Racial and ethnic representation in epigenomic studies of preterm birth: a systematic review

Ai-ris Y Collier^{1,2} , Rachel Ledyard³, Diana Montoya-Williams^{3,4}, Maylene Qiu⁵, Alexandra E Dereix^{3,6}, Minou Raschid Farrokhi^{1,7}, Michele R Hacker^{1,2,8} & Heather H Burris^{*,3,4}

¹Department of Obstetrics & Gynecology, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA

²Department of Obstetrics, Gynecology, & Reproductive Biology, Harvard Medical School, Boston, MA 02115, USA

³Division of Neonatology, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA

⁴Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA

⁵Biomedical Library, University of Pennsylvania, Philadelphia, PA 19104, USA

⁶Loyola University Chicago Stritch School of Medicine, Maywood, IL 60153, USA

⁷Colby College, Waterville, ME 04901, USA

⁸Department of Epidemiology, Harvard TH Chan School of Public Health, Boston, MA 02115, USA

*Author for correspondence: Tel.: +1 215 573 4916; Fax: +1 215 573 5408; hbarris@pennmedicine.upenn.edu

Aim: We conducted a systematic review evaluating race/ethnicity representation in DNA methylomic studies of preterm birth. **Data sources:** PubMed, EMBASE, CINHAL, Scopus and relevant citations from 1 January 2000 to 30 June 2019. **Study appraisal & synthesis methods:** Two authors independently identified abstracts comparing DNA methylomic differences between term and preterm births that included race/ethnicity data. **Results:** 16 studies were included. Black and non-Hispanic Black deliveries were well represented (28%). However, large studies originating from more than 95% White populations were excluded due to unreported race/ethnicity data. Most studies were cross-sectional, allowing for reverse causation. Most studies were also racially/ethnically homogeneous, preventing direct comparison of DNA methylomic differences across race/ethnicities. **Conclusion:** In DNA methylomic studies, Black women and infants were well represented. However, the literature has limitations and precludes drawing definitive conclusions.

First draft submitted: 8 January 2020; Accepted for publication: 27 March 2020; Published online: 2 December 2020

Keywords: DNA methylation • epigenetics • epigenomics • gestational age • health disparities • methylomics • pregnancy • preterm birth • race and ethnicity • systematic review

In the USA, longstanding racial disparities in preterm birth [1] result in an infant mortality rate more than twice as high among Black infants compared with White infants [2]. Preterm birth rates are 52% greater in Black (13.8%) than White (9.0%) women and this disparity has persisted for decades [3]. There is evidence that racial disparities in birth outcomes are largely due to environmental exposures, as opposed to genetic sequence variation by race/ethnicity. In a large US birth cohort, the rate of low birth weight in recently immigrated African-born women resembled the rate of US-born White women more closely than US-born Black women, suggesting that environmental factors play a greater role than genetic factors in racial differences [4]. Similarly, US-born non-Hispanic Black women had greater rates of preterm birth compared with foreign-born non-Hispanic Black women, even after adjusting for maternal sociodemographic factors, health behaviors and medical risk factors [5]. In a large, mixed-race US cohort evaluating the extent of heritable versus environmental exposures that predict timing of birth, environmental sources (particularly factors that change between pregnancies) were largely responsible for the increased heterogeneity in gestational age observed in African-Americans compared with European-Americans [6]. Taken together, these studies support the concept that it is not differences in innate biology, but differences in exposures between Black and White women in the USA that lead to racial disparities in preterm birth.

