHLHE40	3	basic helix-loop-helix family, member e40	1.5E-03	3.4E-04	4.3E-06	1.5E-03	3.0E-04	6.4E-07	1.5E-03	2.2E-04	1.2E-11
MCL1	1	myeloid cell leukemia sequence 1 (BCL2-related)	1.0E-03	2.0E-04	7.5E-07	1.6E-03	3.2E-04	1.5E-06	1.2E-03	1.7E-04	1.4E-11
PRF1	10	perforin 1 (pore forming protein)	2.5E-03	4.1E-04	2.5E-09	1.8E-03	5.3E-04	1.0E-03	2.2E-03	3.3E-04	1.6E-11
GPR56	16	G protein-coupled receptor 56	2.0E-03	3.4E-04	3.5E-09	1.7E-03	5.8E-04	3.0E-03	1.9E-03	2.9E-04	3.9E-11
PPP1R15A	19	protein phosphatase 1, regulatory (inhibitor) subunit 15A	1.5E-03	2.6E-04	1.7E-09	1.3E-03	3.0E-04	2.8E-05	1.4E-03	2.4E-04	1.5E-08
FGFBP2	4	fibroblast growth factor binding protein 2	2.3E-03	5.0E-04	5.8E-06	2.0E-03	6.2E-04	1.5E-03	2.2E-03	3.9E-04	3.3E-08
GNLY	2	granulysin	2.6E-03	6.4E-04	3.6E-05	2.6E-03	7.2E-04	3.0E-04	2.6E-03	4.8E-04	4.0E-08
FOS	14	FBJ murine osteosarcoma viral oncogene homolog	1.7E-03	2.5E-04	1.6E-11	2.6E-03	6.3E-04	3.6E-05	2.3E-03	4.1E-04	4.8E-08
NKG7	19	natural killer cell group 7 sequence	2.3E-03	5.3E-04	1.9E-05	1.4E-03	5.5E-04	8.8E-03	1.9E-03	3.8E-04	9.4E-07
GRAMD1A	19	GRAM domain containing 1A	-6.0E-04	1.4E-04	2.1E-05	-6.7E-04	2.8E-04	1.8E-02	-6.2E-04	1.3E-04	1.1E-06
GLRX5	14	glutaredoxin 5	1.7E-03	3.9E-04	1.3E-05	1.3E-03	6.1E-04	3.5E-02	1.6E-03	3.3E-04	1.5E-06
TMEM43	3	transmembrane protein 43	7.5E-04	2.1E-04	3.0E-04	7.7E-04	2.5E-04	2.4E-03	7.6E-04	1.6E-04	2.3E-06
TIPARP	3	TCDD-inducible poly(ADP-ribose) polymerase	1.2E-03	2.3E-04	1.3E-07	8.6E-04	2.4E-04	3.3E-04	9.5E-04	2.0E-04	2.6E-06
AHNAK	11	AHNAK Nucleoprotein	9.1E-04	2.6E-04	4.1E-04	9.7E-04	3.4E-04	4.0E-03	9.3E-04	2.0E-04	5.2E-06
PIGB	15	phosphatidylinositol glycan anchor biosynthesis, class B	1.1E-03	3.1E-04	5.3E-04	6.7E-04	2.1E-04	1.9E-03	8.0E-04	1.8E-04	6.1E-06
TAGAP	6	T-cell activation RhoGTPase activating protein	1.7E-03	2.5E-04	5.7E-12	1.3E-03	3.7E-04	7.1E-04	1.4E-03	3.1E-04	6.4E-06
—DBP Signature genes											
BHLHE40	3	basic helix-loop-helix family, member e40	2.4E-03	5.1E-04	2.3E-06	2.5E-03	5.2E-04	2.8E-06	2.4E-03	3.6E-04	2.7E-11
ANXA1	9	annexin A1	3.5E-03	5.7E-04	1.2E-09	2.1E-03	7.8E-04	6.3E-03	3.0E-03	4.6E-04	6.5E-11
PRF1	10	perforin 1 (pore forming protein)	3.2E-03	6.2E-04	3.2E-07	3.2E-03	9.4E-04	5.7E-04	3.2E-03	5.2E-04	6.7E-10

(*Continued*)

Table 2. (*Continued*)

