Original Article

Association between placental global DNA methylation and blood pressure during human pregnancy

Sulistyo E. Dwi Putra[a,b,*], Christoph Reichetzeder[c,*], Karoline von Websky[a,c], Corinna Neuber[c], Horst Halle[d], Burkard Kleuser[e], Bernhard K. Krämer[a,f], and Berthold Hocher[a,g,h,i]

Objective: Gene-specific placental DNA methylation patterns differ between normal pregnancies and pregnancies complicated by hypertension. However, whether global placental DNA methylation is associated with maternal blood pressure remains controversial.

Methods: Using multiple linear regression models, we analysed the association between maternal mean arterial pressure (MAP) at the third trimester of pregnancy and global DNA methylation in the placenta in 922 mothers using LC-ESI-MS/MS. To better characterize the contribution of genetic or epigenetic mechanisms, we performed isolated analyses in mothers with and without a family history of hypertension.

Results: Mean placental global DNA methylation was $3.00 \pm 0.46\%$. A significant negative correlation between placental global DNA methylation and mean arterial blood pressure (MAP) in the third trimester could be observed ($P = 0.023$, $r = -0.075$). This association remained significant after adjusting for confounders. In placenta samples from mothers with a family history of hypertension, mean maternal MAP was higher (86.1 ± 8.1 vs. 84.6 ± 7.5, $P < 0.01$) and placental global DNA methylation was lower (2.94 ± 0.43 vs. 3.04 ± 0.47, $P < 0.01$) compared with samples without a family history of hypertension. Furthermore, the significant independent negative correlation between global placental DNA methylation and MAP was only found in mothers without a family history of hypertension.

Conclusion: This study showed an independent negative correlation between placental global DNA methylation and maternal MAP in mothers without a family history of hypertension.

Keywords: family history of hypertension, gestational blood pressure regulation, gestational hypertension, global DNA methylation, placenta

Abbreviations: LC-ESI-MS/MS, liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry; MAP, mean arterial pressure

INTRODUCTION

Adaptations of the maternal cardiovascular system during pregnancy consist of distinct changes in heart rate and blood pressure. Early on in pregnancy systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP) decrease. After this gradual decrease of blood pressure, typically including a mid-trimester drop between week 16 and 20, a gradual rise to the initial levels from before pregnancy occurs [1,2]. These changes in blood pressure are accompanied by other haemodynamic alterations such as an increase in cardiac output and stroke volume, and a marked reduction in systemic vascular resistance in early pregnancy, followed by a continuous increase of heart rate and total vascular resistance at term [3,4]. Several mechanisms have been suggested for these cardiovascular changes during pregnancy [5]. It is known that the placenta has a crucial role in the endocrine, paracrine and autocrine regulation of systemic and local cardiovascular changes during pregnancy [6,7]. A complete understanding of the mechanisms by which the placenta exerts its multiple functions on maternal and foetal circulation remains to be determined, but available data suggest that epigenetic regulation might be involved [8].

Examination of epigenetic alterations and their influence on physiological and pathophysiological processes has received increased interest in current research. Epigenetics is a term for heritable changes in gene activity and expression that occur without any changes in the DNA sequence

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[a]Fifth Department of Medicine (Nephrology/Endocrinology/Rheumatology/Pneumology), University Medical Centre Mannheim, University of Heidelberg, Heidelberg, Germany, [b]University of Surabaya, Surabaya, Indonesia, [c]Institute of Nutritional Science, Department of Nutritional Toxicology, University of Potsdam, Nuthetal, [d]Department of Obstetrics and Gynecology, Charité University Hospital, Campus Mitte, [e]Institute of Pharmacy, Department of Pharmacology, Freie Universität Berlin, Berlin, [f]European Center for Angioscience ECAS, Medical Faculty Mannheim of the University of Heidelberg, Mannheim, Germany, [g]Key Laboratory of Study and Discovery of Small Targeted Molecules of Hunan Province, School of Medicine, Hunan Normal University, [h]Reproductive and Genetic Hospital of CITIC-Xiangya, Changsha, China and [i]Institute of Medical Diagnostics (IMD), Berlin, Germany

Correspondence to Berthold Hocher, Fifth Department of Medicine (Nephrology/Endocrinology/Rheumatology/Pneumology), University Medical Centre Mannheim, University of Heidelberg, 69047 Heidelberg, Germany. E-mail: berthold.hocher@medma.uni-heidelberg.de

*Both Sulistyo E. Dwi Puthra and Christoph Reichetzeder are equally contributing first authors.

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of the gene. DNA methylation is a well known and widely studied epigenetic mechanism [9]. Studies on epigenetic regulation in the placenta, especially in the context of gestational blood pressure regulation, are still scarce. Furthermore, available studies investigating a link between epigenetic regulation in the placenta and maternal blood pressure yielded conflicting results [10,11]. The majority of these studies were mainly focused on preeclampsia, a hypertensive disorder of pregnancy, rather than investigating the association of epigenetic mechanisms and physiologic blood pressure regulation. Therefore, the present study was conducted to elucidate in a large cohort of pregnant women, if the placental methylome may influence or may itself be influenced by physiologic adaptions of maternal blood pressure regulation during pregnancy. Furthermore, the contribution of genetic factors to this association was investigated in subgroups of mothers with and without a family history of hypertension. As the main parameter reflecting maternal blood pressure, MAP was chosen, which is calculated using SBP and DBP. Studies have shown that this blood pressure parameter is better in predicting blood pressure related disease with placental changes such as preeclampsia or gestational hypertension than either SBP or DBP [12,13].

MATERIALS AND METHODS

Clinical study

The study was approved by the Institutional Review Board of the University Hospital of Charité Berlin, Germany. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. Data were collected from 2000 to 2008. This observational study included 1063 mothers delivering at the Charité Obstetrics Department [14–16]. Structured interviews with mothers taking part in the study were conducted after obtaining their written consent. Relevant clinical data were obtained from the 'Mutterpass' (pregnancy health document). As the focus of this study was set on placental methylation in relation to gestational maternal blood pressure regulation, we did not include any mothers with diabetes mellitus before or during pregnancy, with preexisting hypertension or with preeclampsia. Cases with missing data on SBP, DBP measurements or family history of hypertension were also excluded from further analysis. At the end, analyses were performed using data from 922 mothers. Data from a questionnaire were used to obtain ethnicity, smoking status before and during pregnancy and family history of hypertension. Family history of hypertension was defined as at least one of the first-degree relatives suffering from hypertension. Routine antenatal check-ups were used to collect blood pressure data. Blood pressure was measured with a mercury or aneroid sphygmomanometer. DBP was defined based on Korotkoff-phase V. If sounds were still audible when the cuff was fully deflated, Korotkoff -phase IV was used. MAP was calculated as MAP = DBP + 1/3 (SBP − DBP) [17].

Perinatal parameters such as sex of the newborn, birth size and APGAR score were added as well. Gestational age of the newborn was calculated based on the last menstruation. Placental weight was assessed including the umbilical cord. Standardized placenta samples (one cotyledon from similar locations) were obtained and immediately frozen and stored at −20 °C.

DNA extraction and hydrolysis

DNA was extracted using a QIAamp DNA Mini Kit from Qiagen (Hilden, Germany) together with an RNase A digestion according to the manufacturer's protocol. Concentration and quality of DNA solution were determined with a NanoDrop ND-1000 spectrophotometer. DNA hydrolysis was performed using DNA Degradase Plus from Zymo Research (Freiburg, Germany) as indicated in the commercial protocol. Hydrolysis reaction was stopped by adding 75 µl of 0.1% formic acid. The completeness of the reaction was confirmed by agarose gel electrophoresis of digested DNA. Before sample injection to the liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry (LC-ESI-MS/MS) instrument, 70 µl of digested DNA were diluted to 280 µl 0.1% formic acid to yield a final concentration of 2 ng digested DNA/µl.

Liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry

Placental methylation was quantified as described elsewhere [18]. Briefly, LC-ESI-MS/MS was performed usinga HPLC system (Agilent 1200 series) connected to an Accurate-Mass Q-TOF instrument (Agilent 6530) with Jet Stream-Interface (Waldbronn, Germany). Chromatographic separation was done in a Waters X-Bridge column, C18 4.6 mm x 150 mm, with particle size 3.5 µm (Milford, Massachusetts, USA) protected by a Waters X-Bridge column guard, C18 4.6 mm x 20 mm with particle size 5 µm. Two different mobile phases were used: solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol). Prior to sample injection, a linear gradient elution was performed using 4–20% of solvent B for 10 min at a constant flow rate of 0.5 ml/min. The injection volume was 50 µl and typically contained 100 ng digested DNA. The optimized ESI-MS/MS parameters in positive ion mode were as follows: gas temperature, 250°C; drying gas flow, 8 l/min; nebulizer pressure, 60 psig; sheat gas temperature, 300°C; capillary voltage 4000 V; collision energy 7 V for dC, 13 V for 5mdC and 10 V for dG. Selected reaction monitoring (SRM) mode at m/z 228.0979/112.0505 for dC, m/z 242.1135/126.0662 for 5mdC were used to obtain a signal needed for quantification. The percentage of global methylation was calculated as follows: Methylation % = 5-methyl-2′-deoxycytidine (5mdC)/ [5-methyl-2′-deoxycytidine (5mdC) + 2′-deoxycytidine (dC)] × 100%.

Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, New York, USA). Pearson correlation analysis was used to identify correlations between two parameters followed by multiple linear regression analyses, in case two parameters were significantly correlated. Data stratification was conducted whenever necessary. According to normal distribution of the data, Student's *t*-test or Mann–Whitney *U*-test was performed to compare mean values between two groups

TABLE 1. Descriptive data of all mother/child pairs grouped according to family history of hypertension (FH,∗ comparison between 'FH' and 'No FH')

Maternal/Child characteristics	All samples ($N=922$)	Presence of FH		
		No FH ($N=571$)	FH ($N=351$)	P
Placental methylation, %	3.00 ± 0.46	3.04 ± 0.47	2.94 ± 0.43	0.001
Gestational age at delivery (weeks)	38.7 ± 2.1	38.8 ± 1.9	38.8 ± 1.9	0.879
Age of the mother (years)	29.9 ± 5.8	29.6 ± 6.1	30.1 ± 5.5	0.216
BMI at beginning of pregnancy (kg/m^2)	22.8 ± 4.2	24.0 ± 4.9	24.5 ± 4.8	0.080
Mother body weight third trimester (kg)	76.1 ± 12.9	75.7 ± 13.5	76.7 ± 11.7	0.075
SBP third trimester (mmHg)	115.6 ± 10.6	114.9 ± 10.2	116.7 ± 11.2	0.015
DBP third trimester (mmHg)	70 ± 7.2	69.4 ± 7.0	70.8 ± 7.4	0.006
MAP third trimester (mmHg)	85.2 ± 7.7	84.6 ± 7.5	86.1 ± 8.1	0.005
Hypertension during pregnancy, %	3.3	1.2	6.6	<0.001
Smoking before/during pregnancy, %	35.3/13.9	36.7/15.0	32.8/12.0	0.225/0.290
Family history of diabetes, %	36.2	26.6	52.0	<0.001
Ethnicity, White/non-White, %	93.2/6.8	91.7/8.3	96.5/3.5	0.004
Child birth weight (g)	3330.7 ± 629.9	3343.9 ± 623.0	3330.6 ± 585.3	0.750
Child head circumference (cm)	34.7 ± 1.7	34.6 ± 1.8	34.8 ± 1.6	0.217
Child birth length (cm)	50.6 ± 3.2	50.7 ± 3.0	50.6 ± 3.1	0.507
Ponderal index	25.6 ± 4.1	25.5 ± 3.4	25.8 ± 5.1	0.367
Preterm birth, %	9.7	9.1	10.5	0.474
APGAR 5 min	9.3 ± 1	9.3 ± 0.9	9.3 ± 1.1	0.843
APGAR 10 min	9.6 ± 0.8	9.6 ± 0.7	9.6 ± 0.9	0.605
Sex of the child, male/female, %	51.8/48.2	53.4/46.6	50.4/49.6	0.378

Data are given as mean ± SD or %.
FH, family history of hypertension.

in subgroup analyses. The authors had full access to the data and take full responsibility for its integrity.

RESULTS

Nine hundred and twenty-two mothers were included into the study. Detailed descriptive data of the cohort of pregnant women are summarized in Table 1. All study participants were normotensive in the first trimester of pregnancy. The mean maternal MAP was 85.2 ± 7.7 mmHg in the third trimester of pregnancy. SBP and DBP means in the third trimester of pregnancy were 115.6 ± 10.6 and 70 ± 7.2 mmHg, respectively (for details see Table 2). Other parameters including age of the mother (29.9 ± 5.8 years), gestational age at delivery (38.7 ± 2.1 weeks) and BMI at the beginning of pregnancy (22.8 ± 4.2 kg/m^2) were calculated as well. Birth outcome parameters were within normal ranges, including birth weight (3330.7 ± 629.9 gram), head circumference (34.7 ± 1.7 cm), birth length (50.6 ± 3.2 cm), ponderal index (25.6 ± 4.1 g/cm), APGAR 5 min postnatal (9.3 ± 1.0) and APGAR 10 min postnatal (9.6 ± 0.8). All of the above-mentioned parameters are indicative of an inconspicuous cohort of mothers and newborns. The degree of placental methylation ranged between 2.0 and 4.8%, with a mean of 3.00 ± 0.46%.

Placental methylation showed a significant negative correlation with maternal MAP in the third trimester of pregnancy after Pearson correlation analysis ($P=0.023$; $r=-0.075$). Similar findings were made for SBP ($P=0.034$; $r=-0.070$) and DBP ($P=0.029$; $r=-0.072$). Stratifying placental methylation into quintiles, indicated a linear behaviour of MAP in relation to placental methylation (Fig. 1a). To investigate the contribution of genetic factors to the observed association, data were stratified into mothers with and without a family history of hypertension. Details regarding descriptive statistics of these subgroups are summarized in Table 1. Interestingly, the presence or absence of a family history of hypertension had a significant impact on global placental methylation levels. A family history of hypertension was associated with lower placental methylation compared with samples without a family history of hypertension (2.94% ± 0.43 vs. 3.04% ± 0.47%, $P=0.001$, Fig. 2a). MAP was higher in the family history of hypertension group than in the group without a family history of hypertension (86.1 ± 8.1 vs. 84.6 ± 7.5 mmHg, $P=0.005$, Fig. 2b and Table 1). Other parameters that were significantly different between mother with and without a family history of hypertension included SBP (116.7 ± 11.2 vs. 114.9 ± 10.2, $P=0.015$), DBP (70.8 ± 7.4 vs. 69.4 ± 7.0, $P=0.006$), hypertension during pregnancy (6.6 vs. 1.2%, $P<0.001$), family history of diabetes (52.0 vs. 26.6%, $P<0.001$) and ethnicity (white/non-white, 96.5/3.5 vs. 91.7/8.3%, $P=0.004$).

Univariate Pearson correlation analyses showed a significant negative correlation between MAP in the third trimester of pregnancy and placental methylation in mothers without a family history of hypertension ($P=0.021$, $r=-0.098$) but not in mothers with a family history of hypertension ($P=0.675$, $r=-0.023$). This was also observed for SBP (mothers without a family history of hypertension: $P=0.030$, $r=-0.091$; mothers with a family history of hypertension: $P=0.698$, $r=-0.021$) but not for DBP (mothers without a family history of hypertension: $P=0.061$, $r=-0.078$; mothers with a family history of hypertension: $P=0.560$, $r=-0.031$) Stratifying placental methylation data into quintiles showed similar results, with a stronger negative correlation between placental methylation and MAP of third trimester in samples without a family history of hypertension (Fig. 1b) compared with a family history of hypertension (Fig. 1c).

