Transcriptome profiling and network analysis of genetically hypertensive mice identifies potential pharmacological targets of hypertension.

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**Abstract**

Hypertension is a condition with major cardiovascular and renal complications, affecting nearly a billion patients worldwide. Few validated gene targets are available for pharmacological intervention, so there is a need to identify new biological pathways regulating blood pressure and containing novel targets for treatment. The genetically hypertensive “blood pressure high” (BPH), normotensive “blood pressure normal” (BPN), and hypotensive "blood pressure low" (BPL) inbred mouse strains are an ideal system to study differences in gene expression patterns that may represent such biological pathways. We profiled gene expression in liver, heart, kidney, and aorta from BPH, BPN, and BPL mice and determined which biological processes are enriched in observed organ-specific gene signatures. As a result, we identified multiple biological pathways linked to blood pressure phenotype that could serve as a source of candidate genes causal for hypertension. In order to distinguish in the kidney gene signature genes whose differential expression pattern may cause changes in blood pressure, from those genes whose differential expression pattern results from changes in blood pressure, we integrated phenotype associated genes into Genetic Bayesian networks. The integration of data from gene expression profiling and genetics networks is a valuable approach to identify novel potential targets for the pharmacological treatment of hypertension.

**Keywords:** BPH, BPL, blood pressure, gene signature, network analysis
Introduction

Hypertension is a prevalent, chronic, age-related disorder, which may lead to debilitating cardiovascular and renal complications. Essential hypertension affects 25–35% of the adult population, up to 60–70% of those beyond the seventh decade of life (44), and it has become a major burden to health care systems (28). Hypertension is a multifactorial disorder with contributions from lifestyle, race, age, sex, and genetic composition. In order to dissect the complexity of human essential hypertension, research using inbred rats and genetically-engineered mice has been invaluable, providing numerous insights into the regulation of blood pressure (15; 18; 20; 50).

The challenges associated with obtaining human tissues make the use of animal models critical to the study of molecular mechanisms leading to hypertension. The genetically hypertensive “blood pressure high” (BPH) mouse was developed in a selection program resulting in a strain of hypertensive mice inbred to homozygosity (39; 41). The BPH strain closely mimics human hypertension, with elevated blood pressure, high heart rate, endothelial dysfunction and early mortality. The BPH mouse selection program included concurrent selection of a hypotensive mouse strain, the “blood pressure low” (BPL) mouse, and a normotensive control strain, "blood pressure normal" (BPN) (41). A genome scan for blood pressure in these mice identified several candidate loci with possible roles in blood pressure regulation (50).

With the advent of gene expression microarray technology, it is possible to study changes in mRNA expression of large numbers of genes in parallel, in what constitutes a promising way of identifying new targets to treat human diseases (19; 25). For example, Friese and collaborators investigated the global gene expression pattern in adrenal glands
from BPH and BPL mice (15), and identified several biological pathways related to hypertension.

In order to better understand how differences in gene expression lead to effects on blood pressure, and to identify novel targets for the treatment of hypertension, we expanded the analysis of Friese and collaborators by profiling several additional tissues that play a significant role in blood pressure (liver, kidney, heart and aorta) from the BPH, BPL and BPN mice. We used arrays with twice the number of monitored transcripts, and analyzed the data using updated mapping definitions including current genomic annotations (7). In addition, to infer causality relationships we integrated the gene expression signatures into kidney Genetic Bayesian networks (38). Our study provides data on gene expression changes related to hypertension, and identifies several novel potential targets for the pharmacological treatment of hypertension.

**Materials and methods**

*Animals*

Male BPH/2J, BPL/1J and BPN/3J mice used in this study were obtained from Jackson Laboratory. These mice were derived via an eight-way cross of inbred strains, including LP/J, SJL/J, Balb/cJ, C57BL/6J, 129/J, CBA/J, RF/J, and BDP/J mice (39). Following 23 generations of selection, offsprings exhibiting highest blood pressure (BPH), lowest blood pressure (BPL) or normal blood pressure (BPN) were selected and maintained by brother-sister mating. Systolic blood pressure at 20 weeks based on tail-cuff measurements was reported at 130, 93 and 70 mmHg for BPH, BPN and BPL, respectively (40; 41). Systolic blood pressure measurements by others (8; 47) and by us confirmed that at 12-20 weeks age, BPH mice have reproducibly on average ~30 mmHg
higher blood pressure, and BPL have ~20 mmHg lower blood pressure than normal control mice. To avoid introducing gene expression changes due to stress handling, blood pressure was not directly measured in the mice used in this study. Mice were maintained on a 12:12-h light-dark cycle and fed with standard chow *ad libitum* in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. All procedures were in conformance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