Gene	Chr.	Gene Description	FHS Beta	FHS s.e.	FHS pvalue	Illumina Beta	Illumina s.e.	Illumina pvalue	Meta *	Meta s.e.	Meta pvalue
KCNJ2	17	potassium inwardly-rectifying channel, subfamily J, member 2	-2.6E-03	5.6E-04	3.9E-06	-2.0E-03	5.5E-04	2.6E-04	-2.3E-03	3.9E-04	4.9E-09
CLC	19	Charcot-Leyden crystal protein	-4.1E-03	8.6E-04	2.6E-06	-3.6E-03	1.0E-03	5.7E-04	-3.9E-03	6.7E-04	5.8E-09
CD97	19	CD97 molecule	2.3E-03	4.8E-04	1.6E-06	1.9E-03	5.8E-04	1.1E-03	2.1E-03	3.7E-04	7.4E-09
IL2RB	22	interleukin 2 receptor, beta	2.3E-03	4.9E-04	3.0E-06	2.2E-03	7.3E-04	2.4E-03	2.3E-03	4.1E-04	2.5E-08
S100A10	1	S100 calcium binding protein A10	3.2E-03	6.1E-04	2.4E-07	1.6E-03	6.2E-04	9.9E-03	2.4E-03	4.4E-04	4.0E-08
GPR56	16	G protein-coupled receptor 56	2.5E-03	5.2E-04	1.1E-06	2.4E-03	1.0E-03	1.7E-02	2.5E-03	4.6E-04	5.5E-08
TIPARP	3	TCDD-inducible poly(ADP-ribose) polymerase	1.3E-03	3.4E-04	1.3E-04	1.1E-03	3.1E-04	2.8E-04	1.2E-03	2.3E-04	1.4E-07
HAVCR2	5	Hepatitis A Virus Cellular Receptor 2	1.7E-03	4.6E-04	3.8E-04	1.8E-03	4.8E-04	1.8E-04	1.7E-03	3.3E-04	2.4E-07
PTGS2	1	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	-2.1E-03	4.9E-04	2.2E-05	-1.3E-03	5.1E-04	9.0E-03	-1.7E-03	3.5E-04	1.0E-06
MYADM	19	myeloid-associated differentiation marker	2.8E-03	4.9E-04	1.7E-08	4.1E-03	1.0E-03	8.6E-05	3.6E-03	7.4E-04	1.1E-06
ANTXR2	4	anthrax toxin receptor 2	1.5E-03	3.3E-04	5.2E-06	8.3E-04	4.3E-04	5.5E-02	1.3E-03	2.6E-04	1.7E-06
OBFC2A	2	nucleic acid binding protein 1	-1.7E-03	3.9E-04	7.2E-06	-9.6E-04	4.6E-04	3.8E-02	-1.4E-03	3.0E-04	1.8E-06
GRAMD1A	19	GRAM domain containing 1A	-9.3E-04	2.1E-04	1.4E-05	-8.7E-04	5.0E-04	7.8E-02	-9.2E-04	2.0E-04	2.8E-06
ARHGAP15	2	Rho GTPase activating protein 15	-1.3E-03	4.1E-04	1.1E-03	-1.4E-03	4.4E-04	1.5E-03	-1.4E-03	3.0E-04	5.2E-06
FBXL5	4	F-box and leucine-rich repeat protein 5	-1.6E-03	3.7E-04	2.1E-05	-9.4E-04	4.9E-04	5.5E-02	-1.3E-03	2.9E-04	5.3E-06
SLC31A2	9	solute carrier family 31 (copper transporters), member 2	2.8E-03	4.9E-04	1.0E-08	2.4E-03	8.1E-04	2.6E-03	2.6E-03	5.6E-04	5.4E-06
VIM	10	vimentin	1.7E-03	3.8E-04	5.5E-06	7.6E-04	5.9E-04	2.0E-01	1.4E-03	3.2E-04	6.2E-06
—HTN Signature genes											
SLC31A2	9	solute carrier family 31 (copper transporters), member 2	5.9E-02	1.4E-02	1.9E-05	6.4E-02	1.4E-02	2.1E-06	6.1E-02	9.6E-03	1.8E-10
MYADM	19	myeloid-associated differentiation marker	7.8E-02	1.4E-02	1.2E-08	7.3E-02	2.1E-02	6.2E-04	7.4E-02	1.4E-02	3.0E-07
TAGAP	6	T-cell activation RhoGTPase activating protein	4.4E-02	1.1E-02	3.2E-05	3.2E-02	1.2E-02	5.3E-03	3.9E-02	7.8E-03	7.3E-07
GZMB	14	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	1.6E-01	2.3E-02	1.1E-11	1.1E-01	3.5E-02	9.6E-04	1.3E-01	2.6E-02	1.4E-06
KCNJ2	17	potassium inwardly-rectifying channel, subfamily J, member 2	-5.2E-02	1.6E-02	8.4E-04	-4.4E-02	1.3E-02	5.5E-04	-4.7E-02	9.9E-03	1.7E-06

*__Meta__: meta-analysis of all six cohorts.

