Integrative network analysis reveals molecular mechanisms of blood pressure regulation

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一、 Background

1. **Blood pressure (BP) is a highly heritable physiological trait that is regulated through the interactions of numerous genes and environmental factors. Over one billion people worldwide suffer from hypertension (systolic BP [SBP] ≥ 140 mm Hg or diastolic BP [DBP] ≥ 90 mm Hg) (Kearney et al, 2005).**

2. BP elevation contributes to nearly half the deaths from cardiovascular disease (CVD) (Lawe set al, 2006; Ehret & Caulfield, 2013).

一、 Background

Purpose：

BP control in hypertensive individuals , in turn, is an effective intervention for reducing CVD risk (Lewington et al, 2002).

It is hoped that advances from understanding the molecular underpinnings of BP regulation will improve the prediction of CVD susceptibility and offer insights into personalized treatments for hypertension that can reduce the risk of its sequelae。

一、 Background

Developed：

1.Genome-wide association studies (GWAS) have identified numerous loci associated with blood pressure (BP).

2.A recent genome-wide association study (GWAS) meta-analysis of up to 200,000 people identified 29 genetic variants (at 28 loci) associated with BP (Ehret et al, 2011). However, the proportion of interindividual BP variability explained by these genetic variants was only about 1% (Ehret et al, 2011).

It has been increasingly recognized that genes, instead of working in isolation, interact with other genes in complex regulatory networks

二、 Method

We designed a systems biology framework to integrate gene expression (in this study, gene expression refers to mRNA expression) profiles with BP GWAS and cellular network models as a means to explore molecular mechanisms influencing BP regulation

二、 Method

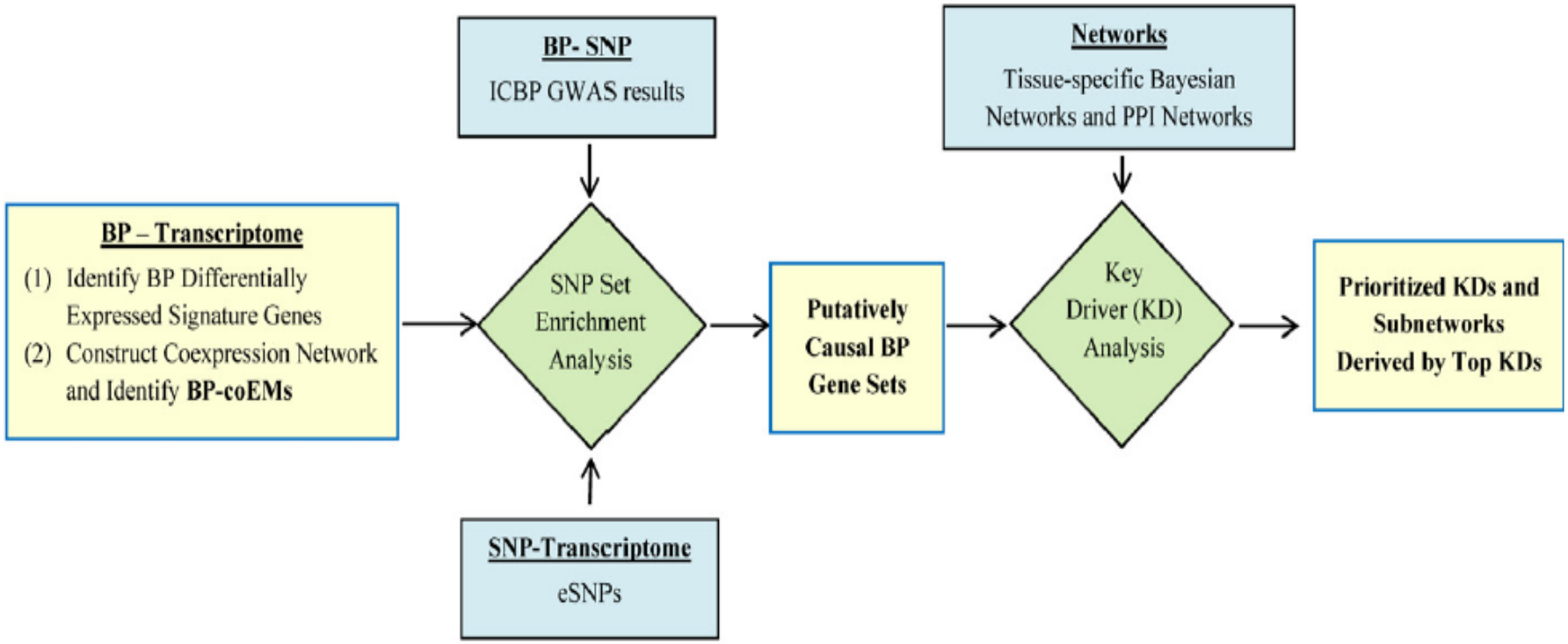


Figure 1. The integrative network-based approach for identifying and prioritizing key drivers of blood pressure regulation.

