**The role of DNA methylation and histone modifications in blood pressure: a systematic review.**

Running title: Epigenetics and blood pressure: systematic review.

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# Abstract

Epigenetic mechanisms might play a role in the pathophysiology of hypertension, a major risk factor for cardiovascular disease and renal failure. We aimed to systematically review studies investigating the association between epigenetic marks (global, candidate-gene or genome-wide methylation of DNA and histone modifications) and blood pressure or hypertension. Five bibliographic databases were searched until December 7th of 2018. Of 2,984 identified references, 26 articles based on 25 unique studies met our inclusion criteria, which involved a total of 28,382 participants. The five studies that assessed global DNA-methylation, generally found lower methylation levels with higher systolic blood pressure, diastolic blood pressure and/or presence of hypertension. Eighteen candidate-gene studies reported, in total, 16 differentially methylated genes including renin-angiotensin-system related genes (*ACE promoter*, and *AGTR1*) and genes involved in sodium homeostasis and extracellular fluid volume maintenance system (*NET promoter*, *SCNN1A*, and *ADD1*). Between the three identified epigenome-wide association studies (EWAS), lower methylation levels of *SULF1*, *EHMT2*, and *SKOR2* were found in hypertensive patients as compared to normotensive subjects and lower methylation levels of *PHGDH*, *SLC7A11* and *TSPAN2*, were associated with higher systolic and diastolic blood pressure. In summary, the most convincing evidence has been reported from candidate-gene studies, which show reproducible epigenetic changes in the interconnected renin-angiotensin and inflammatory systems. Our study highlights gaps in the literature on the role of histone modifications in blood pressure and the need to conduct high-quality studies, in particular, hypothesis-generating studies that may help to elucidate new molecular mechanisms.

# Introduction

Hypertension is a long-term condition in which the blood pressure (BP) in the arteries is persistently elevated. The burden of hypertension remains increasing despite the availability of effective medication, as well as the outcomes associated with it, such as ischemic heart disease, cerebrovascular disease and chronic kidney disease (1, 2).

The etiology of hypertension remains unclear, therefore, a better understanding of the risk factors is key to improve prevention strategies. Several environmental risk factors are contributing to hypertension (3-5). Genetic variants also determine BP and the risk of hypertension, which heritability has been estimated to be up to 30-50% (6). The most recent genome-wide association study (GWAS) on blood pressure phenotypes was conducted among 321,262 participants and found more than 241 loci, of which 44 were newly discovered. This study and previous genetic investigation of the biology of blood pressure regulation, have revealed new opportunities for future drug development and highlighted the shared genetic architecture between blood pressure and lifestyle exposures such as obesity, smoking, alcohol and high salt-intake (7-9). However, these variants explain only a minor fraction (<5%) of the inter-individual variation in the susceptibility for hypertension (10).

Epigenetic modifications might contribute to the pathophysiology of hypertension (11). Epigenetics refers to dynamic and potentially reversible changes that alter gene activity and expression. DNA methylation and histone modifications are the most studied epigenetic mechanisms and have been involved in pathways related to dyslipidemia, type 2 diabetes, and cardiovascular disease, conditions that are strongly correlated with hypertension (11-13).

To date, however, little work has been done to systematically assess the current evidence of the role epigenetic modifications on the risk of high blood pressure. We aimed to systematically review all the available evidence of the association epigenetics with high blood pressure. A critical appraisal of limitations and gaps in the field is also presented.

**Methods**

## Literature search

This review was conducted and reported in accordance with the PRISMA (14) guideline (**Appendix S1**). We sought studies published before December 7th 2018 (date last searched) in five electronic databases: Embase.com, Medline (Ovid), Web-of-Science, Cochrane Central and Google Scholar. The search was done with the help of a medical information specialist. In databases where a thesaurus was available (Embase and Medline), articles were searched by thesaurus terms, title and/or abstract. In other databases, only by title and/or abstract. The search combined terms related to the exposure (e.g. epigenetic, histone acetylation, methylation, demethylation, hypomethylation, hypermethylation, DNA methylation) and outcome (e.g. blood pressure, and hypertension). We did not apply any language restriction, but we restricted the search to studies conducted on humans. The full search strategies of all databases are provided in **Appendix S2**. The study identification also included manual search, based on the screening of the citations of the included studies.