*Tissue collection*

Mice (12 weeks old) were euthanized with CO₂. Liver, heart, and kidney were flash frozen in liquid nitrogen. Aorta from the aortic root to the left renal artery was rinsed with PBS, immersed in 300ul of RNeasy Lysis buffer (Qiagen), and flash frozen in liquid nitrogen.

*RNA preparation*

Tissues (~100mg, 5 mice per strain) were collected independently and homogenized in 2ml of Trizol (Invitrogen). After extraction with 0.4 ml of chloroform, RNA was extracted with SV Total RNA extraction kit (Promega) followed by DNase I treatment and purification using the RNeasy Kit (Qiagen). RNA was assayed for quality (Agilent Bioanalyzer) and yield (Ribogreen). Kidney, heart and liver RNA was amplified and labeled using a custom automated version of the RT/IVT protocol and reagents provided by Affymetrix. Aorta RNA was amplified and labeled using a custom automated version of the NuGEN Ovation WB protocol (NuGEN). Hybridization, labeling and scanning were according to Affymetrix. All samples were processed independently.
Microarray analysis

Merck/Affymetrix mouse 1.0 custom arrays monitoring 38384 individual transcripts (25,846 Entrez genes) were used. A Custom Definition File is available in GEO database (http://www.ncbi.nlm.nih.gov/geo/GPL9734). NCBI build 37 mapping definitions matched probesets in the array with transcripts (7). Raw intensity was normalized using the RMA algorithm (24). Prefiltering removed transcripts not detected (marked as "absent" by using the MAS5 algorithm with p>0.05) in 50% or more of replicates in all treatment groups, which were not considered further (21). ANOVA (p<0.05) between BPH and BPN, or between BPL and BPN, identified differentially expressed genes (the "gene signature"). 1.2 fold change threshold (BPH vs. BPN, or BPL vs. BPN) of the in silico combined biological replicates selected robust changes in gene expression. In addition, for liver, heart and aorta gene expression, changes with opposite directionality in BPH and BPL were selected. False Discovery Rate (FDR) was assessed by permuting 100 times the labels in each dataset prior to any gene filtering, calculating the size of the signature in each permutation, and averaging the results to determine the level of noise. FDR is the ratio between the noise and the size of each signature in percent units. Raw data is available in GEO (GSE19817). Kidney gene expression from Clcn5 KO mice (GSE10162) was analyzed in the same way. Only transcripts present in both Affymetrix 430 2.0 and Merck/Affymetrix mouse 1.0 were compared.

Enrichment for biological processes was performed by comparing each gene signature against the public gene collections Gene Ontology, KEGG, Swissprot and Panther families. Enrichment P values (hypergeometric distribution) were corrected for multiple testing by using Bonferroni correction. Pathway analysis was performed using
Ingenuity Pathway Analysis. Overlap of each gene signature with other publicly available gene signatures was performed by using NextBio libraries (NextBio). Kidney Genetics Bayesian networks (6; 29) were built from two mouse F2 intercrosses (C57BL/6J x A/J, and C57BL/6 ob/ob x BTBR ob/ob, manuscript submitted), which generated six independent networks (male, female and combined for each cross). The total number of nodes and edges is 15326 and 35343, respectively.

Transcription factor binding site enrichment

We used the program MOTIFCLASS from the package CREAD (43). Position weight matrices representing transcription factor binding motifs were obtained from Transfac version 8.2 (31) and Jaspar version 3.0 (49). Promoter sequences were obtained from mouse genome version MM9 and gene annotation from UCSC genome browser. Promoters were defined as 1Kb upstream to 1Kb downstream of transcriptional start site for every annotated transcript. A background set of promoters was generated by randomly selecting 500 promoters from all other mouse promoters that had a similar base composition (±1%) and presence/absence of CpG islands as the foreground sequences. MOTIFCLASS operates as a classifier. Using the foreground and background sequences, it aims to identify those position weight matrices that best classify the promoters into foreground and background. MOTIFCLASS was employed with 10,000 random permutations to obtain a p-value and reported matrices with a specificity larger than 0.5.