It is conceivable that racial disparities in preterm birth have a greater environmental than genetic contribution because there are very few differences in genetic sequences between racial groups. In contrast, adverse physical exposures, and social environmental exposures concentrate in minority neighborhoods in the USA [3,7–10]. For example, Black families are disproportionately exposed to air pollution [11,12] and chronic stressors, such as racial discrimination [13,14] and violent crime [15,16]. Mechanisms connecting physical and social environmental exposures with racial disparities in preterm birth may include inflammation, immunologic and hormonal dysregulation and epigenetics. Epigenetic mechanisms generally refer to heritable (at least mitotically) alterations to regulation of gene expression in the absence of DNA sequence differences. One of the most commonly studied epigenetic mechanisms is DNA methylation, which may be modified by physical and social environmental exposures. It is biologically plausible that differential DNA methylation may explain differences in preterm birth risk between Black and White women in the USA due to the above-mentioned differential exposure by race to physical and environmental stressors. In a controlled human experiment, air pollution altered DNA methylation, specifically lowering blood DNA *TLR4* methylation in circulating leukocytes [17]. Exposure to psychosocial stress also has been shown to increase DNA methylation of the glucocorticoid receptor in rats [18] with similar associations in an observational human study [19].

To determine if sufficient data exist to assess whether DNA methylation could explain racial disparities in preterm birth, we performed a systematic review of epigenomic, specifically DNA methylomic, studies existing in the literature. We then examined the study design, tissue type, geographic location and racial/ethnic composition of the study participants. Racial and ethnic minorities suffer disproportionately from many health conditions, and yet they are under-represented in clinical trials for oncology [20–23], obesity [24] and women's health [25] research despite the 1993 NIH Revitalization act encouraging representation of women and minority patients in research. Recruitment strategies directed at improving enrollment of Black women [24,26] are crucial to improve generalizability and improve equity for clinical studies. Our hypothesis was that, like interventional clinical trials, non-Hispanic Black women and their infants would be under-represented in methylomic studies of preterm birth despite their over-representation among preterm deliveries.

Methods

Protocol & registration

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [27] and performed a search of MEDLINE (PubMed), EMBASE and CINAHL on 2 October 2019 and Scopus on 7 October 2019 using terms for preterm birth in humans and epigenomics (Supplementary Table 1). This systematic review was registered in the PROSPERO database for systematic reviews (protocol #153707) and is available in full on the NIH Research website [28]. References acquired from these searches were uploaded into Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia; available at www.covidence.org).

Eligibility criteria & study selection

Studies were eligible if they were primary publications reporting on epigenomic evaluation of human tissues (maternal blood or tissue, placenta, infant umbilical cord blood or amniotic fluid) to study preterm birth in humans. We focused the scope of this systematic review on epigenomic studies that evaluated DNA methylation; studies had to probe at least 42 methylation sites used by some arrays to qualify as a methylomic study. All study designs with peer-reviewed full-text publication in English were eligible. Studies had to report race/ethnicity and were included if they only included a single racial/ethnic group of participants. Studies had to include both preterm (<37 weeks of gestation) and term (≥ 37 weeks of gestation) participants in order to allow a comparison between preterm birth and term birth.

Two authors (AE Dereix and MR Farrokhi) independently reviewed all abstracts and two authors (AY Collier and R Ledyard) independently reviewed all full-text manuscripts. We excluded animal studies, meta-analyses or reviews that did not contain primary data, case reports, nonmethylomic studies (transcriptomics or genomics) and studies that did not evaluate the outcome of preterm birth. Studies were excluded if they did not contain any information regarding race/ethnicity of the participants in the primary manuscript text, tables or supplementary data. Corresponding authors for each of the studies missing race/ethnicity were contacted in an attempt to acquire data specifying race and/or ethnicity. Conflicts regarding abstract classification and full-text classification were resolved by a third investigator (HH Burris). We also reviewed the reference lists of included manuscripts to identify additional relevant manuscripts meeting the inclusion criteria.

Figure 1. PRISMA flow diagram of the literature search for studies of racial and ethnic representation in human DNA methylomic studies of preterm birth.
PTB: Preterm birth.