## Study selection and inclusion criteria

Studies were eligible for inclusion if they (1) were cross-sectional studies, case-control studies, or cohort studies; (2) were conducted among humans; (3) assessed epigenetic marks (global, site specific or genome-wide methylation of DNA or histone modifications); (4) collected data on blood pressure (systolic and diastolic blood pressure, hypertension, essential hypertension), and (5) reported the association of any of the above-mentioned epigenetic marks with blood pressure. We did not make restriction on the tissue examined for epigenetic marks. We excluded studies that examined epigenetic marks other than DNA methylation and histone modifications, such as noncoding RNAs. We also excluded post-mortem studies.

Two independent reviewers conducted an initial screening of all titles and abstracts and then evaluated all potentially relevant articles based on full text reviews. If no consensus was reached, a third independent reviewer solved discrepancies between the two reviewers.

## Data extraction

A predesigned data collection form was prepared to extract the relevant information from the selected studies, including study design, characteristics of the study population, location of the study, sample size, and degree of adjustment. Furthermore, we extracted, for each study, the tissue type and methods used to determine DNA methylation, the specific CpGs sites, the directions of the associations, and, when possible, the reported measures of associations (e.g., correlation coefficients, beta-coefficients, relative risks, and confidence intervals).

## Assessing the risk of bias

Two reviewers independently rated the quality of the studies based on the Newcastle-Ottawa Scale (NOS) (15), a semi-quantitative scale designed to evaluate the quality of case-control or cohort studies. We evaluated cross-sectional studies using an adapted version of the scale. Studies that received a score of nine stars were judged to have good quality and to be at low risk of bias; studies that scored eight or seven stars were considered medium risk of bias and those that scored less than seven were considered to be at high risk of bias.

## Outcome assessment and statistical methods

For each study, we defined whether an association was reported, and when applicable, direction and effect sizes were reported. Heterogeneity permitting, we sought to pool the results using a random effects meta-analysis model. However, due to differences in exposure and outcomes, and input parameters, it was not feasible to pool the data quantitatively.

# Results

In total, we identified 2,984 unique references (**Fig 1**). Based on the title and abstract, we selected full texts of 55 articles for detailed evaluation. After full-text assessment, 26 of these articles, based on 25 unique studies, met our eligibility criteria and were included in this review. The other 29 articles were excluded for reasons presented in **Fig 1**.

## Characteristics of the included studies

Detailed characteristics of the 25 included studies are summarized in **Tables 1-3**. Combined, the 25 studies included data from 28,382 individuals. Five studies assessed global DNA-methylation. From those, two studies also used candidate-gene approach (16, 17). Sixteen studies assessed the DNA methylation only in specific candidate genes, three studies used genome-wide approaches, and one study assessed histone modification in relation to BP. One study included South Asian and European population (18), and another one included individuals of European, African American, and Hispanic ancestry from different countries. Twelve studies included participants from China, three from Canada, two from USA, and the rest included participants from Brazil, Egypt, the Netherlands, Poland, Spain and Switzerland. The majority (n=22) of the studies assessed epigenetic signatures in blood, two in visceral adipose tissue (VAT) and one in saliva. Eight studies were judged to be at medium risk of bias whereas the rest at high risk of bias.

## Outcome definition and assessment

The studies reported the outcomes in two different ways: measures of blood pressure (expressed as continuous variables) (n=7) or diagnosis status (presence or absence of essential hypertension) (n=14). The remaining four studies reported both types of outcomes. Although studies that reported diagnosis status, used different cut-off to define the presence of essential hypertension, the majority (n=11) used the same criteria based on the European Society of Hypertension-European Society of Cardiology Guidelines of 2003 (19) (**Table S3**). Studies that assessed the blood pressure levels, usually measured it in a standardized way. That is after at least 10 minutes of rest, with multiple measures taken with waiting intervals of 10 minutes between them, either in different days or in different arms, in order to finally obtain an average measure (**Table S3**).