TABLE 2. Regression analyses of the association between placental methylation (%) and mean arterial pressure of the third trimester of pregnancy (mmHg) as dependent variable

(a) Entire population ($r^2 = 0.204$)					
Independent variable	**B**	**Beta**	***P***	**(95% CI for B)**	
Placental methylation, %	−1.26	−0.08	0.025	−2.35	−0.16
Age of the mother (years)	−0.06	−0.04	0.235	−0.15	0.04
Gestational age at delivery (weeks)	−0.14	−0.04	0.332	−0.43	0.15
BMI at beginning of pregnancy (kg/m^2)	−0.07	−0.04	0.533	−0.30	0.16
Mother body weight third trimester (kg)	0.21	0.34	<0.001	0.14	0.28
Sex of the child	−0.92	−0.06	0.093	−2.00	0.15
Ethnicity	−2.04	−0.07	0.047	−4.05	−0.03
Hypertension during pregnancy	10.40	0.24	<0.001	7.36	13.44
Family history of diabetes	0.61	0.04	0.296	−0.53	1.75
(b) No FH subgroup ($r^2 = 0.137$)					
Independent variable	**B**	**Beta**	***P***	**(95% CI for B)**	
Placental methylation, %	−1.54	−0.10	0.027	−2.89	−0.18
Age of the mother (years)	−0.04	−0.03	0.511	−0.15	0.07
Gestational age at delivery (weeks)	−0.09	−0.02	0.633	−0.46	0.28
BMI at beginning of pregnancy (kg/m^2)	0.03	0.01	0.849	−0.24	0.29
Mother body weight third trimester (kg)	0.17	0.31	<0.001	0.09	0.26
Sex of the child	−0.81	−0.05	0.243	−2.17	0.55
Ethnicity	−2.19	−0.09	0.064	−4.52	0.13
Hypertension during pregnancy	11.85	0.18	<0.001	5.74	17.95
Family history of diabetes	−0.16	−0.01	0.837	−1.73	1.40
(c) FH subgroup ($r^2 = 0.231$)					
Independent variable	**B**	**Beta**	***P***	**(95% CI for B)**	
Placental methylation, %	−0.41	−0.02	0.681	−2.35	1.54
Age of the mother (years)	−0.10	−0.07	0.253	−0.27	0.07
Gestational age at delivery (weeks)	−0.26	−0.06	0.273	−0.74	0.21
BMI at beginning of pregnancy (kg/m^2)	−0.28	−0.12	0.221	−0.73	0.17
Mother body weight third trimester (kg)	0.29	0.41	<0.001	0.15	0.42
Sex of the child	−1.09	−0.07	0.236	−2.89	0.72
Ethnicity	−1.67	−0.05	0.431	−5.85	2.51
Hypertension during pregnancy	9.52	0.30	<0.001	5.71	13.34
Family history of diabetes	1.34	0.08	0.155	−0.51	3.20

Multiple linear regression models were used to further analyse the correlation between placental methylation and maternal MAP in the third trimester of pregnancy. The following confounders were used based on literature: age and ethnicity of the mother, and sex of the child [19–22]. In addition, maternal BMI, body weight in the third trimester of pregnancy, family history of diabetes and presence or absence of hypertension during pregnancy were included into the models, as these parameters are known to correlate with birth outcomes and blood pressure and/or were significantly different in mothers with and without FH [23–25].

In the multiple linear regression model including the entire study population, placental methylation was significantly correlated with maternal MAP in the third trimester of pregnancy ($P = 0.025$, model A, Table 2). However, multiple linear regression analyses highlighted an independent negative correlation between MAP in the third trimester of pregnancy and placental methylation for mothers without FH ($P = 0.027$, Table 2). In contrast, no correlation between placental methylation and MAP in the third trimester of pregnancy was observed in the group with a family history of hypertension ($P = 0.681$, Table 2). In multiple linear regression models, global placental DNA methylation was significantly associated with DBP ($P = 0.030$, supplemental Table 3, http://links.lww.com/HJH/B884), whereas only a trend was observed for SBP ($P = 0.054$ supplemental Table 2, http://links.lww.com/HJH/B884), when the entire population was analysed. Subgroup analyses stratified according to presence or absence of FH demonstrated a significant association of global placental DNA methylation with SBP ($P = 0.041$, supplemental Table 3, http://links.lww.com/HJH/B884) and with DBP ($P = 0.041$, supplemental Table 3, http://links.lww.com/HJH/B884). No significant association could be observed between global placental DNA methylation and SBP ($P = 0.894$ supplemental Table 2, http://links.lww.com/HJH/B884) or DBP in mothers with a family history of hypertension ($P = 0.574$, supplemental Table 3, http://links.lww.com/HJH/B884).

DISCUSSION

This study showed an independent negative correlation between placental methylation and maternal MAP in the third trimester of pregnancy in women without family history of hypertension.

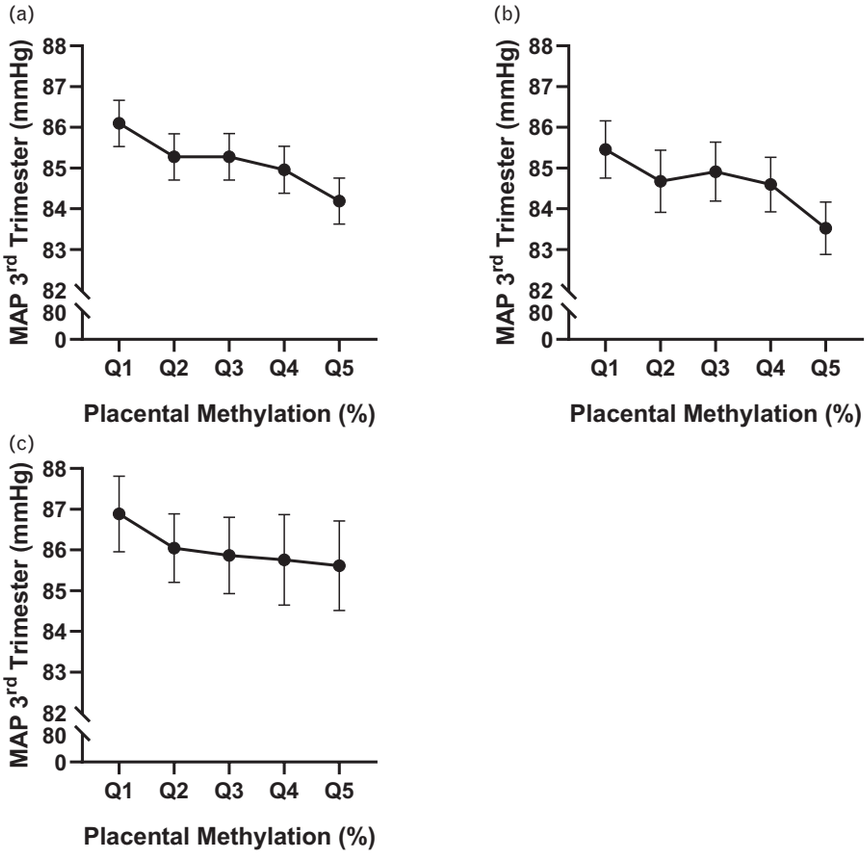

FIGURE 1 Placental methylation (%) and mean arterial pressure in the third trimester (mmHg). (a—c) show global placental DNA methylation data stratified into and the according MAP in the third trimester (mmHg) of each quintile for the whole cohort, for placental samples of mothers without FH and for placental samples of mothers with FH, respectively. Data are given as mean ± SEM.