Results

Global variation of gene expression
We first identified global gene transcription changes correlated with blood pressure phenotype by looking for genes coregulated in all analyzed tissues in either BPH or BPL strains. mRNA levels for these genes move in the same direction in all tissues in BPH or BPL strains when compared to the BPN baseline (for example upregulated in BPH vs. BPN in all tissues). We found 222 transcripts regulated more than 20% in all tissues (False Discovery Rate (FDR) 0.6%, Figure 1 and Supplementary Table S1) and several of them have been previously implicated in hypertension. For example, natriuretic peptide receptor A/guanylate cyclase A (Npr1) is upregulated in BPH mice vs. BPN mice in all tissues, ranging from +1.5 fold in kidney up to +5 fold in aorta (Supplementary Table S1); the chemokine receptor CCR5 exhibits reduced expression in all tissues in BPH mice; Cyp2J2 expression is increased in all tissues in the BPH mice.

Of the 222 genes regulated in all tissues in association with blood pressure phenotype only two change in opposite direction in BPH and BPL when compared to BPN: Pcdh17, a protocadherin, and NM_001012326, a transcript with unknown function (Supplementary Figure S1). We investigated if any of the gene clusters in Figure 1 are enriched for specific biological processes, but found none (data not shown). Next, we investigated whether genes in these clusters are enriched in regulatory motifs, like transcription factor binding sites or miRNA seed matches. We found no enrichment for miRNA seed region matches within the 3'UTR of these transcripts, but we did find enrichment for binding sites of several transcription factors (Supplementary Table S2). The most prominent one is serum response factor (SRF), whose binding sites are overrepresented in the promoters of genes in cluster four in figure 1 (Supplementary Table S2).
We investigated whether this gene signature shares common features with other gene expression datasets by using Nextbio, a database containing results from all published expression studies. The BPH/BPL signature common for all tissues overlaps significantly (P<10e-8) with the Clcn5 (a chloride/proton exchanger) knock-out mouse kidney signature (GSE10162 in GEO)(51). In order to investigate further the connection between BPH mice and Clcn5 KO mice, we downloaded the original array data from Wright et al(51), and compared the gene expression patterns in kidneys of BPH and Clcn5 mice. 307 transcripts are co-regulated with at least 1.2 fold change in these strains, and move in the opposite direction in BPL mice (FDR 10%, Supplementary Figure S2 and Supplementary Table S3).

**Tissue specific variation in gene expression**

Next we determined whether there were any patterns of gene expression changes that are tissue specific and anticorrelated (upregulated in BPH vs. BPN, and simultaneously downregulated in BPL vs. BPN, or vice versa) in aorta, heart, and liver. During this analysis, in addition to the previous criteria applied to identify global gene expression changes (ANOVA P<0.05 and at least 1.2 fold change in one of the strains, BPL or BPH, when compared to BPN), we set a criterion related to directionality of change. This allowed us to identify genes whose expression level changed in opposite direction in BPL and BPH mice. This approach selects only for robust signatures occurring in aorta, heart or liver in both mouse strains, and simplifies the analysis by greatly reducing the number of transcripts that show differential expression.

Since kidney has a major role regulating blood pressure, we identified any gene expression changes occurring in either BPH or BPL when compared to BPN as baseline.
This way, in addition to identifying anticorrelated changes, the signature is expanded with gene changes that occur only in one of the strains but not in the other (e.g. upregulated in BPH vs. BPN, unchanged in BPL vs. BPN). We reasoned that this approach would result in greater specificity for aorta, liver and heart and greater sensitivity for kidney. The resulting gene signatures contained 1690, 972, 806 and 2146 transcripts in aorta, heart, liver and kidney respectively (Figure 2, Supplementary Figure S3 and Supplementary Tables S4-S8).

**Aorta**

1690 transcripts are differentially expressed more than 1.2 fold change in the aortas of BPH vs. BPN mice or in BPL vs. BPN mice, and show opposite regulation in BPH vs. BPL mice (FDR 0.4%, Figure 2). This gene signature contains multiple genes related to blood pressure regulation. For example, Serpina1e (an alpha-1-antitrypsin), Plat (14), Acvr1l (17), Ece1 (3), NOS3 (34) and Rock1 (42) are most highly expressed in BPH mice (Supplementary Table S4). Genes upregulated in BPL mice are enriched for "secreted proteins" (Supplementary Table S9). Genes upregulated in BPH mice are enriched for "cell motion", "extracellular space" and "cytoskeleton families" (Supplementary Table S9). Ingenuity Pathway Analysis confirmed the enrichment for these biological processes (data not shown).