## Global DNA methylation and blood pressure

Five studies examined the association between global DNA methylation and BP (**Table 1**). Four of them used blood samples to assess DNA methylation and only one was conducted in VAT (20). Three of the five studies assessed global DNA methylation in the repeat sequences and transposable elements in the genome. A large portion of methylation sites within the genome is found in these sequences, and is shown to correlate with total genomic methylation content (21). Of these three, one study (reported in two articles) (16, 22) assessed both long-interspersed nuclear element (LINE-1) and ALU transposable repeated elements, one study assessed solely LINE-1 methylation (20) and one solely ALU methylation (17). The remaining two studies assessed global DNA methylation as a percentage of total cytosine (methylcytosine/cytosine ratio) (23) or the level of 5-methylcytosine (5mC) (24). Two studies assessed BP as outcome, one study assessed hypertension and two additional studies (reported in three articles) (16, 22, 23) assessed both BP and hypertension.

The studies that assessed LINE-1 methylation showed and association of lower methylation level with higher diastolic blood pressure (DBP) and hypertension (16, 20, 22). From the two studies that assessed methylation of ALU transposable repeated elements, one showed results consistent with the previous two studies, lower ALU methylation with higher DBP (17), whereas the other study reported both systolic blood pressure (SBP) and DBP to be positively associated with the degree of methylation of the gene for ALU (16).

Of the studies that measured methylcytosine, one reported higher levels of 5mC in healthy controls compared to patients with hypertension (24), whereas the other one reported no association between methylcytosine/cytosine ratio and BP(23).

## Gene-specific DNA methylation and blood pressure

Eighteen studies examined methylation sites in specific candidate genes (**Table 2**).The rational and criteria for the selection of the candidate genes varied across studies. Some of the studies investigated genes (*ADRB3, ABCG1, GALNT2* and *HMGCR*) that were previously identified in genome- or epigenome- wide association studies on hypertension or cardiovascular disease (18, 25, 26). Other investigations studied pro-inflammatory genes (*TRl2, iNOS, IFNγ, F3, GCR, ICAM-1, TLR4,* *NFKB1,* *PPARγ* and *IL-6*) (16, 17, 27-29), or renin-angiotensin-system (RAS) genes (*ACE promoter, and AGTR1*) (30-33). Some others chose genes involved in the physiology of hypertension, e.g. related to the sympathetic nervous system, sodium homeostasis, extracellular fluid volume maintenance or proliferation of vascular smooth muscle cells (*NET promoter, SCNN1A, ADD1* and *Mfn2*) (34-38).

Of the eighteen studies, one measured DNA methylation in VAT(20) and one in saliva (32), whereas the other studies used blood samples. Four of the studies did not report any level of adjustment or control for confounders, while the others controlled for age and additional confounders such as sex, body mass index, lipid levels, and smoking. Five studies assessed BP as outcome and twelve assessed hypertension. One additional study assessed both BP levels and hypertension as outcome (18).

Among the studies that assessed BP levels, three of them found hypomethylation of the genes (*TLR4*, *ACE* promoterand *NFKB1*) at higher levels of SBP (17, 28, 30) and one found hypermethylation of the gene (*ADRB3*) at higher levels of SBP (25). There was also no consensus for DBP (**Table S4**).

Overall, among the other 13 studies whose outcome was hypertension, 12 studies found hypertension to be associated with hypomethylation of the candidate genes (*ADD1, ADD1 promoter, GCK, AGTR1, IL-6, NET promoter,* *IFNγ promoter and* *Mfn2*). Each of the genes *ADD1* and *AGTR1* were assessed by two studies, finding congruent results that showed hypomethylation in patients with hypertension (**Table S4**). Only one study found higher levels of methylation of the gene among hypertensive patients (39).

## Epigenome-wide analysis and blood pressure

Three studies investigated genomic DNA methylation in a hypothesis-free approach (**Table 3**). One of them adjusted only for age and the other two, additionally, for sex, body mass index, and ethnicity, among others. The studies assessed DNA from blood and used replication cohorts to validate their findings. Wang et al., found seven out of the 10 differentially methylated top genes to be hypomethylated in American hypertensive patients (40). The top two CpG sites (one located in *SULF1* and one in *PRCP*) could not be replicated in two independent cohorts. The study of Boström et al. was performed among patients that underwent gastric surgery. They found differentially methylated genes correlated with changes in SBP before and after the surgery. The association of the top CpGs with essential hypertension was evaluated (41). The replication cohort showed two CpGs (one in *EHMT2* and one in *SKOR2*) to be significantly hypomethylated in cases compared to controls.