doi:10.1371/journal.pgen.1005035.t002

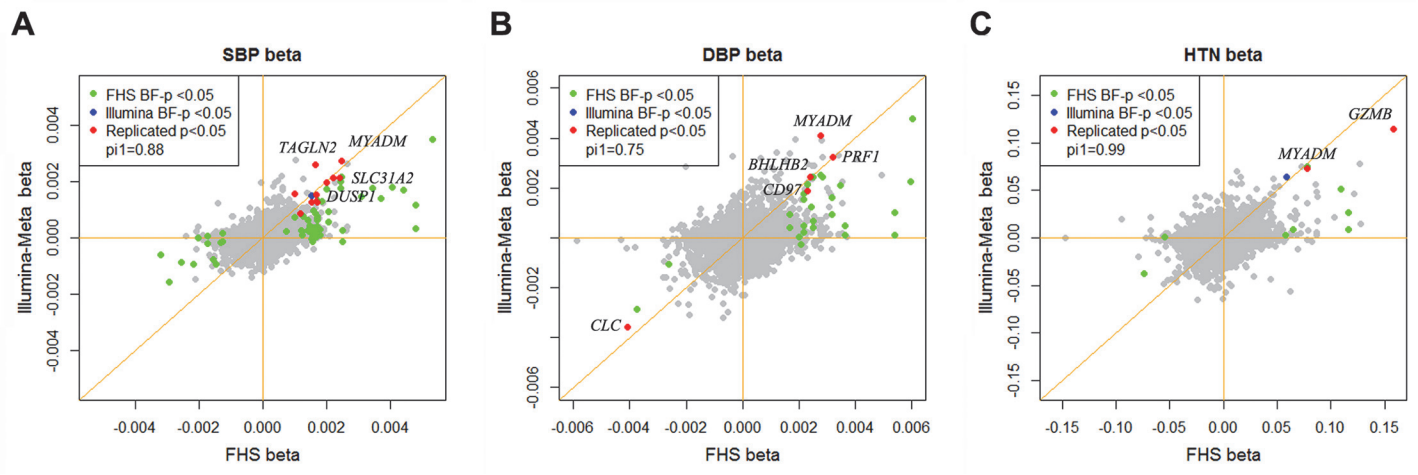

Fig 1. Effect size of differentially expressed BP genes in the Framingham Heart Study and the Illumina cohorts. A) SBP; B) DBP; C) HTN. The x-axis is the effect size of the differentially expressed genes in the FHS cohort and the y-axis is the effect size in the Illumina cohorts. The BP signature genes identified both in the FHS and the Illumina cohorts at $p<0.05$ (Bonferroni corrected) are highlighted. *pi1* values indicate the proportion of significant signals among the tested associations [11] (See details in the Methods section).

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Meta-analysis of the six cohorts identifies differentially expressed BP signature genes

A meta-analysis of differential expression across all six cohorts revealed 34 differentially expressed BP genes at $p<0.05$ (Bonferroni corrected for 7717 genes that were measured and passed quality control in the FHS and Illumina cohorts), including 21 for SBP, 20 for DBP, and 5 for HTN (Table 2 and S2 Fig.). All of the 34 differentially expressed BP signature genes showed directional consistency in the FHS and the Illumina cohorts (Table 2). The 34 BP signature genes included all 13 genes that were cross-validated between the FHS and the Illumina cohorts. Of the 34 BP signature genes, 27 were positively correlated with BP and only 7 genes were negatively correlated. *MYADM* and *SLC31A2* were top signature genes for SBP, DBP, and HTN. At FDR<0.2, 224 unique genes were differentially expressed in relation BP phenotypes including 142 genes for SBP, 137 for DBP, and 45 for HTN (details are reported in the S1–S2 Text, and S3–S5 Table).

Functional analysis of differentially expressed BP signature genes

We used gene set enrichment analysis (GSEA) to identify the biological process and pathways associated with gene expression changes in relation to SBP, DBP, and HTN in order to better understand the biological themes within the data. As shown in Table 3, the GSEA of genes whose expression was positively associated with BP showed enrichment for antigen processing and presentation ($p<0.0001$), apoptotic program ($p<0.0001$), inflammatory response ($p<0.0001$), and oxidative phosphorylation ($p = 0.0018$). The negatively associated genes showed enrichment for nucleotide metabolic process ($p<0.0001$), positive regulation of cellular metabolic process ($p<0.0001$), and positive regulation of DNA dependent transcription ($p = 0.0021$).

Genetic effects on expression of BP signature genes

Among the 34 BP signatures genes from the meta-analysis of all 6 studies, 33 were found to have *cis*-eQTLs and 26 had *trans*-eQTLs (Fig. 2A and S2 Table) based on whole blood

Table 3. Gene set enrichment analysis for BP associated gene expression changes.

Name	Pos / Neg associated gene expression changes	Database	Number of genes in pathway	NES*	p value	FDR
- ***DBP signature***						
Antigen processing and presentation	Positive	KEGG	37	2.0	<1E-4	0.01
Nature killer cell mediated cytotoxicity	Positive	KEGG	71	1.8	<1E-4	0.07
Porphyrin and chlorophyll metabolism	Positive	KEGG	15	1.7	0.01	0.13
Rho protein signaling transduction	Negative	GO-BP	18	-1.8	3.9E-3	0.10
Receptor mediated endocytosis	Negative	GO-BP	16	-1.8	3.9E-3	0.17
Detection of stimulus	Negative	GO-BP	18	-1.9	9.8E-3	0.20
- ***SBP signature***						
Natural killer cell mediated cytotoxicity	Positive	KEGG	71	1.9	1.7E-3	0.05
Apoptotic program	Positive	GO-BP	37	1.9	<1E-4	0.03
Inflammatory response	Positive	GO-BP	72	2.0	<1E-4	0.05
Nucleotide metabolic process	Negative	GO-BP	32	-1.9	<1E-4	0.04
Translation	Negative	GO-BP	79	-1.8	<1E-4	0.05
- ***HTN signature***						
Antigen processing and presentation	Positive	KEGG	37	1.8	<1E-4	0.04
Oxidative phosphorylation	Positive	KEGG	52	1.8	1.8E-3	0.05
Apoptotic program	Positive	GO-BP	37	1.9	1.8E-3	0.14
Positive regulation of nucleic acid metabolic process	Negative	GO-BP	71	-1.9	<1E-4	0.08
Positive regulation of cellular metabolic process	Negative	GO-BP	105	-1.8	<1E-4	0.08
Positive regulation of transcription DNA dependent	Negative	GO-BP	56	-1.8	2.1E-3	0.09

***NES**: normalized enrichment score;

GO-BP: Gene ontology- biological process;

KEGG: Kyoto encyclopedia of genes and genomes.