**Heart**

972 transcripts show opposite regulation in heart (FDR 1.3%, Supplementary Figure S3A, Supplementary Table S5). Among them, the PPARγ regulated genes Ucp1, Pck1, Angptl4, and adiponectin are upregulated in the heart of BPL mice (Supplementary Table S5). Biological processes enriched in this signature are "fatty acid oxidation" and
"mitochondrion" upregulated in BPL mice, and "cytoskeletal proteins" upregulated in BPH mice (Supplementary Table S10).

Liver

In liver, 806 transcripts show opposite regulation (FDR 1%, Supplementary Figure S3B, Supplementary Table S6). Several have known roles in the regulation of blood pressure: Atp4a (10), Corin (52), Nox4 (2), Ece1 (3) and Enpp1 (9) (Supplementary Table S6). Gene families enriched in the liver signature are "lipid, steroid and fatty acid metabolism", which is upregulated in BPL mice, and "transporters", with 14 members upregulated in BPH (Supplementary Table S11).

Kidney

The kidney signature contains 2146 transcripts that are differentially expressed more than 1.2 fold in BPH vs. BPN, or BPL vs. BPN (FDR 5%, Supplementary Figure S3C and Supplementary Table S7), and of these, 749 transcripts are anticorrelated in BPH and BPL strains (Supplementary Figure S3D and Supplementary Table S8). Several of the genes in the kidney signature are important regulators of blood pressure: ACE, Npr1 (55), Sgk1 (26), ENaC (26) components, Scnn1g and Scnn1b, and HSD11B2 (37). In terms of the biological processes enriched in the full kidney signature, the most prominent gene family is "TGF beta signaling", a signaling pathway critical in kidney physiology, which is downregulated in BPH mice. Some examples of other enriched gene families are "mitochondrial genes", downregulated in BPL, and "sterol and lipid metabolism", upregulated in BPL.

Validation of gene expression changes and data integration with literature
To validate the gene expression changes we detected by microarrays we analyzed several of the changes by qPCR. This analysis confirmed the microarray data (Table 1).

To validate the biological significance of the patterns of gene expression changes we detected by microarray, we compared the set of genes contained in the kidney signature with a set of genes obtained by searching PubMed with the words "hypertension" and "blood pressure". Of the 268 genes identified as candidate genes related to blood pressure regulation by the PubMed search, 42 were found in the kidney signature set and eight of them have been found genetically linked to blood pressure elevation in humans according to the OMIM database (Supplementary Table S12).

**Gene regulatory networks in the kidney**

Gene expression analysis alone cannot be used to determine whether the expression changes associated with a phenotype are potentially causal or reactive to the trait of interest. A way to overcome this problem is to analyze gene expression changes in the context of Genetic Bayesian networks, which provide causality links (edges) between each gene (node) in the network, and between causally linked genes (nodes) and the trait of interest. Genetic Bayesian networks are built from gene-gene co-expression analyses and genotype association with specific traits, and have been used to identify candidate susceptibility genes that are directly implicated in the development of several multifactorial diseases (11; 12; 54). When a gene expression signature is analyzed in the context of Genetic Bayesian networks associations are generated between signature genes and known biological pathways, disease traits, and other genes that are linked to common genetic loci (6; 11; 12; 29; 38). In this way, one can identify those key groups of genes that are perturbed by genetic loci that lead to disease. Thus, juxtaposition of signature
genes within the Genetic Bayesian networks provides a powerful means to select putative therapeutic targets from among the myriad of expression signature genes which include both causal and reactive genes.

In order to integrate the kidney gene expression signature and the kidney Genetic Bayesian networks, the set of genes in the signature was intersected with the set of nodes in the kidney Bayesian networks. Sub-networks containing more than 10 nodes formed by genes which occur in both the kidney signature and in the kidney network were studied further.

