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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 the 7th of December 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 aetiology 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 dyslipidaemia, 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 the 7th of December 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* promoter and *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 correlates 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 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 aetiology 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.

ADD1 is a protein coding gene, part of a family of cytoskeletal proteins (48), known to increase renal sodium reabsorption and involved in the pathophysiology of hypertension in the Asian population (49). The renin angiotensin system is a crucial mechanism in the aetiology of hypertension. The epigenetic variability found in genes involved in this system, such as AGTR1 and ACE, encourages the design of better approaches at both population and experimental level to get more insight into these mechanisms.

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 (50). 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 (51). 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 (52). 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 (52).

DNA methylation may differ by race or ethnicity, challenging replication across individuals of varying descent in epigenetic studies (53). Previous epigenome wide association studies of several cardio metabolic risk factors for example, C-reactive protein, have been able to provide trans-ethnic replication of the differentially methylated genes (54). 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, epigenome equivalent of GWAS, alongside with the development of microarray technologies, has allowed the interrogation of DNA methylation sites at single-nucleotide resolution (55).

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 catalyses the hydrolysis of the 6-O-sulfate group attached to glucosamine residues in heparin sulfate proteoglycans (56). The pathways controlled by this protein are closely related with inflammation through the production of interleukin-6 (57). *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 (58). *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 (59, 60). *SKOR2* gene is an homolog to the SKI family of transcriptional corepressors (61) and has been mainly identified as a potential tumour suppressor in neck squamous cell carcinomas (62). *PHGDH* encodes phosphoglycerate dehydrogenase, a key enzyme for de-novo sphingolipid synthesis, membrane lipids involved in lipid metabolism(63). *SLC7A11* encodes a sodium-independent cysteine/glutamate antiporter resulting in protection from oxidative stress and ferroptotic cell death (64). 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 (65). Several studies reported higher global DNA methylation levels in males (66), whereas studies on gender-associated differences in DNA methylation at specific loci have yielded contrasting results (67). 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 also seen in this review, 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 over time 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.

References

1. Forouzanfar MH, Liu P, Roth GA, Ng M, Biryukov S, Marczak L, et al. Global Burden of Hypertension and Systolic Blood Pressure of at Least 110 to 115 mm Hg, 1990-2015. JAMA. 2017;317(2):165-82.
2. Collins R, Peto R, MacMahon S, Hebert P, Fiebach NH, Eberlein KA, et al. Blood pressure, stroke, and coronary heart disease. Part 2, Short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. Lancet. 1990;335(8693):827-38.
3. Jiang S-Z, Lu W, Zong X-F, Ruan H-Y, Liu Y. Obesity and hypertension. Experimental and Therapeutic Medicine. 2016;12(4):2395-9.
4. Graudal NA, Hubeck-Graudal T, Jurgens G. Effects of low sodium diet versus high sodium diet on blood pressure, renin, aldosterone, catecholamines, cholesterol, and triglyceride. Cochrane Database Syst Rev. 2017;4:CD004022.
5. Diaz KM, Shimbo D. Physical Activity and the Prevention of Hypertension. Current hypertension reports. 2013;15(6):659-68.
6. Kupper N, Willemsen G, Riese H, Posthuma D, Boomsma DI, de Geus EJC. Heritability of Daytime Ambulatory Blood Pressure in an Extended Twin Design. Hypertension. 2005;45(1):80-5.
7. Evangelou E, Warren HR, Mosen-Ansorena D, Mifsud B, Pazoki R, Gao H, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. Nature genetics. 2018;50(10):1412.
8. Warren HR, Evangelou E, Cabrera CP, Gao H, Ren M, Mifsud B, et al. Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. Nat Genet. 2017;49(3):403-15.
9. Wain LV, Vaez A, Jansen R, Joehanes R, van der Most PJ, Erzurumluoglu AM, et al. Novel Blood Pressure Locus and Gene Discovery Using Genome-Wide Association Study and Expression Data Sets From Blood and the Kidney. Hypertension. 2017.
10. Munroe PB, Barnes MR, Caulfield MJ. Advances in Blood Pressure Genomics. Circulation Research. 2013;112(10):1365-79.
11. Muka T, Koromani F, Portilla E, O'Connor A, Bramer WM, Troup J, et al. The role of epigenetic modifications in cardiovascular disease: A systematic review. Int J Cardiol. 2016;212:174-83.
12. Braun KV, Voortman T, Dhana K, Troup J, Bramer WM, Troup J, et al. The role of DNA methylation in dyslipidaemia: A systematic review. Prog Lipid Res. 2016;64:178-91.
13. Muka T, Nano J, Voortman T, Braun KVE, Ligthart S, Stranges S, et al. The role of global and regional DNA methylation and histone modifications in glycemic traits and type 2 diabetes: A systematic review. Nutr Metab Cardiovasc Dis. 2016;26(7):553-66.
14. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.
15. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol. 2010;25(9):603-5.
16. Alexeeff SE, Baccarelli AA, Halonen J, Coull BA, Wright RO, Tarantini L, et al. Association between blood pressure and DNA methylation of retrotransposons and pro-inflammatory genes. Int J Epidemiol. 2013;42(1):270-80.
17. Bellavia A, Urch B, Speck M, Brook RD, Scott JA, Albetti B, et al. DNA hypomethylation, ambient particulate matter, and increased blood pressure: Findings from controlled human exposure experiments. J Am Heart Assoc. 2013;2(3).