Data collection process

The ability to extract information on race and ethnicity into prespecified categories was limited, in part, by lack of consensus guidelines on how race and ethnicity should be reported. We used broad categories of race alone, such as White, Black, Asian; ethnicity alone, such as Hispanic or mutually exclusive race and ethnicity categories, such as non-Hispanic Black. When individual studies used more specific terms, including Chinese, Indian, Malay, Pakistani, western and non-western geographic region, we included these specific ethnicities, as described. Details of each study, including study design, birth cohort, tissue type, number of DNA methylation sites evaluated, number of DNA methylation sites associated with preterm birth and number of preterm and term deliveries by race and ethnicity category were collected by two investigators (AY Collier and R Ledyard). Institutional review board approval was not required as identifiable data were not utilized in the course of this project.

Data analysis

After a study was included, each woman who delivered was counted as a single ‘delivery’. Each infant born was counted as a ‘birth’. From each delivery or birth, either a single specimen or multiple specimens could be collected (maternal blood, cord blood, placenta, etc.). The proportion of deliveries represented by each race/ethnicity was the primary outcome for this systematic review. As a secondary analysis, studies excluded due to lack of race/ethnicity reporting alone were further evaluated to determine whether their inclusion would have changed the findings regarding race/ethnicity representation. For these studies, the corresponding authors were contacted for further unpublished details about race/ethnicity. If the corresponding author did not respond, or the corresponding author confirmed that race/ethnicity data were unknown, the current estimates of the racial and ethnic demographics of the country of the study were obtained from an independent, nonpolitical online resource (<http://worldpopulationreview.com/>) to infer the likely racial and ethnic representation of the study.

Results

Study selection

999 references were imported from the search. Of the 74 abstracts that met initial inclusion criteria, the full-text review led to inclusion of 16 studies [29–44] that drew from 13 birth cohorts and exclusion of 58 studies (Figure 1 & Table 1). Among excluded studies, two were excluded because they were transcriptomic [45] and metabolomic [46] studies. Five studies were epigenomic studies, but were excluded because they investigated epigenomic mechanisms other than DNA methylation including long noncoding RNA [47–49] and miRNA [50,51], and six analyzed candidate DNA methylation sites (<42) [52–57]. Four studies were excluded because they were

Table 1. Characteristics of 16 studies evaluating DNA methylomic differences in preterm birth with description of race and/or ethnicity and number of CpG sites evaluated.