In the current study, MAP was chosen as the main parameter reflecting blood pressure, as studies have demonstrated that this blood pressure parameter is better in predicting blood disease affecting gestational blood pressure control such as preeclampsia or gestational hypertension than either SBP or DBP [12,13]. In regards to the predictive value of SPB and DBP for hypertensive disorders during pregnancy, traditionally, more emphasis has been given to DBP, as pregnant women tend to be of younger age. SBP, which is largely influenced by aortic stiffness, gains more importance with advancing age [26]. However, in recent decades, a gradual increase in the age of pregnant women has occurred, with a higher frequency of pregnant women over the age of 35 [27], a trend that was also reflected in the cohort of the current study. Mounting evidence from studies in nonpregnant populations demonstrates that with advancing age a shift from DBP to SBP as the main predictor of the adverse cardiovascular consequences of hypertension occurs [28]. Consequently, also in pregnant women of more advanced age, an increase in the contribution of SBP in pregnancy-related hypertension might occur. On the basis of the fact that a fairly large proportion of women in the cohort of the current study were of more advanced age, the usage of MAP, a blood pressure parameter that reflects both SBP and DPB, appeared best suited. Furthermore, a large GWAS study on the general population has demonstrated that MAP revealed loci influencing blood pressure phenotypes that may not have been detected by studying SBP and DBP in a separate fashion. Moreover, a recent genome wide DNA methylation study investigating associations between normal blood pressure variation and placental CpG methylation also showed that SBP and DBP were associated with a different number of CpGs [29,30].

To the best of our knowledge, this is the first large-scale study investigating the association of gestational maternal blood pressure and global placental DNA methylation. All studies available so far, investigating placental methylation were much smaller, with sample sizes ranging between 50 and 100. Most likely due to the small sample size, the results of previous studies were conflicting. Nomura *et al.* [11] analysed placental methylation in 50 placenta samples and showed that mothers with preeclampsia display a lower global placental DNA methylation compared with healthy controls, whereas Kulkarni *et al.* [10] reported higher placental methylation in preeclamptic pregnancies by comparing global DNA methylation in 87 placenta samples of hypertensive preeclamptic mothers to normotensive healthy controls. This study additionally demonstrated a positive association between global DNA methylation and

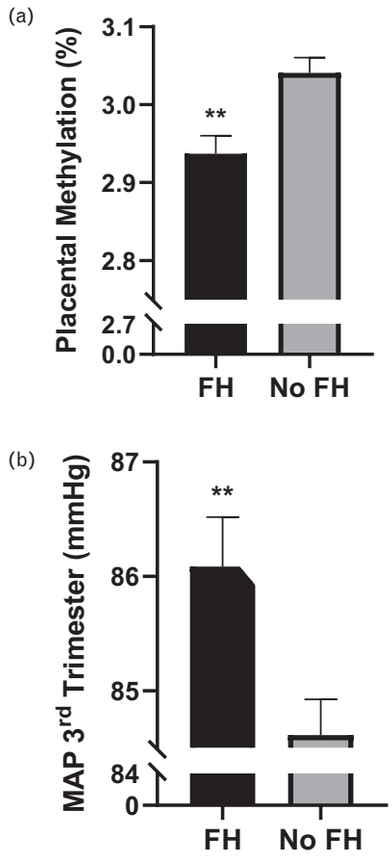

FIGURE 2 The impact of family history of hypertension on placental methylation and mean arterial pressure during pregnancy. Degree of placental methylation (%) (a) and MAP (mmHg) (b) according to the presence or absence of FH. $^{*}P < 0.05$, $^{**}P < 0.01$.

blood pressure in a subset of 30 term preeclamptic pregnancies.

Whereas there is only a small set of studies available regarding the association between placental DNA methylation and blood pressure, several studies have been conducted to explore the association between global DNA methylation and blood pressure in nonplacental tissues [31–33]. Also, these studies showed conflicting results regarding the association between blood pressure and global DNA methylation. Differences in employed methods of measuring global DNA methylation, sample sizes and the use of surrogate parameters for global DNA methylation, such as Alu and LINE-1, could be important contributing factors to the conflicting results [34]. Furthermore, as observed in the current study, the association between blood pressure and global DNA methylation is rather weak. Other confounders such as type II diabetes that are often associated with hypertension may interact stronger with DNA methylation than blood pressure itself [35,36]. In addition to this, the diverse cohort composition of the available studies could also be accountable for a different overall state of global DNA methylation. Cohort composition ranged from preeclamptic mothers to obese patients with essential hypertension to elderly patients, each one of them bearing putative additional factors that could influence DNA methylation [10,11,20,32,33]. However, in the present currently largest study using the gold standard for global DNA methylation quantification, multivariable analyses substantiated an independent negative correlation between maternal MAP and placental global DNA methylation.

Due to the nature of this study, it can only be speculated about the mechanism behind the observed association between global placental DNA methylation and MAP. It has been demonstrated that hypertension is associated with increased oxidative stress [37]. Furthermore, several studies have shown that overproduction of reactive oxygen species can be associated with global hypomethylation [38,39]. A putative link between higher blood pressure and lower methylation may thus be oxidative stress [31]. This hypothesis would suggest that changes in global DNA methylation are a consequence of hypertension associated increases in ROS. However, adequately designed studies that investigate the placenta in early stages of pregnancy are still needed to solve the chicken and egg dilemma behind many so far observed associations between different maternal diseases and global placental DNA methylation [40]. Similarly, the potential biological effects of changes in global placental DNA methylation remain very poorly understood. An interesting characteristic of the placental methylome, that is shared with many neoplasms, is the presence of global DNA hypomethylation in comparison to somatic tissues [41,42]. However, it was demonstrated that hypermethylation of certain genomic regions, such as Alu elements, appears to be important for proper placental and foetal growth [43,44]. This finding is supported by animal experiments, which could show that treatment with the DNA methyltransferase inhibitor 5-Azacitidine impairs placental growth and function, underscoring the putative role of global placental DNA methylation in placental and foetal growth [45]. However, probably due to the study design of the current study, we did not observe any effects on foetal outcomes. Also in regards to effects of alterations of global placental DNA methylation on offspring outcomes, more studies are still needed.

Our data suggest that there may be two forms of gestational hypertension: a form of gestational hypertension characterized by primary epigenetic changes in the placenta and another form of gestational hypertension caused by genetic variants that contribute to essential hypertension in the nonpregnant population. These risk genes (positive family history of hypertension) unmask hypertension that would have occurred much later in life without pregnancy. In this sense, pregnancy could be seen as a provocation test to unmask essential hypertension that would otherwise occur later in life. To be distinguished from this is gestational hypertension without positive family history. Here, epigenetic changes in the placenta may have greater etiological significance. This hypothesis needs to be independently tested in further studies. We hope that our data will stimulate this.

Limitations and strength

In this study, analyses were performed regardless of any specification for placental cell types. This has to be mentioned because DNA methylation patterns are different in

different cell types [46–49]. However, placental DNA methylation patterns for several genes were shown to be almost the same and independent of sites and depths of placental sampling [20,50,51]. Moreover, global DNA methylation levels in different regions in the placenta seem to be almost the same [52].

The advantage of the current study is that a large cohort was analysed by one of the most sensitive methods of global DNA methylation quantification [53]. To the best of our knowledge, this is the largest study ($N = 922$) investigating placental methylation in pregnancies. The LC-MS/MS method measures absolute methyl-cytosine content precisely and is considered the current gold standard [53]. Comparable studies investigating global placental DNA methylation so far all used smaller sample sizes and/or different methods for quantifying global DNA methylation [10,11,54].

In conclusion, this study showed an independent negative correlation between placental global DNA methylation and maternal MAP in the third trimester of pregnancy in women without familiar history of hypertension.

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Conflicts of interest

The authors report no conflicts of interest.