18. Kato N, Loh M, Takeuchi F, Verweij N, Wang X, Zhang W, et al. Trans-ancestry genome-wide association study identifies 12 genetic loci influencing blood pressure and implicates a role for DNA methylation. Nat Genet. 2015;47(11):1282-93.
19. European Society of Hypertension-European Society of Cardiology Guidelines C. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. J Hypertens. 2003;21(6):1011-53.
20. Turcot V, Tchernof A, Deshaies Y, Perusse L, Belisle A, Marceau S, et al. LINE-1 methylation in visceral adipose tissue of severely obese individuals is associated with metabolic syndrome status and related phenotypes. Clin Epigenetics. 2012;4(1):10.
21. Ehrlich M, Gama-Sosa MA, Huang LH, Midgett RM, Kuo KC, McCune RA, et al. Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells. Nucleic Acids Res. 1982;10(8):2709-21.
22. Baccarelli A, Wright R, Bollati V, Litonjua A, Zanobetti A, Tarantini L, et al. Ischemic heart disease and stroke in relation to blood DNA methylation. Epidemiology. 2010;21(6):819-28.
23. Luttmer R, Spijkerman AM, Kok RM, Jakobs C, Blom HJ, Serne EH, et al. Metabolic syndrome components are associated with DNA hypomethylation. Obes Res Clin Pract. 2013;7(2):e106-e15.
24. Smolarek I, Wyszko E, Barciszewska AM, Nowak S, Gawronska I, Jablecka A, et al. Global DNA methylation changes in blood of patients with essential hypertension. Med Sci Monit. 2010;16(3):CR149-55.
25. Guay SP, Brisson D, Lamarche B, Biron S, Lescelleur O, Biertho L, et al. ADRB3gene promoter DNA methylation in blood and visceral adipose tissue is associated with metabolic disturbances in men. Epigenomics. 2014;6(1):33-43.
26. Peng P, Wang L, Yang X, Huang X, Ba Y, Chen X, et al. A preliminary study of the relationship between promoter methylation of the ABCG1, GALNT2 and HMGCR genes and coronary heart disease. PLoS ONE. 2014;9(8).
27. Mao SQ, Sun JH, Gu TL, Zhu FB, Yin FY, Zhang LN. Hypomethylation of interleukin-6 (IL-6) gene increases the risk of essential hypertension: a matched case-control study. J Hum Hypertens. 2017;31(8):530-6.
28. Macias-Gonzalez M, Martin-Nunez GM, Garrido-Sanchez L, Garcia-Fuentes E, Tinahones FJ, Morcillo S. Decreased blood pressure is related to changes in NF-kB promoter methylation levels after bariatric surgery. Surg Obes Relat Dis. 2018;14(9):1327-34.
29. Bao XJ, Mao SQ, Gu TL, Zheng SY, Zhao JS, Zhang LN. Hypomethylation of the Interferon gamma Gene as a Potential Risk Factor for Essential Hypertension: A Case-Control Study. Tohoku J Exp Med. 2018;244(4):283-90.
30. Rangel M, Dos Santos JC, Ortiz PHL, Hirata M, Jasiulionis MG, Araujo RC, et al. Modification of epigenetic patterns in low birth weight children: Importance of hypomethylation of the ACE gene promoter. PLoS ONE. 2014;9(8).
31. Fan R, Mao S, Zhong F, Gong M, Yin F, Hao L, et al. Association of AGTR1 Promoter Methylation Levels with Essential Hypertension Risk: A Matched Case-Control Study. Cytogenet Genome Res. 2015;147(2-3):95-102.
32. Lin J, Lin S, Wu Y, Wang X, Wu S, Li H. Hypomethylation of the Angiotensin II Type I Receptor (AGTR1) Gene Along with Environmental Factors Increases the Risk for Essential Hypertension. Cardiology. 2017;137(2):126-35.
33. Fan R, Wang WJ, Zhong QL, Duan SW, Xu XT, Hao LM, et al. Aberrant methylation of the GCK gene body is associated with the risk of essential hypertension. Mol Med Rep. 2015;12(2):2390-4.

