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Molecular markers in arterial hypertension

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Abstract

Hypertension is a complex disease determined by the interconnection between multiple molecular, pathophysiological and environmental factors. This paper reviews the potential role of various molecular markers in the mechanisms associated with the development and consequences of arterial hypertension. Molecular markers are effective tools that have been used to identify small changes (particular DNA sequences) within the mapping population allowing for segregation of traits and identity. The importance of genetic determination in hypertension is best represented by the fact that family history increases the risk of developing hypertension by approximately 4 times. Genomic technologies make it possible today to genotype millions of genetic variants across the human genome. So far the most powerful approaches for finding genetic variants influencing susceptibility to hypertension have been genome wide association studies (GWAS) between single nucleotide polymorphisms (SNPs) and disease phenotypes. Not all features of gene regulation are encoded in genes or DNA sequences. DNA methylation, histone modification, and alteration of microRNA expression - referred to as epigenetics - may also contribute to blood pressure gene regulation. MicroRNAs, as essential gene expression regulators, modulate cardiovascular development and disease and are emerging as potential biomarkers and therapeutic targets in cardiovascular disease. The molecular mechanisms of arterial hypertension are still incompletely understood and further research in the field will help discover new early detection techniques and novel treatment strategies therefore reducing mortality risk.

Keywords: arterial hypertension, molecular markers, DNA

Molecular markers in arterial hypertension

Hypertension is a major public health problem in the developing as well as in developed countries due to its high prevalence and its association with coronary heart disease, renal disease, stroke, peripheral vascular disease, and related disorders[1].

Arterial hypertension is a complex disease determined by the interconnection between multiple molecular, pathophysiological and environmental factors. It is among the leading global risks for mortality with 7.5 million deaths worldwide in 2004, (i.e. 12.8% of the global total), while in 2010 reaching 9.4 million deaths worldwide [2]. Unfortunately, it still remains nowadays an incurable disease associated with a high early mortality, despite advances in treatment options.

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Since hypertension is clinically silent at the beginning and symptoms occur late in its natural history, early diagnosis is a key element, being associated with improved long term survival and screening of at risk population is a good strategy to improve outcome. Therefore novel tools for the early detection are urgently needed.

Molecular markers are effective tools that have been used to identify small changes (particular DNA sequences) within the mapping population[3]. The importance of genetic determination in hypertension is best represented by the fact that family history increases the risk of developing hypertension by approximately 4 times.

But from a genetic viewpoint, is blood pressure considered as a quantitative trait or a dichotomous disease phenotype? How does this affect upcoming studies of genetic causation?

Regarding the nature of hypertension, a quite famous dispute took place in the 1950s between two prominent figures in the history of blood pressure research: Robert Platt and Sir George White Pickering. The Platt Pickering debate focused on the observed Gaussian distribution of blood pressure, or the lack of a bimodal distribution to support a polygenic basis for the blood pressure trait[4]. Platt suggested that hypertension occurred in a discrete subpopulation and was caused by a single, heritable genetic mutation (a simple mendelian disease). On the contrary, Pickering argued that there was a range of blood pressure levels in the population, and that there was no clear dividing line between hypertension and normotension; instead, hypertension represented the end of a continuum and was therefore polygenic in origin (non mendelian)[5]. After many years of epidemiological studies, it is now obvious that hypertension exists as a polygenic trait with rare syndromes representing the extreme end of the blood pressure distribution [6].

In order to reveal the genetic basis of essential hypertension, two main approaches have been used: association studies and linkage analysis, respectively. The association studies use unrelated individuals, hence the ease to gather a large amount of samples, while linkage analysis is pedigree-based, using related individuals diagnosed with hypertension [7]. Genetic-association studies gather a large number of individuals affected by a particular trait (hypertension in this case) and search for individual marker alleles (or genotypes) that are more frequent in individuals with disease than in healthy control individuals.