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Original Article

Association between placental global DNA methylation and blood pressure during human pregnancy

Sulistyo E. Dwi Putra[a,b,*], Christoph Reichetzeder[c,*], Karoline von Websky[a,c], Corinna Neuber[c], Horst Halle[d], Burkard Kleuser[e], Bernhard K. Krämer[a,f], and Berthold Hocher[a,g,h,i]

Objective: Gene-specific placental DNA methylation patterns differ between normal pregnancies and pregnancies complicated by hypertension. However, whether global placental DNA methylation is associated with maternal blood pressure remains controversial.

Methods: Using multiple linear regression models, we analysed the association between maternal mean arterial pressure (MAP) at the third trimester of pregnancy and global DNA methylation in the placenta in 922 mothers using LC-ESI-MS/MS. To better characterize the contribution of genetic or epigenetic mechanisms, we performed isolated analyses in mothers with and without a family history of hypertension.

Results: Mean placental global DNA methylation was $3.00 \pm 0.46\%$. A significant negative correlation between placental global DNA methylation and mean arterial blood pressure (MAP) in the third trimester could be observed ($P = 0.023$, $r = -0.075$). This association remained significant after adjusting for confounders. In placenta samples from mothers with a family history of hypertension, mean maternal MAP was higher (86.1 ± 8.1 vs. 84.6 ± 7.5, $P < 0.01$) and placental global DNA methylation was lower (2.94 ± 0.43 vs. 3.04 ± 0.47, $P < 0.01$) compared with samples without a family history of hypertension. Furthermore, the significant independent negative correlation between global placental DNA methylation and MAP was only found in mothers without a family history of hypertension.

Conclusion: This study showed an independent negative correlation between placental global DNA methylation and maternal MAP in mothers without a family history of hypertension.

Keywords: family history of hypertension, gestational blood pressure regulation, gestational hypertension, global DNA methylation, placenta

Abbreviations: LC-ESI-MS/MS, liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry; MAP, mean arterial pressure

INTRODUCTION

Adaptations of the maternal cardiovascular system during pregnancy consist of distinct changes in heart rate and blood pressure. Early on in pregnancy systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP) decrease. After this gradual decrease of blood pressure, typically including a mid-trimester drop between week 16 and 20, a gradual rise to the initial levels from before pregnancy occurs [1,2]. These changes in blood pressure are accompanied by other haemodynamic alterations such as an increase in cardiac output and stroke volume, and a marked reduction in systemic vascular resistance in early pregnancy, followed by a continuous increase of heart rate and total vascular resistance at term [3,4]. Several mechanisms have been suggested for these cardiovascular changes during pregnancy [5]. It is known that the placenta has a crucial role in the endocrine, paracrine and autocrine regulation of systemic and local cardiovascular changes during pregnancy [6,7]. A complete understanding of the mechanisms by which the placenta exerts its multiple functions on maternal and foetal circulation remains to be determined, but available data suggest that epigenetic regulation might be involved [8].

Examination of epigenetic alterations and their influence on physiological and pathophysiological processes has received increased interest in current research. Epigenetics is a term for heritable changes in gene activity and expression that occur without any changes in the DNA sequence

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[a]Fifth Department of Medicine (Nephrology/Endocrinology/Rheumatology/Pneumology), University Medical Centre Mannheim, University of Heidelberg, Heidelberg, Germany, [b]University of Surabaya, Surabaya, Indonesia, [c]Institute of Nutritional Science, Department of Nutritional Toxicology, University of Potsdam, Nuthetal, [d]Department of Obstetrics and Gynecology, Charitè University Hospital, Campus Mitte, [e]Institute of Pharmacy, Department of Pharmacology, Freie Universität Berlin, Berlin, [f]European Center for Angioscience ECAS, Medical Faculty Mannheim of the University of Heidelberg, Mannheim, Germany, [g]Key Laboratory of Study and Discovery of Small Targeted Molecules of Hunan Province, School of Medicine, Hunan Normal University, [h]Reproductive and Genetic Hospital of CITIC-Xiangya, Changsha, China and [i]Institute of Medical Diagnostics (IMD), Berlin, Germany

Correspondence to Berthold Hocher, Fifth Department of Medicine (Nephrology/Endocrinology/Rheumatology/Pneumology), University Medical Centre Mannheim, University of Heidelberg, 69047 Heidelberg, Germany. E-mail: berthold.hocher@-medma.uni-heidelberg.de

[*]Both Sulistyo E. Dwi Puthra and Christoph Reichetzeder are equally contributing first authors.

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of the gene. DNA methylation is a well known and widely studied epigenetic mechanism [9]. Studies on epigenetic regulation in the placenta, especially in the context of gestational blood pressure regulation, are still scarce. Furthermore, available studies investigating a link between epigenetic regulation in the placenta and maternal blood pressure yielded conflicting results [10,11]. The majority of these studies were mainly focused on preeclampsia, a hypertensive disorder of pregnancy, rather than investigating the association of epigenetic mechanisms and physiologic blood pressure regulation. Therefore, the present study was conducted to elucidate in a large cohort of pregnant women, if the placental methylome may influence or may itself be influenced by physiologic adaptions of maternal blood pressure regulation during pregnancy. Furthermore, the contribution of genetic factors to this association was investigated in subgroups of mothers with and without a family history of hypertension. As the main parameter reflecting maternal blood pressure, MAP was chosen, which is calculated using SBP and DBP. Studies have shown that this blood pressure parameter is better in predicting blood pressure related disease with placental changes such as preeclampsia or gestational hypertension than either SBP or DBP [12,13].

MATERIALS AND METHODS

Clinical study

The study was approved by the Institutional Review Board of the University Hospital of Charité Berlin, Germany. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. Data were collected from 2000 to 2008. This observational study included 1063 mothers delivering at the Charité Obstetrics Department [14–16]. Structured interviews with mothers taking part in the study were conducted after obtaining their written consent. Relevant clinical data were obtained from the 'Mutterpass' (pregnancy health document). As the focus of this study was set on placental methylation in relation to gestational maternal blood pressure regulation, we did not include any mothers with diabetes mellitus before or during pregnancy, with preexisting hypertension or with preeclampsia. Cases with missing data on SBP, DBP measurements or family history of hypertension were also excluded from further analysis. At the end, analyses were performed using data from 922 mothers. Data from a questionnaire were used to obtain ethnicity, smoking status before and during pregnancy and family history of hypertension. Family history of hypertension was defined as at least one of the first-degree relatives suffering from hypertension. Routine antenatal check-ups were used to collect blood pressure data. Blood pressure was measured with a mercury or aneroid sphygmomanometer. DBP was defined based on Korotkoff-phase V. If sounds were still audible when the cuff was fully deflated, Korotkoff -phase IV was used. MAP was calculated as MAP = DBP + 1/3 (SBP − DBP) [17].

Perinatal parameters such as sex of the newborn, birth size and APGAR score were added as well. Gestational age of the newborn was calculated based on the last menstruation. Placental weight was assessed including the umbilical cord. Standardized placenta samples (one cotyledon from similar locations) were obtained and immediately frozen and stored at −20 °C.

DNA extraction and hydrolysis

DNA was extracted using a QIAamp DNA Mini Kit from Qiagen (Hilden, Germany) together with an RNase A digestion according to the manufacturer's protocol. Concentration and quality of DNA solution were determined with a NanoDrop ND-1000 spectrophotometer. DNA hydrolysis was performed using DNA Degradase Plus from Zymo Research (Freiburg, Germany) as indicated in the commercial protocol. Hydrolysis reaction was stopped by adding 75 µl of 0.1% formic acid. The completeness of the reaction was confirmed by agarose gel electrophoresis of digested DNA. Before sample injection to the liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry (LC-ESI-MS/MS) instrument, 70 µl of digested DNA were diluted to 280 µl 0.1% formic acid to yield a final concentration of 2 ng digested DNA/µl.

Liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry

Placental methylation was quantified as described elsewhere [18]. Briefly, LC-ESI-MS/MS was performed usinga HPLC system (Agilent 1200 series) connected to an Accurate-Mass Q-TOF instrument (Agilent 6530) with Jet Stream-Interface (Waldbronn, Germany). Chromatographic separation was done in a Waters X-Bridge column, C18 4.6 mm x 150 mm, with particle size 3.5 µm (Milford, Massachusetts, USA) protected by a Waters X-Bridge column guard, C18 4.6 mm x 20 mm with particle size 5 µm. Two different mobile phases were used: solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol). Prior to sample injection, a linear gradient elution was performed using 4–20% of solvent B for 10 min at a constant flow rate of 0.5 ml/min. The injection volume was 50 µl and typically contained 100 ng digested DNA. The optimized ESI-MS/MS parameters in positive ion mode were as follows: gas temperature, 250°C; drying gas flow, 8 l/min; nebulizer pressure, 60 psig; sheat gas temperature, 300°C; capillary voltage 4000 V; collision energy 7 V for dC, 13 V for 5mdC and 10 V for dG. Selected reaction monitoring (SRM) mode at m/z 228.0979/112.0505 for dC, m/z 242.1135/126.0662 for 5mdC were used to obtain a signal needed for quantification. The percentage of global methylation was calculated as follows: Methylation % = 5-methyl-2′-deoxycytidine (5mdC)/ [5-methyl-2′-deoxycytidine (5mdC) + 2′-deoxycytidine (dC)] × 100%.

Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, New York, USA). Pearson correlation analysis was used to identify correlations between two parameters followed by multiple linear regression analyses, in case two parameters were significantly correlated. Data stratification was conducted wherever necessary. According to normal distribution of the data, Student's *t*-test or Mann–Whitney *U*-test was performed to compare mean values between two groups

TABLE 1. Descriptive data of all mother/child pairs grouped according to family history of hypertension (FH,* comparison between 'FH' and 'No FH')

Maternal/Child characteristics	All samples ($N=922$)	Presence of FH		
		No FH ($N=571$)	FH ($N=351$)	P
Placental methylation, %	3.00 ± 0.46	3.04 ± 0.47	2.94 ± 0.43	0.001
Gestational age at delivery (weeks)	38.7 ± 2.1	38.8 ± 1.9	38.8 ± 1.9	0.879
Age of the mother (years)	29.9 ± 5.8	29.6 ± 6.1	30.1 ± 5.5	0.216
BMI at beginning of pregnancy (kg/m^2)	22.8 ± 4.2	24.0 ± 4.9	24.5 ± 4.8	0.080
Mother body weight third trimester (kg)	76.1 ± 12.9	75.7 ± 13.5	76.7 ± 11.7	0.075
SBP third trimester (mmHg)	115.6 ± 10.6	114.9 ± 10.2	116.7 ± 11.2	0.015
DBP third trimester (mmHg)	70 ± 7.2	69.4 ± 7.0	70.8 ± 7.4	0.006
MAP third trimester (mmHg)	85.2 ± 7.7	84.6 ± 7.5	86.1 ± 8.1	0.005
Hypertension during pregnancy, %	3.3	1.2	6.6	<0.001
Smoking before/during pregnancy, %	35.3/13.9	36.7/15.0	32.8/12.0	0.225/0.290
Family history of diabetes, %	36.2	26.6	52.0	<0.001
Ethnicity, White/non-White, %	93.2/6.8	91.7/8.3	96.5/3.5	0.004
Child birth weight (g)	3330.7 ± 629.9	3343.9 ± 623.0	3330.6 ± 585.3	0.750
Child head circumference (cm)	34.7 ± 1.7	34.6 ± 1.8	34.8 ± 1.6	0.217
Child birth length (cm)	50.6 ± 3.2	50.7 ± 3.0	50.6 ± 3.1	0.507
Ponderal index	25.6 ± 4.1	25.5 ± 3.4	25.8 ± 5.1	0.367
Preterm birth, %	9.7	9.1	10.5	0.474
APGAR 5 min	9.3 ± 1	9.3 ± 0.9	9.3 ± 1.1	0.843
APGAR 10 min	9.6 ± 0.8	9.6 ± 0.7	9.6 ± 0.9	0.605
Sex of the child, male/female, %	51.8/48.2	53.4/46.6	50.4/49.6	0.378

Data are given as mean ± SD or %.
FH, family history of hypertension.

in subgroup analyses. The authors had full access to the data and take full responsibility for its integrity.

RESULTS

Nine hundred and twenty-two mothers were included into the study. Detailed descriptive data of the cohort of pregnant women are summarized in Table 1. All study participants were normotensive in the first trimester of pregnancy. The mean maternal MAP was 85.2 ± 7.7 mmHg in the third trimester of pregnancy. SBP and DBP means in the third trimester of pregnancy were 115.6 ± 10.6 and 70 ± 7.2 mmHg, respectively (for details see Table 2). Other parameters including age of the mother (29.9 ± 5.8 years), gestational age at delivery (38.7 ± 2.1 weeks) and BMI at the beginning of pregnancy (22.8 ± 4.2 kg/m^2) were calculated as well. Birth outcome parameters were within normal ranges, including birth weight (3330.7 ± 629.9 gram), head circumference (34.7 ± 1.7 cm), birth length (50.6 ± 3.2 cm), ponderal index (25.6 ± 4.1 g/cm), APGAR 5 min postnatal (9.3 ± 1.0) and APGAR 10 min postnatal (9.6 ± 0.8). All of the above-mentioned parameters are indicative of an inconspicuous cohort of mothers and newborns. The degree of placental methylation ranged between 2.0 and 4.8%, with a mean of 3.00 ± 0.46%.

Placental methylation showed a significant negative correlation with maternal MAP in the third trimester of pregnancy after Pearson correlation analysis ($P=0.023$; $r=-0.075$). Similar findings were made for SBP ($P=0.034$; $r=-0.070$) and DBP ($P=0.029$; $r=-0.072$). Stratifying placental methylation into quintiles, indicated a linear behaviour of MAP in relation to placental methylation (Fig. 1a). To investigate the contribution of genetic factors to the observed association, data were stratified into mothers with and without a family history of hypertension. Details regarding descriptive statistics of these subgroups are summarized in Table 1. Interestingly, the presence or absence of a family history of hypertension had a significant impact on global placental methylation levels. A family history of hypertension was associated with lower placental methylation compared with samples without a family history of hypertension (2.94% ± 0.43 vs. 3.04% ± 0.47%, $P=0.001$, Fig. 2a). MAP was higher in the family history of hypertension group than in the group without a family history of hypertension (86.1 ± 8.1 vs. 84.6 ± 7.5 mmHg, $P=0.005$, Fig. 2b and Table 1). Other parameters that were significantly different between mother with and without a family history of hypertension included SBP (116.7 ± 11.2 vs. 114.9 ± 10.2, $P=0.015$), DBP (70.8 ± 7.4 vs. 69.4 ± 7.0, $P=0.006$), hypertension during pregnancy (6.6 vs. 1.2%, $P<0.001$), family history of diabetes (52.0 vs. 26.6%, $P<0.001$) and ethnicity (white/non-white, 96.5/3.5 vs. 91.7/8.3%, $P=0.004$).

Univariate Pearson correlation analyses showed a significant negative correlation between MAP in the third trimester of pregnancy and placental methylation in mothers without a family history of hypertension ($P=0.021$, $r=-0.098$) but not in mothers with a family history of hypertension ($P=0.675$, $r=-0.023$). This was also observed for SBP (mothers without a family history of hypertension: $P=0.030$, $r=-0.091$; mothers with a family history of hypertension: $P=0.698$, $r=-0.021$) but not for DBP (mothers without a family history of hypertension: $P=0.061$, $r=-0.078$; mothers with a family history of hypertension: $P=0.560$, $r=-0.031$) Stratifying placental methylation data into quintiles showed similar results, with a stronger negative correlation between placental methylation and MAP of third trimester in samples without a family history of hypertension (Fig. 1b) compared with a family history of hypertension (Fig. 1c).