三、 Results

1. Clinical characteristics of study participants

Table 1. Clinical characteristics of FHS participants.

Phenotypes/ Covariates	Offspring cohort $N = 1{,}102$ (examination cycle 8: 2005–2008)[a] Mean ± SD	Third-generation cohort $N = 2{,}577$ (examination cycle 2: 2008–2011)[a] Mean ± SD
Male (%)	38	44
Age (years)	63 ± 9	45 ± 8
Body mass index (kg/m^2)	27.1 ± 5.0	27.2 ± 5.4
Systolic BP (mm Hg)	126 ± 16	115 ± 14
Diastolic BP (mm Hg)	75 ± 10	74 ± 9
Hypertension (%)	21	7

[a]Individuals who were receiving antihypertensive treatment were excluded in this study.

High-throughput gene expression profiles from whole-blood-derived RNA were generated in 5,626 individuals of European ancestry from the FHS offspring (n = 2,446) and the third-generation (n = 3,180) cohorts

三、 Results

2. Influence of blood cell types on BP-associated gene expression differences

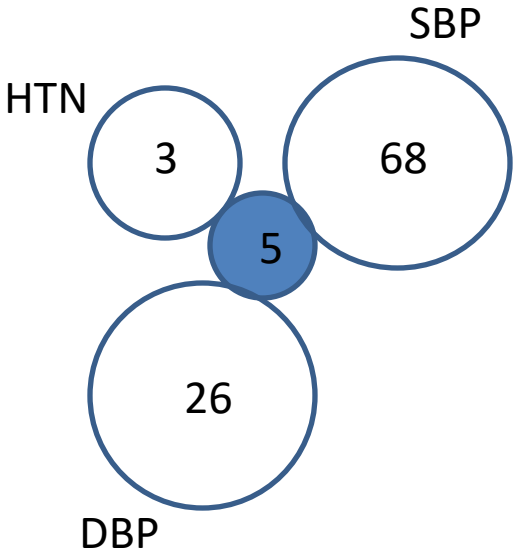
Supplementary Table S1: Summary of genes associated with cell type proportions

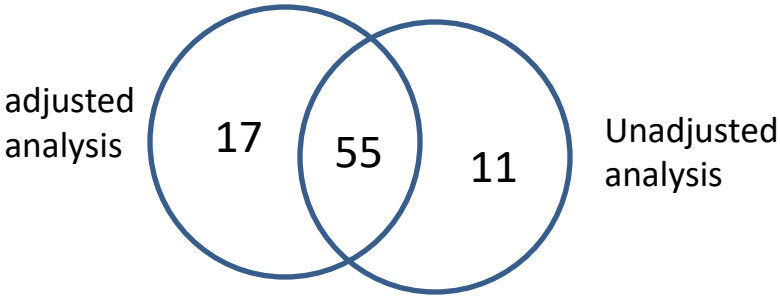
	Associated genes at p <0.05	Associated genes at Bonferroni corrected p <0.05 (corrected for 17,318 measured genes)
White blood cell	10,388	5,160
Neutrophils	12,751	7,114
Lymphocytes	12,905	7,499

We found that approximately 42% of genes were significantly correlated with cell-type proportions at Bonferroni-corrected P < 0.05, suggesting a major impact of blood cell types on gene expression。 As results from both cell type-adjusted and cell type-unadjusted analyses could be biologically relevant， we report both sets of results but focus our discussions on the adjusted analysis to simplify results interpretation。