34. Meng L, Chen D, Pei F, Hui R, Zheng Y, Chen J. DNA methylation in the norepinephrine transporter gene promoter region is not associated with depression and hypertension. Clin Exp Hypertens. 2017;39(6):539-45.
35. Mao S, Fan R, Gu T, Zhong Q, Gong M, Dong C, et al. Hypermethylation of SCNN1A gene-body increases the risk of essential hypertension. Int J Clin Exp Pathol. 2016;9:8047-56.
36. Zhang LN, Liu PP, Wang L, Yuan F, Xu L, Xin Y, et al. Lower ADD1 Gene Promoter DNA Methylation Increases the Risk of Essential Hypertension. PLoS ONE. 2013;8(5).
37. Bayoumy NMK, El-Shabrawi MM, Leheta OF, Omar HH. alpha-Adducin gene promoter DNA methylation and the risk of essential hypertension. Clin Exp Hypertens. 2017:1-5.
38. Jin F, Li X, Wang Z, Liu Y, Liu J, Sun D, et al. Association of mitofusin 2 methylation and essential hypertension: a case-control study in a Chinese population. Hypertens Res. 2018;41(8):605-13.
39. Xu M, Li J, Chen X, Han L, Li L, Liu Y. MTHFD1 promoter hypermethylation increases the risk of hypertension. Clin Exp Hypertens. 2018:1-6.
40. Wang X, Falkner B, Zhu H, Shi H, Su S, Xu X, et al. A Genome-Wide Methylation Study on Essential Hypertension in Young African American Males. PLoS ONE. 2013;8(1).
41. Bostrom AE, Mwinyi J, Voisin S, Wu W, Schultes B, Zhang K, et al. Longitudinal genome-wide methylation study of Roux-en-Y gastric bypass patients reveals novel CpG sites associated with essential hypertension. BMC Med Genomics. 2016;9:20.
42. Richard MA, Huan T, Ligthart S, Gondalia R, Jhun MA, Brody JA, et al. DNA Methylation Analysis Identifies Loci for Blood Pressure Regulation. Am J Hum Genet. 2017;101(6):888-902.
43. Kresovich JK, Zhang Z, Fang F, Zheng Y, Sanchez-Guerra M, Joyce BT, et al. Histone 3 modifications and blood pressure in the Beijing Truck Driver Air Pollution Study. Biomarkers. 2017;22(6):584-93.
44. Gu Z, Wang H, Nekrutenko A, Li W-H. Densities, length proportions, and other distributional features of repetitive sequences in the human genome estimated from 430 megabases of genomic sequence. Gene. 2000;259(1):81-8.
45. Yang AS, Estécio MRH, Doshi K, Kondo Y, Tajara EH, Issa JPJ. A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. Nucleic acids research. 2004;32(3):e38-e.
46. Su J, Shao X, Liu H, Liu S, Wu Q, Zhang Y. Genome-wide dynamic changes of DNA methylation of repetitive elements in human embryonic stem cells and fetal fibroblasts. Genomics. 2012;99(1):10-7.
47. Hopkins PN, Hunt SC. Genetics of hypertension. Genetics in Medicine. 2003;5(6):413-29.
48. Matsuoka Y, Li X, Bennett V. Adducin: structure, function and regulation. Cellular and Molecular Life Sciences CMLS. 2000;57(6):884-95.
49. Liao X, Wang W, Zeng Z, Yang Z, Dai H, Lei Y. Association of alpha-ADD1 Gene and Hypertension Risk: A Meta-Analysis. Medical science monitor: international medical journal of experimental and clinical research. 2015;21:1634.
50. Ferrari P, Krozowski Z. Role of the 11beta-hydroxysteroid dehydrogenase type 2 in blood pressure regulation. Kidney Int. 2000;57(4):1374-81.
51. Mariniello B, Ronconi V, Sardu C, Pagliericcio A, Galletti F, Strazzullo P, et al. Analysis of the 11beta-hydroxysteroid dehydrogenase type 2 gene (HSD11B2) in human essential hypertension. Am J Hypertens. 2005;18(8):1091-8.
52. Huan T, Esko T, Peters MJ, Pilling LC, Schramm K, Schurmann C, et al. A meta-analysis of gene expression signatures of blood pressure and hypertension. PLoS Genet. 2015;11(3):e1005035.
53. Barfield RT, Almli LM, Kilaru V, Smith AK, Mercer KB, Duncan R, et al. Accounting for population stratification in DNA methylation studies. Genet Epidemiol. 2014;38(3):231-41.

