Pediatric RESEARCH

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POPULATION STUDY ARTICLE

Can social support during pregnancy affect maternal DNA methylation? Findings from a cohort of African-Americans

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BACKGROUND: While stress and the absence of social support during pregnancy have been linked to poor health outcomes, the underlying biological mechanisms are unclear.

METHODS: We examined whether adverse experiences during pregnancy alter DNA methylation (DNAm) in maternal epigenomes. Analyses included 250 African-American mothers from the Boston Birth Cohort. Genome-wide DNAm profiling was performed in maternal blood collected after delivery, using the Infinium HumanMethylation450 Beadchip. Linear regression models, with adjustment of pertinent covariates, were applied.

RESULTS: While self-reported maternal psychosocial lifetime stress and stress during pregnancy was not associated with DNAm alterations, we found that absence of support from the baby's father was significantly associated with maternal DNAm changes in *TOR3A, IQCB1*, *C7orf36*, and *MYH7B* and that lack of support from family and friends was associated with maternal DNA hypermethylation on multiple genes, including *PRDM16* and *BANKL*.

CONCLUSIONS: This study provides intriguing results suggesting biological embedding of social support during pregnancy on maternal DNAm, warranting additional investigation, and replication.

Pediatric Research (2020) 88:131–138; https://doi.org/10.1038/s41390-019-0512-7

INTRODUCTION

While psychosocial stress has been linked to adverse health associations, social support can promote resilience or indirectly buffer stress during pregnancy.[1] In spite of the important role of support and of psychosocial stress during pregnancy,[2,3] it is unclear how either of these factors affect maternal epigenomes. Regarding stress-induced changes in maternal DNA methylation (DNAm), studies have found changes in the glucocorticoid receptor gene (*NR3C1*) among women who were victims of violence[4] and who were exposed to the Tutsi genocide during pregnancy.[5] Other research has linked maternal depressed mood in the second trimester of pregnancy to changes in maternal DNAm levels in the *SLC6A4* gene,[6] a gene that encodes a membrane protein that transports the neurotransmitter serotonin from synaptic spaces into presynaptic neurons[7] and may play a role in obsessive-compulsive disorder[8] and anxiety.[9] These results suggest that maternal stress may influence neurological functions through epigenetic regulation of genes involved in neurotransmission. To our knowledge, no research has explored self-perceived stress or social support during pregnancy in relation to epigenetic changes in mothers' DNAm.

Given prior studies on major stressors and DNAm changes, it is plausible that stressors also including less severe types of stress (captured as self-perceived stress) might also impact the epigenome. Because stress and lack of social support increase the risk of a number of health outcomes (e.g., pregnancy complications, preterm, and low birth weight births),[1,3,10] it follows that social support may contribute to resiliency in pregnant women. However, it is yet to be investigated whether this translates to biological changes detected in DNAm. If so, epigenetic changes resulting from social support are likely to be particularly important for disadvantaged groups like African-Americans, who disproportionately experience stressors like racism and poverty.[11] Therefore, using data from a cohort of African-Americans, the aim of this study was to examine whether prenatal maternal social support or psychosocial stressors are associated with alternations in DNAm of mothers. We hypothesized that both maternal stress during pregnancy and social support from a woman's partner and family and friends would be associated with changes in DNAm.

METHODS

The Boston Birth Cohort (BBC) and the study population

Study participants come from the BBC, which was initiated in 1998 with a rolling enrollment in Boston, MA, as detailed elsewhere.[12] Briefly, mothers who delivered singleton live preterm or low birth weight (<2500 g) newborns or mothers who delivered term and normal birth weight newborns at Boston Medical Center were invited to join the parent study. Mothers with pregnancies that

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Received: 15 April 2019 Revised: 25 June 2019 Accepted: 29 June 2019
Published online: 26 July 2019

were a result of in vitro fertilization, fetal chromosomal abnormalities, or that resulted from multiple gestations or in major birth defects were excluded from the parent study. Enrolled mothers provided written informed consent and completed a questionnaire to obtain information on maternal characteristics, lifestyle, and diet. Maternal blood samples were obtained within 24–72 h after delivery. The study was approved by the Institutional Review Boards of Boston University Medical Center and Johns Hopkins Bloomberg School of Public Health.

The BBC has 8494 mothers who were enrolled during years 1998–2013 including 4026 African-Americans. Three-hundred of those African-American mothers had genome-wide DNAm profiling as a part of an epigenetic study on preterm birth. These enrolled mothers and the complete sample of African-American participants in the BBC were largely comparable with regard to maternal characteristics, as shown in Supplementary Table S1.

