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Epigenomics of Hypertension

Mingyu Liang*, Allen W. Cowley Jr.* , David L. Mattson* , Theodore A. Kotchen[#], and Yong Liu*

*Department of Physiology, Medical College of Wisconsin, Milwaukee, WI

[#]Department of Medicine, Medical College of Wisconsin, Milwaukee, WI

Abstract

Multiple genes and pathways are involved in the pathogenesis of hypertension. Epigenomic studies of hypertension are beginning to emerge and hold great promise of providing novel insights into the mechanisms underlying hypertension. Epigenetic marks or mediators including DNA methylation, histone modifications, and non-coding RNA can be studied at a genome or near-genome scale using epigenomic approaches. At the single gene level, several studies have identified changes in epigenetic modifications in genes expressed in the kidney that correlate with the development of hypertension. Systematic analysis and integration of epigenetic marks at the genome scale, demonstration of cellular and physiological roles of specific epigenetic modifications, and investigation of inheritance are among the major challenges and opportunities for future epigenomic and epigenetic studies of hypertension.

Essential hypertension is a multifactorial disease involving multiple genetic and environmental factors and mediated by alterations in multiple biological pathways. Because the non-genetic mechanisms may involve epigenetic modifications, epigenomics is one of the latest concepts and approaches brought to bear on hypertension research. In this article, we summarize briefly the concepts and techniques for epigenomics, discuss the rationale for applying epigenomic approaches to study hypertension, and review the current state of this research area.

Concepts and techniques of epigenomics

Epigenetics is the study of molecular changes (epigenetic marks or mediators) and associated phenotypes that are mitotically or meiotically inheritable but do not involve changes in DNA nucleotide sequence. Epigenetic marks or mediators contribute to biological regulation by influencing gene expression. Epigenomics is the study of epigenetic marks at a genome or near-genome scale. DNA methylation, histone modification, and non-coding RNA are three major types of epigenetic mediators, all of which can be studied at a genome or near-genome scale. Several examples of techniques for epigenomic analysis are listed in Table 1, and key features of these techniques are briefly discussed below. For a more detailed introduction of these techniques, refer to recent reviews published elsewhere.^{1,2,3,4}

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Correspondence: Mingyu Liang, Tel: 4149558539; Fax: 4149556546; mliang@mcw.edu.

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It is important to recognize that epigenomic analysis has very different requirements than genomic analysis even though epigenomic analysis often involves examination of DNA. With a few exceptions, genomic sequence is identical across all cells in an individual and remains the same throughout an individual's lifetime. The epigenomes, however, are different between cell types and may undergo dynamic changes in response to environmental cues. Many of the variables that one typically considers in a physiological or gene expression study but might not take into account in a genomic study, such as cell type, age, disease, treatment, and so on, should be considered in an epigenomic study.

One of the most widely studied epigenetic marks is 5-methylcytosine (5mC) found in cytosine-guanine dinucleotides (CpG).^{5,6} Of particular interest are CpG sites located in CpG islands which are clusters of CpG sites showing dynamic variations of methylation levels and often colocalized with *cis* regulatory elements such as gene promoters. Increased DNA methylation (hypermethylation) in CpG islands within or close to gene promoters is typically, although not always, associated with suppression of transcriptional activity. Genomic segments of interest can be enriched by affinity purification using antibodies or other proteins that recognize methylated DNA. Alternatively, genomic segments of interest, such as segments enriched for CpG islands, can be obtained by digestion using specific endonucleases. The genomic segments can then be identified or mapped to single-base resolution using microarray hybridization or next-generation sequencing.^{1,2,7} Other types of nucleotide modifications, notably 5-hydroxymethylcytosine (5hmC), may also be important for epigenetic regulation.^{8,9} 5hmC is one of the intermediates in the process of 5mC demethylation catalyzed by the ten-eleven translocation family of dioxygenases. 5hmC could play a role in gene regulation distinct from 5mC. Methods for genome-wide analysis of 5hmC at single-base resolution have been reported recently.^{10,11}

Post-translational modifications of histones, such as methylation and acetylation, are another major type of epigenetic marks. Proteomic techniques can be used to analyze a large number of histone modifications simultaneously.¹² DNA segments that bind to a histone with a specific protein modification can be examined by cross-linking DNA with histones followed by immunoprecipitation using an antibody recognizing the histone with the specific modification. The isolated DNA segments can then be identified with microarray or sequencing.⁴ Histones may affect gene expression by altering chromatin structure. Several techniques are available for analyzing chromatin structure at a genome-scale.¹³

Regulatory, non-coding RNA may contribute importantly to epigenetic regulation.^{14,15} Non-coding RNA, particularly long non-coding RNA, may play a crucial role in conferring site- or gene-specificity to epigenetic modification. Non-coding RNA can be identified by RNA-seq, sometimes with further support from identification of transcriptionally active genomic segments based on chromatin immunoprecipitation analysis.^{3,16,17,18} Some non-coding RNA molecules do not have a poly(A) tail and can only be identified with RNA-seq methods that do not use poly(A) selection.