Seven gene networks larger than 10 nodes each were identified, containing a total of 264 nodes (Networks A to G in Supplementary Figures S4 and S5). One of them, Network D composed of 23 genes, was analyzed further (Figure 3). Causality relationships for nodes in the Bayesian networks are indicated by the directionality of the edges connecting them (arrows in the figure). For example, quantitative expression trait loci analysis indicates changes in mRNA levels in Irs2 cause changes in mRNA levels in Osbp11a that can be linked to a blood pressure phenotype (green circle). Three of the genes with causality links to signature genes have previously been reported as being directly related to blood pressure regulation (Cxc12 (30)), vascular function (ceruloplasmin (13), Cp) or having genetic polymorphisms that confer susceptibility to hypertension (Irs2 (48)). Two additional causally linked genes belong to protein families with key roles in blood pressure regulation (Rgs14 and Carboxypeptidase E, Cpe).

Several nodes show dramatic changes between BPH and BPL mice. For example, lumican is upregulated 3.5 fold in BPL mice and Plekhb1 is upregulated 6.8 fold in BPH mice.
Discussion

The hypertensive (BPH), normotensive (BPN) and hypotensive (BPL) mouse strains were developed from a selection program based solely on blood pressure levels (39). The BPH mice mimic human hypertension with elevated blood pressure, and at 20 weeks they exhibit co-morbidities such as increased heart rate, increased kidney and heart size, and early mortality (41). A previous profiling study of the adrenal glands from BPH mice identified common mechanisms of blood pressure elevation with that of SHR rats (16) and wide-spread alterations in patterns of gene expression in diverse systems including sympathetic, vasoconstrictor/vasodilator, carbohydrate metabolism or oxidative stress (15). To date, no profiling study focusing on the kidney, liver, heart and aorta from BPH/BPL mice strains has been reported. These organs are central regulators of blood pressure, so an analysis of differentially expressed genes in them should lead to improved understanding of the regulation of blood pressure and to the identification of potential gene targets for pharmacological intervention. With these goals in mind, we characterized these mouse strains at the level of gene expression: we determined the set of genes differentially expressed (gene signatures) in BPH and BPL mice, identifying gene changes in common among the different tissues as well as specific for each analyzed organ. At 12 weeks of age there are no obvious phonotypical pathologies (enlarged organs or increased mortality) beyond changes in blood pressure. We used arrays with twice the number of transcripts than the previous study and improved mapping annotations (7; 15).

Observed gene signatures are very robust, with many of the affected genes changing expression level by over 50%, and with some genes being upregulated or downregulated.
several fold. A number of the differentially expressed genes were already known to have links with blood pressure regulation. Among the genes differentially expressed in all tissues and with known links to blood pressure regulation are, for example, Npr1, Ccr5 and Cyp2J2. Natriuretic peptide receptor A/guanylate cyclase A (Npr1) antagonizes the physiological effects mediated by the renin-angiotensin system. Mice deficient for Npr1 have 30-35 mmHg higher systolic blood pressure, and show increased adrenal angiotensin II and aldosterone levels (55), so an upregulation in all tissues of BPH mice could be a mechanism to compensate for gene expression changes that lead to increases in blood pressure. Ccr5 expression in monocytes is upregulated by Telmisartan (45), and a polymorphism in this gene is associated with high blood pressure in several studies (35). Cyp2J2 metabolizes arachidonic acid to cis-epoxyeicosatrienoic acid, playing a central role in regulating renal tubular fluid-electrolyte transport and vascular tone and several studies have associated Cyp2J2 to hypertension (53). A look at common regulatory motifs in the promoters of those genes pointed out to serum response factor (SRF). SRF is a transcription factor that is critical in the regulation of actin cytoskeleton and muscle contraction (32), which may directly affect blood pressure.

Tissue-specific differentially expressed genes with known links to blood pressure regulation are, for example, in aorta Plat (14), Acvrl1 (17), NOS3 (34) and Rock1 (42); in liver Serpina1e, Atp4a (10), Corin (52), Nox4 (2) and Enpp1 (9); and in kidney ACE, adrenergic receptors alpha and beta, Adm (27), Sgk1 (26), ENaC (26) components Scnn1g and Scnn1b, and HSD11B2 (37). These results validate our experimental approach by confirming that the identified gene expression signatures represent perturbations in molecular pathways that result from, or cause, the observed blood

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pressure differences. These molecular pathways can be mined to identify putative new
targets in the genes that interact with, or belong to, the same biological pathways as the
known blood pressure regulators.