Association studies examine unrelated individuals in a population, using genotypes of a large number of polymorphic markers in subjects with marked hypertension compared to healthy controls [7].

The strategy of linkage mapping in affected families to identify loci from which candidate genes and genotypes are selected has not achieved similar success for hypertension because high blood pressure involves multiple genes and gene environment interactions [7].

Genomic technologies make it possible today to genotype millions of genetic variants across the human genome. There are several genetic methods used to indentify blood pressure genes and examine the linkage of genes or chromosome regions to hypertension: study of rare Mendelian forms of hypertension, candidate genes association studies, genome wide linkage study and genome wide association studies (GWAS).

The genetic contribution to blood pressure regulation is of two different types: monogenic hypertension and rare genetic variants on one hand and primary hypertension and common genetic variants on the other hand [6].

Monogenic hypertension and rare genetic variants represent rare mutations in families that can cause secondary hypertension even in the absence of other risk factors [8]. The rare Mendelian forms, where mutations in single genes cause variations in blood pressure, provided a molecular basis for understanding the pathogenesis of hypertension [7, 9].

So far 9 different monogenic hypertensive syndromes have been found (also called Mendelian blood pressure phenotypes) determined by 13 specific genetic mutations. These blood pressure phenotypes are characterized by a major gene that affects a single pathway. The identified genes are linked to renal salt and potassium excretion (Bartter, Gitelman, Gordon, Liddle syndromes), steroids/aldosterone synthesis and sympathetic nervous system[7, 10].

Given the diversity of physiological systems that affect blood pressure, it is surprising that the mutated gene products in all cases act in the same physiological pathway in the kidneys, altering renal salt reabsorption [6]. Interestingly, different mutations, even in the same gene, may cause hyper or hypotension.

Mutations that increase sodium reabsorption and cause hypertension include mutations in the mineralo-