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profiling [12,13]. Of these, six master *trans*-eQTLs mapped to either five or six BP signature genes (no master *cis*-eQTL was identified). Five master *trans*-eQTLs (rs653178, rs3184504, rs10774625, rs11065987, and rs17696736) were located on chromosome *12q24* within the same linkage disequilibrium (LD) block (r^2 >0.8, **Fig. 2B**). We retrieved a peak *cis*- and *trans*-eQTL for each BP signature gene. The peak *cis*-eQTL explained 0.2–20% of the variance in the corresponding transcript levels, in contrast, the peak *trans*-eQTL accounted for very little (0.02–2%) of the corresponding transcript variance. Westra *et al*. also reported a similar small proportion of variance in transcript levels explained by *trans*-eQTLs [12].

We then linked the *cis*- and *trans*-eQTLs of the 34 BP signature genes with BP GWAS results from the ICBP Consortium [3] and the NHGRI GWAS Catalog [14] (**Fig. 2** and **S2 Table**). We did not find any *cis*-eQTLs for the top BP signature genes that also were associated with BP in the ICBP GWAS [3]. However, the 6 master *trans*-eQTLs were all associated with BP at $p<5e-8$ in the ICBP GWAS [3] and were associated with multiple complex diseases or traits (**Table 4**). For example, rs3184504, a nonsynonymous SNP in *SH2B3* that was associated in GWAS with BP, coronary heart disease, hypothyroidism, rheumatoid arthritis, and type 1 diabetes [12], is a *trans*-eQTL for 6 of our 34 BP signature genes from the meta-analysis (*FOS*, *MYADM*, *PP1R15A*, *TAGAP*, *S100A10*, and *FGBP2*; **Fig. 2A-B** and **Table 4**). These 6 genes are all highly expressed in neutrophils, and their expression levels are correlated significantly

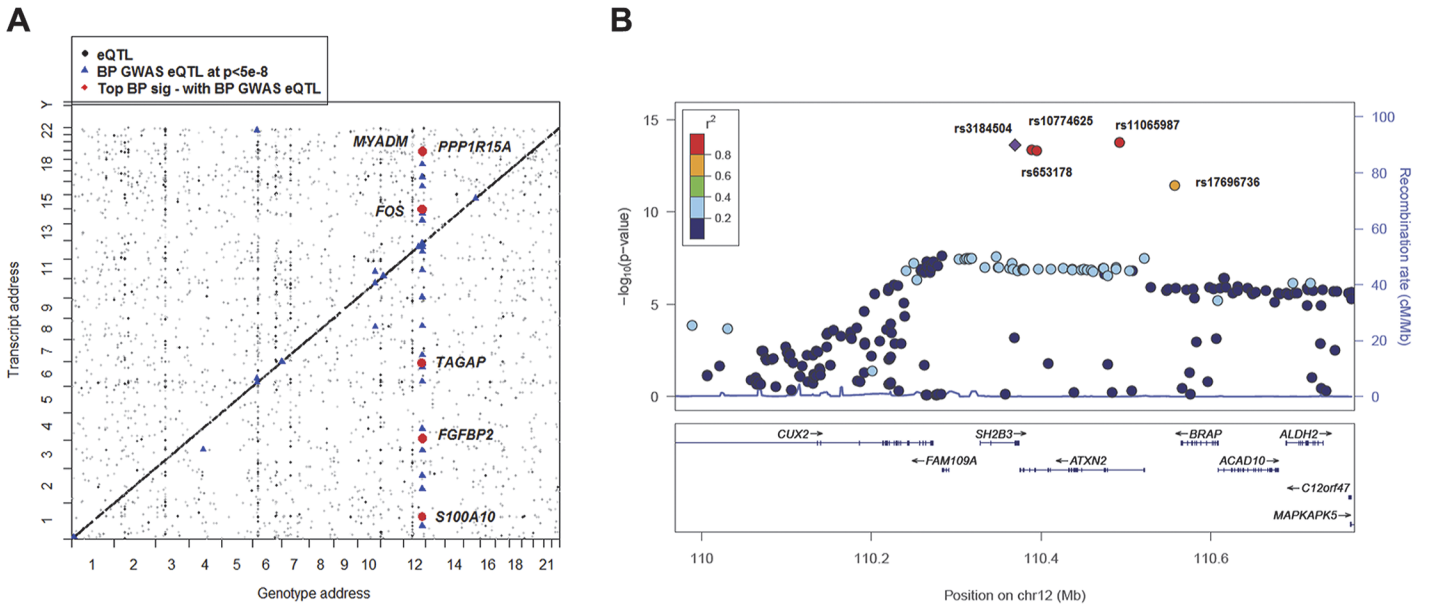

Fig 2. Global view of BP eQTLs effects on differentially expressed BP signature genes. A) 2-Dimensional plot of in whole blood eQTLs vs. transcript position genome wide. eQTL-transcript pairs at FDR<0.1 are shown in black dots; those that fall along the diagonal are cis eQTLs and all others are trans eQTLS. eQTL-transcript pair SNPs that are associated with BP in GWAS [3] are highlighted with blue triangles. eQTL-transcript pair genes that are BP signature genes from analysis of differential gene expression in relation to BP are depicted by red circles. B) Regional association plots for rs3184504 proxy QTLs that showing association with BP in ICBP GWAS [3]. –log10(p) indicated the –log10 transformed DBP association *p* values in ICBP GWAS [3]. Color coding indicates the strength (measured by r^2) of LD of each SNP with the top SNP (rs3184504). Five master *trans*-eQTLs (also BP GWAS SNPs) for BP signature genes are labeled in the figure. This figure was drawn by LocusZoom [32].