Cohort	Tissue type	Publication title Included in systematic review	Study	Year	Location	Study Design	Total	Preterm	Number of CpG sites evaluated	CpG sites associated with preterm birth	Racial/ethnic group represented	Ref.
Nashville Birth Cohort (NBC)	UCB	Methylation differences reveal heterogeneity in preterm pathophysiology: results from bipartite network analyses	Bhavnani et al.	2018	Nashville, TN, USA	Cross-sectional	50	22	>485,000	10	Non-Hispanic Black (n = 50)	[29]
		Fetal DNA methylation associates with early spontaneous preterm birth and gestational age	Parets et al.	2013	Nashville, TN, USA	Cross-sectional	50	22	479,808	9637	Non-Hispanic Black (n = 50)	[30]
	Maternal blood	DNA methylation provides insight into intergenerational risk for preterm birth in African Americans	Parets et al.	2015	Nashville, TN, USA	Prospective	40	16	483,830	17,829	Non-Hispanic Black (n = 40)	[37]
Women & Infants Hospital 2008–2009	Placenta	Placental DNA methylation alterations associated with maternal tobacco smoking at the <i>RUNX3</i> gene are also associated with gestational age	Maccani et al.	2013	Providence, RI, USA	Cross-sectional	206	22	21,551	2	White (n = 106), non-White (n = 100)	[38]
Women & Infants Hospital	Placenta	Effect of prematurity on genome wide methylation in the placenta	Schuster et al.	2019	Providence, RI, USA	Cross-sectional	9	6	427	21	Non-Hispanic White (n = 7), Hispanic (n = 1), other (n = 1)	[39]
Boston Birth Cohort (BBC)	Maternal blood, UCB	Genome-wide DNA methylation associations with spontaneous preterm birth in US Blacks: findings in maternal and cord blood samples	Hong et al.	2018	Boston, MA, USA	Cross-sectional	410	205	432,857 maternal blood	45 maternal blood	Black/African-American (n = 410)	[44]
Growing Up in Singapore toward Healthy Outcomes (GUSTO) birth cohort study	UCB and tissue	Analysis of two birth tissues provides new insights into the epigenetic landscape of neonates born preterm	Wu et al.	2019	Singapore	Cross-sectional	1019	68	134,676	67	Chinese (n = 591), Indian (n = 178), Malay (n = 250)	[31]
The Rotterdam Periconceptual Cohort (Predict Study)	UCB, placenta, HUVECs	Early- and late-onset pre-eclampsia and the tissue-specific epigenome of the placenta and newborn	Herzog et al.	2017	The Netherlands	Cross-sectional	112	33	43,488 UCB, 134,700 placenta, 42,352 HUVEC	12,040 UCB, 5668 placenta, 1 HUVEC	Western origin (n = 86), non-western origin (n = 26)	[32]
Tracking Health Related to Environmental Exposures (THREE) Study	UCB	DNA methylation shows genome-wide association of <i>NPFX</i> , <i>RAPGEF2</i> and <i>MSRB3</i> with gestational age at birth	Lee et al.	2012	Baltimore, MD, USA	Cross-sectional	141	18	2,100,000	8611	White (n = 32), Black (n = 102), Asian (n = 7)	[33]
University of North Carolina PTB Biobank	Placenta	Epigenetic regulation of the nitric oxide pathway, 17-alpha hydroxyprogesterone caproate and recurrent preterm birth	Manuck et al.	2018	Chapel Hill, NC, USA	Cross-sectional	17	7	850,000	850	Non-Hispanic White (n = 3), non-Hispanic Black (n = 7), Hispanic (n = 7)	[34]
HUVEC: Human umbilical vein endothelial cell; PTB: Preterm birth; UCB: Umbilical cord blood.												

Table 1. Characteristics of 16 studies evaluating DNA methylomic differences in preterm birth with description of race and/or ethnicity and number of CpG sites evaluated (cont.).

Cohort	Tissue type	Publication title included in systematic review	Study	Year	Location	Study Design	Total	Preterm	Number of CpG sites evaluated	CpG sites associated with preterm birth	Racial/ethnic group represented	Ref.
Women's Mental Health Program (WMHP), Conditions Affecting Neurocognitive Development	UCB	Neonatal DNA methylation patterns associate with gestational age	Schroeder et al.	2011	Atlanta, GA & Shelby County, TN, USA	Cross-sectional	453	30	27,578	994	White (n = 316), Black (n = 137)	[40]
Penn Center for the Study of Epigenetics in Reproduction as well as the University of Pennsylvania March of Dimes Prematurity Research Center	Placenta	Epigenetic changes in preterm birth placenta suggest a role for ADAMTS genes in spontaneous preterm birth	Mani et al.	2019	Philadelphia, PA, USA	Cross-sectional	71	23	850,000	11,700	White (n = 46), Black (n = 22), other (n = 3)	[41]
Tampa General Hospital	UCB	Racial differences in DNA-methylation of CpG sites within preterm-promoting genes and gene variants	Salihu et al.	2016	Tampa, FL, USA	Cross-sectional	89	12	42	6	Black (n = 21), non-Black (n = 68)	[42]
University of Iowa and Instituto de Maternidad y ginecología Nuestra Señora de las Mercedes in Argentina	Amnion of the placenta	Genome-wide analysis of DNA methylation in human amnion	Kim et al.	2013	IA, USA & Tucumán, Argentina	Cross-sectional	24	6	2334	65	White (n = 10), Black (n = 1), other (n = 13)	[43]
University of Texas Health Science Center (San Antonio) & London, UK	Uterine myometrium	Epigenetics of human myometrium: relationship to the length of gestation and the onset of labor	Mitsuya et al.	2014	San Antonio, TX, USA & London, UK	Cross-sectional	53	23	2,100,000	13 genes	Hispanic White (n = 8), non-Hispanic White (n = 1), Pakistani (n = 1); White (n = 27), Black (n = 5), Asian (n = 2), missing race/ethnicity (n = 9)	[35]
Royal Women's Hospital, Melbourne in 1991 or 1992	Neonatal blood	Analysis of epigenetic changes in survivors of preterm birth reveals the effect of gestational age and evidence for a long-term legacy	Cruickshank et al.	2013	Australia	Prospective	24	12	485,000	1555	White (n = 24)	[36]
HUVCE: Human umbilical vein endothelial cell; PTB: Preterm birth; UCB: Umbilical cord blood.												