Association between epigenetic marks, especially DNA methylation, and a phenotype can be investigated at the genome scale in epigenome-wide association studies (EWAS).¹⁹ Similar to genome-wide association studies (GWAS), EWAS often require a sample size of several hundred or more.¹⁹ The sample size of an EWAS is primarily determined by the magnitude of methylation rate differences among individuals, the impact of the methylation difference on the phenotype, and the number of methylation marks analyzed.

Epigenomics of hypertension

Hypertension affects 29% of the adult population in the US and costs 93.5 billion dollars in 2010.^{20,21} Hypertension is a major risk factor for stroke, myocardial infarction, heart failure,

and end-stage renal disease. About 50% of hypertensive patients in the US do not have their blood pressure adequately controlled, in many cases due to non-adherence and other disease management issues.^{20,22} Importantly, about 5 million patients in the US are apparently resistant to current anti-hypertensive treatments (hypertensive despite taking at least three antihypertensive medications).²³ Patients with apparent treatment-resistant hypertension are more likely to be blacks and have obesity, chronic kidney disease, and Framingham 10-year coronary risk > 20%.²³

Arterial blood pressure is the product of cardiac output and total peripheral vascular resistance. Several physiological and neurohormonal mechanisms regulate cardiac output and/or peripheral resistance and contribute to maintain the homeostasis of arterial blood pressure. These mechanisms involve several organ systems including the kidney and the cardiovascular, nervous, endocrine, and immune systems. The kidney plays a key role in this regulatory network.^{24,25,26} Genes that underlie Mendelian forms of human hypertension typically affect tubular transport in the kidney directly or indirectly.²⁷ The molecular mechanisms underlying common forms of essential hypertension, however, remain poorly understood.

Although the multifactorial nature of hypertension is widely recognized, much of hypertension research has been focused on single genes or single regulatory pathways. The approach is useful despite its simplicity. Abnormalities in one gene or one pathway may very well make measurable contribution to the development of hypertension in a specific, defined setting (a specific animal model, a well-defined subset of patients). It would be valuable to identify these abnormalities. The approach also has a strong practical appeal since numerous methods are available for analyzing and experimentally manipulating one gene or one mechanism. It is often challenging to interpret network data and difficult (if possible at all) to experimentally manipulate a regulatory network. Nonetheless, understanding molecular regulatory networks on a genome-wide scale is undoubtedly what is needed to truly understand the disease mechanism of hypertension and its associated comorbidities.

Analysis of transcriptomes and proteomes is a powerful approach for studying molecular regulatory networks.^{28,29} Interestingly, transcriptome and proteome analysis has provided evidence for broad, genome-wide relevance of epigenetic regulatory mechanisms in hypertension. Analysis of transcriptomes and proteomes has been carried out in several organs or cell types relevant to blood pressure regulation in several models of hypertension.^{30,31} Enhanced sensitivity of blood pressure to salt intake is observed in a majority of hypertensive patients, especially in African Americans.^{32,33} The Dahl salt-sensitive (SS) rat is a commonly used genetic model that recapitulates the increased blood pressure salt-sensitivity and exacerbated renal injury observed in African American hypertensive patients.^{34,35,36}

Studies have been performed to analyze transcriptomes or proteomes in the renal cortex, renal medulla, or the medullary thick ascending limb of the Loop of Henle in SS, SS.13^{BN}, SS.13^{BN5}, and/or SS.13^{BN26} rats.^{37,38,39,40,41,42,43,44,45} The genomic sequences of the consomic SS.13^{BN} rat and congenic SS.13^{BN5} and SS.13^{BN26} rats are identical to SS except chromosome 13 (in SS.13^{BN}) or segments of chromosome 13 (in SS.13^{BN5} and SS.13^{BN26}) are replaced by the corresponding genomic segments derived from the Brown Norway (BN) rat.^{46,47} While genes differentially expressed between the consomic rat and the SS rat are enriched for genes located in the substituted chromosome, numerous genes located on other chromosomes are also differentially expressed.^{39,44} The magnitude of transcriptome difference between a consomic or congenic rat and the SS rat correlates with the magnitude of genetic difference to some extent but also with phenotypic differences.⁴¹ Moreover,

replacement of different genomic segments can impact on a shared set of genes and pathways, while replacement of a small genomic segment can affect genes or pathways that are not affected by replacement of a larger genomic segment that encompasses the smaller segment.⁴¹