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(average $r^2 = 0.04$, $p<1e\text{-}16$). rs653178, intronic to *ATXN2* and in perfect LD with rs3184504 ($r^2 = 1$), also is associated with BP and multiple other diseases in the NHGRI GWAS Catalog [14]. It also is a *trans*-eQTL for the same 6 BP signature genes (**Table 4**). These two SNPs are *cis*-eQTLs for expression *SH2B3* in whole blood (FDR<0.05), but not for *ATXN2* (FDR = 0.4). We found that the expression of *SH2B3* is associated with expression of *MYADM*, *PP1R15A*, and *TAGAP* (at Bonferroni corrected $p<0.05$), but not with *FOS*, *S100A10*, or *FGBP2*. The expression of *ATXN2* was associated with expression of 5 of the 6 genes (*PP1R15A* was not associated). **S3 Fig.** shows the coexpression levels of the eight genes that were *cis*- or *trans*-associated with rs3184504 and rs653178 genotypes. These results suggest that there may be a pathway or gene co-regulatory mechanism underling BP regulation involving these genes that is driven by this common genetic variant (rs3184504; minor allele frequency 0.47) or its proxy SNPs.

We further checked whether the *cis*- or *trans*-eQTLs for the top 34 BP signature genes are associated with other diseases or traits in the NHGRI GWAS catalog [14]. We identified 12 *cis*-eQTLs (for 8 genes) and 6 *trans*-eQTLs (for 6 genes) that are associated with other diseases or traits in the NHGRI GWAS catalog [14] (**Table 4**).

Discussion

Our meta-analysis of gene expression data from 7017 individuals from six studies identified and characterized whole blood gene expression signatures associated with BP traits. Thirty-four BP signature genes were identified at Bonferroni corrected $p<0.05$ (224 genes were identified at FDR<0.2, reported in the **S1 Text**). Thirteen BP signature genes replicated between the FHS and Illumina cohorts. The top BP signature genes identified in the FHS (55 genes for SBP

Table 4. GWAS eQTLs for the top differentially expressed BP signature genes.

		SNP—Trait Association			SNP-Gene Association			Gene-Trait Association		
SNP ID	SNP. Location	ICBP-SBP pval	ICBP-DBP pval	Other Traits in GWAS Catalog	Gene	Chr. Gene	Cis/ Trans	SBP pval	DBP pval	HTN pval
rs3184504*	chr12 (missense, SH2B3)	1.70E-09	2.30E-14	Coronary heart disease; Rheumatoid arthritis; Type 1 diabetes	MYADM	chr19	trans	**<1e-16**[&]	**1.1e-6**	**3.0e-7**
					FOS	chr14	trans[§]	**4.9e-8**	3.2e-4	7.9e-5
					PPP1R15A	chr19	trans[§]	**1.6e-8**	1.2e-5	6.1e-4
					TAGAP	chr6	trans	**6.4e-6**	1.3e-4	**7.3e-7**
					S100A10	chr1	trans[§]	2.6e-4	**4.0e-8**	7.0e-5
					FGFBP2	chr4	trans[§]	**3.3e-8**	1.8e-5	5.1e-3
rs10187424	chr2 (intergenic)	-	-	Prostate cancer	GNLY	chr2	cis[§]	**4.0e-8**	2.8e-5	2.2e-4
rs411174	chr5 (intron, ITK)	-	-	Personality dimensions	HAVCR2	chr5	cis[§]	1.6e-4	**2.4e-7**	1.5e-3
rs3758354	chr9 (intergenic)	-	-	Schizophrenia, bipolar disorder and depression	ANXA1	chr9	cis	1.8e-3	**6.5e-11**	7.5e-3
rs1950500	chr14 (intergenic)	-	-	Height	GZMB	chr14	cis	7.8e-5	6.0e-5	**1.4e-6**
rs8017377	chr14 (missense, NYNRIN)	-	-	LDL cholesterol	GZMB	chr14	cis	7.8e-5	6.0e-5	**1.4e-6**
rs8192917	chr14 (missense, GZMB)	-	-	Vitiligo	GZMB	chr14	cis	7.8e-5	6.0e-5	**1.4e-6**
rs2284033	chr22 (intron, IL2RB)	-	-	Asthma	IL2RB	chr22	cis[§]	1.6e-4	**2.5e-8**	9.3e-3
rs11724635[+]	chr4 (intergenic)	-	-	Parkinsons disease	FBXL5	chr4	cis	5.9e-5	**5.3e-6**	0.07
rs4333130[$]	chr4 (intron, ANTXR2)	-	-	Ankylosing spondylitis	ANTXR2	chr4	cis	2.8e-4	**1.7e-6**	0.04
rs8005962	chr14 (intergenic)	-	-	Tuberculosis	GLRX5	chr14	cis	**1.5e-6**	0.13	0.09
rs7995215	chr13 (intron, GPC6)	-	-	Attention deficit hyperactivity disorder	TAGAP	chr6	trans	**6.4e-6**	1.3e-4	**7.3e-7**
rs12047808	chr1 (intron, C1orf125)	-	-	Multiple sclerosis (age of onset)	FOS	chr14	trans[§]	**4.9e-8**	3.2e-4	7.9e-5
rs2894207	chr6 (intergenic)	-	-	Nasopharyngeal carcinoma	AHNAK	chr11	trans	**5.2e-6**	6.8e-5	1.8e-3
rs3763313	chr6 (neargene 5, BTNL2)	-	-	HIV-1 control	PPP1R15A	chr19	trans	**1.6e-8**	1.2e-5	6.1e-4
rs9376092	chr6 (intergenic)	-	-	Beta thalassemia/hemoglobin E disease	GPR56	Chr16	trans	**3.9e-11**	**5.5e-8**	4.9e-4

* rs653178, intronic to *ATXN2* and in tight linkage disequilibrium with rs3184504 ($r^2 = 1$), was also associated with BP in ICBP GWAS and all the 6 genes;

[+] A proxy SNP rs4698412 at LD $r^2 = 1$ associated with the same trait;

$ A proxy SNP rs4389526 at LD $r^2 = 1$ associated with the same trait;

[§] indicated eQTL were identified from[12].