Table 2. Summary of mother-infant delivery participants in DNA methylomic studies of preterm birth by racial and ethnic category.

	Deliveries n = 2711	%
Race alone		
White	561	21
Black	698	26
Asian	9	0.3
Other:		
Unspecified	25	0.9
Non-Black	68	3
Non-White	100	4
Ethnicity alone		
Hispanic	8	0.3
Non-Hispanic	34	1
Chinese	591	22
Indian	178	7
Malay	250	9
Pakistani	1	0.04
Western geographic origin	86	3
Non-western geographic origin	26	1
Combined race/ethnicity		
Non-Hispanic White	10	0.4
Non-Hispanic Black	57	2
Hispanic	8	0.3
Asian	0	0
Other	1	0.04

methylomic studies utilizing the extreme low gestational age newborns (ELGAN) cohort or other cohorts including only samples from preterm infants and thus lacking infants born at term for comparison [58–61]. 14 of the 16 included studies used a cross-sectional study design [29–35,38,39,44] and two were prospective [36,37]. Only three of the 16 studies evaluated epigenomic changes in maternal specimens [35,37,44], whereas the remaining utilized samples of neonatal and placental origin (both derived from the fetal compartment).

Racial & ethnic representation within individual studies

The Boston Birth Cohort (BBC) [44] and the Nashville Birth Cohort (NBC) [29,30,37] were two large cohorts that contributed to four separate articles included in the systematic review (Table 1). These cohorts included only non-Hispanic Black (NBC cohort) or Black women (BBC cohort) and contributed 500 participant deliveries. Two of the included studies utilized cohorts that were uniformly White or European [32,36]. Only nine studies included both Black or non-Hispanic Black participants and White or non-Hispanic White participants [33–35,38–43], and of those, only seven had more than five individuals from both groups to allow for potential comparison by race and ethnicity [33–35,38,40–42].

Racial & ethnic representation across all studies

There were 2711 deliveries from the 16 eligible studies (Table 2). Non-Hispanic Black and Black women accounted for 755 (28%) of the deliveries. White and non-Hispanic White women accounted for 571 (21%) of all. Hispanic women accounted for less than 1% (n = 16) of all deliveries. A methylomic study conducted in Singapore by Wu *et al.* [31] contributed the largest number of deliveries (n = 1019) of all 16 studies. Thus, Chinese women accounted for 22% of all deliveries. Given that some cohorts were used for multiple analyses of various tissues from the same delivery, we also report the total number and proportion of specimens by race/ethnicity in a supplementary file (Supplementary Table 2).