Finally, Richard et al. conducted a study using data from CHARGE consortium. After replication, 13 CpG sites were associated with BP. All replicated CpG sites demonstrated associations of decreased DNA methylation with increases in BP. The top CpG sites for both SBP and DBP were located at *PHGDH* locus and *SLC7A11* locus (42).

## Histone modifications and blood pressure

Only one study examined the association between histone modifications and BP (43). The authors assessed histone 3 acetylation and methylation levels in whole blood of Beijing workers and found higher levels of both acetylation and methylation associated with lower SBP and DBP.

# Discussion

The present work is the first to systematically assess the current evidence of the association between epigenetic modifications and BP. We observed an association between a generalized hypomethylation status and high levels of DBP and SBP. Our findings suggest that epigenetic variations, mainly DNA methylation, may play an important role in the regulation of molecular mechanisms of BP. Accordingly, we showed that the genes reported in these findings are important regulators of inflammatory mechanisms (*NFKB1, IFNγ, MFN2, SULF1*), and RAS activity (*PRCP, ACE, AGTR1* genes). However, no overlap was found between the findings from EWAs and the studies that used candidate-gene approach. Conclusive evidence in alterations of histones in BP is still lacking.

## Global DNA methylation

Global DNA methylation in DNA repetitive elements, such as ALU and LINE-1 are the most widely used in population-based studies (44). There are 1.4 million ALU repetitive elements and half a million LINE-1 elements interspersed throughout the human genome, which represents up to 50% of global genomic methylation (45).

Consistent trend of demethylation was observed with both LINE-1 and ALU. The studies that used LINE-1 concluded a significant association between decreased methylation levels and high SBP and DBP (16, 20, 22). Hypomethylation at ALU elements was related with higher BP (16). These findings are in line with other studies showing that hypomethylation at LINE-1, inversely correlate with coronary artery disease and stroke (11). In contrast, global DNA hypermethylation at LINE-1 appears to be associated with vascular inflammatory response to endothelial injury and an increased mortality from chronic kidney disease (46).

## Gene-specific DNA methylation

The assessment of DNA methylation in candidate genetic regions provides further insight into the importance of relevant genes and pathways in the etiology of BP (47). Our review expands current knowledge of blood pressure-related pathways by supporting the role of (epi) genetic dysregulation of a specific set of genes in the development of abnormal BP levels. Several pieces of evidence included in this review are consistent regarding the role of hypomethylation in *ADD1* (Adducin1), *AGTR1* (angiotensin II receptor type 1) and *ACE* (angiotensin I-converting enzyme) in the pathogenesis of hypertension.

Genetic factors of blood pressure regulation are still not very well elucidated. Evidence suggests a key role for 11β-hydroxysteroid dehydrogenase (11βHSD) on the pathogenesis of EH (48). Patients with EH show a decreased production of the enzyme, related with a prolonged half-life of cortisol and an increased ratio of urinary cortisol to cortisone metabolites. Genetic variants in the coding gene, *HSD11B2,* contribute to the enhanced blood pressure response to salt in humans (49). However, the percentage of people with essential hypertension is low and efforts have been focused in investigating overall blood pressure regulation and the influence of environmental factors.

The evaluation of genes whose expression is associated with blood pressure may shed light on novel mechanisms associated with blood pressure regulation as well as unravel how transcripts mediate genetic and environmental effects on blood pressure variability (50). Huan et al. evaluated the global expression signatures of blood pressure and hypertension in 7,017 individuals who were not receiving antihypertensive drug treatment. They identified 34 differentially expressed genes in relation to blood pressure in which some of them explain 5%–9% of inter-individual variance in blood pressure. The genes identified are involved in inflammatory response and apoptosis pathways (50).

DNA methylation may differ by race or ethnicity, challenging replication across individuals of varying descent in epigenetic studies (51). Previous epigenome wide association studies of several cardiometabolic risk factors for example, C-reactive protein, have been able to provide trans-ethnic replication of the differentially methylated genes (52). Current evidence supports the notion that despite differing baseline epigenetic profiles, different ethnicities may have consistent epigenetic association.