[&] highlighted p values indicated passing transcriptome-wide significance at Bonferroni corrected $p<0$

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and 22 genes for DBP) explained 5–9% of interindividual variation in BP in the Illumina cohorts on average.

Among the 34 BP signature genes (at Bonferroni corrected $p<0.05$), only *FOS* [15] and *PTGS2 [16]* have been previously implicated in hypertension. We did not find literature

support for a direct role of the remaining signature genes in BP regulation. However, we found several genes involved in biological functions or processes that are highly related to BP, such as cardiovascular disease (*GZMB*, *ANXA1*, *TMEM43*, *FOS*, *KCNJ2*, *PTGS2*, and *MCL1*), angiogenesis (*VIM* and *TIPARP*), and ion channels (*CD97*, *ANXA1*, *S100A10*, *PRF1*, *ANTXR2*, *SLC31A2*, *TIPARP*, and *KCNJ2*). We speculate that these genes may be important for BP regulation, but further experimental validation is needed.

Seven of the 34 signature genes, including *KCNJ2*, showed negative correlation of expression with BP. *KCNJ2* is a member of the potassium inwardly-rectifying channel subfamily; it encodes the inward rectifier K+ channel Kir2.1, and is found in cardiac, skeletal muscle, and nervous tissue [17]. Most outward potassium channels are positively correlated with BP. Loss-of-function mutations in *ROMK* (*KCNJ1*, the outward potassium channel) are associated with Bartter's syndrome, and *ROMK* inhibitors are used in the treatment of hypertension [18,19]. Previous studies reported that greater potassium intake is associated with lower blood pressure [20,21,22,23]. These data suggest that *KCNJ2* up-regulation may be a means of lowering BP.

By linking the BP signature genes with eQTLs and with BP GWAS results, we found several SNPs that are associated with BP in GWAS and that also are *trans* associated with several of our top BP signature genes. For example, rs3184504, a non-synonymous SNP located in exon 3 of *SH2B3*, is associated in GWAS with BP, coronary heart disease, hypothyroidism, rheumatoid arthritis, and type I diabetes [12]. rs3184504 is a common genetic variant with a minor allele frequency of approximately 0.47; the rs3184504-T allele is associated with an increment of 0.58 mm Hg in SBP and of 0.48 mm Hg in DBP [2]. rs3184504 is a *cis*-eQTL for *SH2B3*, expression of this gene was not associated with BP or hypertension in our data. However, rs3184504 also is a *trans*-eQTL for 6 of our 34 BP signature genes: *FOS*, *MYADM*, *PP1R15A*, *TAGAP*, *S100A10*, and *FGBP2*. These 6 genes are highly expressed in neutrophils [12], and are coexpressed. Prior studies have suggested an important role of neutrophils in BP regulation [24]. We speculate that these 6 BP signature genes, all driven by the same BP-associated eQTL, point to a critical and previously unrecognized mechanism involved in BP regulation. Further experimental validation is needed.

One limitation of our study is the use of whole blood derived RNA for transcriptomic profiling. GSEA showed that the top enriched biological processes for the differentially expressed BP genes include inflammatory response. Numerous studies have shown links between inflammation and hypertension [25,26,27]. The top ranked genes in inflammatory response categories provide a guide for further experimental work to recognize the contributions of inflammation to alterations in BP regulation. We speculate that using similar approaches in other tissues might identify additional differentially expressed BP signature genes.

In conclusion, we conducted a meta-analysis of global gene expression profiles in relation to BP and identified a number of credible gene signatures of BP and hypertension. Our integrative analysis of GWAS and gene expression in relation to BP can help to uncover the genetic and genomic architecture of BP regulation; the BP signature genes we identified may represent an early step toward improvements in the detection of susceptibility, and in the prevention and treatment of hypertension.

Materials and Methods

Study population and ethics statement

This investigation included six studies (the Framingham Heart Study (FHS), the Estonian Biobank (EGCUT), the Rotterdam Study (RS) [8], the InCHIANTI Study, the Cooperative Health Research in the Region of Augsburg (KORA F4) Study [9], and the Study of Health in Pomerania (SHIP-TREND) [10], each of which conducted genome-wide genotyping, mRNA

expression profiling, and had extensive BP phenotype data. Each of the six studies followed the recommendations of the Declaration of Helsinki. The FHS: Systems Approach to Biomarker Research (SABRe) in cardiovascular disease is approved under the Boston University Medical Center's protocol H-27984. Ethical approval of EGCUT was granted by the Research Ethics Committee of the University of Tartu (UT REC). Ethical approval of the InCHIANTI study was granted by the Instituto Nazionale Riposo e Cura Anziani institutional review board in Italy. Ethical approval of RS was granted by the medical ethics committee of the Erasmus Medical Center. The study protocol of SHIP-TREND was approved by the medical ethics committee of the University of Greifswald. KORA F4 is a population-based survey in the region of Augsburg in Southern Germany which was performed between 2006 and 2008. KORA F4 was approved by the local ethical committees. Informed consent was obtained from each study participant.