Maternal social support and perceived stress status

Maternal variables during pregnancy, including social support and perceived stress, were collected through questionnaires. Perceived support during pregnancy: Two variables represented social support that the mother perceived during pregnancy: (1) support from the baby's father and (2) support from family members and friends. Regarding the former, mothers were asked "How would you rate the amount of social support you received from the father of your baby during your pregnancy?" For the latter, mothers were asked "How would you rate the amount of social support you received during your pregnancy from your other family members and your friends?" Response categories for both questions (0 = none; 1 = a little; 2 = a good amount; 3 = an excellent amount) were further categorized as none (0 = none) versus at least some support (including "a little," "a good amount," or "an excellent amount," referred as "some support" in the text) as reported previously.[3] Perceived stress: Two stress variables were collected based on the maternal questionnaires, including: (1) lifetime stress, for which mothers were asked "How would you characterize the amount of stress in your life in general?" (2) stress during pregnancy, for which mothers were asked "How would you characterize the amount of stress in your life during this pregnancy? (both questions had response categories "0 = not stressful; 1 = average; 2 = very stressful"). Both of these measures have been used previously in the BBC and are based on standard questions.[13,14]

Other maternal covariates

Other maternal characteristics were also collected from each of the mothers based on questionnaires and archived electronic medical records. Maternal pre-pregnancy body mass index (BMI) was calculated as self-reported pre-pregnancy weight in kilograms divided by self-reported height in meters squared. Pre-pregnancy BMI was missing for 18 mothers and was imputed based on the means of the mothers for whom data were available. Maternal smoking during pregnancy was categorized as never smoker, quitter, or continuous smoker.[12] Maternal educational attainment was classified as high school or lower versus college or above. Self-reported maternal marital status was classified as married versus unmarried. Gestational age at delivery was determined based on the first day of the last menstrual period and early prenatal ultrasonographic results,[12] and preterm birth was defined as gestational age at delivery <37 complete weeks. Information regarding parity and Cesarean section was abstracted from the electronic medical record.

DNAm measurements in maternal/cord blood and quality control steps

Genomic DNA was isolated from EDTA-treated peripheral white blood cells from each enrolled mother, which were stored in a −20 °C freezer until use. For DNAm profiling, each DNA sample, diluted to 50–100 ng/μL, was randomly located on a plate and shipped to the Center for Genetic Medicine, Northwestern University Feinberg School of Medicine for methylation profiling, using the Infinium HumanMethylation450 BeadChip, as previously described.[15] For each sample, a raw intensity file (.idat) was processed, and several quality control steps were performed using the R/Bioconductor package "minfi,"[16] as reported previously,[15] leading to high-quality DNAm data for 432,857 sites (including 10,107× chromosome sites) among 290 maternal samples.

With the "minfi" package,[16] we then performed a stratified quantile normalization procedure. Normalized beta values (β), ranging from 0 to 1 for 0–100% methylated, were generated for the 290 maternal samples. To account for potential batch effects, beta values (or β) and the corresponding M values (logit-transformed β values) were ComBat-transformed[17] using the "sva" package[18] with the array number as a surrogate for the batches. ComBat-transformed M values, reportedly superior to β values for identification of differential methylation,[19] were utilized for downstream analyses. We use the intuitive, ComBat-transformed β values for plotting the results.

Cell composition estimation

Using the estimateCellCounts() function in the "minfi" package,[16] a method of deconvolution, the distribution of six cell types (CD4+, CD8+ T cells, B cells, monocytes, granulocytes, and natural killer cells) was inferred for each maternal sample,[20,21] based on adult reference DNAm signatures of the constituent cell types in whole blood.

Data analyses

After removing individuals with missing data on both social support variables and smoking during pregnancy ($n = 40$), we focused our main data analyses on 250 mothers. Characteristics of the sample were compared across groups of mothers perceiving different amounts of social support during pregnancy, using χ^2 tests (or Fisher Exact Test, when the sample size in a specific subgroup was small, i.e., <5) and analysis of variance, respectively, for categorical and continuous variables. To identify differentially methylated sites in maternal blood that were associated with each social support variable, we fit a linear regression model with the ComBat-transformed M value at each site as the outcome and lack of social support as the exposure, adjusting for covariates associated with DNAm such as maternal age (a continuous variable), pre-pregnancy BMI (a continuous variable), maternal smoking (never smoker/quitter/continuous smoker), gestational age (a continuous variable), parity (dichotomized as 0 versus ≥1), cell composition (continuous variables), and three principal components to account for genetic ancestry (continuous variables), as well as other maternal factors that were associated with social support (i.e., marital status, classified as married versus unmarried). In the analyses, lack of social support was analyzed as a four-level categorical variable (1 = a little, 2 = good amount, 3 = excellent amount, compared to 0 = none) as well as a binomial variable (1 = some support, versus 0 = none). We reported the results for social support analyzed as a binomial variable since we found that the DNAm levels were comparable among the mothers with little support, with good support, or with excellent support (Supplementary Fig. S1).