54. Ligthart S, Marzi C, Aslibekyan S, Mendelson MM, Conneely KN, Tanaka T, et al. DNA methylation signatures of chronic low-grade inflammation are associated with complex diseases. Genome Biol. 2016;17(1):255.
55. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. Nature Reviews Genetics. 2011;12(8):529-41.
56. Morimoto-Tomita M, Uchimura K, Werb Z, Hemmerich S, Rosen SD. Cloning and characterization of two extracellular heparin-degrading endosulfatases in mice and humans. Journal of Biological Chemistry. 2002;277(51):49175-85.
57. Schelwies M, Brinson D, Otsuki S, Hong YH, Lotz MK, Wong CH, et al. Glucosamine-6-sulfamate Analogues of Heparan Sulfate as Inhibitors of Endosulfatases. ChemBioChem. 2010;11(17):2393-7.
58. Fyhrquist F, Metsärinne K, Tikkanen I. Role of angiotensin II in blood pressure regulation and in the pathophysiology of cardiovascular disorders. Journal of human hypertension. 1995;9:S19-24.
59. Lu Z, Tian Y, Salwen HR, Chlenski A, Godley LA, Raj JU, et al. Histone lysine methyltransferase EHMT2 is involved in proliferation, apoptosis, cell invasion and DNA methylation of human neuroblastoma cells. Anti-cancer drugs. 2013;24(5):484.
60. Shinkai Y, Tachibana M. H3K9 methyltransferase G9a and the related molecule GLP. Genes & development. 2011;25(8):781-8.
61. Minaki Y, Nakatani T, Mizuhara E, Inoue T, Ono Y. Identification of a novel transcriptional corepressor, Corl2, as a cerebellar Purkinje cell-selective marker. Gene Expression Patterns. 2008;8(6):418-23.
62. Bennett KL, Lee W, Lamarre E, Zhang X, Seth R, Scharpf J, et al. HPV status-independent association of alcohol and tobacco exposure or prior radiation therapy with promoter methylation of FUSSEL18, EBF3, IRX1, and SEPT9, but not SLC5A8, in head and neck squamous cell carcinomas. Genes, Chromosomes and Cancer. 2010;49(4):319-26.
63. Worgall TS. Sphingolipids: major regulators of lipid metabolism. Curr Opin Clin Nutr Metab Care. 2007;10(2):149-55.
64. Lewerenz J, Hewett SJ, Huang Y, Lambros M, Gout PW, Kalivas PW, et al. The cystine/glutamate antiporter system x(c)(-) in health and disease: from molecular mechanisms to novel therapeutic opportunities. Antioxid Redox Signal. 2013;18(5):522-55.
65. Boks MP, Derks EM, Weisenberger DJ, Strengman E, Janson E, Sommer IE, et al. The relationship of DNA methylation with age, gender and genotype in twins and healthy controls. PLoS One. 2009;4(8):e6767.
66. Fuke C, Shimabukuro M, Petronis A, Sugimoto J, Oda T, Miura K, et al. Age related changes in 5-methylcytosine content in human peripheral leukocytes and placentas: an HPLC-based study. Ann Hum Genet. 2004;68(Pt 3):196-204.
67. El-Maarri O, Becker T, Junen J, Manzoor SS, Diaz-Lacava A, Schwaab R, et al. Gender specific differences in levels of DNA methylation at selected loci from human total blood: a tendency toward higher methylation levels in males. Hum Genet. 2007;122(5):505-14.

Figure title and legend

Fig 1. Flowchart of studies included in the systematic review.