Hypertension (HTN) was defined as SBP $\geq$140 mm Hg or DBP $\geq$90 mm Hg. We excluded individuals receiving anti-hypertensive treatment because of the possibility that some of the differentially expressed genes we identified would reflect treatment effects. The eligible study sample included 7017 individuals: 3679 from FHS, 972 from EGCUT, 604 from RS, 597 from InCHIANTI, 565 from KORA F4, and 600 from SHIP-TREND.

Gene expression profiling

RNA was isolated from whole blood samples that were collected in PaxGene tubes (PreAnalytiX, Hombrechtikon, Switzerland) in FHS, RS, InCHIANTI, KORA F4 and SHIP-TREND, and in Blood RNA Tubes (Life Technologies, NY, USA) in EGCUT. Gene expression in the FHS samples used the Affymetrix Exon Array ST 1.0. EGCUT, RS, InCHANTI, KORA F4, and SHIP-TREND used the Illumina HT12v3 (EGCUT, InCHANTI, KORA F4, and SHIP-TREND) or HT12v4 (RS) array. Raw data from gene expression profiling are available online (FHS [http://www.ncbi.nlm.nih.gov/gap; accession number phs000007], EGCUT [GSE48348], RS [GSE33828], InCHIANTI [GSE48152], KORA F4 [E-MTAB-1708] and SHIP-TREND [GSE36382]). The details of sample collection, microarrays, and data processing and normalization in each cohort are provided in the **S2 Text**.

Identification and replication of differentially expressed genes associated with BP

The association of gene expression with BP was analyzed separately in each of the six studies (Equation 1). A linear mixed model was used in the FHS in order to account for family structure. Linear regression models were used in the other five studies. In each study, gene expression level, denoted by geneExp, was included as the dependent variable, and explanatory variables included blood pressure phenotypes (SBP, DBP, and HTN), and covariates included age, sex, body mass index (BMI), cell counts, and technical covariates. A separate regression model was fitted for each gene. The general formula is shown below, and the details of analyses for each study are provided in the **S2 Text** and **S6 Table**.

$$geneExp = BP + \sum_{j=1}^{m} covariates$$

The overall analysis framework is provided in **S1 Fig**. We first identified differentially expressed genes associated with BP (BP signature genes) in the FHS samples (Set 1) and attempted replication in the meta-analysis results from the Illumina cohorts (Set 2, see Methods,

Meta-analysis). We next identified BP signature genes in the Illumina cohorts (Set 2), and then attempted replication in the FHS samples (Set 1). The significance threshold for pre-selecting BP signature genes in discovery was at Bonferroni corrected $p = 0.05$ (in FHS, corrected for 17,318 measured genes [17,873 transcripts], and in illumina cohorts, corrected for 12,010 measured genes [14,222 transcripts] that passed quality control). Replication was established at Bonferroni corrected $p = 0.05$, correcting for the number of pre-selected BP signatures genes in the discovery set. We computed the *pi1* value to estimate the enrichment of significant *p* values in the replication set (the Illumina cohorts) for BP signatures identified in the discovery set (the FHS) by utilizing the R package *Qvalue* [11]. *Pi1* is defined as *1-pi0*. *Pi0* value provided by the *Qvalue* package, represents overall probability that the null hypothesis is true. Therefore, *pi1* value represents the proportion of significant results. For genes passing Bonferroni corrected $p<0.05$ in the discovery set for SBP, DBP and HTN, we calculated *pi1* values for each gene set in the replication set.

Meta-analysis

We performed meta-analysis of the five Illumina cohorts (for discovery and replication purposes), and then performed meta-analysis of all six cohorts. An inverse variance weighted meta-analysis was conducted using fixed-effects or random-effects models by the *metagen()* function in the R package Meta (http://cran.r-project.org/web/packages/meta/index.html). At first, we tested heterogeneity for each gene using Cochran's Q statistic. If the heterogeneity p value is significant ($p<0.05$), we will use a random-effects model for the meta-analysis, otherwise use a fixed-effects model. The Benjamini-Hochberg (BH) method [28] was used to calculate FDR for differentially expressed genes in relation to BP following the meta-analysis of all six cohorts. We also used a more stringent threshold to define BP signature genes by utilizing $p<6.5e\text{-}6$ (Bonferroni correction for 7717 unique genes [7810 transcript] based on the overlap of FHS and illumina cohort interrogated gene sets).

Estimating the proportion of variance in BP attributable to BP signature genes

To estimate the proportion of variances in SBP or DBP explained by a group of differentially expressed BP signature genes (gene 1, gene 2, . . ., gene n), we used the following two models:

Full model:

$$BP = \sum_{i=1}^{n} gene\ i + \sum_{j=1}^{m} covariates$$

Null model:

$$BP = \sum_{j=1}^{m} covariates$$

The proportion of variance in BP attributable to the group of differentially expressed BP signature genes ($h^2_{BP\_sig}$) was calculated as:

$$h^2_{BP\_sig} = \max\left(0, \frac{\sigma^2_{G.null} + \sigma^2_{err.null} - \sigma^2_{G.full} - \sigma^2_{err.full}}{\sigma^2_{BP}}\right)$$

where σ^2_{BP} is the total phenotypic variance of SBP or DBP, $\sigma^2_{G.full}$ and $\sigma^2_{err.full}$ are the variance and

error variance when modeling with the tested group of gene expression traits (gene 1, gene 2, . . ., gene n), and $\sigma^2_{G.null}$ and $\sigma^2_{err.null}$ are the variance and error variance when modeling without the tested group of gene expression traits.