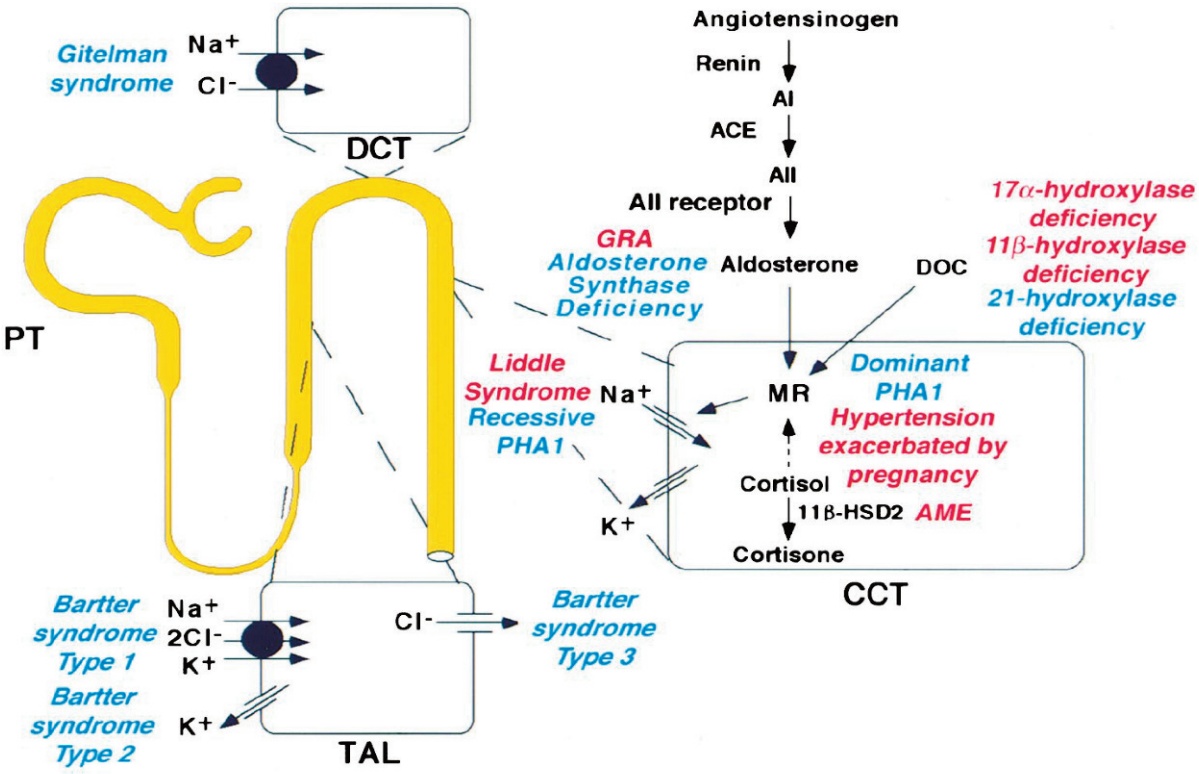

Figure 1. Mendelian forms of hypertension (modified after Lifton et al, Cell 2001) [11].

corticoid receptor (hypertension exacerbated by pregnancy), aldosterone synthase (glucocorticoid-remediable aldosteronism), other enzymes synthesizing steroids that activate the mineralocorticoid receptor (11β-hydroxysteroid dehydrogenase, 17α-hydroxylase, and 11β-hydroxylase), the β- and γ-subunits of the renal epithelial sodium channel (Liddle's syndrome), and the serine-threonine kinases (WNK1 and WNK4 in pseudohypoaldosteronism type 2) [7] (Fig. 1).

Hypertension exacerbated by pregnancy is a very rare condition characterized by large blood pressure increases in pregnancy. With this mutation, the mineralocorticoid receptor can be activated by progesterone in addition to aldosterone [6, 10].

Glucocorticoid-remediable aldosteronism (familial hyperaldosteronism type I) is a form of mineralocarticoid hypertension accompanied by hypokalaemia, an elevated plasma aldosterone level and a suppressed renin level. Hypertension is caused by increased aldosterone secretion determined by ACTH. These patients have a chimeric gene formed from portions of the 11-beta-hydroxylase gene and the aldosterone synthase gene that is stimulated by adrenocorticotropic hormone (ACTH), resulting in the production of aldosterone [6, 10].

11-beta-hydroxylase deficiency causing **congenital adrenal hyperplasia** is a disorder associated with mutations of the CYP11B1 gene and hypertension often occuring during the first years of life [6, 10].

17-alpha-hydroxylase deficiency causing **congenital adrenal hyperplasia** is a very rare defect usually associated with hypogonadism/adrogen deficiency [6, 10].

11-beta-hydroxysteroid dehydrogenase mutations can cause **the syndrome of apparent mineralocorticoid excess**. Patients with the disorder cannot metabolize cortisol to its inactive metabolite cortisone normally, resulting in a prolonged half-life of cortisol and a characteristic increase in urinary cortisol. It is a low renin syndrome accompanied by kipokaliemia and metabolic acidosis [6, 10].

Liddle's syndrome is a disorder caused by a gain of function mutation in epithelial sodium channel causing increased sodium reabsorption that leads to hypertension, low plasma renin and aldosterone levels as well as hypokalemia, all of which responding to amiloride, an inhibitor of the epithelial sodium channel (ENaC) [6, 10].

Gordon's syndrome (Pseudohypoaldosteronism type 2) causes elevated blood pressure accompanied by hyperkalaemia, despite normal renal glomerular filtration and low or low-normal plasma renin activity and aldosterone concentrations. Mutations in WNK kinases 1 and 4 result in increased chloride reabsorption with sodium, thereby producing volume expansion, hypertension, and, due to reduced distal sodium delivery, hyperkalemia [6, 10].

Familial hyperaldosteronism type III is an extremely rare defect produced by loss-of-function mutations of a potassium channel. The typical presentation is hypertension with hypokalemia and elevated aldosterone [6, 10].