Similar analyses were also performed to identify methylated sites in maternal blood that were associated with perceived lifetime stress (a three-category exposure, with "not stressful" as the reference) and stress during pregnancy (a three-category exposure, with "not stressful" as the reference). The false discovery rate (FDR) was estimated to correct for multiple testing with an FDR < 0.05 as the cut-off for statistical significance.

Table 1. Sample characteristics of two indicators of maternal perceived lack of support during pregnancy

Characteristics	Total sample	Support from baby's father		Support from family or friends	
		At least some	None	At least some	None
n	250	228	19	241	8
Gestational age (weeks), mean (sd)	35.1 (5.8)	35.2 (5.8)	33.7 (5.5)	35.2 (5.7)	31.8 (7)
Maternal age (years), mean (sd)	27.7 (6.3)	27.9 (6.3)	26.5 (6)	27.7 (6.3)	28.6 (5.7)
Maternal perceived stress scale, 4 items (PSS-4), mean (sd)	6.1 (3.0)	6.1 (3)	8 (0)	6.1 (3)	NA
Maternal BMI, mean (sd)	26.3 (6.8)	26.6 (7)	23.9 (3.4)	26.3 (6.9)	26.1 (4.1)
Lifetime stress, *n* (%)					
Not stressful	89 (35.6)	83 (36.4)	4 (21.1)	87 (36.1)	2 (25)
Average	125 (50)	114 (50)	11 (57.8)	121 (50.2)	3 (37.5)
Very stressful	36 (14.4)	31 (13.6)	4 (21.1)	33 (13.7)	3 (37.5)
Stress during pregnancy, *n* (%)					
Not stressful	91 (36.4)	83 (36.4)	6 (31.6)	88 (36.5)	2 (25)
Average	103 (41.2)	95 (41.7)	8 (42.1)	100 (41.5)	3 (37.5)
Very stressful	55 (22)	49 (21.5)	5 (26.3)	52 (21.6)	3 (37.5)
Unknown	1 (0.4)	1 (0.4)	0 (0)	1 (0.4)	0 (0)
Maternal smoking, *n* (%)					
Never	179 (71.6)	165 (72.4)	12 (63.2)	173 (71.7)	5 (62.5)
Quit	24 (9.6)	21 (9.2)	3 (15.8)	24 (10)	0 (0)
Continuous	47 (18.8)	42 (18.4)	4 (21)	44 (18.3)	3 (37.5)
Education, *n* (%)					
High school or lower	156 (62.4)	141 (61.8)	13 (68.4)	150 (62.2)	5 (62.5)
College or above	92 (36.8)	85 (37.3)	6 (31.6)	89 (37)	3 (37.5)
Unknown	2 (0.8)	2 (0.9)	0 (0)	2 (0.8)	0 (0)
Marital status, *n* (%)					
Married	66 (26.4)	66 (28.9)	0 (0)*	66 (27.4)	0 (0)
Unmarried	180 (72)	158 (69.3)	19 (100)	171 (71)	8 (100)
Unknown	4 (1.6)	4 (1.8)	0 (0)	4 (1.6)	0 (0)
Nulliparity status, *n* (%)	103 (41.2)	91 (39.9)	10 (52.6)	101 (41.9)	2 (25)
Preterm, *n* (%)	121 (48.4)	108 (47.4)	12 (63.2)	115 (47.7)	5 (62.5)

BMI body mass index, *NA* not available
*$P < 0.05$ for the difference of each variable between mothers with and without support from the baby's father or between mothers with and without support from family or friends during pregnancy, tested based on χ^2 tests (or Fisher exact test, when the sample size in a specific subgroup was small, i.e., <5) and *t* test, respectively, for categorical and continuous variables

RESULTS

Population characteristics

The current study included 250 African-American mothers with data available on social support and epigenome-wide DNAm. These enrolled mothers were largely comparable to all the available African-American mothers in the BBC, except that they were younger and were more likely to have a preterm delivery ($P < 0.05$, Supplementary Table S1). Among these 250 enrolled mothers (the mean age was 27.7 ± 6.3 years at the time of delivery), 7.6% reported no support from the baby's father, and 3.2% reported no support from other family or friends during pregnancy (Table 1). When stratified by the level of social support, we found that mothers who lacked support from the baby's father were more likely to be unmarried compared to those having some support from the baby's father, but other factors, including maternal age, maternal smoking during pregnancy, education level, parity, and BMI, were not statistically different between these two groups (Table 1). Mothers with and without support from family or friends during pregnancy were also comparable on these maternal characteristics (Table 1).