三、 Results

3. Identification of transcriptome-wide gene expression signatures for BP





三、 Results

4. Construction of coexpression networks and identification of BP-associated gene coexpression modules

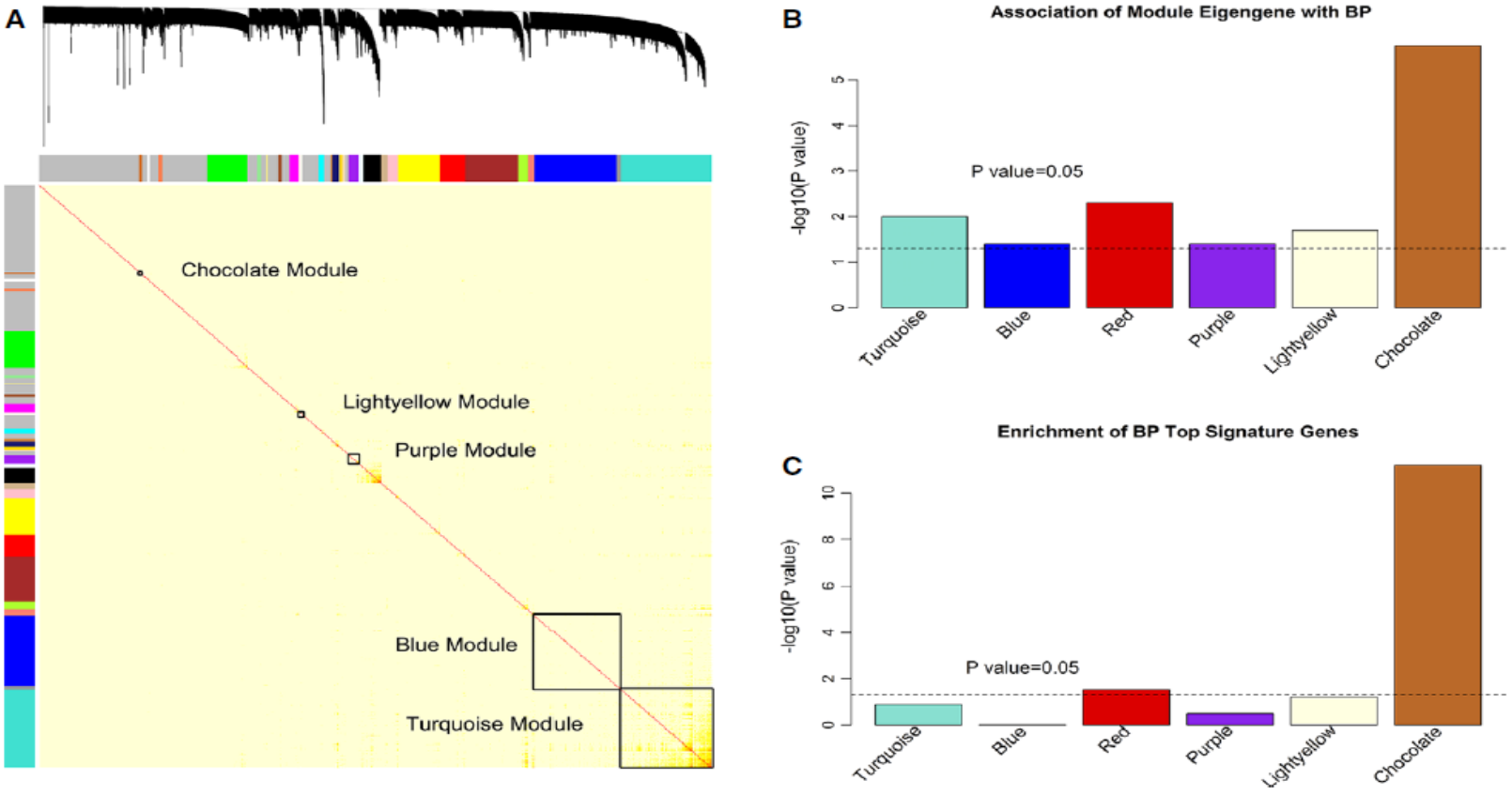
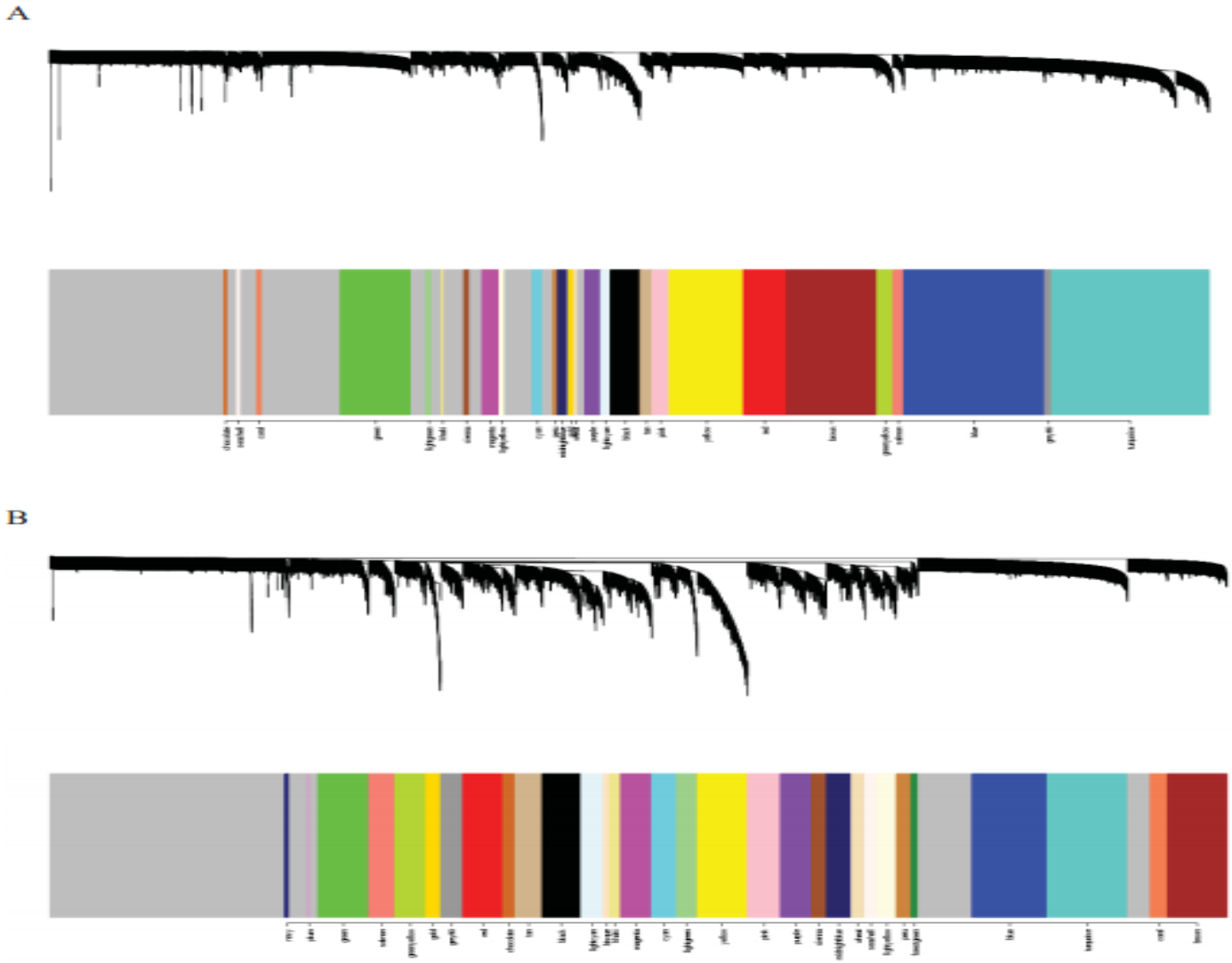