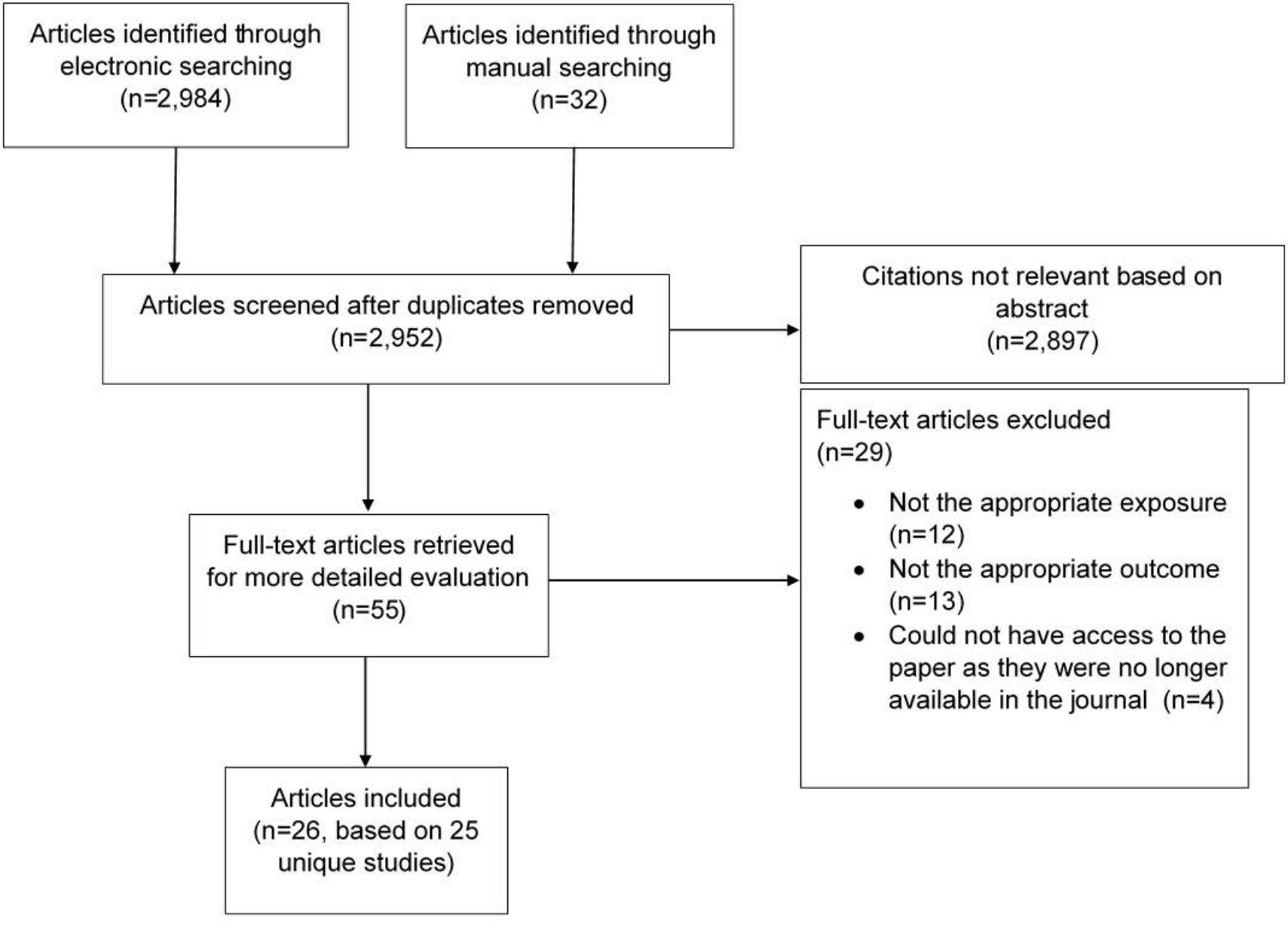
Articles identified through electronic searching (n=2,984)
Articles identified through manual searching (n=32)
Articles screened after duplicates removed (n=2,952)
Citations not relevant based on abstract (n=2,897)
Full-text articles retrieved for more detailed evaluation (n=55)
Full-text articles excluded (n=29)
Not the appropriate exposure (n=12)
Not the appropriate outcome (n=13)
Could not have access to the paper as they were no longer available in the journal (n=4)
Articles included (n=26, based on 25 unique studies)

Table 1. Global DNA methylation and blood pressure.

Author, year, quality*	Study design	Outcome	%male/Age/Sample size/ Country	Methylation sites/ method	Tissue type	Adjustment level	Main findings
LINE-1 methylation							
Baccarelli et al., 2010, 6/9 (22)	CS and PS	Hypertension	100/55-92/n=712/USA	Bisulfite PCR-Pyrosequencing	WB	Age	Inverse association. 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, 7/9 (20)	CS	Blood pressure	18.28/35.1±7.73/n=186/ Canada	Bisulfite PCR-Pyrosequencing	VAT	Age, sex, smoking, and waist circumference	Inverse association. 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, 7/9 (16)	CS and PS	Blood pressure	100/74.1±6.7**/n=789/ USA	Bisulfite PCR-Pyrosequencing	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 association. LINE-1 methylation was inversely associated with DBP (β=-0.7, 95% CI: -1.2, -0.2). The association with SBP was weaker, with the 95% CI including zero.
ALU							
Alexeeff et al., 2013, 7/9 (16)	CS and PS	Blood pressure	100/74.1±6.7**/n=789/ USA	Bisulfite PCR-Pyrosequencing	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 association. ALU methylation was positively associated with both SBP and DBP. An increase in inter-quartile range (IQR) in the methylation was associated with an increase of 0.97mmHg 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, 4/9 (17)	CS	Blood pressure	53.3/27.7±8.6/ n=15/Canada	*TLR4, IL-12, IL-6, iNOS/* Bisulfite PCR-Pyrosequencing	WB		Inverse association. 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, 5/9 (24)	CC	Essential hypertension	63.33/ 36.74± 10.59/ n=90/Poland	TLC analysis of the DNA nucleotide composition	Blood	Age, sex, BMI, duration of disease, smoking, concentration of cholesterol, ALT, AST, glucose, and others (not specified).	Inverse association. 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 hypertension, and 0.99±0.42 in patients with stage 2 hypertension.

mCyt/tCyt ratio							
Luttmer et al., 2013, 7/9 (23)	CS	Blood pressure, hypertension	49.5/68.7±7.2 /n=738/ The Netherlands	Liquid chromatography–tandem mass spectrometry.	PBL	Age, sex, and 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; WB: whole blood; VAT: visceral adipose tissue; BMI: body mass index; DM: diabetes mellitus; IHD: ischemic heart disease; DBP: diastolic blood pressure; SBP: systolic blood pressure; CC: case-control; TLC: thin-layer chromatography; ALT: alanine aminotransferase; AST: aspartate aminotransferase; PBL: peripheral blood leukocytes.