Epigenome-wide association study among mothers

At an FDR < 5%, we identified three CpG sites (cg07923746 in the *TOR3A* gene, cg22187826 in the *IQCB1* gene, and cg05735009 in the *C7orf36* gene) that were significantly hypomethylated and one CpGs site (cg06175927 in the *MYH7B* gene) that was significantly hypermethylated in mothers with lack of support from the baby's father compared with mothers who received some support from the baby's father during pregnancy (Fig. 1a and Table 2). When family/friend support during pregnancy was analyzed as the exposure, we found another eight autosomal CpG sites with significantly higher methylation levels in mothers lacking family/friend support compared to mothers having some support from family/friends (Fig. 1b and Table 2). These eight identified CpG sites included two nearby sites in the *PRDM16* gene, one in the *MCF2L2* gene (or in the 5′ untranslated region of the *B3GNT5* gene), one in the *GP5* gene, two in the *BANK1* gene, one in the *BCL9L* gene, and one in the *CDAN1* gene. The magnitude of DNAm differences at these sites ranged from 1.1% to 11.1% between the two groups (Table 2).

Given the significantly higher prevalence of preterm delivery in the enrolled mothers, we thus performed stratified analyses to test

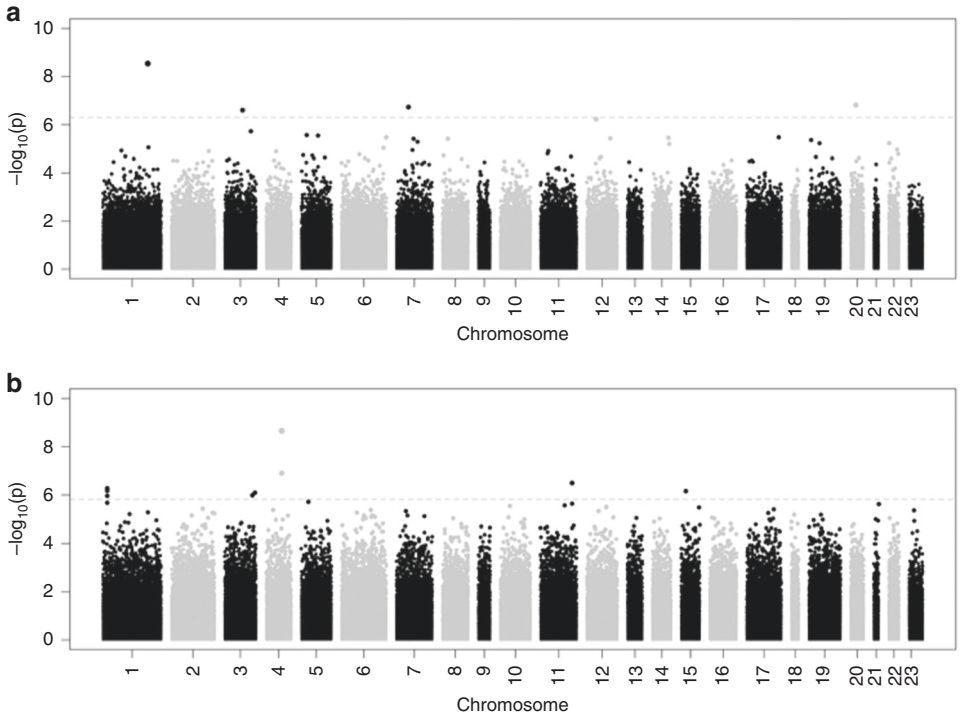

Fig. 1 Manhattan plots for epigenome-wide associations in mothers lacking support from the baby's father and lacking support from family or friends during pregnancy. **a** is for lack of support from baby's father, and **b** is for lack of support from family or friends during pregnancy. The dashed line represents the epigenome-wide significance threshold (false discovery rate = 5%)

Table 2. The top CpG sites whose DNA methylations were significantly associated with lack of maternal social support in the BBC

CpG	CHR[a]	Position	Gene	Location	Mean diff[a], %	P^b	FDR[c]
Lack of support from baby's father as the exposure							
cg07923746	1	179050803	*TOR3A*	TSS1500	−2.40	2.89E−09	1.25E−03
cg22187826	3	121554169	*IQCB1;EAF2*	TSS1500; first exon	−0.88	2.50E−07	0.03
cg05735009	7	39606071	*C7orf36*	First exon	−0.76	1.86E−07	0.03
cg06175927	20	33563009	*MYH7B*	TSS200	1.87	1.53E−07	0.03
Lack of support from family or friends as the exposure							
cg21848084	1	3264381	*PRDM16*	Body	5.90	5.32E−07	0.05
cg06060874	1	3269315	*PRDM16*	Body	11.13	6.59E−07	0.05
cg04468081	3	182983386	*MCF2L2;B3GNT5*	Body; 5′UTR	5.42	1.03E−06	0.05
cg03022680	3	194117679	*GP5*	Body	6.75	8.05E−07	0.05
cg05935311	4	102711702	*BANK1*	TSS200	1.06	1.25E−07	0.03
cg00332153	4	102712010	*BANK1*	5′UTR; first exon	3.46	2.20E−09	9.51E−04
cg04075973	11	118781608	*BCL9L*	First exon; 5′UTR	5.10	3.19E−07	0.05
cg02122467	15	43027820	*CDAN1*	Body	2.16	6.93E−07	0.05