Sensitivity analysis of representation

The nine studies excluded solely due to lack of data on race or ethnicity in a table, manuscript text or in Supplementary materials are detailed in Table 3. We contacted the corresponding authors for details regarding race and ethnicity and obtained the following information by personal communication. One birth cohort [62,63] did not obtain self-reported race/ethnicity, and instead used 57 ancestry informative marker SNPs and determined that none of the samples were of majority African ancestry. The authors of one study [64] stated that race/ethnicity data are rarely considered due to the homogeneity of the Danish population, and estimate that their cohort is more than 95% Danish/European. Similarly, the authors for the Norwegian study [65] stated that the cohort contains only 2% non-White participants. The authors of the ARIES study [66] confirmed that their cohort was 100% White. In another study [67], all participants were confirmed to be of Chinese ancestry, without any ethnic minorities included. Contacting the corresponding authors for the remaining three birth cohorts [68–70] did not lead to any further details about race or ethnicity. With exception of the study from Hawaii [70], these nine studies drew from populations with homogeneous race and ethnicity. The addition of these studies to our analysis would yield an additional 4179 deliveries – a 2.5-fold increase in the total number of deliveries. Studies from Norway, Denmark and the UK contributed 94% (3930) of these additional deliveries and each of these cohorts included over 95% White participants. Based on personal communications and available country demographic data, we estimated that the nine studies from Table 3 would include predominantly White (95%) and very few Black (0.05%) participants (Table 4). A sensitivity analysis combining these additional nine studies with the 16 included studies, would reduce the representation of Black women to 11% of all deliveries in DNA methylomic studies. However, because some non-western Norwegian and Danish women (categorized in Table 4 as ‘Other’) could be Black, the representation of Black women may be slightly greater than 11%.

Discussion

We found that Black mother–infant pairs were well represented among DNA methylomic studies of preterm birth. However, the primary dataset was potentially limited by reporting bias due to the lack of race/ethnicity data published in large studies from Denmark, Norway and the UK where approximately 90% of the population is White [71].

Our findings are in agreement with a prior systematic review evaluating DNA methylomic changes in preterm birth [73] that concluded that existing studies are limited by small sample size, repeated use of samples from the same biobanks, inconsistent definition of preterm birth, cross-sectional study design and limited inclusion of Black participants. One major limitation of DNA methylomic studies of preterm birth is the lack of prospective study design. Only two studies, with a mere 64 deliveries, analyzed maternal specimens prior to delivery [36,37]. The remaining studies analyzed specimens (maternal blood, cord blood or placenta) collected at the time of birth (cross-sectional). Cross-sectional studies are problematic for two reasons. First, epigenetic mechanisms are part of the regulatory signaling that is involved in developmental biology. Patterns of DNA methylation may vary as gestational age progresses, and as such, samples among infants born preterm may have different DNA methylation patterns that reflect development as opposed to the pathology that led to the preterm birth. Second, cross-sectional studies can suffer from reverse causation; the pathology that caused the preterm birth may also cause the differences in DNA methylation observed between term and preterm specimens. Thus, these differences would not be involved in the pathogenesis of preterm birth and cannot be used to predict preterm birth.

Studies also were limited with respect to their within-study diversity, which precludes analysis of DNA methylation differences by race that might contribute to preterm birth disparities. Even though there was adequate representation of Black and non-Hispanic Black deliveries across studies, the largest studies did not include non-Black participants as a comparison group [29,30,37,44]. While it is theoretically possible to compare DNA methylation across studies, such analyses can be problematic due to batch effects and other sources of confounding between studies. Thus, within-study inclusivity is crucial for analysis of DNA methylation between racial and ethnic groups.

Even within-study analysis of DNA methylation between race/ethnicity groups can be difficult due to differential SNP frequencies by race. While race is a social construct and not due to a specific genetic sequence, there are continental ancestral sequences that track, although incompletely, with self-identified race [72]. Cytosine nucleotides followed by guanines are CpG sites and are potential DNA methylation sites. If an SNP occurs in a CpG site, it may affect levels of DNA methylation. While methylation of that site may not be involved in the pathogenesis of preterm birth, it may differ by race/ethnicity due to differences in SNP frequency between groups. Thus, even if there are detected differences in DNA methylation by race, they may not be responsible for racial disparities in

Table 3. Study characteristics and estimated race/ethnicity and number of CpG sites evaluated for nine DNA methylomic studies of preterm birth lacking published race/ethnicity data.