Autosomal dominant hypertension with brachydactyly is a syndrome caused by a mutation of the phosphodiesterase 3A gene. Severe hypertension that occurs at older age is associated with brachydactyly [6, 10].

Loss-of-function mutations were found to impair renal sodium reabsorption thus causing hypotension. They include genes encoding the mineralocorticoid receptor (autosomal dominant pseudohypoaldosteronism type 1), aldosterone synthase, 21-hydroxylase, the β- and γ-subunits of the epithelial sodium channel (EnaC; recessive pseudohypoaldosteronism type 1), the ATP-sensitive potassium channel ROMK (Bartter's syndrome type 2), and chloride channel CLC-NKB (Bartter's syndrome type 3) [10].

Pseudohypoaldosteronism type I is characterized by life-threatening dehydration in the neonatal period, hypotension, salt wasting, hyperkalaemia, metabolic acidosis, and marked elevation of renin and aldosterone. The autosomal recessive form is due to inactivating mutations in one of the genes SCNN1A, SCCN1B or SCNN1G, encoding (respectively) the α, β, and γ subunits of the epithelial sodium channel, while the autosomal dominant form is due to loss-of-function mutations in the gene (NR3C2) encoding the mineralocorticoid receptor [6, 10].

Gitelman's syndrome is caused by inactivating mutations in the gene encoding the renal thiazide-sensitive Na-Cl cotransporter (SLC12A3) leading to hypotension, hypokalaemia, hypomagnesaemia, hypocalciuria, metabolic alkalosis, neuromuscular abnormalities in adolescence or early adulthood [6, 10].

Bartter's syndrome caused by mutations in one of the genes that encode regulators of chloride transport within the thick ascending loop of nephron determines hypotension or normal blood pressure, hypovolaemia and polyuria, elevated prostaglandin levels and nephrocalcinosis with early onset [6, 10].

Monogenic disorders of blood pressure regulations are rare and do not explain blood pressure variability in the population at large [7]. Nevertheless, these rare single gene mutations are still of importance because they give insight into the pathways through which common genetic variations may influence blood pressure. However, no single genetic variant has emerged from linkage or association analyses as consistently related to an elevated blood pressure level in every sample and in all populations [7].

Primary hypertension cannot be determined by only one or few genetic variants meaning that there is no such thing as the primary hypertension gene! There are hundreds, even thousands of common genetic hypertension risk variants that are individually associated with small effect sizes (approximately 1 mmHg or less). The probability of primary hypertension occurring grows larger with the number of risk alleles present and is modulated by environmental factors such as age, body mass index (BMI), sex, salt consumption, and others [10].

The most powerful approaches for finding genetic variants linked to hypertension susceptibility found so far have been GWAS between single nucleotide polymorphisms (SNPs) associated with hypertension or blood pressure regulation [5].

Recent GWAS meta-analysis [9, 10, 12, 13] including almost 300000 people identified so far 34 differentially expressed blood pressure genes and over 60 loci associated with blood pressure or hypertension. However, these loci only explain a small proportion of interindividual blood pressure variation [12, 13].

The heritability of BP varies from 30% to 50% [9], but the collective effect of all BP loci identified through GWAS explains aprox 2% of blood pressure heritability and disease phenotypes [6, 12].

GWAS concluded that there are hundreds, possibly thousands of blood pressure loci and only a small fraction of the heritability of blood pressure is explained by the loci discovered so far (~ 4 - 6 %) [10]. The other genetic determinants of blood pressure heritability still remain unknown. Also the effect of any specific individual loci on blood pressure is small, ~ 1 mmHg for systolic pressure and 0.5 mmHg for diastolic pressure [8]. And there is still no evidence of gene-gene interactions, meaning that there is no evidence thet

blood pressure loci are dependent upon other blood pressure loci but they do depend upon some environmental factors, such as age [10].