The proportion of the variance in BP phenotypes attributable to the FHS BP signature genes was estimated in the five Illumina cohorts, respectively, and then the average proportion values were reported. In turn, the proportion of the variance in BP phenotypes attributable to the Illumina BP signature genes was estimated in the FHS.

Identifying eQTLs and estimating the proportion of variance in gene expression attributable to single *cis*- or *trans*-eQTLs

SNPs associated with altered gene expression (i.e. eQTLs) were identified using genome-wide genotype and gene expression data in all available FHS samples (n = 5257) at FDR<0.1 (Joehanes R, submitted, 2014, and a brief summary of methods and results are provided in the **S2 Text**). A *cis*-eQTL was defined as an eQTL within 1 megabase (MB) flanking the gene. Other eQTLs were defined as *trans*-eQTLs. We combined the eQTL list generated in the FHS with the eQTLs generated by meta-analysis of seven other studies (n = 5300) that were also based on whole blood expression[12].

For every BP signature gene, we estimated the proportion of variance in the transcript attributable to the corresponding *cis*- or *trans*-eQTLs (h^2_{eQTL}) using the formula:

$$h^2_{eQTL} = max\left(0, \frac{\sigma^2_{eQTL.null} + \sigma^2_{err.null} - \sigma^2_{eQTL.full} - \sigma^2_{err.full}}{\sigma^2_{gene}}\right)$$

where σ^2_{gene} was the total phenotypic variance of a gene expression trait; $\sigma^2_{eQTL.full}$ and $\sigma^2_{err.full}$ were the variance and the residual error, respectively, when modeling with the tested eQTL; $\sigma^2_{eQTL.null}$ and $\sigma^2_{err.null}$ were the variance and the residual error when modeling without the tested eQTL.

Functional category enrichment analysis

In order to understand the biological themes within the global gene expression changes in relation to BP, we performed gene set enrichment analysis[29] to test for enrichment of any gene ontology (GO) biology process[30] or KEGG pathways[31]. "Metric for ranking gene" parameters were configured to the beta value of the meta-analysis, in order to look at the top enriched functions for BP associated up-regulated and down-regulated gene expression changes respectively. One thousand random permutations were conducted and the significance level was set at FDR$\leq$ 0.25 to allow for exploratory discovery [29].

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Supporting Information

S1 Fig. Overall analysis framework. At first, we identified BP differentially expressed genes in six cohorts (FHS, EGCUT, RS, InCHIANT, KORA F4 and SHIP-TREND) respectively. Second, we conducted a meta-analysis of the Illumina cohorts (EGCUT, RS, InCHIANT, KORA F4 and SHIP-TREND). Third, for discovery and replication purpose, we replicated the BP

signature genes identified in the FHS cohort in the Illumina cohorts. And in turn, we replicated the BP signature genes identified in Illumina cohorts in FHS cohort. Fourth, we conducted a meta-analysis in the six cohorts and reported the BP signature genes passing Bonferroni corrected $p<0.05$ (corrected for 7717 genes). And finally, we cross-analyzed the BP signature genes with blood eQTLs as well as with BP GWAS results to identify the BP signature genes having BP GWAS eQTLs.
(TIF)

S2 Fig. Volcano plots of the meta-analysis results of differentially expressed genes of BP. A) SBP; B) DBP; C) HTN. The x-axis is the effect size (beta values) of meta-analysis and the y-axis is the –log10 transformed p values.
(TIF)

S3 Fig. Coexpression of the eight genes associated in *cis* or *trans* with rs3184504 or rs653178 in the FHS. The numbers in the Heatmap indicate Pearson correlations between pairs of genes.
(TIF)

S1 Table. Differentially expressed genes of BP at Bonferroni corrected p<0.05 in the FHS cohort.
(XLSX)

S2 Table. BP signature genes at Bonferroni corrected p<0.05 with *cis/trans* eQTLs.
(XLSX)

S3 Table. BP differentially expressed genes at FDR<0.2 in the meta-analysis of all six cohorts.
(XLSX)

S4 Table. Gene ontology enrichment analysis of BP signatures at FDR<0.2.
(XLSX)

S5 Table. BP signature genes at FDR<0.2 with *cis* eQTLs in ICBP GWAS.
(XLSX)

S6 Table. Technical covariates utilized for gene expression data normalization.
(XLSX)

S1 Text. Supplementary Results.
(DOCX)

S2 Text. Supplementary Materials and Methods.
(DOCX)

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Performed the experiments: NR RW PL PC MC. Analyzed the data: TH TE MJP LCP KS CS BHC CL RJ. Wrote the paper: TH TE MJP LCP KS CS DL LF JBJvM HP UV XY. Designed, directed, and supervised the project: DL LF JBJvM HP UV XY CH. Participated in revising and editing the manuscripts: ADJ CY SxY LM NR ER AD AH AGU DGH SB AS DM AM MC HG CH TM AP MR MW MD SBF TZ RV CJO PJM.

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