

Transcriptomics in Twins Separates Genetic From Environmental Effects on Gene Expression and Blood Pressure

Brian J. Morris

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Identification of all of the genes differentially expressed in essential hypertension is highly relevant to elucidation of the mechanisms involved in this complex polygenic condition. Key to this includes the tissues investigated, the genes differentially expressed in each, and sorting out whether the differences in expression in hypertension stem from (1) polymorphism(s) in regulatory DNA affecting transcription of the gene, (2) the expression change being a downstream effect of the latter, (3) a response to an environmental factor, (4) a counter-regulatory response to the elevated blood pressure (BP), or (5) another factor driving both the BP response and gene expression. Although any simple straightforward study documenting transcriptome-wide expression changes should identify the major genes that are differentially expressed in a particular tissue in hypertension, such data are, by themselves, unable to discriminate between each of the aforementioned possibilities.

In the current issue of *Hypertension*, Huang et al¹ at Augusta University, University of Helsinki, the National Heart, Lung, and Blood Institute's Framingham Heart Study, and University of Mississippi performed a transcriptome-wide analysis of peripheral blood leukocytes of 391 twins (57% monozygous) from the Finnish Twin Cohort study, and a replication study involving the Framingham cohort, looking for transcripts whose expression was associated with BP.¹

Although this study is not the first to use peripheral blood leukocytes to determine such transcriptome-wide expression differences, it not only represents an important confirmation but also distinguishes genetic and environmental influences.

Huang et al¹ refer to 2 previous blood transcriptome-wide expression studies. One, in 2015 by Huan et al,² found 83 genes whose expression levels correlated with BP (73 with systolic, 31 with diastolic, and 8 with hypertension).² They found 65 were positively correlated with BP traits and 8 were negatively correlated. After adjustment for multiple factors, these formed 27 BP-associated gene coexpression networks. Six networks showed association with either systolic or diastolic BP at $P < 0.05$

and a false discovery rate cutoff of < 0.02 . Among the genetically inferred causal coexpression networks, they found that blood expression SNPs (single nucleotide polymorphisms) of 15 genes had shown genome-wide significance in a prior BP genome-wide association study. Multiple key drivers were identified, and many of these had been reported previously in relation to BP regulation. The top key driver, Src homology 2 domain-containing adaptor protein 3 (gene: *SH2B3*), was validated in *Sh2b3* knockout mice and was shown to exacerbate angiotensin II-induced hypertension via inflammation and T-cell activation. Huan et al³ performed a meta-analysis of results from 6 studies of transcriptome-wide expression profiles of BP and hypertension in blood from 7017 subjects not on antihypertensive medication.³ They found 34 differentially expressed genes—21 for systolic BP, 20 for diastolic BP, and 5 for hypertension. Some were associated with multiple BP parameters. Together they explained 5% to 9% of BP variability. Even though one of the top BP loci from genome-wide association studies, *SH2B3*, was not among the differentially expressed genes, it is a *trans*-regulator of expression of 6 (*FOS*, *MYADM*, *PP1R15A*, *TAGAP*, *S100A10*, and *FGBP2*) of the 34 signature genes. The high coexpression in neutrophils of these 6 genes, all driven by the same BP locus, pointed to the important role neutrophils have in BP regulation.

The other study, by Zeller et al⁴ in 2017 of >4500 subjects, identified 8 transcripts (*CRIP1*, *MYADM*, *TIPARP*, *TSC22D3*, *CEBPA*, *F12*, *LMNA*, and *TPPP3*) that jointly accounted for up to 13% of BP variability. Differences in expression of *CRIP1*, *MYADM*, *TIPARP*, *LMNA*, *TSC22D3*, *CEBPA*, and *TPP3* were associated with variation in BP. These genes are expressed in various other tissues besides blood. *CRIP1*, *MYADM*, *TIPARP*, *F12*, and *TSC22D3* had, moreover, been implicated in hypertension. Expressions of *CRIP1*, *MYADM*, *TIPARP*, and *TPPP3* were strongly associated with genetic variants in the *SH2B3/LNK* locus implicated as a master regulator of BP. *Sh2b3* knockout mice are hypertensive. *SH2B3* suppresses growth factors and cytokine signaling. Furthermore, *CRIP1* expression correlated with indicators of cardiac hypertrophy. *CRIP1* (cysteine-rich protein 1) level in blood was, moreover, a biomarker of increased risk of stroke. Renin is crucial for BP regulation and, interestingly, *CRIP1* expression in renin-expressing juxtaglomerular cells is high.