While most of the GWAS studies addressed the blood pressure in a quantitative manner, there were 2 studies analysing hypertension as a dichotomous feature; as a result, new or well-known pathways of blood pressure regulation were identified, involving uromodulin gene and endothelial nitric oxide synthase gene respectively [14, 15].

The Mosaic Theory of hypertension, proposed by Paige in 1960 [16], considered interactions among genes, environment, adaptive, mechanical, hormonal and neural perturbations as foundation of hypertension. In 2014 the updated Paige model emphasized the huge contribution made by genetics in understanding blood pressure regulation while highlighting the major issue regarding GWAS, namely the difficulty in linking the associated SNPs to causal genes [6].

Discovering the genetic basis for hypertension has proven to be challenging and only a small part of the total variation in blood pressure can be explained by common genetic variants associated signals. A part of this ''missing heritability'' is likely to be due to as yet unknown common or low-frequency variants, and a fraction of it could be identified by increasing sample size [17].

The speculation about the missing heritability has focused on the possible contribution of variants of low minor allele frequency (0.5%-5%) or rare variants (<0.5%). These variants are not frequent enough to be captured by existing association studies arrays, nor do they carry appropriately large effect sizes to be identified by conventional linkage analysis in family studies [18].

Studies have showed that when minor allele frequency is lower than 0.5%, detection of associations is improbable except if effect sizes are very large, as in monogenic conditions; in this case, low frequency variants could have significant effect sizes by increasing disease risk two- to threefold and could considerably contribute to missing heritability [18, 19].

Epigenetics

But not all features of gene regulation are encoded in genes or DNA sequences [20].

Epigenetics refers to all heritable changes to the regulation of gene activity as well as chromatin remodeling, without altering the DNA sequence itself [21].

Blood pressure genes can also be differentially regulated by epigenetic mechanisms including: DNA methylation, histone modification, alteration of non-coding RNA expression [20]. In some cases, epigenetic modifications are stable and passed on to future generations, but in other instances they are dynamic and change in response to environmental stimuli. Nearly every aspect of biology is influenced by epigenetics, making it one of the most important fields in science. Epigenetic modifications can be determined by many factors, including but not limited to environmental influences, chemical exposure, aging, dietary habits, use of recreational drugs, various medications [22].

Different sources present in the environment (exercise, microbiome, alternative medicine, exposure to toxic chemicals and drugs of abuse) have various epigenetic influences on humans, some beneficial for health and beahaviour, other harmful, creating imbalance [23].

Hence the need for extended research in the field, in order to direct these influences in a beneficial way

An epigenetic factor can modify the epigenome either directly or indirectly, leading to altered gene expression.

In the direct manner, the epigenetic factor either can tamper the epigenetic enzymes altering their bioavailability in the cell, or interfere with a biochemical pathway leading to altered availability of a metabolite essential for constituting an epigenetic tag. Eventually, an altered epigenetic profile occurs [23].

As for the indirect effect, it occurs under acute or chronic exposure to an epigenetic factor. The acute exposure causes altered expression of growth factors, receptors or ion channels with impaired cellular processes and compromised status of transcriptional apparatus [23]. If uncorrected, these disruptive exposure will persist and escalate in severity over time, becoming chronic; the compromised transcriptional apparatus will persists, causing altered gene expression and abnormal recruitment of epigenetic enzymes, leading to permanent addition or removal of epigenetic tags to specific promoter/enhancers. As a result, an altered epigenetic profile occurs [23].

Non coding RNAs

Essentially, epigenetics allows the same genome to show alternative phenotypes based on different epigenetic influences.

Non coding RNAs (ncRNAs) have changed the view of the 'central dogma' of molecular biology, that all DNA is transcribed into RNA and then translated into proteins.