BBC Boston Birth Cohort, *FDR* false discovery rate, *UTR* untranslated region
[a]β_{diff}%: methylation level (or beta value) difference in percentage between the two groups. $\beta_{diff}\% < 0$ means that the methylation level was lower in the mothers/newborns lacking social support than in those with some support, while $\beta_{diff}\% > 0$ means the methylation level was higher in the mothers/newborns lacking social support
[b]The associations were adjusted for maternal age, pre-pregnancy BMI, parity, maternal smoking during pregnancy, marital status, gestational age at delivery, cell composition, and three principal components to account for genetic ancestry
[c]FDR correction for multiple tests on the epigenome-wide CpG sites

whether the identified associations were confounded by term/preterm delivery. As shown in Supplementary Table S2, we found that the associations between the four identified CpG sites and lack of support from the baby's fathers remained comparable in mothers with term deliveries and in mothers with preterm delivery, and there were no significant interactions between preterm deliveries and lack of support associated with methylation level at the four sites identified. For the eight CpGs sites that were identified related to lack of support from family/friends, we noticed that the effect size for the association between lack of support from family/friends and methylation level of cg04468081 tended to be larger in mothers with preterm deliveries; however,

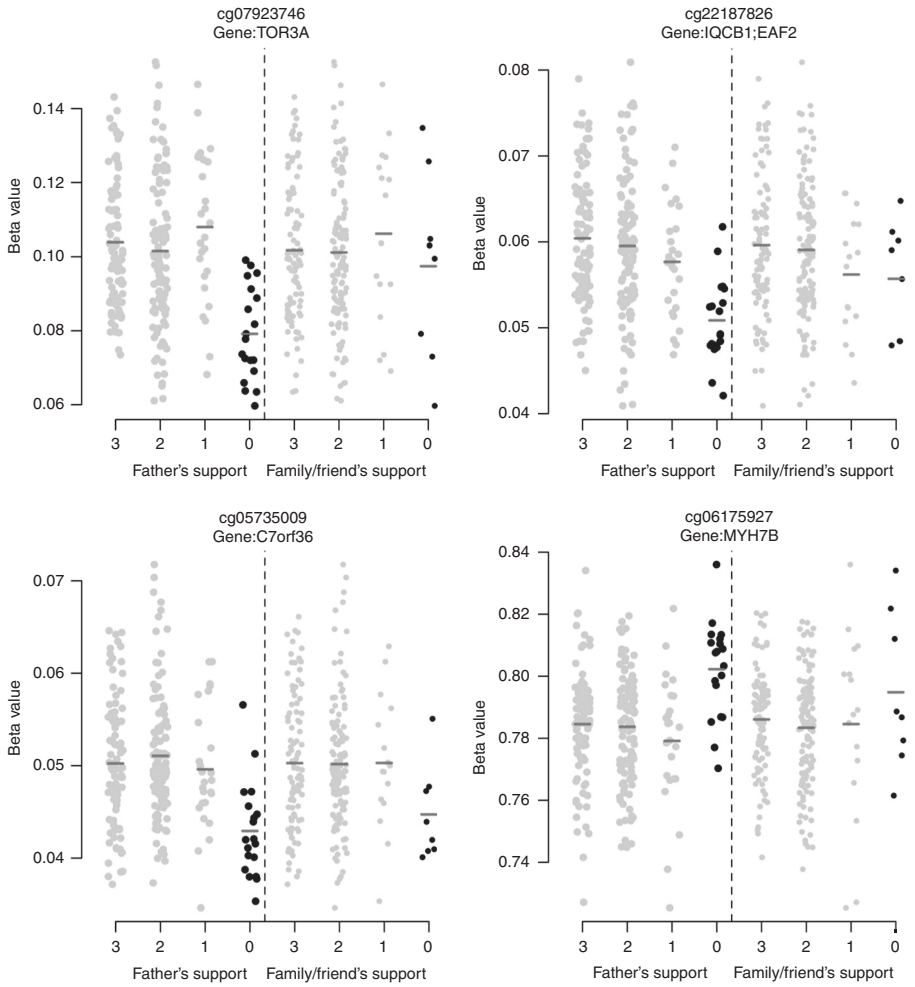

Fig. 2 Distribution of methylation levels for the four CpG sites identified for lack of mothers' support from the baby's fathers, stratified by different levels of social support (i.e., father's support as well as friend/family support) in the Boston Birth Cohort. Social support level: 0 = no support; 1 = a little support; 2 = a good amount of support; and 3 = an excellent amount of support

the interaction association between lack of support from family/friend and preterm delivery ($P = 0.03$, FDR = 0.28) was not significant after FDR correction for multiple testing (Supplementary Table S2).