## Epigenome-wide association studies

The implementation of EWAS, which are the large scale, systematic design, epigenomic equivalent of GWAS, alongside with the development of microarray technologies, has allowed the interrogation of DNA methylation sites at single-nucleotide resolution (53). In the current review, the three EWAS reported significantly hypomethylated CpGs in association with increase in BP (40-42). The hypomethylated CpG sites are located in the genes *SULF1* (Sulfatase 1), *PRCP* (Prolylcarboxypeptidase), *EHMT2* (Histone H3-K9 Methyltransferase 3), *SKOR2* (SKI Family Transcriptional Corepressor 2), *PHGDH* (Phosphoglycerate Dehydrogenase)and *SLC7A11* (Solute Carrier Family 7 Member 11). *SULF1* is a protein coding gene which catalyzes the hydrolysis of the 6-O-sulfate group attached to glucosamine residues in heparin sulfate proteoglycans (54). The pathways controlled by this protein are closely related with inflammation through the production of interleukin-6 (55). *PRCP* gene encodes a member of the peptidase S28 involved in the degradation of angiotensin II, one of the main regulators of BP and electrolyte balance (56). *EHMT2* encodes a methyltransferase that methylates lysine residues of histone H3 which is also associated with cellular responses to starvation, negative regulation of transcription from RNA polymerase II promoter and regulation of DNA replication (57, 58). *SKOR2* gene is an homolog to the SKI family of transcriptional corepressors (59) and has been mainly identified as a potential tumor suppressor in neck squamous cell carcinomas (60). *PHGDH* encodes phosphoglycerate dehydrogenase, a key enzyme for de-novo sphingolipid synthesis, membrane lipids involved in lipid metabolism(61). *SLC7A11* encodes a sodium-independent cysteine/glutamate antiporter resulting in protection from oxidative stress and ferroptotic cell death (62). Further research is needed to determine the functional relevance of *EHMT2*, *SKOR2, PHGDH* and *SLC7A11* genes in the pathogenesis of hypertension.

## Age and gender-specific effects on epigenetic variations

DNA methylation gradually changes with age while gender-specific methylation patterns have been observed over the lifespan (63). Several studies reported higher global DNA methylation levels in males (64), whereas studies on gender-associated differences in DNA methylation at specific loci have yielded contrasting results (65). Among twenty studies, only three articles (with overlapping participants) stratified the analyses by gender (27, 31, 36). In Chinese Han population, DNA methylation of *ADD1* gene was significantly higher in females as compared to males, yet, *ADD1* promotor methylation was a risk factor in both, males (CpG2-5) and females (CpG1) (36). Similarly, *AGTR1* CpG1 methylation was a significant predictor of hypertension in both genders (31). Finally, at CpG1 and CpG2 sites of IL-6 promoter males were hypomethylated as compared to females, yet, only hypomethylation of CpG3 site was significantly associated with hypertension risk in both genders (27). Gender stratification in epigenetics is lacking, as seen in the current review as well, thus we are not able to make any conclusions regarding the role of gender-specific methylation patterns in hypertension risk.

In the context of aging, chronological age is one of the main determinants for functional impairments in blood pressure regulation. Until now, there is no evidence of the potential impact of the ‘epigenetic age’ on blood pressure. Considering that DNA methylation patterns change overtime and are highly correlated with age, they may contribute to age-related traits such as blood pressure. Therefore, further research on the impact of ‘biological age’ on blood pressure variability is warranted.

## Strengths and limitations

The strengths and limitations of the findings from this study merit careful consideration. The present analysis, involving data from nearly 28,382 individuals, is the first to systematically assess the evidence on the subject following an a priori designed protocol with clearly defined inclusion and exclusion criteria. However, as mentioned above, the majority of studies included are cross-sectional, making it difficult to determine whether epigenetic marks are a cause or a consequence of BP. Moreover, many epigenetic studies are often limited by the fact that, since it is the most accessible tissue in epidemiologic studies, only blood is studied rather than other more relevant tissues. Although the use of standardized and validated protocols allowed us to undertake a comprehensive search of the literature, we cannot exclude the possibility of publication bias from underreporting negative findings.