*Quality assessment based on the Newcastle-Ottawa Scale. Highest score: 9/9.

Mean age from the original cohort from which the patients were taken.**

Table 2. Gene-specific DNA methylation and blood pressure

Author, year, quality*	Study design	Outcome	Tissue type	%male /Age/ Sample size/Country	Methylation sites/ method	Adjustment level	Main findings
Bellavia et al., 2013, 4/9 (17)	CS	Blood pressure	WB	53.3/27.7±8.6/ n=15/Canada	*TLR4*/Bisulfite PCR-Pyrosequencing		Inverse association. Decreased *TLR4* methylation was associated with significant increases of both diastolic (β=0.84, p=0.02) and systolic blood pressure (β=1.45, p=0.01).
Alexeeff et al., 2013, 7/9 (16)	CS and PS	Blood pressure	WB	100/74.1±6.7**/n=789/USA	*TRL2, iNOS, IFNγ, F3, GCR, ICAM-1*/ Bisulfite PCR-Pyrosequencing	Age, BMI, smoking, pack-years of smoking, DM, alcohol consumption, race, IHD, number of neutrophils in with blood count, season, day of week.	They found a positive association between DBP and methylation of *TLR2* and iNOS, and a negative association between DBP and methylation of *IFNγ*. No clear associations were observed between SBP/DBP and methylation level of *ICAM-1, GCR* or *F3*.
Zhang et al., 2013, 6/9 (36)	CC	Essential hypertension (EH)	PB	50.1/50.2±5.3/ n=61/China	*ADD1*/Bisulfite PCR-Pyrosequencing	Adjusted for age, sex, smoking, and drinking.	Inverse association. *ADD1* CpG2-5 methylation levels were significantly associated with essential hypertension (cases versus controls (%): 27.54±7.48 versus 31.44±5.30, adjusted p=0.026).
Guay et al., 2014, 4/9 (25)	CS	Blood pressure	VAT	100/-/n=30/Canada	*ADRB3*/Bisulfite PCR-Pyrosequencing		Positive correlations. Partial Pearson´s correlations (r) between mean *ADRB3* DNA methylation in visceral adipose tissue and SBP and DBP: r=0.43, p=0.05 and r=00.45, p=0.04, respectively.
Peng et al., 2014, 8/9 (26)	CS	Hypertension	PB	64/59.39±9.14/ n=139/China	*ABCG1, GALNT2, HMGCR*/ Bisulfite PCR-Pyrosequencing	Age, sex, smoking, lipid level, history of hypertension and history of diabetes.	Treating gene methylation as a dichotomous variable (methylated or unmethylated), none statistically significant difference was found between patients with or without hypertension.
Rangel et al., 2014, 7/9 (30)	CS	Blood pressure	PBL	52/8.99±0.22/ n=115/Brazil	*ACE promoter*/ Bisulfite PCR-Pyrosequencing	Age, sex, birth weight, prematurity and family history of CVD.	Inverse association. Hypomethylation of the *ACE* promoter was associated with changes in SBP as well as *ACE* activity, even after adjustment for confounders. Pearson´s correlation coefficient: -0.206, p=0.031.
Fan et al., 2015, 6/9 (33)	CC	Essential hypertension	PB	M and W***/ 59.28±7.41/ n=94/China	*GCK,* 4CpGs/ Bisulfite PCR-Pyrosequencing	Age-matched	Significantly lower CpG 1-3 methylation (cases vs. controls, 49.13±5.72 vs. 53.49±7.53%; adjusted p=0.006) and significantly higher CpG4 methylation (cases vs. controls, 46.34±6.48 vs. 34.74±12.73%; adjusted p=0.002) were observed in patients with hypertension.