Figure 2 presents the distribution of the methylation levels at the four identified CpG sites for lack of social support from the baby's father. When mothers with some support from the baby's father were further classified into three subgroups (mothers with a little support, mothers with a good amount of support, and mothers with an excellent amount of support), we found that these three subgroups all had comparable methylation levels at the four sites (although they all were differentially methylated compared to mothers with lack of support from the baby's father), indicating no significant dose-responsive effects (Fig. 2). Similarly, we found no dose–response trends in the associations of the identified eight CpG sites and lack of social support from family or friends (Fig. 3).

To further explore whether there were shared CpG sites between support from the baby's father and support from family or friends, we then tested whether the four significant CpG sites for lack of support from the baby's father were also significantly associated with lack of family/friend support and vice versa. Although similar patterns were observed, methylation levels at these four CpG sites (Fig. 2) were not significantly associated with lack of family/friend support. Similarly, among the eight CpG sites identified for lack of support from family/friend support, none were significantly associated with lack of support from the baby's father, as shown in Fig. 3.

Epigenome-wide association study for perceived stress variables

We further investigated the associations between two perceived stress variables (lifetime stress and stress during pregnancy) and DNAm changes in mothers (Supplementary Fig. S1); however, no significant signals were noticed after FDR adjustment.

DISCUSSION

While social support during pregnancy has been found as protective for prenatal complications, postnatal mental health, and other maternal health conditions,[22–24] to our knowledge, this is the first study to examine associations between social support during pregnancy and DNAm changes in African-American mothers. Our findings suggested that social support, both from family/friends and the baby's father, was related to DNAm changes in maternal blood.

We found that maternal DNAm at four CpG sites were modestly related to social support from the baby's father (located in the *TOR3A*, *IQCB1*, *EAF2*, *C7orf36* and *MYH7B* genes). *TOR3A* (Torsin family 3 member A) encodes the torsin-3A protein, which is a member of Torsin family ATPases.[25] A recent study identified an essential role of Torsin in cellular lipid metabolism.[26] Another study suggested a functional link