# Conclusions

The emerging evidence highlights the importance of epigenetic variation in the regulation and maintenance of blood pressure levels. The most convincing evidence has been reported from candidate-gene studies, where mechanisms related to RAS activation and inflammation can be assumed to represent a substrate for epigenetic regulation. Further studies integrating the systematic analysis of epigenetic markers at genomic scale, as well as the demonstration of the exact cellular and physiological role of target epigenetic modifications, will be needed to elucidate alternative molecular pathways.

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The contributions of the authors were as follows: VG, EP and MG screened title/abstract. VG obtained full text, determined eligibility of articles and participated in data extraction. VG and EP assessed the quality of the included studies. EP participated in data synthesis/analysis and interpretation of the data. VG, EP and JN drafted the final manuscript. All authors contributed to the critical revision of the manuscript and approved the final version.

# Conflicts of Interest

The authors declare no conflict of interest.

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**Table 1.** Global DNA methylation and blood pressure.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Author, year** | **Study Design** | **Outcome** | **PopulationGender/Age/Population/****Country** | **Tissue type** | **Adjustment level** | **Main findings** |
| **LINE-1 methylation** |  |  |  |  |
| Baccarelli et al., 2010(22) | CS and PS | Hypertension | M/55-92/n=712/USA | WB | Age | Inverse. LINE-1 methylation was inversely associated with an existing diagnosis of hypertension at baseline (age-adjusted OR=0.6 [0.3 to 1.0] for subjects in the lowest vs. highest quartile-based category of LINE-1 methylation). |
| Turcot et al., 2012(20) | CS | Blood pressure | M and W/35.1±7.73/n=186/ Canada | VAT | Age, sex, smoking, waist circumference | Inverse. Multiple linear regression analyses revealed that LINE-1% methylation was negatively associated with diastolic blood pressure (β =-0.65; P = 0.03) after adjustments for the effects of age, sex, waist circumference and smoking. |
| Alexeeff et al., 2013(16) | CS and PS | Blood pressure | M/74.1±6.7\*/n=789/ USA | WB | Age, BMI, smoking, pack-years of smoking, DM, alcohol consumption, race, IHD or stroke, number of neutrophils in white blood count, season, and day of week. | Inverse. The methylation of the gene for LINE-1 was inversely associated with DBP (β=-0.7, 95% CI -1.2, -0.2), yet the association with SBP was weaker, with the 95% CI including zero. |
| **ALU** |  |  |  |
| Alexeeff et al., 2013(16) | CS and PS | Blood pressure | M/74.1±6.7\*/n=789/ USA | WB | Age, BMI, smoking, pack-years of smoking, DM, alcohol consumption, race, IHD or stroke, number of neutrophils in white blood count, season, and day of week. | Positive. Both SBP and DBP were positively associated with the degree of methylation of the gene for Alu. An increase in inter-quartile range (IQR) in the methylation was associated with an increase of 0.97mm Hg in DBP (95% CI 0.32–1.57) and with an increase of 1.51mmHg in SBP (95% CI 0.36-2.61 ) |
| Bellavia et al., 2013(17) | CS | Blood pressure | M and W/27.7±8.6/ n=15/Canada | WB |  | Inverse. Decreased Alu methylation was associated with significantly increased DBP (β=0.41, P=0.04) and non-significantly increased SBP (β =0.40, P=0.15). |
| **5mC** |  |  |  |  |  |
| Smolarek et al., 2010 | CC | Essential hypertension | M and W/ 36.74± 10.59/ n=90/Poland | Blood | Age, sex, BMI, duration of disease, smoking, concentration of cholesterol, ALT, AST, glucose, and others (not specified). | Inverse. The mean level of 5mC was 1.80±0.69 in the healthy subjects, 1.14±0.48 in the whole group of patients with essential hypertension, 1.29±0.50 in the patients with stage 1, and 0.99±0.42 with stage 2 hypertension. |
| **mCyt/tCyt ratio** |  |  |  |  |  |
| Luttmer et al., 2013(23) | CS | Blood pressure, hypertension | M and W/68.7±7.2 /n=738/ The Netherlands | PBL | Age, sex, use of antihypertensive medication. | No association. Mean systolic and diastolic blood pressure were not associated to MC/C ratio, nor was the presence of hypertension, with or without adjustment for antihypertensive treatment. |