These data indicate extensive convergence and divergence of genomic effects that cannot be explained solely by the genomic sequence. It is likely that both genetic factors and non-genetic mechanisms contribute to the alterations of gene expression and eventually the development of salt-sensitive hypertension in these rats. Some of the non-genetic mechanisms may involve epigenetic modifications that occur as direct or indirect consequences of genomic sequence variations and their interactions with dietary and other environmental factors.

We have proposed a tree-like paradigm to serve as a framework for integrating multiple genes and pathways that underlie the development of common forms of hypertension such as salt-sensitive hypertension (Figure 1).⁴⁸ Multiple causal genes would resemble numerous leaves on a tree. A causal gene may impact on one or more intermediate molecular pathways (the small branches of the tree), which converge gradually to form larger branches, or intermediate physiological pathways. The various pathways eventually converge at final common pathways, which in the case of hypertension could be impairment of renal pressure natriuresis and diuresis, and result in the development of salt-sensitive hypertension and renal injury. The regulatory network operates within specific genomic contexts and interacts with environmental factors such as dietary salt levels. A thorough understanding of these complex phenotypes requires that most of the genes and pathways involved are identified and the regulatory network constructed. Epigenetic mechanisms may have an important role in this tree-like paradigm (Figure 1), and it is highly likely that epigenetic marks relevant to hypertension are distributed widely throughout the genome, many of which probably have not been identified. Application of epigenomic approaches, therefore, holds great promise of revealing novel mechanisms underlying the development of hypertension.

Epigenomic studies of hypertension are just beginning to emerge, even though the concept has been proposed earlier.⁴⁹ Wang and colleagues conducted a genome-wide methylation analysis in leukocytes obtained from 8 hypertensive cases and 8 normotensive age-matched controls.⁵⁰ The methylation analysis was performed using the HumanMethylation27 BeadChip from Illumina. None of the CpG sites examined reached statistical significance following multiple testing corrections, with the most significant CpG site showing a false discovery rate of 0.75. This is not surprising given the small number of subjects with varying genetic background and possible phenotypic variations, complex cellular composition of the leukocyte samples, and the large number of CpG sites tested. Nevertheless, some of the most significantly different CpG sites (according to p values without multiple testing correction), including a CpG site in the sulfatase 1 gene (*SULF1*), were experimentally validated by sequencing in additional cohorts of hypertensive and control subjects.

An epigenomic study was performed in our laboratory to examine the influence of diet on the development of hypertension in the SS rat. As we previously described, the progression of salt-sensitive hypertension and renal disease is dramatically altered in SS rats fed diets containing different sources of protein.^{51,52} In the recent study, we confirmed our earlier observations that indicated that SS rats obtained from a breeding colony maintained on an AIN-76A diet (a purified rodent diet containing casein as its protein source) develop more severe salt-sensitive hypertension and renal disease when compared to age-matched Dahl SS rats obtained from a colony fed a grain-based diet (LabDiet 5L79). A genome-wide methylation analysis using the reduced representation bisulfite sequencing method¹⁷ and an

RNA-seq analysis subsequently demonstrated multiple genes associated with inflammation, cell growth, and cell matrix formation or degradation with reciprocal DNA methylation and mRNA expression patterns in the renal outer medulla. These exciting data indicate that DNA methylation may be a mechanism leading to the protective effect of different diets on the development of disease phenotypes; as a corollary, epigenomic studies have the potential to generate unique insight into disease mechanisms.

Epigenetic regulation of kidney genes relevant to hypertension

While epigenome-wide studies of hypertension are just beginning to emerge, a number of studies have reported changes in epigenetic modification (presumably mitotically inheritable) of specific genes associated with development of hypertension. Changes in DNA methylation and histone modification have been shown to correlate with the expression of several genes involved in the development of hypertension or hypertensive tissue injury as discussed below. Non-coding RNA, especially microRNAs, may also influence the development of hypertension and hypertensive tissue injury,^{53,54} although it remains to be determined whether mitotically heritable changes are induced by non-coding RNA in hypertension. Some of the epigenetic modifications associated with hypertension have been reviewed elsewhere.^{55,56} We will highlight several examples here with a focus on genes particularly relevant to the role of the kidney in blood pressure regulation or the development of hypertension. Most of these examples are summarized in Figure 2.