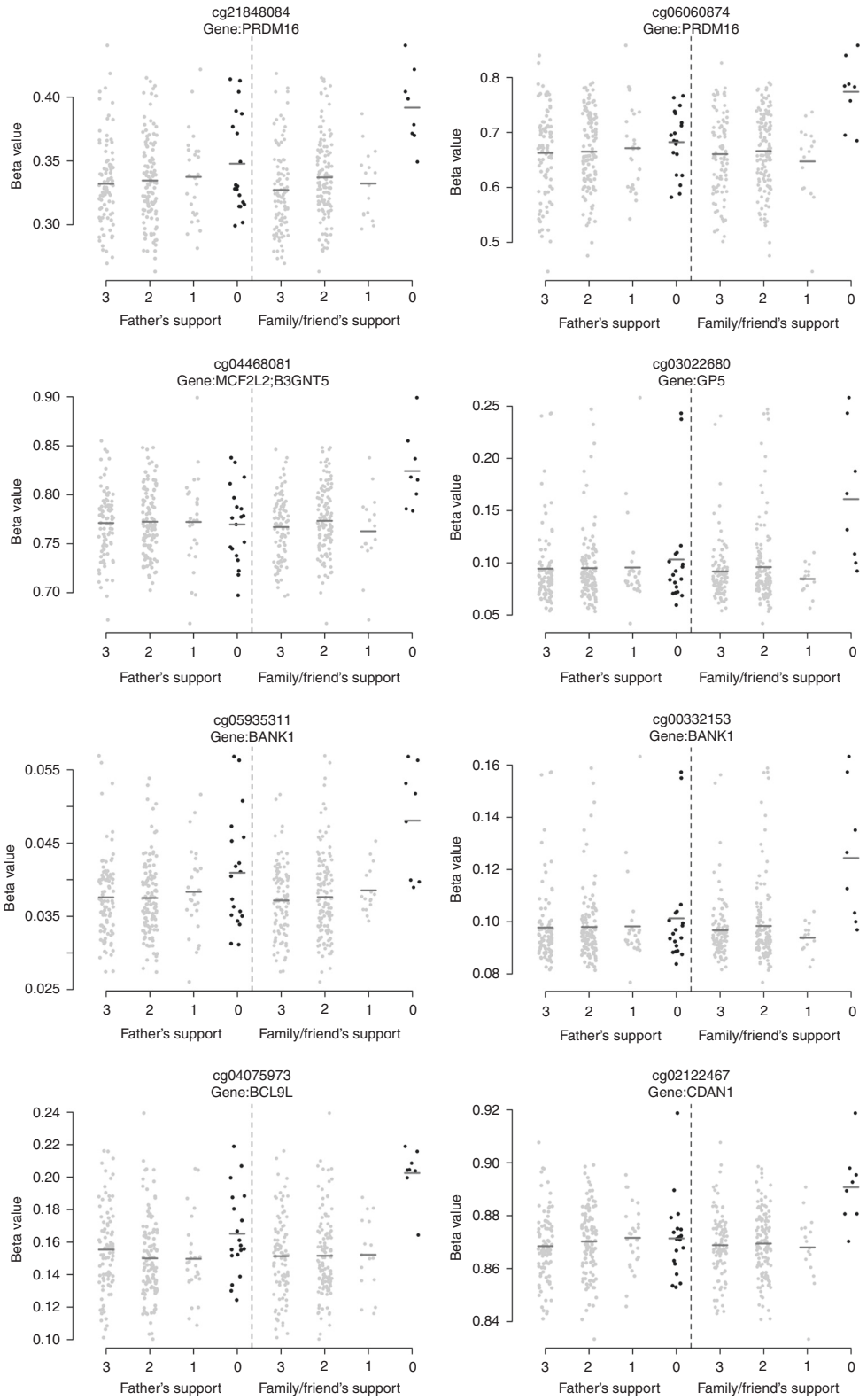

Fig. 3 Distribution of the eight CpG sites identified for lack of mothers' support from friends and/or family members, stratified by different levels of social support in the Boston Birth Cohort

between the Torsin/cofactor system and nuclear envelope/nuclear pore complex biogenesis or homeostasis.[27] However, the biological significance of the DNAm changes in the *TOR3A* gene related to social support from the baby's father, as identified in our study, remains to be clarified.

We also observed significant changes in maternal DNAm related to absence of support from family and friends, including CpG sites on *MCF2L2*, *B3GNT5*, *GP5*, *BCL9L*, *CDAN1*, *BANK1*, and *PRDM16*. Of note, 3 CpG sites in the *PRDM16* genes were identified, all with relatively large effect size (having 5–11% DNAm changes).

Although *PRDM16* is generally known as a transcription factor that regulates brown fat development, a recent study found that *PRDM16* promotes stem cell maintenance in multiple organ systems, including the hematopoietic and central nervous systems.[28] Yang et al. found that decreased promoter methylation of mouse *PRDM16* and its correlated increased gene expression occurs during the course of early brown adipogenesis.[29] Interestingly, the induction of *PRDM16* gene expression was modulated by activation of AMP-activated protein kinase (AMPK), a key regulator in intracellular energy metabolism (especially nerve cell metabolism). This finding suggests that modulation of AMPK activity may contribute to epigenetic regulation of *PRDM16* gene expression. A recent study reported that chronically stressed mice showed heightened symptoms of anxiety and depression, accompanied by decreased AMPK activity in the brain.[30] It is possible that chronic stress-associated AMPK suppression may inhibit *PRDM16* gene expression via increased promoter methylation. Thus, although replication studies and functional studies are needed,[31,32] our results suggest that a lack of social support in mothers might decrease AMPK activity, leading to increased DNAm levels of *PRDM16* in mothers.

The present study was focused on understanding associations between maternal exposure and gene methylation changes. Interestingly, some of the genes we identified are involved in various biological pathways (such as metabolism) that may ultimately affect maternal health (Supplementary Table S3), e.g., *B3GNT5* in the synthesis of lacto-series glycolipids,[33] *PRDM16* in energy balance and brown fat development,[34] and *TOR3A* in lipid metabolism.[26] Given our findings and those of others demonstrating associations between stress responses and epigenetic regulation of genes involved in cell metabolism and mental health disorders (e.g., *NR3C1* and *SLC6A4*),[4–6] we speculate that lack of support may contribute to dysregulated metabolic pathways and mental health disorders, which could be a topic of future study.

Our study did not show evidence for associations between perceived stress and DNAm in mothers, which is inconsistent with some previous studies. Prior studies suggested that different genes might be affected by varied types of stress (e.g., *NR3C1* for violence and *SLC6A4* for depressed mood).[5,6] In addition, Devlin et al. assessed the association between maternal DNAm levels in the *SLC6A4* gene and maternal depressed mood during the second and the third trimester of pregnancy.[6] Interestingly, an association was found only with depressed mood in the second trimester. Given our measure of perceived stress likely reflected the average level throughout the pregnancy, it could be that the difference in timing or the type of stress may partly explain the inconsistency between our findings and that of others. Lack of more robust and validated maternal stress measures may be another possible reason we did not find an association between stress and DNAm. Self-report of stress levels may be less accurate than for social support, as the latter is potentially easier to recall and more quantifiable.