A ncRNA is a functional RNA molecule that is transcribed from DNA but not translated into proteins. Although protein is the link between genotype and phenotype, many factors determine the presence and abundance of proteins, and therefore their function. NcRNAs have a fundamental role in regulating protein levels by modulating transcription and translation to either increase or decrease protein levels [24]. NcRNAs are involved in epigenetic processes and can influence histone modification and cytosine methylation (implicated in gene expression regulation) [25]. Even though most ncRNAs have no identified function so far, it is feasible to assume that their role in epigenetic regulation is yet to be revealed.

Recently, several small and mid-sized ncRNAs have been described and are known to have a role in the regulation of transcription, post-transcription and translation [22].

Small ncRNAs include PIWI-interacting RNAs (piRNAs), transcription initiation RNAs (tiRNAs) and miRNAs. Mid-size ncRNAs include small nucleolar RNAs (snoRNAs), promoter upstream transcripts (PROMPTs), transcription start sites (TSS)-associated RNAs (TSSaRNAs) and promoter-associated small RNAs. Long ncRNAs include circular RNAs (circRNAs), transcribed ultra-conserved regions (T-UCRs) and large intergenic ncRNAs (lincRNAs)) and are most commonly associated with a reduction in transcription but may also have a role in the regulation of miRNA levels [24].

So far no studies have been reported on long ncRNAs and blood pressure or hypertension. They are mostly known to modulate chromatin complexes, and thus change DNA condensation, where more condensation results in less transcription.

Small and mid-size ncRNAs mostly regulate transcription, posttranscription and translation.

ncRNAs function is to regulate gene expression at the transcriptional and post-transcriptional level. Both major groups are shown to play a role in heterochromatin formation, histone modification, DNA methylation targeting, and gene silencing [24].

Epigenetic related ncRNAs include microRNAs (miRNAs), short interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs) and long noncodingRNA [24].

Micro RNAs

MicroRNAs, as essential gene expression regulators, modulate cardiovascular development and disease and are emerging as potential biomarkers and therapeutic targets in cardiovascular disease [20].

MiRNAs are the most commonly studied singlestranded small ncRNA; currently there is no research available regarding the involvement of other types of small and mid-sized ncRNAs in essential hypertension [22]. They are non-coding RNA molecules, 22 nucleotides in length acting as post-transciptional regulators of gene expression by reducing the expression of specific target genes.

They are most abundant class of gene expression regulators that bind complementarily to transcripts to repress their translation or messenger RNA degradation. Also being remarkably stable in the blood they appear in concentrations measurable by current techniques and often show tissue specific expression.

MiRNAs are increasingly implicated as regulators of cardiovascular system including modulation of arterial pressure [26].

It has been found to participate in cell proliferation, differentiation and death. For example the loss of miRNAs in juxtaglomerular cells and vascular smooth muscle cells (VSMCs) causes significant reductions of blood pressure [20].

The reduction in blood pressure can be attributed to reduced renin production in case of juxtaglomerular cells whereas in VSMCs is determined by decreased vascular contractility, attributed mostly to the lack of miR-145. This observations has been made in mice lacking miR-143 and miR-145 with significant reductions in blood pressure resulting from modulation of actin dynamics [20] .

In a paper published in 2011, Li and collaborators showed that human cytomegalovirus-encoded miRNA is associated with essential hypertension independently of other hypertension risk factors [24].

Moreover, miRNAs regulate renin-angiotensinal-dosterone system; for example, mineralocorticoid receptor gene NR3C2 is downregulated by miRNAs miR-135a and miR-124 [27] but there are also genes controlled by some other miRNAs [28, 29].

All these data emphasize the role played by the miRNAs in blood pressure regulation and the necessity for outspread exploration in this area.

Histone modification

Alternative facet of epigenetics is represented by histone modifications which are indicators of active or repressed chromatin; combinations of specific histone modifications define chromatin regulation and gene transcription [20, 30, 31].

Posttranscriptional modifications regulate gene expression by controlling the dynamics of chromatin. Modifications occuring at residues in histone tails include the following: acetylation, methylation, phosphorilation, biotinylation [22]. Epigenetic histone modification occurs when the N terminal tail is subjected to post translational modification – aprox 60 possible modifications [32].