Cohort	Tissue type	Publication title	Study	Year	Country	Study design	Total	Preterm	Number of CpG sites evaluated	CpG sites associated with preterm birth	Race/ethnicity % (n)	Ref.
BC Women's Hospital	UCB	Cord blood hematopoietic cells from preterm infants display altered DNA methylation patterns	de Goede et al.	2017	British Columbia, Canada	Cross-sectional	10	5	485,577	11,210	European 67% (n = 82); East Asian 15% (n = 18); other 18% (n = 22); African 0%	[63]
	Placenta	DNA methylation profiling of acute chorioamnionitis-associated placentas and fetal membranes: insights into epigenetic variation in spontaneous preterm births	Konwar et al.	2018	British Columbia, Canada	Cross-sectional	122	44	866,895	NS	European 67% (n = 82); East Asian 15% (n = 18); other 18% (n = 22); African 0%	[62]
Pre-eclampsia and Nonpre-eclampsia Database (PANDA) Project	UCB	The idiopathic preterm delivery methylation profile in UCB DNA	Fernando et al.	2015	The Netherlands	Cross-sectional	22	11	443,190	1855	Estimated [†] Dutch or European 85% (n = 19); Caribbean and other 5% (n = 1)	
MINERVA cohort	Dried neonatal blood spot	Variable DNA methylation in neonates mediates the association between prenatal smoking and birth weight	Hannon et al.	2019	Denmark	Cross-sectional	1263	NS	430,676	4299	Danish >95% (n = 1200); non-western 5% (n = 63)	[64]
Norwegian Mother and Child Cohort (MoBa)	UCB	Prediction of gestational age based on genome-wide differentially methylated regions	Bohlin et al.	2016	Norway	Cross-sectional	1753	NS	485,577	5474	Norwegian or European 98% (n = 1726); non-western 2% (n = 27)	[65]
Women and Children's Hospitals of Guangdong Province, China	Placenta, UCB	Comparison of DNA methylation profiles associated with spontaneous preterm birth in placenta and cord blood	Wang et al.	2019	Guangdong Province, China	Cross-sectional	48	32	551,326 placenta, 538,304 UCB	43,638 placenta, 72,711 UCB	Chinese 100% (n = 48)	[67]
Ewha Preterm Birth and Case-Control Study	Amnion of the placenta	Differential expression and methylation of integrin subunit alpha 11 and thrombospondin in the amnion of preterm birth	Yoo et al.	2018	Seoul, Korea	Cross-sectional	10	5	485,577	35,850	Estimated [†] Korean 99% (n = 10)	[69]
ARIES (sub-cohort of ALSPC group)	UCB	Longitudinal analysis of DNA methylation associated with birth weight and gestational age	Simpkin et al.	2015	United Kingdom	Cross-sectional	914	27	466,432	224	White 100% (n = 914)	[66]
Hawaii Biorepository (HIBR)	Chorioamniotic membranes	Genome-wide hypermethylation coupled with promoter hypomethylation in the chorioamniotic membranes of early onset pre-eclampsia	Ching et al.	2014	Hawaii, USA	Cross-sectional	47	30	>485,000	9995	Estimated [†] Asian 48% (n = 23), White 25% (n = 12), Black 2% (n = 1)	[70]
[†] Proportion and number of race/ethnicity categories based on country census data [74,75]. NS: Not specified; UCB: Umbilical cord blood.												

Table 4. Sensitivity analysis of racial/ethnic representation in DNA methylomic studies of preterm birth.

Analyzed studies	Total deliveries	White	Black	Asian [‡]	Hispanic/other
16 included studies	2711	571 (21)	755 (28)	1028 (38)	357 (13)
Nine studies missing race/ethnicity [†]	4179	3953 (95)	2 (0.05)	99 (2)	125 (3)
Combined 25 studies	6890	4524 (66)	757 (11)	1127 (16)	469 (7)

Data presented as n (row %).