TABLE 2. Regression analyses of the association between placental methylation (%) and mean arterial pressure of the third trimester of pregnancy (mmHg) as dependent variable

(a) Entire population ($r^2 = 0.204$)					
Independent variable	**B**	**Beta**	***P***	**(95% CI for B)**	
Placental methylation, %	−1.26	−0.08	0.025	−2.35	−0.16
Age of the mother (years)	−0.06	−0.04	0.235	−0.15	0.04
Gestational age at delivery (weeks)	−0.14	−0.04	0.332	−0.43	0.15
BMI at beginning of pregnancy (kg/m^2)	−0.07	−0.04	0.533	−0.30	0.16
Mother body weight third trimester (kg)	0.21	0.34	<0.001	0.14	0.28
Sex of the child	−0.92	−0.06	0.093	−2.00	0.15
Ethnicity	−2.04	−0.07	0.047	−4.05	−0.03
Hypertension during pregnancy	10.40	0.24	<0.001	7.36	13.44
Family history of diabetes	0.61	0.04	0.296	−0.53	1.75
(b) No FH subgroup ($r^2 = 0.137$)					
Independent variable	**B**	**Beta**	***P***	**(95% CI for B)**	
Placental methylation, %	−1.54	−0.10	0.027	−2.89	−0.18
Age of the mother (years)	−0.04	−0.03	0.511	−0.15	0.07
Gestational age at delivery (weeks)	−0.09	−0.02	0.633	−0.46	0.28
BMI at beginning of pregnancy (kg/m^2)	0.03	0.01	0.849	−0.24	0.29
Mother body weight third trimester (kg)	0.17	0.31	<0.001	0.09	0.26
Sex of the child	−0.81	−0.05	0.243	−2.17	0.55
Ethnicity	−2.19	−0.09	0.064	−4.52	0.13
Hypertension during pregnancy	11.85	0.18	<0.001	5.74	17.95
Family history of diabetes	−0.16	−0.01	0.837	−1.73	1.40
(c) FH subgroup ($r^2 = 0.231$)					
Independent variable	**B**	**Beta**	***P***	**(95% CI for B)**	
Placental methylation, %	−0.41	−0.02	0.681	−2.35	1.54
Age of the mother (years)	−0.10	−0.07	0.253	−0.27	0.07
Gestational age at delivery (weeks)	−0.26	−0.06	0.273	−0.74	0.21
BMI at beginning of pregnancy (kg/m^2)	−0.28	−0.12	0.221	−0.73	0.17
Mother body weight third trimester (kg)	0.29	0.41	<0.001	0.15	0.42
Sex of the child	−1.09	−0.07	0.236	−2.89	0.72
Ethnicity	−1.67	−0.05	0.431	−5.85	2.51
Hypertension during pregnancy	9.52	0.30	<0.001	5.71	13.34
Family history of diabetes	1.34	0.08	0.155	−0.51	3.20

Multiple linear regression models were used to further analyse the correlation between placental methylation and maternal MAP in the third trimester of pregnancy. The following confounders were used based on literature: age and ethnicity of the mother, and sex of the child [19–22]. In addition, maternal BMI, body weight in the third trimester of pregnancy, family history of diabetes and presence or absence of hypertension during pregnancy were included into the models, as these parameters are known to correlate with birth outcomes and blood pressure and/or were significantly different in mothers with and without FH [23–25].

In the multiple linear regression model including the entire study population, placental methylation was significantly correlated with maternal MAP in the third trimester of pregnancy ($P = 0.025$, model A, Table 2). However, multiple linear regression analyses highlighted an independent negative correlation between MAP in the third trimester of pregnancy and placental methylation for mothers without FH ($P = 0.027$, Table 2). In contrast, no correlation between placental methylation and MAP in the third trimester of pregnancy was observed in the group with a family history of hypertension ($P = 0.681$, Table 2). In multiple linear regression models, global placental DNA methylation was significantly associated with DBP ($P = 0.030$, supplemental Table 3, http://links.lww.com/HJH/B884), whereas only a trend was observed for SBP ($P = 0.054$ supplemental Table 2, http://links.lww.com/HJH/B884), when the entire population was analysed. Subgroup analyses stratified according to presence or absence of FH demonstrated a significant association of global placental DNA methylation with SBP ($P = 0.041$, supplemental Table 3, http://links.lww.com/HJH/B884) and with DBP ($P = 0.041$, supplemental Table 3, http://links.lww.com/HJH/B884). No significant association could be observed between global placental DNA methylation and SBP ($P = 0.894$ supplemental Table 2, http://links.lww.com/HJH/B884) or DBP in mothers with a family history of hypertension ($P = 0.574$, supplemental Table 3, http://links.lww.com/HJH/B884).

DISCUSSION

This study showed an independent negative correlation between placental methylation and maternal MAP in the third trimester of pregnancy in women without family history of hypertension.

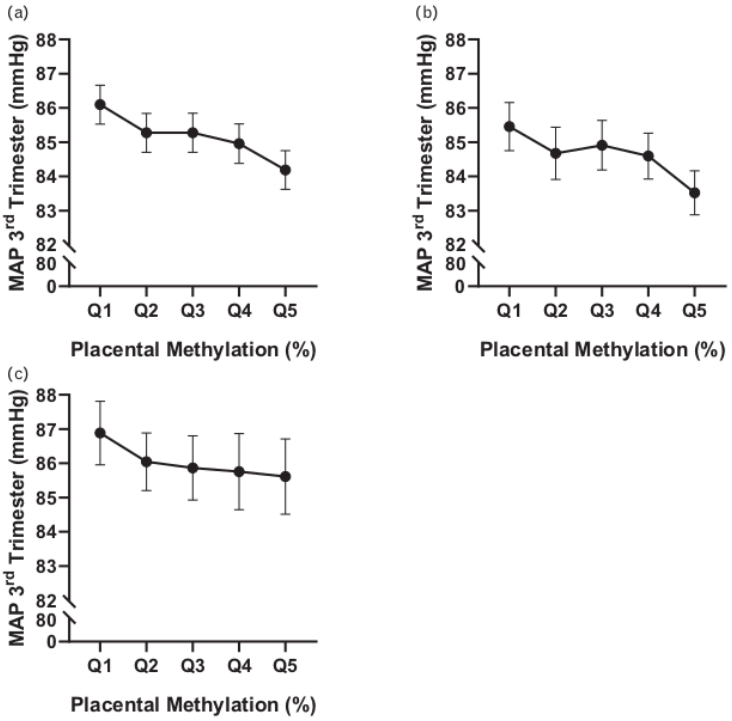

FIGURE 1 Placental methylation (%) and mean arterial pressure in the third trimester (mmHg). (a—c) show global placental DNA methylation data stratified into and the according MAP in the third trimester (mmHg) of each quintile for the whole cohort, for placental samples of mothers without FH and for placental samples of mothers with FH, respectively. Data are given as mean ± SEM.

In the current study, MAP was chosen as the main parameter reflecting blood pressure, as studies have demonstrated that this blood pressure parameter is better in predicting blood disease affecting gestational blood pressure control such as preeclampsia or gestational hypertension than either SBP or DBP [12,13]. In regards to the predictive value of SPB and DBP for hypertensive disorders during pregnancy, traditionally, more emphasis has been given to DBP, as pregnant women tend to be of younger age. SBP, which is largely influenced by aortic stiffness, gains more importance with advancing age [26]. However, in recent decades, a gradual increase in the age of pregnant women has occurred, with a higher frequency of pregnant women over the age of 35 [27], a trend that was also reflected in the cohort of the current study. Mounting evidence from studies in nonpregnant populations demonstrates that with advancing age a shift from DBP to SBP as the main predictor of the adverse cardiovascular consequences of hypertension occurs [28]. Consequently, also in pregnant women of more advanced age, an increase in the contribution of SBP in pregnancy-related hypertension might occur. On the basis of the fact that a fairly large proportion of women in the cohort of the current study were of more advanced age, the usage of MAP, a blood pressure parameter that reflects both SBP and DPB, appeared best suited. Furthermore, a large GWAS study on the general population has demonstrated that MAP revealed loci influencing blood pressure phenotypes that may not have been detected by studying SBP and DBP in a separate fashion. Moreover, a recent genome wide DNA methylation study investigating associations between normal blood pressure variation and placental CpG methylation also showed that SBP and DBP were associated with a different number of CpGs [29,30].