Kato et al., 2015, 6/9 (18)	CS	SBP, DBP, and hypertension	PB	74.2/54.6± 9.99/ n=6,757/South Asian and European population.	28 CpG/Bisulfite PCR-Pyrosequencing		Based on their GWAS analysis on five blood pressure phenotype, 35 sentinel SNPs were identified. Then, they investigated the relationship of them with local DNA methylation and found that 28 of the 35 SNPs were associated with local methylation markers. Then, using Mendelian randomization, they showed that the observed effects of SNPs on blood pressure were correlated with the effects predicted through association with methylation (r=0.52, p=0.005).
Fan et al., 2015, 7/9 (31)	CC	Essential hypertension (EH)	PB	40/ 56.52±8.47/ n=192/China	*AGTR1 promoter,* 5 CpGs/Bisulfite PCR-Pyrosequencing	Age, gender, smoking, drinking, BMI, triglycerides, HDL, uric acid and homocysteine.	Inverse association. A significantly lower CpG1 methylation level was identified in EH cases compared to controls (cases vs. controls: 6.74 ± 4.32% vs. 9.66 ± 5.45%, p = 0.007), and no significant association was observed in the remaining analyses. Receiver operating characteristic curves showed that CpG1 methylation was a significant predictor of EH.
Mao et al., 2016, 7/9 (35)	CC	Essential hypertension (EH)	PB	35/ 57.83±7.74/ n=180/China	*SCNN1A*/Bisulfite PCR-Pyrosequencing	Age, sex, gender, BMI, TC, TG, glucose, ALT, smoking and drinking.	Positive association. Incident cases had a higher *SCNN1A* methylation level than the non-EH controls (16.15±4.51 versus 13.66±4.08, p=0.041) and prevalent cases (16.15±4.51 versus 13.77±3.90, p=0.002). Logistic regression analysis results showed that *SCNN1A* hypermethylation was the risk factor of EH in incident cases compared with non-EH (OR=1.157, p=0.01), and in incident cases compared with prevalent cases (OR=1.149, p=0.013).
Bayoumy et al., 2017, 5/9 (37)	CC	Essential hypertension (EH)	WB	48/52.6±5.02/ n=250/Egypt	*ADD1 promoter*/ Bisulfite PCR-Pyrosequencing		Inverse association. Lower methylation of *AAD1* CpG2-5 was found among EH cases (29.21±6.81) compared to the healthy group (34.63±7.5).
Lin et al., 2017, 6/9 (32)	CC	Essential hypertension	Saliva	51.4/40.76± 16.92*/ n=326/China	*AGTR1*/Bisulfite PCR-Pyrosequencing	Age, sex, education level, marital status, physical activity, diet regularity, smoking and drinking status, and sleep duration and quality.	Inverse association. There was a decrease in DNA methylation in the hypertensive group compared to the control group.

Mao et al., 2017, 6/9 (27)	CC	Essential hypertension (EH)	PB	40/ 56.5± 8.5/ n=192/China	*IL-6*/Bisulphite pyrosequencing	Age- and gender-matched	Inverse association. CpG2 and CpG3 had lower methylation in EH group compared with controls (58.43 ± 7.53 versus 62.34 ± 9.65, p=0.004 and 51.52 ± 6.18 versus 57.45 ± 8.29, p<0.001, respectively). Logistic regression analysis found that CpG3 hypomethylation was a risk factor of EH (OR= 1.11, adjusted p=0.004). Receiver operating characteristic curve analysis showed that CpG2 (area under the curve: 0.638, p=0.001) and CpG3 (area under the curve: 0.704, p<0.001) had a diagnostic value to predict the risk of EH.
Meng et al., 2017, 6/9 (34)	CS	Hypertension	PBL	85.4/ 45.1±7.43/ n=162/China	*NET promoter*/ Pyrophosphate sequencing	Age and BMI	Inverse association. The average and specific methylation levels were higher in non-hypertensive subjects except for CpG2.
Bao et al., 2018, 6/9 (29)	CC	Essential hypertension (EH)	PB	39.6/ 56.5±8.43/ n=192/China	*IFNγ promoter*, 6 CpGs/ pyrosequencing	Age, sex, smoking, drinking, uric acid, HDL and BMI	CpG2 was significantly hypomethylated among cases compared controls (p=0.032) and it was found to be an effective marker of EH based on the area under the curve.
Jin et al., 2018, 7/9 (38)	CC	Essential hypertension	WB or serum	59.2/ 50.6±2.54/ n=76/China	*Mfn2*/ Bisulphite DNA sequencing	Age- and sex-matched	The DNA methylation level of *Mfn2* was significantly lower in hypertensive patients than in controls.
Macías-González et al., 2018, 6/9 (28)	PS	Blood pressure	PBMC	34.6/ 44.68±9.27/ n=60/Spain	*PPARγ, SLC19A1, IL-6, NFKB1*/ pyrosequencing	Age, sex, bariatric procedure, weight loss (%)	There was no statistically significant difference between the DNA methylation patterns of the *PPARγ, SLC19A1* and *IL-6* genes before and 6 months after bariatric surgery. The promoter methylation levels of the *NFKB1* gene were increased after surgery. This change of methylation level was associated with changes in both SBP and DBP (r=-0.513, p=0.003 and r=-0.544, p=0.002, respectively).
Xu et al., 2018, 6/9 (39)	CC	Essential hypertension	Serum	53.3/ 65.9±9.2 /n=461/China	*MTHFD1 promoter*/ methylation-specific PCR	Age, gender, total homocysteine, uric acid, TG, BMI, glucose, waist circumference, hip circumference, SBP, DBP, drinking, smoking.	The *MTHFD1* promoter methylation was higher in hypertensive patients than healthy controls (median PMR were 8.97% and 5.69%, respectively, p<0.001). Multivariable analysis showed that *MTHFD1* promoter hypermethylation increases the risk of essential hypertension (OR=1.336; 95% CI: 1.235, 1.446; p< 0.001). The area under the curve of *MTHFD1* promoter methylation was 0.739 in total patients with essential hypertension.