In the current study, Huang et al¹ replicated 12 of 40 genes whose expression levels were reported in the Huan et al² and Zeller et al⁴ studies to be associated with BP. Expression of each of the 12 genes (*CD97*, *CRIP1*, *F12*, *LMNA*, *MYADM*, *S100A10*, *SLC31A2*, *TAGAP*, *TAGLN2*, *TIPARP*, *TPPP3*, and *TSC22D3*) was positively correlated with BP. Eight of these

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From the School of Medical Sciences and Bosch Institute, The University of Sydney, New South Wales, Australia.

Correspondence to Brian J. Morris, School of Medical Sciences and Bosch Institute, Anderson Stuart Bldg (Bldg F13), The University of Sydney, Sydney, NSW 2006, Australia. E-mail brian.morris@sydney.edu.au

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were among the 34 differentially expressed transcripts found by Huan et al² and 7 were among the transcripts noted by Zeller et al.⁴ One of the genes found by Huang et al¹, *MOK* (mitogen-activating protein kinase/male germ-cell associated kinase/mixed lineage kinase-related overlapping kinase gene), was novel, and they suggested that it could be involved in inflammation.¹ Another, *SLC31A2* (solute carrier family 31 member 2 gene), was one that emerged in the study by Huan et al.² Expression of *MOK* and *SLC31A2* was associated with systolic BP at a significant false discovery rate cutoff of <0.10.

Twins tend to share a common environment, at least during their early life of cohabitation. Thus, twin studies allow dissection of genetic and environmental effects on BP. Huang et al¹ found that association of *LMNA*, *SLC31A2*, and *TSC22D3* with BP and of *TAGLN2* with just systolic BP was completely determined by shared environmental factors. In contrast, the association of *TIPARP* (encoding TCDD inducible poly(ADP-ribose) polymerase), *CD97* (encoding leukocyte antigen CD97, a 7-transmembrane heterodimeric receptor associated with inflammation), and *TPPP3* (encoding tubulin polymerization promoting protein family member 3) expression with systolic and diastolic BPs was completely a result of shared genetic factors. Bivariate twin modeling showed that *MOK* overexpression, which was associated with both systolic and diastolic BPs, correlated with 52% of the shared genetic contribution to systolic BP. Association of *MOK* expression with systolic and diastolic BPs was, moreover, replicated in the Framingham Heart Study cohort.

Pathway analyses showed enrichment of terms for inflammation and autoimmune response.¹ In accord with this, Zeller et al⁴ had suggested that *CRIP1*, *TSC22D3*, *CEBPA*, *TPPP*, and *F12* contribute to BP regulation and hypertension via their role in the immune system.

These kinds of studies have used peripheral blood leukocytes for convenience. Blood cells are involved in immune function. The immune system and inflammation have long been thought to be involved in the pathogenesis of hypertension.

Two recent studies have demonstrated how dietary factors—high fiber⁵ and NaCl⁶—via the gut microbiome influence the immune system, vascular inflammation, and thence BP.

The environmental effects of a diet low in fiber and high in NaCl on gut microbiome composition might alter gene expression in various tissues.⁷ “Good” gut bacteria generate acetate and propionate, which bind to the G-protein-coupled receptor OR51E2 in vascular smooth muscle, autonomic nerves, heart, gut, and juxtaglomerular cells.⁷ Propionate reduced renin secretion from juxtaglomerular cells of *Olfcr78* knockout mice. A gut-sympathetic nervous system axis affecting BP might also exist.⁷