Figure 2. Identification of BP-associated coexpression network modules (coEMs).

adjusted analysis

三、 Results



adjusted analysis

Unadjusted analysis

三、Results

5.Gene ontology (GO) enrichment analysis

adjusted analysis

Table 2. Gene ontology enrichment analysis of the BP coexpression modules.

Gene set	Biological process terms	Gene count	Fold change	*P*-value	Bonferroni-corrected *P*
Turquoise	Chromatin modification	89	2.5	4.6e-17	3.8e-14
	Intracellular transport	156	1.8	1.3e-14	1.1e-11
	Regulation of gene expression	382	1.4	7.1e-14	5.9e-11
Purple	Hemostasis	14	5.1	6.1e-7	5.0e-4
	Platelet activation	9	7.8	2.2e-6	1.8e-3
	Wound healing	14	4.1	9.1e-6	7.5e-3
Chocolate	Immune cell-mediated cytotoxicity	7	54.9	3.1e-11	2.6e-8
	Cellular defense response	10	12.4	4.7e-9	3.9e-6
	Inflammatory response	14	6.3	1.6e-8	1.3e-5

These results suggest that genes involved in multiple biological processes are tightly coregulated in relation to BP.

三、Results

Unadjusted analysis

	A	B	C	D	E	F
1	**Supplementary Table S2: Gene ontology enrichment analysis of BP coEMs from cell count unadjusted gene expression data**					
2	**Module**	**Ontology category**	**Overlap size**	**Category**	**Fold Change**	**P value**
3	**Green**	natural killer cell mediated cytotoxicity	11	27	11	1.17E-09
4		immune cell mediated cytotoxicity	12	35	9.3	2.13E-09
5		natural killer cell activation	13	45	7.8	4.93E-09
6	**Greenyellow**	macromolecule biosynthesis	44	902	1.9	1.36E-05
7		protein biosynthesis	39	808	1.9	5.61E-05
8	**Magenta**	DNA packaging	35	359	3.9	3.18E-12
9		chromosome organization and biogenesis (sensu Eukaryota)	37	401	3.7	3.82E-12
10		DNA metabolism	53	780	2.7	1.12E-11
11	**Tan**	ribonucleoside monophosphate metabolism	6	20	14.6	2.15E-06
12		ribonucleoside monophosphate biosynthesis	6	20	14.6	2.15E-06
13		nucleoside monophosphate biosynthesis	6	23	12.7	5.32E-06
14		mRNA metabolism	16	251	3.1	6.09E-05
15	**Cyan**	integrin-mediated signaling pathway	11	146	4	9.62E-05
16		RNA splicing	12	211	3	0.000643
17	**Midnightblue**	nucleosome assembly	10	96	5.6	1.19E-05
18		chromatin assembly	10	148	3.6	0.000463
19	**Lightgreen**	platelet activation	17	82	11.2	1.00E-13
20		hemostasis	22	194	6.1	8.42E-12
21		blood coagulation	21	181	6.3	1.66E-11
22						

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6.Inferring causal modules using SNP set enrichment analysis (SSEA)

adjusted analysis

Table 3. SNP set enrichment analysis of BP coexpression modules and BP signature gene set.

Module	SBP GWAS				DBP GWAS			
	KS *P*	Permutation-based KS *P*[a]	Fisher *P*	Permutation-based Fisher *P*[a]	KS *P*	Permutation-based KS *P*	Fisher *P*	Permutation-based Fisher *P*[a]
BP signature	0.98	0.96	1	1	0.20	0.23	1	1
Turquoise	2.8e-45	< 0.001	7.8e-115	< 0.001	1.8e-28	< 0.001	3.0e-39	< 0.001
Blue	1.4e-44	< 0.001	7.0e-54	< 0.001	1.3e-8	< 0.001	3.4e-15	< 0.001
Red	8.0e-5	< 0.001	1.7e-17	< 0.001	2.2e-15	< 0.001	6.7e-19	< 0.001
Purple	0.65	0.71	0.58	0.61	1	1	1	1
Lightyellow	1.6e-3	0.004	1	1	0.12	0.16	1	1
Chocolate	2.3e-14	< 0.001	5.0e-5	< 0.001	0.07	0.06	1	1

[a]Permutation-based *P* is empirically derived based on 1,000 permutations (see Materials and Methods). < 0.001 indicates none of the 1,000 random gene sets of matching size had *P*-values lower than the observed test *P*-values.

To explore which top genes contributed to the overall enrichment for BP-related genetic variants in the four genetically inferred causal coEMs, we retrieved the ICBP GWAS P-values for the blood eSNPs within these gene sets.

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Table 4. Genes in the genetically inferred causal BP gene sets whose blood eSNPs show significant association with BP in GWAS at $P < 5e\text{-}8$.

SNP (Genomic location)	SNP Chr	ICBP GWAS SBP *P*-value	ICBP GWAS DBP *P*-value	*cis* or *trans*	Gene symbol	Gene chr	Gene set
rs3184504 (Coding, *SH2B3*)[a]	chr12	9.3e-10	2.3e-14	*cis*	*ALDH2*	chr12	Turquoise
					SH2B3	chr12	Turquoise
					NAA25	chr12	Blue
				trans[b]	*IL8*	chr4	Turquoise
					TAGAP	chr6	Blue
rs3742004 (3UTR, *FAM109A*)	chr12	1.0e-6	2.2e-8	*cis*	*ATXN2*	chr12	Turquoise
rs17367504 (Intron, *MTHFR*)	chr1	2.1e-10	1.3e-8	*cis*	*CLCN6*	chr1	Turquoise
rs17249754 (Coding, *ATP2B1*)	chr12	9.7e-13	5.3e-9	*cis*	*GALNT4*	chr12	Blue
rs198846 (3downstream, *HIST1H1T*)	chr6	2.2e-5	3.8e-8	*cis*	*HIST1H4B*	chr6	Turquoise
					BTN3A2	chr6	Turquoise
					HIST1H4C	chr6	Turquoise
					HIST1H2BF	chr6	Turquoise
					HIST1H4F	chr6	Turquoise
					HIST1H3B	chr6	Blue
rs17115100 (Intron, *CYP17A1*)	chr10	9.2e-10	1.4e-5	*cis*	*SFXN2*	chr10	Blue