Histone modification affecting arterial pressure has been documented in a variety of human and animal tissue animal tissues, including vascular smooth muscle.

Vascular oxidative stress can contribute to endothelial dysfunction and the development of hypertension. It is common knowledge that endothelial nitric oxide synthase (eNOS) is predominantly responsible for the production of nitric oxide in the vascular endothelium [33], therefore playing a fundamental role in regulating vascular tone, cellular proliferation, leukocyte adhesion and platelet aggregation [34]. It has been found that eNOS activity and expression, thought to be downregulated in cardiovascular disease, is controlled by cell-specific histone modifications [35].

Also the Na+-K+-2Cl– cotransporter 1 (NKCC1) is upregulated via histone modification in the aortas of Ang II-induced hypertensive rats. This ion transporter is epigenetically upregulated by histone modification or DNA demethylation upon the development of hypertension [36].

Histone modification and/or DNA demethylation have a role in the epigenetic up-regulation of NKCC1 during hypertension development. Acetylated histone H3 (an activating histone), was significantly increased together with greatly decreased trimethylated histone H3 (a deactivating histone) in hypertensive patients [36].

Histone modification also plays an important role in the epigenetic of epithelial sodium channel-α subunit (ENaCα) gene expression and modulation of WNK4 transcription in the development of salt sensitive hypertension, mechanisms which have been extensively discussed in two recent reviews [20, 22]. A nuclear repressor complex can regulate histone H3 Lys-79 methylation of chromatin associated with the ENaCα promoter and suppress its transcriptional activity [37].

Histone modification is involved in decreasing transcription of the WNK4 gene induced by β2-adrenergic receptor stimulation in mice on a high salt diet; in normotensive mice, a low-sodium diet decreases expression of renal WNK4, whereas a high sodium diet increases its expression [38].

DNA methylation

DNA methylation is intrinsically linked to the regulation of gene expression. Epigenetic DNA methylation occurs when a methyl group derived from S-adenosyl-L-metionine is bound to position 5 of the cystone ring, forming 5-methyl-cytosine. This takes place at specific sites called CpGs, that are short DNA sequences. DNA methylation suppresses gene transcription, meaning that DNA hypermethylation results in gene silencing [22].

Different degrees of DNA methylation have been correlated with variable onset, timing, and severity of hypertension [32]. Genomic DNA methylation can be quantified by measuring the amount of 5- methyl cytosine present in a DNA sample. Several studies observed that global DNA methylation levels decrease as the severity of hypertension increases [39].

Methylation changes of genes have also been linked to a wide range of other complex diseases, including diabetes, autoimmune disorders, obesity, heart disease, and mental disorder [40].

More than 1.000 hypermethylated CpG sites were identified in the kidneys of salt-sensitive rats compared with normotensive Brown Norway rats, pyrosequencing of the promoter of renin genes showed that 10 CpG sites were significantly hypermethylated in saltsensitive

rats, consistent with the reduced renin expression in this strain [40].

Loss-of-function mutations of 11beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2) genes lead to a form of salt-sensitive monogenic hypertension. Methylation modulation of this gene has recently been demonstrated in both a rodent model and cultured human cell lines [20].

The cis-regulation of DNA methylation by genetic variation may reflect the existence of a common pathway that acts on both genetic and environmental effects and represents a potential mechanism for gene-envirnment interaction [20].

Small changes in blood pressure have been observed from changes in DNA methylation of pro-inflammatory genes; also a stronger association between methylation and diastolic rather than systolic blood pressure has been found [41].

Conclusions

Unfortunately, despite considerable research, the molecular mechanisms of arterial hypertension remain incompletely understood. The quest to solving the genetic origins and molecular pathways of hypertension has not yet come to an end, and further research in this field will help improve the understanding of blood pressure pathophysiology and may eventually lead to the development of novel treatment approaches for hypertension.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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