CS: cross-sectional; PS: prospective; M: men; WB: whole blood; W: women; VAT: visceral adipose tissue; BMI: body mass index; DM: diabetes mellitus; IHD: ischemic heart disease; SBP: systolic blood pressure; DBP: diastolic blood pressure; ALT: alanine aminotransferase; AST: aspartate aminotransferase; PBL: peripheral blood leukocytes.
\*Mean age from the original cohort from which the patients were taken.

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| **ALU** |  |  |  |
| Alexeeff et al., 2013(16) | CS and PS | Blood pressure | M/74.1±6.7\*/n=789/ USA | WB | Age, BMI, smoking, pack-years of smoking, DM, alcohol consumption, race, IHD or stroke, number of neutrophils in white blood count, season, and day of week. | Positive. Both SBP and DBP were positively associated with the degree of methylation of the gene for Alu. An increase in inter-quartile range (IQR) in the methylation was associated with an increase of 0.97mm Hg in DBP (95% CI 0.32–1.57) and with an increase of 1.51mmHg in SBP (95% CI 0.36-2.61 ) |
| Bellavia et al., 2013(17) | CS | Blood pressure | M and W/27.7±8.6/ n=15/Canada | WB |  | Inverse. Decreased Alu methylation was associated with significantly increased DBP (β=0.41, P=0.04) and non-significantly increased SBP (β =0.40, P=0.15). |
| **5mC** |  |  |  |  |  |
| Smolarek et al., 2010 | CC | Essential hypertension | M and W/ 36.74± 10.59/ n=90/Poland | Blood | Age, sex, BMI, duration of disease, smoking, concentration of cholesterol, ALT, AST, glucose, and others (not specified). | Inverse. The mean level of 5mC was 1.80±0.69 in the healthy subjects, 1.14±0.48 in the whole group of patients with essential hypertension, 1.29±0.50 in the patients with stage 1, and 0.99±0.42 with stage 2 hypertension. |
| **mCyt/tCyt ratio** |  |  |  |  |  |
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CS: cross-sectional; PS: prospective; M: men; WB: whole blood; W: women; VAT: visceral adipose tissue; BMI: body mass index; DM: diabetes mellitus; IHD: ischemic heart disease; SBP: systolic blood pressure; DBP: diastolic blood pressure; ALT: alanine aminotransferase; AST: aspartate aminotransferase; PBL: peripheral blood leukocytes.
\*Mean age from the original cohort from which the patients were taken.

Table 3. Gene methylation in candidate gene approach and blood pressure.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome**  | **Sample** | **Hypomethylated genes** | **Hypermethylated genes** | **Null association** |
|  | WB | *TLR4* |  | *F3, GCR, ICAM-1* |
| **SBP** | PBL | *ACE promoter* |  |  |
|  | VAT |  | *ADRB3* |  |
| **DBP** | WB | *TLR4, IFNγ* | *TRL2, iNOS,* | *F3, GCR, ICAM-1* |
| VAT |  | *ADRB3* |  |
|  | WB | *ADD1* |  |  |
| **Hypertension** | PB | *ADD1, ADD1 promoter, GCK, AGTR1, IL-6, NET promoter* |  | *ABCG1, GALNT2, HMGCR* |
|  | Saliva | *AGTR1* |  |  |

SBP: systolic blood pressure; WB: whole blood; PBL: peripheral blood leukocytes; VAT: visceral fat tissue;

DBP: diastolic blood pressure; PB: peripheral blood.