Environmental stress in early life or during critical periods of fetal development, the latter of which often leads to low birth weight, is correlated with the development of hypertension (and several other conditions) in adult life.⁵⁷ Epigenetic modifications are commonly cited as a possible mechanism for this phenomenon of developmental programming. Although specific epigenetic modifications associated with developmental programming of adult hypertension remains largely unidentified, several cases related to genes expressed in the kidney have been reported. For example, uteroplacental insufficiency was found to specifically decrease CpG methylation of the BstU I digestion site in the promoter of p53 gene in the kidneys of a rat model of intrauterine growth retardation.⁵⁸ The hypomethylation of renal p53 promoter was associated with up-regulation of p53 mRNA and increased apoptosis in the kidney.⁵⁸ Fetal exposure to excess glucocorticoids leads to the development of hypertension in adult offspring. It was reported that maternal treatment with dexamethasone increased renal expression of glucocorticoid receptor in 6-month-old offspring by several fold.⁵⁹ Methylation-sensitive PCR analysis indicated that this up-regulation of renal glucocorticoid receptor was associated with hypomethylation of the glucocorticoid receptor promoter.⁵⁹

Activated glucocorticoid receptors can bind to negative glucocorticoid-responsive elements in the promoter regions and suppress target gene expression.⁶⁰ One of the target genes of glucocorticoid receptors appears to be WNK4, a regulator of sodium reabsorption in the kidney.⁶¹ It was reported that stimulation of α -adrenergic receptor resulted in cyclic AMP-dependent inhibition of histone deacetylase-8 activity in the kidney, increased histone acetylation, increased accessibility and binding of glucocorticoid receptors to a negative glucocorticoid-responsive element in WNK4 promoter, and decreased transcription of WNK4. This pathway may contribute to increased sodium reabsorption in the kidney and the development of salt-sensitive hypertension in mouse and rat models.⁶¹

Glucocorticoids (primarily cortisol in human and corticosterone in rodents) can bind to mineralocorticoid receptors with equal affinity as mineralocorticoids (primarily aldosterone). The binding would stimulate sodium reabsorption in the distal nephron and, when excessive, could lead to the development of hypertension. The high expression level of

11 β -hydroxysteroid dehydrogenase type 2 in the distal nephron, which converts active glucocorticoids to inactive glucocorticoids, is crucial for preventing abnormal stimulation of mineralocorticoid receptors by glucocorticoids. The tissue-specific enrichment pattern of 11 β -hydroxysteroid dehydrogenase type 2 is inversely correlated with the degree of methylation of its promoter.⁶² It suggests that promoter methylation could contribute to the role of renal 11 β -hydroxysteroid dehydrogenase type 2 in the regulation of volume and blood pressure homeostasis. Indeed, it was reported that renal 11 β -hydroxysteroid dehydrogenase type 2 was down-regulated from birth through postnatal day 21 in an intrauterine growth retardation rat model of hypertension. The down-regulation was correlated with altered DNA methylation at birth in a gender-specific manner and decreased trimethylation of H3K36 in exon 5 at birth and at postnatal day 21 in both genders.⁶³ Renal expression of the type 1 isoform of 11 β -hydroxysteroid dehydrogenase, which converts inactive glucocorticoids back to active glucocorticoids, has also been shown to contribute to the development of salt-sensitive hypertension.⁴⁸

Stimulation of mineralocorticoid receptors leads to increased sodium reabsorption in the distal nephron in part by up-regulating the expression of epithelial sodium channels (ENaC). Kone and colleagues reported that the transcriptional activity of ENaC was regulated by histone H3 Lys-79 methyltransferase disruptor of telomeric silencing-1a (Dot1a) and the sequence-specific DNA binding protein ALL1 fused gene from chromosome 9 (Af9).⁶⁴ They proposed that, in the absence of aldosterone, Dot1a would form a complex with Af9 and bind to ENaC 5'-flanking region, which presumably facilitates methylation of Lys79 of histone H3 and suppresses ENaC transcription. Aldosterone could disrupt this repressor complex by down-regulating or phosphorylating Dot1a and/or Af9, leading to up-regulation of ENaC.