A unique feature of our study was the ability to couple information on social support during pregnancy with epigenetic data from mothers in a predominantly urban minority population, where two thirds of our sample had a high school degree or less. Women with these socio-demographic characteristics are often at risk of having low levels of social support.[35] Our study also included a high proportion of single mothers, who generally get less support from the baby's father. Our findings provide intriguing evidence for biological embedding of maternal social support during pregnancy and suggest that lack of social support from either the baby's father or from family and friend both can have impact on maternal DNAm, although the targeted genes may be different. These findings, if confirmed, suggest that efforts to mobilize support from these multiple sources during pregnancy may offer an effective intervention. Given that US non-Hispanic African-American fathers are more likely to be unmarried compared to other racial groups such as non-Hispanic Whites, garnering support from family and friends may be especially important among African-Americans.[36] Presently, African-American teens are more likely to receive support from their extended families during pregnancy compared to that from the baby's father,[37] suggesting that efforts to further engage family and friends to provide support during pregnancy in similar populations might be feasible.

Our study also has limitations. Although this study is among the first and largest study of this kind, the relatively small sample size did not allow for more in-depth analyses. Since our sample is a small subset of the BBC, with significantly higher prevalence of preterm delivery (Supplementary Table 1), we performed stratified analyses by preterm delivery and found that similar patterns of the associations remained in both mothers with term delivery and mothers with preterm delivery (Supplementary Table 2). This finding suggests that selection bias, if it exists, is minimized. Another limitation is the study's cross-sectional design, since interviews and blood samples were collected simultaneously. Although women recalled a period prior to when the blood samples were collected, the study was nonetheless retrospective (even if information was collected very soon after delivery), allowing the possibility that recall could be influenced by a mother's postpartum mood. Given the timing of data collection, it is possible that women with adverse birth outcomes, such as preterm birth, would be more likely to report stress or absence of support. To take this into account, we adjusted for gestational age in our analyses.

Also, information on social support and some of the stress measures was gathered using single broad questions, precluding examination of the type of support received/stress experienced, although an advantage was that we could differentiate from whom the support was received. Another potential limitation was that the DNAm changes identified in this study were not validated by pyrosequencing, and changes in gene expression were not assessed. In addition, the interpretability of several of the DNAm changes we observed are unclear, given that there was a modest (i.e., <5%) difference in methylation levels between the exposure and reference groups. However, it is possible that the methylation changes in other tissues might be larger than the changes observed in maternal blood samples. Previous studies have shown that small changes in methylation can have a strong functional effect on transcriptional activity.[38] Finally, because DNAm was assessed only at one point in time, it is not possible to know whether these methylation changes occurred pre-pregnancy, during pregnancy (during which trimester), or post-pregnancy. Our data represent methylation changes resulting from all possible exposures during the entire pregnancy. Given the fact that methylation changes are time-sensitive and gene-specific, future studies should focus on different exposure windows during pregnancy.

In summary, this is the first study to investigate lack of social support during pregnancy in relation to maternal DNAm changes. Our results are suggestive of potential novel associations between social support from family/friends and baby's father and gene-specific DNAm changes in maternal blood. However, these findings need confirmation. If confirmed in larger studies with more robust measures of social support and stress, this work could be extended to examine subsequent maternal health conditions associated with the observed DNAm changes and ultimately to investigate whether strengthening social support could serve as an effective strategy to improve maternal health outcomes.

ACKNOWLEDGEMENTS

We thank the study participants for their help with the study. We are also grateful for the dedication and hard work of the field team at the Department of Pediatrics, Boston University School of Medicine and for the help and support of the obstetric nursing staff at Boston Medical Center. This work was supported in part by the National Institutes of Health (NIH) (grants R21HD085556, R21ES011666,

R21HD066471, R01HD086013, and 2R01HD041702) and the March of Dimes PERI (grants 20-FY02-56 and 21-FY07-605). X.H. is also partially supported by Hopkins Population Center (grant NICHD R24HD042854). Dr. Hongkai Ji is partially supported by NIH (grant R01HG006282).

AUTHOR CONTRIBUTIONS

All authors meet the journal's authorship requirements and approved the final manuscript. P.J.S. and X.W. developed the concept for this analysis. P.J.S., X.H., and N. N. drafted the manuscript. B.Z. conducted the data analysis. C.P. supervised the field data collection. G.W. supervised biospecimen processing and biomarker assays. X.H. maintained and managed the database. All authors contributed to the analysis plan, edited the manuscript, and interpreted data. X.W. was responsible for the initiation, overall development, and oversight of the study and its measures.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41390-019-0512-7) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

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