Careful inspection of the gene expression signatures helps identify a second tier of
genes: those with connections to blood pressure, but with an unknown role in the process.
These are promising candidates as novel targets for hypertension. For example, Meprin
1a (-12.6 fold in BPL kidney), a zinc-dependent metalloproteinase highly expressed in
kidney and intestine. Mep1a-deficient mice have lower blood pressure than many other
mouse strains and are resistant to cisplatin-induced kidney injury (23). Spondin 1 (+6.54
fold in BPH kidney), is an extracellular matrix protein that regulates angiogenesis and is
upregulated in SHRSP rats under conditions that increase blood pressure (5). Mme (+13
fold in BPH liver), is an enzyme that degrades the Atrial natriuretic factor, a peptide with
beneficial effects in several cardiovascular disorders (22), so its up-regulation would have
a negative impact on blood pressure. These are just few examples of the power of gene
expression analysis to identify candidate genes contributing to hypertension.

Analysis for enrichment of biological processes and pathways showed that the vast
majority of the biological processes and pathways related to chronic changes in blood
pressure are tissue specific. This suggests that each tissue reacts to changes in blood
pressure by activating and inhibiting different molecular pathways, and that hypertension
results from interactions between very diverse molecular pathways across body tissues. In
heart, "fatty acid oxidation" and "mitochondrion" gene families are enriched in the blood
pressure signature, and some of the genes present in these families are PPARγ regulated
genes (PGC1α, Ucp1, Angptl4, Pck1 and adiponectin). All these genes are upregulated in
the heart of BPL mice and concomitantly downregulated in heart of BPH mice. PPARγ itself is also differentially expressed in both mice strains (up 24% in BPL and down 20% in BPH). This suggests that certain metabolic functions in heart could have an impact on blood pressure, which is further supported by the finding that PPARγ agonists lower blood pressure (36). Since PPARγ regulates expression of renin (46), our findings suggest that the PPARγ-renin-angiotensin receptor system may have important roles in heart function.

A second gene family showing coregulation in heart is a group of "cytoskeletal proteins", upregulated in BPH and downregulated in BPL mice when compared to BPN mice. This gene family includes genes like Kif1b, Mapre1, Arpc2, the muscle proteins Tmod4 and Myot, and the Wiskott-Aldrich syndrome proteins Was and Wasf2. All these proteins participate in several aspects of cytoskeleton function, and may have a role in heart muscle contraction, which directly impacts blood pressure regulation. In aorta, genes with increased expression in BPH (and low expression in BPL) are enriched for "cell motion", "extracellular space" and "cytoskeleton families" suggesting a link between increased blood pressure in BPH mice and changes in arterial contractility and stiffness. These are some examples of how gene expression profiling in model organisms can be used to infer relationships between the molecular pathways differentially expressed and the phenotype of interest.

An interesting finding is the similarity between Clcn5 KO and BPH gene expression signatures in kidney, with a statistically significant overlap in gene expression changes between these strains that is reversed in BPL mice. Clcn5 is a kidney chloride/proton exchanger and the knock-out mouse mimics the phenotype of Dent disease, which is
characterized by low-molecular-weight proteinuria, aminoaciduria, phosphaturia, and glycosuria, all of them attributed to proximal tubule malfunction (51). Genes like Uromodulin, Nephrosis1 and Nephrosis2, known markers of renal function, are among the genes that are upregulated in both BPH and Clcn5 KO, and downregulated in BPL mice, so these genes or others in the same pathways could be potential new targets for pharmacological intervention. Our results suggest common molecular mechanisms between chloride homeostasis and blood pressure regulation. Interestingly, Clcn5 is upregulated in kidney of BPH mice, so it is possible that this could be driven by a compensatory mechanism as well.

A limitation of our study is that BPH, BPN and BPL mouse strains do not share identical genetic backgrounds. Thus it seems likely that some of the gene expression changes we see are not responsible for blood pressure regulation. A way to partially overcome this limitation is by integrating data from other sources. The intersection of the kidney signature with a set of genes linked to hypertension in the literature resulted in an overlap of 42 genes, eight of them linked to blood pressure elevation in humans. It is likely that new pharmacological targets for hypertension are found among signature genes present in biological pathways that contain the known 42 blood pressure related genes.