[a]A proxy SNP rs653178 ($r^2 = 1$ with rs3184504) showing same *cis*- and *trans*-associations with genes listed for rs3184504. rs653178 is significantly associated with both SBP and DBP in ICBP GWAS, too (SBP $P = 9.3e\text{-}10$, and DBP $P = 1.6e\text{-}14$).
[b]The *trans*-associations between rs3184504 and those genes identified from Westra *et al* (2013).

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Unadjusted analysis

	A	B	C	D	E	F	G	H	I	J
1	Supplementary Table S3: SNP set enrichment analysis of BP coEMs from cell count unadjusted gene expression data									
2		DBP-GWAS			SBP-GWAS					
3	MODULE	KS P	Permutaion-based KS P*	Fisher P	Permutation-based Fisher P*	KS P	Permutaion-based KS P*	Fisher P	Permutation-based Fisher P*	
4	cyan	3.05E-12	<0.001	3.00E-10	<0.001	2.81E-07	<0.001	7.15E-23	<0.001	
5	green	4.99E-07	<0.001	0.011299485	<0.001	6.85E-21	<0.001	9.81E-11	<0.001	
6	greenyellow	5.34E-10	<0.001	0.841223893	0.846	0.109047	0.096	0.985388935	0.993	
7	lightgreen	0.174206799	0.167	0.09956956	0.112	3.27E-07	<0.001	5.67E-10	<0.001	
8	magenta	1.21E-63	<0.001	4.31E-159	<0.001	1.05E-24	<0.001	1.21E-95	<0.001	
9	midnightblue	1.11E-48	<0.001	9.63E-18	<0.001	1.42E-46	<0.001	2.58E-31	<0.001	
10	tan	1.20E-16	<0.001	7.02E-20	<0.001	5.99E-14	<0.001	1.54E-25	<0.001	
11	*Permutation-based p is empirically derived based on 1000 permutations (see Methods). <0.001 indicates none of the 1000 random gene sets of ma									
12										

三、Results

7. Identification of key drivers (KDs)

原因：Recent studies have shown that disease genes (or functionally correlated genes) are not distributed randomly in cellular or molecular interaction networks

假设：the graphic structure of the corresponding network models may help prioritize candidate genes for disease

网络模型：Bayesian networks (BNs)、protein–protein interaction (PPI) networks

过程：we took each gene in a given network as a candidate KD and tested whether the network neighborhood of the candidate KD was enriched for gene members of the genetically inferred causal BP gene set using Fisher's exact test

准确性：We further tested the reliability of these PPI KDs using an independent PPI database

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7. Identification of key drivers (KDs)

Table 5. Top key drivers (KDs).

KD	Cellular network		TWAS	GWAS		
	KD *P*-value, corrected for subnetwork size	Tissue / network	*P*-value[a] for BP TWAS	eSNP ID	*P*-value[b] in ICBP GWAS	BP coEM
Top BP GWAS KDs						
SH2B3	4.4e-4	HPRD		rs653178	1.6e-14	Turquoise
ATXN2[c]	2.2e-5	HPRD		rs3742004	2.2e-8	Turquoise
NMT1	1.6e-5	HPRD		rs12946454	8.9e-8	Turquoise
NSF	5.0e-9	HPRD		rs17608766	7.3e-7	Turquoise
HSPA1B	2.1e-9	HPRD		rs805303	1.3e-6	Blue
BAT2	5.4e-7	HPRD		rs805303[d]	1.3e-6	Turquoise
MAPKAPK5[c]	2.8e-8	HPRD		rs4767293	1.5e-6	Turquoise
Top BP TWAS KDs						
GZMB[c]	2.0e-23	Blood	4.8e-22			Chocolate
PRF1	2.0e-26	Blood	2.5e-9			Chocolate
GPR56	1.2e-26	Blood	3.5e-9			Chocolate
RAB11FIP1	1.3e-3	Blood	4.0e-8			Turquoise
HIPK1[c]	3.1e-8	HPRD	9.1e-8			Turquoise
GZMH[c]	5.4e-24	Blood	3.3e-7			Chocolate
VIM	6.0e-3	HPRD	4.2e-7			Turquoise
BCL2L11[c]	1.8e-10	HPRD	1.7e-6			Blue
BHLHE40[c]	3.8e-7	HPRD	2.2e-6			Turquoise
KLRD1	1.2e-30	Blood	2.5e-6			Chocolate
TGFBR3	1.1e-26	Blood	2.5e-6			Chocolate
Top multi-tissue/ network KDs						
DCLRE1C[c]	1.1e-16	HPRD, Blood				Blue
ERCC6	8.8e-14	HPRD, Blood				Turquoise

[a] *P*-values passing transcriptome-wide significance at Bonferroni-corrected $P < 0.05$ (corrected for 17,318 measured genes).