Activation of systemic and/or local renin-angiotensin systems leads to the development of hypertension. Angiotensin converting enzyme 1 was up-regulated in several organs including the kidney in spontaneous hypertensive rats compared to Wistar-Kyoto rats.⁶⁵ This up-regulation was associated with increased binding of histone 3 acetylation (H3Ac) and fourth lysine trimethylation (H3K4me3) but decreased binding of histone ninth lysine dimethylation (H3K9me2) to the promoter of the angiotensin converting enzyme 1 gene in the kidney and several other organs.⁶⁵ Changes in promoter methylation have also been reported to correlate with changes in the expression of genes in the renin-angiotensin system in non-kidney organs.⁶⁶ For example, maternal exposure to low protein diet, which leads to hypertension in adult offspring, was reported to cause up-regulation of angiotensin type 1 receptor and hypomethylation of its promoter in the adrenal gland.⁶⁷

A major challenge in epigenetic research is demonstrating a causal contribution of specific epigenetic marks to the determination of a phenotype. The enzymes that catalyze DNA methylation, demethylation, or histone modifications do not in themselves confer any site-specificity. Targeting these enzymes will affect many epigenetic marks in a largely non-specific manner. Several methods have been developed to enable targeted changes in DNA methylation at specific genomic sites.^{68,69,70} The methods typically involve fusing a DNA methylation enzyme with a DNA binding protein engineered to recognize a specific genomic site. The DNA binding protein guides the methylation enzyme and introduces changes in methylation at the specific genomic site. Further improvement of these methods, especially their in vivo application, will be highly valuable to epigenetic research including epigenetic studies of hypertension.

Hypertension and epigenetic inheritance

Essential hypertension has a significant level of heritability estimated to be up to 50% based on studies of twins. GWAS have identified numerous DNA polymorphisms that can account for parts of many quantitative traits. Hypertension, however, is one of the most notable examples in which the DNA polymorphisms identified by GWAS account for only a small fraction of the trait.⁷¹ The missing heritability may be due to several reasons, one of which being epigenetic inheritance.⁷¹

Whether and how epigenetic marks and any associated phenotypes are inherited transgenerationally in mammalian species remains an intensively investigated question. DNA methylation is erased following fertilization and then again during the formation of primordial germ cells.⁷² Some evidence suggests that the erasure is not complete, leaving behind methylation marks that might help to maintain aspects of the epigenetic pattern through generations.^{73,74,75} Trans regulatory factors in zygotes, such as RNA, might also help to transmit epigenetic patterns through generations.^{76,77}

Because of the possibility of incomplete erasure of epigenetic patterns during re-programming, epigenetic marks and associated phenotypes have to be transmitted from the F0 generation (the exposed generation) to F3 to be considered transgenerationally inheritable and not the consequence of initial environmental exposure. The primordial germ cell that gives rise to an F2 individual was formed when F1 developed as an embryo in the uterus of F0 and could have been exposed to any environmental cue that the F0 individual was exposed to (Figure 3). Transmission from F0 to F2 would be sufficient evidence for transgenerational inheritance if the environmental exposure of F0 took place prior to pregnancy (Figure 3). Developmental programming that involves only one or two generations, as discussed earlier in the article, could still be direct results of initial environmental exposure and does not require or prove transgenerational inheritance. Clear evidence for transgenerational inheritance of epigenetic marks and associated phenotypes remains scarce in all areas of research and absent in hypertension research.

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Figure 1. A tree-like paradigm for understanding the gene and pathway network underlying the development of salt-sensitive hypertension

Figure 2. Several genes expressed in the kidney exhibit changes in epigenetic modifications that correlate with hypertension

mC, DNA methylation; Hm, histone methylation; Ha, histone acetylation; ACE1, angiotensin converting enzyme 1; GC, glucocorticoids; HSD, hydroxysteroid dehydrogenase; GR, glucocorticoid receptor; AR, adrenergic receptor; HDAC, histone deacetylase; MR, mineralocorticoid receptor; ENaC, epithelial sodium channel.

Figure 3. Transgenerational inheritance of epigenetic traits

(*Left*) Exposure to environmental cues occurs during pregnancy in F0. PGCs that give rise to F2 are exposed. (*Right*) Exposure occurs in F0 prior to pregnancy. Zygotes in F0 forming the embryo that develops to become F1 are exposed in this scenario. But PGCs that give rise to F2 are not exposed. PGC, primordial germ cell.

Table 1
Examples of methods for genome-wide analysis of epigenetic marks

See the text for further detail and references.

Epigenetic mark or mediator	Genome-wide analysis method (examples)
DNA methylation	Affinity purification and sequencing Endonuclease digestion and bisulfite sequencing
Histone modification	Chromatin immunoprecipitation and sequencing PTM-sensitive proteomics
Non-coding RNA	RNA-seq