Blood pressure phenotype related gene changes can be also separated from unrelated changes by cross referencing gene signatures with a Genetic Bayesian network established for hypertension. Genetic Bayesian networks have been successfully used in the identification of genes with causative roles in complex diseases like obesity and atherosclerosis (11; 12; 54), and are not susceptible to the citation bias that can occur
when gene expression changes are characterized using only existing literature databases. Cross referencing the murine kidney Genetic Bayesian network with the kidney signature we identified seven Bayesian sub-networks with more than 10 nodes each. These networks contain several nodes with known roles in blood pressure regulation, or with links to hypertension. For example ACE (network E), is a key molecule in the angiotensin receptor pathway. Abcb1b (network F), has a critical role in aldosterone metabolism and is associated to hypertension (56). Ccr5 (network A) and Knyu (network F) polymorphisms are associated to hypertension (33; 35). Insulin signaling through Irs2 regulates angiotensin signaling (48). These networks can be used to identify new potential targets by providing biological context to less known genes. For example, network D contains three genes with connections to blood pressure regulation (Cxcl12 (30), ceruloplasmin (13), Irs2 (48)) and two that belong to protein families with key roles in blood pressure regulation (Rgs14 and Carboxypeptidase E). In this context, any of these genes is a potential new target for hypertension. However, genes with no currently known association with hypertension, but connected in the Bayesian sub-network to the hypertension associated genes are also potential new targets. For example, Plekhb1, a protein with a role in signal transduction, is another node in network D and to our knowledge, it has not been associated with hypertension. However, it is a good candidate to susceptibility locus of hypertension: 1) it is upregulated +6.8 fold in the BPH mice; 2) it is part of a genetic network with known blood pressure related genes (network D); 3) it is genetically associated with coronary artery disease and rheumatoid arthritis(1); and it interacts with TGFβ receptor, SMAD1 and activin receptor(4), key molecules in vascular development and function. Altogether, these results suggest that increased levels of
Plekhb1 are linked to high blood pressure and that drug treatments that reduce Plekhb1 expression could have beneficial effects for hypertensive patients. Our team is currently assessing Plekhb1 and other genes mentioned in this manuscript as potential targets for pharmacological intervention.

In summary, our results integrating data from gene expression, the literature, and genetics networks help identify novel targets and to provide insights into the complex molecular mechanisms of hypertension.
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All authors in this manuscript work at Merck Research Laboratories.


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Figure 1. Heatmap of gene expression changes (222 reporters) common in all tissues grouped in a Kmeans cluster with K=4. The Y axis represents samples and the X axis transcripts. The intensity of each pixel is proportional to the relative mRNA levels of one transcript in one sample. Measurements are relative because they are compared to a baseline, which was calculated independently by pooling in silico the intensities of BPN samples for that tissue. Log ratios between the intensity of each sample and the baseline are shown. Upregulated genes are shown in magenta, downregulated genes are shown in cyan. The gene expression changes can be grouped in 4 major clusters that behave in a similar way among all tissues. For example, cluster 1 shows gene expression changes for upregulated genes in BPL samples across all tissues.

Figure 2. Aorta specific gene signatures. Log ratios between each sample and the pool of BPN samples, used as baseline, are represented. Upregulated genes are shown in magenta, downregulated genes in cyan.

Figure 3. Genetic Bayesian networks in the kidney signature. The set of transcripts in the kidney signature was intersected with the set of nodes in the kidney Genetic Bayesian networks, and subnetworks were built interconnecting the resulting nodes by using edges from the kidney Bayesian networks. An example is shown where each node is identified as a gene, if known, or a Riken identification number. Causality relationships (edges) for nodes in the Bayesian networks are indicated by the directionality of the arrows connecting them. For example, changes in mRNA levels in Irs2 cause changes in mRNA levels in Osbpl1a that can be linked to a blood pressure phenotype (green circle). The colors of the nodes represent the degree to which the transcript mRNA levels changed in
either BPH or BPL mice, with red being most up-regulated and blue being most down-
regulated. White nodes do not change (P>0.05). Plekhb1 (blue circle in network D) is a
candidate causal gene for hypertension.
Table 1. qPCR confirms changes detected by microarrays.

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* arrays and qPCR do not correlate
Common changes (222 transcripts)

Figure 1
Aorta (1690 transcripts)

Figure 2
Figure 3