**S1 Appendix.** PRISMA checklist

|  |  |  |  |
| --- | --- | --- | --- |
| **Section/topic**  | **#** | **Checklist item**  | **Reported on page #**  |
| **TITLE**  |  |
| Title  | 1 | Identify the report as a systematic review, meta-analysis, or both.  | 1 |
| **ABSTRACT**  |  |
| Structured summary  | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.  | 2 |
| **INTRODUCTION**  |  |
| Rationale  | 3 | Describe the rationale for the review in the context of what is already known.  | 4 |
| Objectives  | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).  | 5 |
| **METHODS**  |  |
| Protocol and registration  | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.  | 5 |
| Eligibility criteria  | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.  | 6 |
| Information sources  | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.  | 5 |
| Search  | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.  | 5, Supplement material 2 |
| Study selection  | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).  | 6 |
| Data collection process  | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.  | 6-7 |
| Data items  | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.  | 7 |
| Risk of bias in individual studies  | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.  | 7 |
| Summary measures  | 13 | State the principal summary measures (e.g., risk ratio, difference in means).  | NA\* |
| Synthesis of results  | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I2) for each meta-analysis.  | NA\* |
| Risk of bias across studies  | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).  | NA\* |
| Additional analyses  | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.  | NA\* |
| **RESULTS**  |  |
| Study selection  | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.  | 8, Figure 1 |
| Study characteristics  | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.  | Table 1 and 2 |
| Risk of bias within studies  | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | 8 |
| Results of individual studies  | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.  | 8-11 |
| Synthesis of results  | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency.  | NA\* |
| Risk of bias across studies  | 22 | Present results of any assessment of risk of bias across studies (see item 15). | NA\* |
| Additional analysis  | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see item 16]).  | NA\* |
| **DISCUSSION**  |  |
| Summary of evidence  | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).  | 12-19 |
| Limitations  | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).  | 17-18 |
| Conclusions  | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research.  | 19-20 |
| **FUNDING**  |  |
| Funding  | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.  | 1 |

\*NA: not applicable

**S2 Appendix.** Search strategy

embase.com

(epigenetics/exp OR 'DNA methylation'/exp OR 'histone modification'/exp OR 's adenosylmethionine'/exp OR 'CpG island'/exp OR (((histone\* OR dna OR 'long interspersed') NEAR/3 (acetylat\* OR demethylat\* OR methylat\* OR phosphorylat\* OR ubiquitinat\* OR modif\*)) OR 's adenosylmethionine' OR cpg OR epigenetic\* OR epigenomic\*):ab,ti) AND ('abnormal blood pressure'/exp OR 'blood pressure'/exp OR (hypertensi\* OR hypotensi\* OR 'blood pressure\*'):ab,ti) NOT ([animals]/lim NOT [humans]/lim) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Editorial]/lim)

Medline (Ovid)

(Epigenomics/ OR DNA methylation/ OR S-Adenosylmethionine/ OR CpG Islands/ OR (((histone\* OR dna OR long interspersed) ADJ3 (acetylat\* OR demethylat\* OR methylat\* OR phosphorylat\* OR ubiquitinat\* OR modif\*)) OR s adenosylmethionine OR cpg OR epigenetic\* OR epigenomic\*).ab,ti.) AND (exp Hypertension/ OR exp Hypotension/ OR exp blood pressure/ OR (hypertensi\* OR hypotensi\* OR blood pressure\*).ab,ti.) NOT (exp animals/ NOT humans/) NOT (letter OR news OR comment OR editorial OR congresses OR abstracts).pt.

Cochrane

((((histone\* OR dna OR 'long interspersed') NEAR/3 (acetylat\* OR demethylat\* OR methylat\* OR phosphorylat\* OR ubiquitinat\* OR modif\*)) OR 's adenosylmethionine' OR cpg OR epigenetic\* OR epigenomic\*):ab,ti) AND ((hypertensi\* OR hypotensi\* OR 'blood pressure\*'):ab,ti)

Web-of-science

TS=(((((histone\* OR dna OR "long interspersed") NEAR/2 (acetylat\* OR demethylat\* OR methylat\* OR phosphorylat\* OR ubiquitinat\* OR modif\*)) OR "s adenosylmethionine" OR cpg OR epigenetic\* OR epigenomic\*)) AND ((hypertensi\* OR hypotensi\* OR "blood pressure\*")) NOT ((animal\* OR rat OR rats OR mouse OR mice OR murine OR dog OR dogs OR canine OR cat OR cats OR feline) NOT (human\* OR patient\* OR man OR men OR woman OR women))) AND DT=(Article)

Google scholar

"histone|dna methylation|modification"|epigenetics hypertension|hypotension|"blood pressure"