CS: cross-sectional; WB: whole blood; PS: prospective; BMI: body mass index; DM: diabetes mellitus; IHD: ischemic heart disease; DBP: diastolic blood pressure; SBP: systolic blood pressure; CC: case-control; PB: peripheral blood; VAT: visceral adipose tissue; PBL: peripheral blood leukocytes; CVD: cardiovascular disease; M: men; W: women; HDL: high-density-lipoprotein; TC: total cholesterol; TG: triglycerides; ALT: alanine aminotransferase; PBMC: peripheral blood mononuclear cells; PMR: percentage of methylated reference.

*Quality assessment based on the Newcastle-Ottawa Scale. Highest score: 9/9.

**Mean age from the original cohort from which the patients were taken.

***Percentage of men not described.

Table 3. Epigenome-wide association and histone modification in relation to blood pressure.

Author, year, quality*	Study design	Outcome	Tissue type	%male/Age/ Sample size/Country	Methylation sites/ method	Adjustment level	Main findings
Epigenome-Wide Association Study							
Wang et al., 2013, 6/9 (40)	CC	Essential Hypertension (EH)	PB	100/14-23/n=16/ USA	Illumina HumanMethylation 27K BeadChip	Age	7 out of the 10 most significant CpG sites were hypomethylated in cases. The two most significant CpGs (one CpG site in *SULF1* gene and one in *PRCP* gene) were replicated in 96 patients. CpG in *SULF1* remained significant even after adjustment for age (p=0.038). Validation of the CpG sites in the *SULF1* gene was further conducted in a second replication sample of 70 patients and it was not found to be significantly different methylated among cases vs controls.
Boström et al., 2016, 6/9 (41)	CS and PS	Blood pressure and essential hypertension (EH)	WB	49.8/46.9±11.9/n =11/Switzerland	Illumina HumanMethylation 450K BeadChip	Age, sex, BMI and ethnicity.	In case of 24 CpG sites, changes in methylation was significantly correlated with the percentile change in SBP six months after RYGB surgery. Those CpG were further investigated for an association with EH in the verification cohort (n=539, aged 19 to 101 years), finding two CpG (one in *EHMT2* and one in *SKOR2*) significantly hypomethylated in EH.
Richard et al., 2017, 8/9 (42)	CS	Blood pressure (BP)	WB and CD4+ T cells**	M and W***/ mean age between 46.3 and 76.0/n=17,010/C onsortia	Illumina HumanMethylation 450K BeadChip	Age, sex, blood cell counts, BMI, smoking, ancestry and technical covariates.	In the discovery stage, they conducted genome-wide associations of DNA methylation with SBP and DBP in nine cohort studies (n=9,828). Multiethnic meta-analyses identified methylation at 31 CpG sites associated with BP after Bonferroni correction. They replicated those 31 CpG in multiethnic meta-analyses of six additional cohorts (n=7,182). Methylation at 13 of the 31 discovery CpG sites (corresponding to 8 genes) was associated with BP at p<0.0016 in the replication meta-analysis (0.05/31). All of the 13 CpG 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. The investigators found a mediation of a causal relationship of cg23999170 with BP through expression of *TSPAN2.*
Histone modification							

Kresovich et al., 2017, 6/9 (43)	CS	Blood pressure	WB	67/18-46/ n=240/China	Histone 3 lysine 9 acetylation (H3K9ac), histone 3 lysine 9 tri-methylation (H3K9me3), histone 3 lysine 27 tri-methylation (H3K27me3), and histone 3 lysine 36 tri-methylation (H3K36me3)	Age, sex, occupational group, BMI, work hours per week, day of the week, smoking habits, number of cigarettes smoked during examination time, alcohol drinking status, temperature, and 8-day ambient PM10.	Inverse association. In all participants, a one fold increase in H3K9ac was associated with 2.52mmHg lower mean SBP (95%CI: -4.22, -0.81, p<0.01) and 1.54 mmHg lower mean MAP (95%CI: -2.95, -0.14, p=0.03). A one-fold increase in H3K9me3 was associated with 2.04mmHg lower mean SBP (95%CI: -3.32, -0.77, p<0.01), 1.68 mmHg lower mean DBP (95%CI: -2.84, -0.52, p=0.01), and 1.75 mmHg lower mean MAP (95%CI:-2.86, - 0.64, p<0.01). Finally, the authors observed a one-fold increase in H3K27me3 associated with 2.2 8mmHg lower SPB (95%CI:-4.42, -0.13, p=0.04).

CC: case-control; PB: peripheral blood; CS: cross-sectional; PS: prospective; WB: whole blood; BMI: body mass index; RYGB: Roux-en-Y gastric bypass surgery; M: men; W: women; SBP: systolic blood pressure; DBP: diastolic blood pressure.

*Quality assessment based on the Newcastle-Ottawa Scale. Highest score: 9/9.

**Of the 14 cohorts, 13 used whole blood samples to measure DNA methylation. One cohort (GOLDN) used CD4+ T cells.

***Percentage of men not described.