To the best of our knowledge, this is the first large-scale study investigating the association of gestational maternal blood pressure and global placental DNA methylation. All studies available so far, investigating placental methylation were much smaller, with sample sizes ranging between 50 and 100. Most likely due to the small sample size, the results of previous studies were conflicting. Nomura *et al.* [11] analysed placental methylation in 50 placenta samples and showed that mothers with preeclampsia display a lower global placental DNA methylation compared with healthy controls, whereas Kulkarni *et al.* [10] reported higher placental methylation in preeclamptic pregnancies by comparing global DNA methylation in 87 placenta samples of hypertensive preeclamptic mothers to normotensive healthy controls. This study additionally demonstrated a positive association between global DNA methylation and

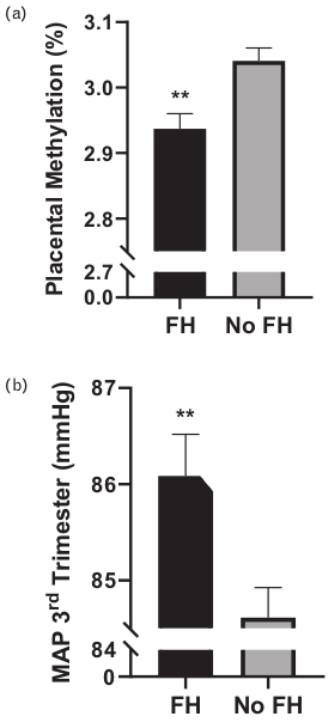

FIGURE 2 The impact of family history of hypertension on placental methylation and mean arterial pressure during pregnancy. Degree of placental methylation (%) (a) and MAP (mmHg) (b) according to the presence or absence of FH. $^{*}P < 0.05$, $^{**}P < 0.01$.

blood pressure in a subset of 30 term preeclamptic pregnancies.

Whereas there is only a small set of studies available regarding the association between placental DNA methylation and blood pressure, several studies have been conducted to explore the association between global DNA methylation and blood pressure in nonplacental tissues [31–33]. Also, these studies showed conflicting results regarding the association between blood pressure and global DNA methylation. Differences in employed methods of measuring global DNA methylation, sample sizes and the use of surrogate parameters for global DNA methylation, such as Alu and LINE-1, could be important contributing factors to the conflicting results [34]. Furthermore, as observed in the current study, the association between blood pressure and global DNA methylation is rather weak. Other confounders such as type II diabetes that are often associated with hypertension may interact stronger with DNA methylation than blood pressure itself [35,36]. In addition to this, the diverse cohort composition of the available studies could also be accountable for a different overall state of global DNA methylation. Cohort composition ranged from preeclamptic mothers to obese patients with essential hypertension to elderly patients, each one of them bearing putative additional factors that could influence DNA methylation [10,11,20,32,33]. However, in the present currently largest study using the gold standard for global DNA methylation quantification, multivariable analyses substantiated an independent negative correlation between maternal MAP and placental global DNA methylation.

Due to the nature of this study, it can only be speculated about the mechanism behind the observed association between global placental DNA methylation and MAP. It has been demonstrated that hypertension is associated with increased oxidative stress [37]. Furthermore, several studies have shown that overproduction of reactive oxygen species can be associated with global hypomethylation [38,39]. A putative link between higher blood pressure and lower methylation may thus be oxidative stress [31]. This hypothesis would suggest that changes in global DNA methylation are a consequence of hypertension associated increases in ROS. However, adequately designed studies that investigate the placenta in early stages of pregnancy are still needed to solve the chicken and egg dilemma behind many so far observed associations between different maternal diseases and global placental DNA methylation [40]. Similarly, the potential biological effects of changes in global placental DNA methylation remain very poorly understood. An interesting characteristic of the placental methylome, that is shared with many neoplasms, is the presence of global DNA hypomethylation in comparison to somatic tissues [41,42]. However, it was demonstrated that hypermethylation of certain genomic regions, such as Alu elements, appears to be important for proper placental and foetal growth [43,44]. This finding is supported by animal experiments, which could show that treatment with the DNA methyltransferase inhibitor 5-Azacitidine impairs placental growth and function, underscoring the putative role of global placental DNA methylation in placental and foetal growth [45]. However, probably due to the study design of the current study, we did not observe any effects on foetal outcomes. Also in regards to effects of alterations of global placental DNA methylation on offspring outcomes, more studies are still needed.

Our data suggest that there may be two forms of gestational hypertension: a form of gestational hypertension characterized by primary epigenetic changes in the placenta and another form of gestational hypertension caused by genetic variants that contribute to essential hypertension in the nonpregnant population. These risk genes (positive family history of hypertension) unmask hypertension that would have occurred much later in life without pregnancy. In this sense, pregnancy could be seen as a provocation test to unmask essential hypertension that would otherwise occur later in life. To be distinguished from this is gestational hypertension without positive family history. Here, epigenetic changes in the placenta may have greater etiological significance. This hypothesis needs to be independently tested in further studies. We hope that our data will stimulate this.

Limitations and strength

In this study, analyses were performed regardless of any specification for placental cell types. This has to be mentioned because DNA methylation patterns are different in

different cell types [46–49]. However, placental DNA methylation patterns for several genes were shown to be almost the same and independent of sites and depths of placental sampling [20,50,51]. Moreover, global DNA methylation levels in different regions in the placenta seem to be almost the same [52].

The advantage of the current study is that a large cohort was analysed by one of the most sensitive methods of global DNA methylation quantification [53]. To the best of our knowledge, this is the largest study ($N=922$) investigating placental methylation in pregnancies. The LC-MS/MS method measures absolute methyl-cytosine content precisely and is considered the current gold standard [53]. Comparable studies investigating global placental DNA methylation so far all used smaller sample sizes and/or different methods for quantifying global DNA methylation [10,11,54].

In conclusion, this study showed an independent negative correlation between placental global DNA methylation and maternal MAP in the third trimester of pregnancy in women without familiar history of hypertension.

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Conflicts of interest

The authors report no conflicts of interest.

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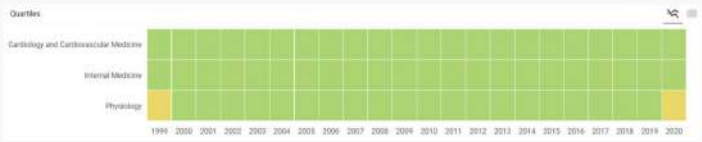

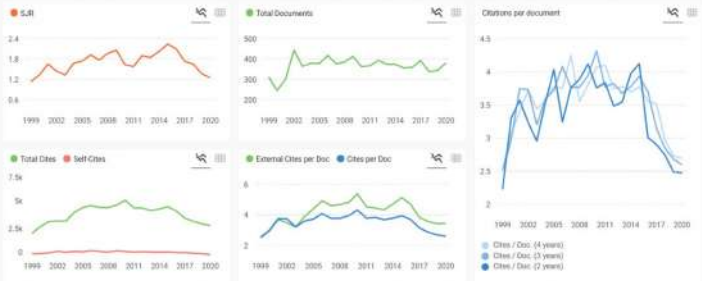

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