三、Results

7. Inferring BP gene regulatory subnetworks driven by top key drivers

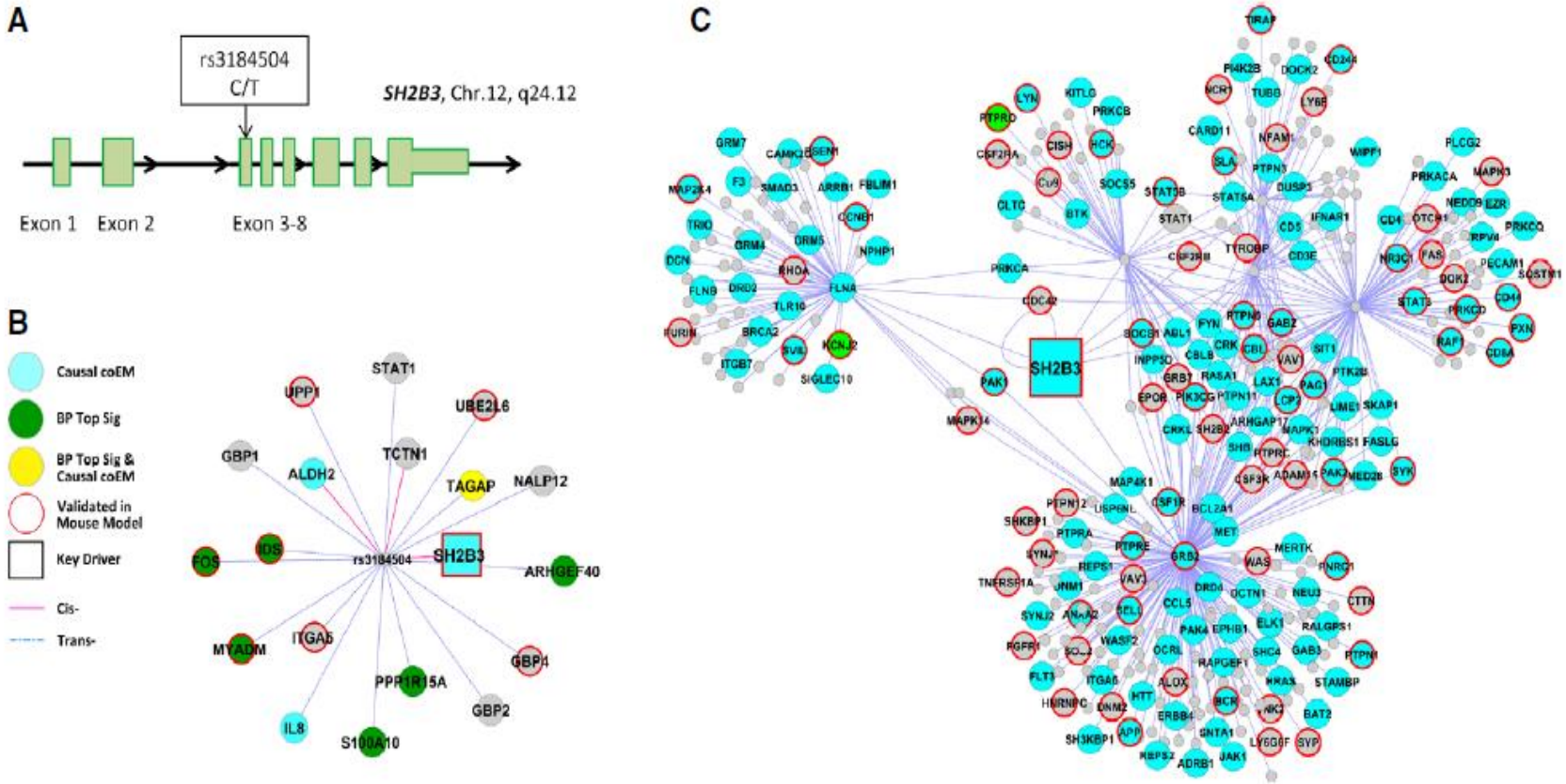

Figure 3. *SH2B3*-related genetic and protein–protein interaction subnetworks.