Blood has been used for transcriptome-wide studies in humans because it is easily obtainable. But other tissues have important roles in hypertension. Any tissue is amenable in experimental animals. Transcriptome-wide differential expression of mRNAs in relevant tissues of rat and mouse models of hypertension should be examined to see whether there are transcripts common to those found in human blood. The kidney in particular has long been thought to be a key player in hypertension pathogenesis. The first study of transcriptome-wide differential expression of mRNAs in human kidney in hypertension used mRNA extracted from the healthy renal tissue from hypertensive and normotensive subjects who had undergone nephrectomy for kidney cancer.⁸ Marques et al⁸ found 14 differentially expressed transcripts in the renal medulla and 46 in the renal cortex. None of these were observed in the current study of leukocytes,¹ suggesting the existence of substantial tissue-specific differences. The kidney study also used microRNA (miRNA) microarrays, finding that in hypertension 11 and 13 miRNAs were differentially expressed in medulla and cortex, respectively.⁷ Several of these miRNAs were able to downregulate expression of luciferase reporter constructs containing *REN*, *APOE*, *NR4A2*, and *AIFM1* 3'-untranslated regions targeted by miRNA. The renin finding in particular offered the first evidence to implicate downregulation of miR-181a in renin overexpression intrarenally in hypertension.⁸

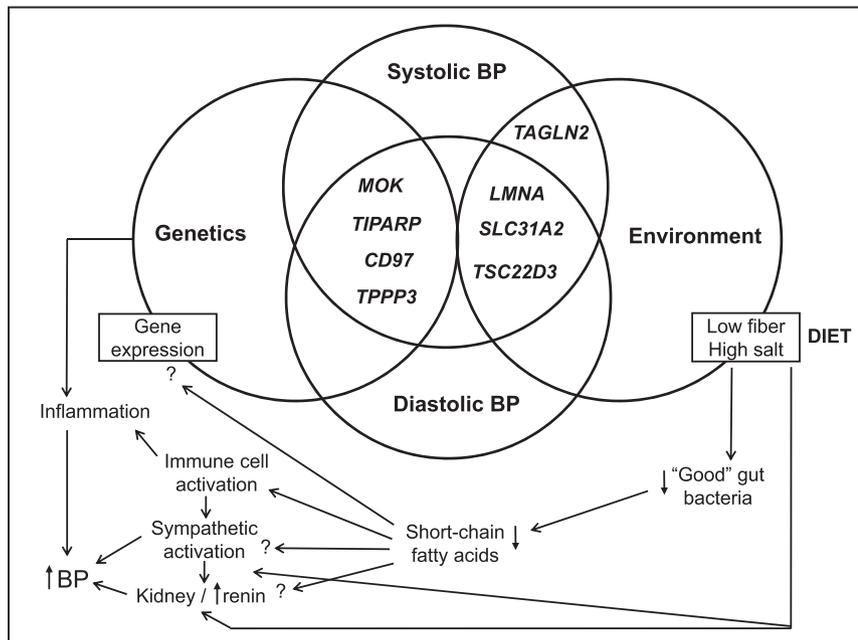


Figure. Genes whose differential expression was associated with systolic blood pressure (BP), diastolic BP, or both and showing those found by Huang et al¹ to be influenced by environmental factors and those influenced by genetic factors. Also shown is a scheme whereby new data suggesting how dietary factors may lead to hypertension by causing colonic dysbiosis and immune system activation.

MiR-181a was, moreover, colocalized with renin in collecting duct cells.⁹ Sympathetic activity activated the miR-181a–renin mechanism.¹⁰ The findings implicated an intrarenal renin–angiotensin system mechanism in hypertension pathogenesis. RNA sequencing further revealed association with downregulation of mitochondrial pathways and upregulation of signaling cascades of adaptive immunity and inflammation,¹⁰ adding to the current immune system findings.

Discovery of transcripts whose differential expression correlates with BP offers valuable insights into the mechanisms involved in hypertension. The present study of twins has helped differentiate genetic and environmental contributors (Figure). It has, moreover, added to evidence of an important role for immune system–mediated inflammation in hypertension. Because dietary factors affect BP via alterations in gut microbiota composition affecting vascular inflammation, future research should consider combining genetic and transcriptomic data with gut microbiome analyses, especially as the latter may be influenced by the former.⁷ Thus, although studies to date, including the current one by Huang et al,¹ provide valuable data, much more research is needed to understand the molecular basis for the changes seen and the role of each transcript and its protein product in the pathogenesis of hypertension. Integration of data on gene expression from multiple tissues—blood, kidney, heart, blood vessels, brain, etc—with genome-wide association study data, as well as other relevant information, will improve knowledge about this complex polygenic condition and how environmental factors impact genetic and other mechanisms responsible.

Disclosures

None.

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