三、Results

7. Inferring BP gene regulatory subnetworks driven by top key drivers

1. In order to systematically check whether the SH2B3-derived PPIsubnetwork showed any enrichment for literature-based BP-related genes, we created a list of 657 BP-related by searching Gene-Rif .GeneRif includes literature descriptions of 14,069 unique human genes in total.
2. We found that 41 of the 657 genes were present in the SH2B3-derived PPI subnetwork, which consisted of 362 genes in total.Comparison of the two ratios 656/14,069 and 41/362 yielded P = 5.5e-8 (by the hypergeometric test) and 2.43-fold enrichment.

3.This result indicates that the SH2B3- derived PPI subnetwork is enriched for known BP-related genes.

三、Results

8. Validation of the SH2B3 subnetworks in a Sh2b3/ mouse model

1. As stated above, we identified SH2B3 as a putative KD for BP, and subnetworks based on SH2B3 revealed molecular interactions between this KD and many genes and multiple pathways related to BP regulation.
2. In a related study (Saleh et al, 2015), we found that Sh2b3/Mice had normal baseline BP but markedly elevated blood pressure in response to a low dose of angiotensin II (Ang II; 140 ng/kg/min) that did not affect BP in wild-type (WT) mice.

3. This suggests a key role of Sh2b3 in BP regulation, and that loss or changes to this gene exacerbate response to hypertensive stimuli.

三、Results

Table 6. Summary of the overlap between gene signatures of *Sh2b3*$^{-/-}$ mice and the predicted SH2B3 subnetworks.

SH2B3 subnetwork	Number of genes in the subnetwork	Number of overlapping genes	Fold enrichment	*P*-value
Genetic subnetwork	19	8	2.5	1.2e-5
PPI subnetwork	362	78	1.3	2.2e-14

these signature genes significantly overlapped with those in the SH2B3 genetic subnetwork

三、Results

Supplementary Table S4: Overlapping genes between the SH2B3-related subnetworks and the differentially expressed genes in *Sh2b3* knockout mice

Network	Overlap NO	Genes
SH2B3-related genetic subnetwork.	8	*FOS//GBP4//IDS//ITGA5//MYADM//SH2B3//UBE2L6//UPP1*
SH2B3-related protein-protein interaction subnetwork.	78	*ADAM15//ALOX5//ANXA2//APP//BCR//CBL//CCNB1//CD244//CD44//CD8A//CD9//CDC42//CISH//CSF1R//CSF2RA//CSF2RB//CSF3R//CTTN//DNM2//DOK2//EPOR//FAS//FGFR1//FURIN//GAB2//GRB2//GRB7//HCK//HNRNPC//KCNJ2//LCP2//LY6E//LY6G6F//LYN//MAP2K4//MAPK14//MAPK3//NCR1//NFAM1//NOTCH1//NR3C1//PAG1//PAK1//PAK2//PIK3CG//PNRC1//PRKCD//PSEN1//PTPN1//PTPN12//PTPN6//PTPRC//PTPRE//PTPRO//PXN//RAF1//RHOA//SELL//SH2B2//SH2B3//SHKBP1//SLA//SOCS1//SOS2//SQSTM1//STAT3//STAT5B//SVIL//SYK//SYNJ1//SYP//TIRAP//TNFRSF1A//TNK2//TYROBP//VAV1//VAV3//WAS*

It showed significant enrichment for intracellular signaling cascade (P = 9.4e-16) and T-cell activation (P = 5.0e-6).

三、Results

8. Validation of the SH2B3 subnetworks in a Sh2b3/ mouse model

1. These results strongly support our predicted SH2B3 subnetworks.

2. Consistent with our prediction, Saleh et al (2015) also confirmed the exacerbation of inflammation and T-cell activation in Sh2b3/mice.

四、Discussion

结论：In conclusion, our integrative and systems biology analysis, which leveraged transcriptional profiling, GWAS, and network modeling, revealed multiple biological processes that contribute to BP regulation

创新点：用多种假设，最后还增加了一个概念验证，增加研究的可信度；把微观分子机制网络结构化，使之间的关系更加明朗。

启发：处理数据要多方面考虑，还要用多种检验方法；学会网络模块化处理数据，从大方向了解整体关系。

需要改进的问题：数据量太少，并且应该增加药物影响的对照组。