[†]Number of White, Black, Asian and Hispanic/other participants estimated based on personal communications from study authors [62,64–67] and population census data for Denmark [64], Korea [69] and Hawaii [70]. 'Non-western' ethnicity for Norway and Denmark and missing race/ethnicity estimates for Hawaii [70] and Netherlands studies [68] were categorized as 'Other'.

[‡]Combined categories Asian, Chinese, Indian, Malaysian and Korean.

preterm birth. Rigorous analysis to exclude the role of SNPs in DNA methylation differences by race, as well as demonstration of the effect of DNA methylation within multiple race/ethnicities, is necessary before comparing DNA methylation contributions to racial disparities in preterm birth.

Conclusion & future perspective

In conclusion, given the limitations in study design, whether epigenomics and specifically DNA methylomics can predict preterm birth is unknown. Further, given the lack of within-study racial and ethnic representation, it currently is not possible to determine whether racial disparities in preterm birth could be attributable to differences in DNA methylation. Future studies will require adequate within-study representation across racial/ethnic groups and careful attention to study design to address this important research question. A longitudinal, prospective study design with similar sample sizes of non-Hispanic Black and White participants that examines DNA methylation, independent of ancestral SNPs, prior to the outcome of preterm birth would be the ideal study design for understanding the role of differential DNA methylation in preterm birth between racial groups.

Executive summary

- Black infants are born preterm more than 50% more often than White infants in the USA.
- Exposures to stressors, such as pollution and social discrimination differ by race in USA and these exposures may affect DNA methylation patterns.
- Whether DNA methylation is involved in the pathogenesis of preterm birth remains an active area of investigation.
- Whether DNA methylation explains the biology underlying racial disparities in preterm birth is not established, and cannot be determined due to the following limitations in the literature:
 - Studies of DNA methylation and preterm birth are largely cross-sectional, making causal links difficult to ascertain.
 - While overall, Black women are well represented in studies that specify race/ethnicity in the USA, globally, they are under-represented and studies do not have adequate numbers of both Black and White women in the same study to allow comparison.
 - Future longitudinal studies with representation of both Black and White women with samples obtained in advance of delivery will be required to determine if DNA methylomic differences could help to explain racial disparities in preterm birth.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/epi-2020-0007

Financial & competing interests disclosure

AY Collier received support from the grant K12HD000849, awarded to the Reproductive Scientist Development Program by the Eunice Kennedy Shriver National Institute of Child Health & Human Development and Burroughs Wellcome Fund. MR Hacker received support from Harvard Catalyst The Harvard Clinical and Translational Science Center (National Center for Advancing Translational Sciences, NIH Award UL 1TR002541) and financial contributions from Harvard University and its affiliated academic healthcare centers. HH Burris received support from the Department of Pediatrics at Children's Hospital of Philadelphia. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic healthcare centers, or the National Institutes of Health.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Martin J, Hamilton B, Osterman M, Driscoll A. Births: final data for 2018. *Natl Vital Stat. Reports* 68(13), 68 (2019).
2. Ely DM, Driscoll AK. Infant mortality in the United States, 2017: data from the period linked birth/infant death file. *Natl Vital Stat. Reports* 68(10), 1–20 (2019).
3. Burris HH, Lorch S, Kirpalani H *et al.* Racial disparities in preterm birth in USA: a biosensor of physical and social environmental exposures. *Arch. Dis. Child.* 104(10), 931–935 (2019).
4. David RJ, Collins JW. Differing birth weight among infants of U.S.-born Blacks, African-born Blacks, and U.S.-born Whites. *N. Engl. J. Med.* 337, 1209–1214 (1997).
- **This study was the first to show that disparities in birth outcomes between White and Black women could be more attributable to environmental exposures rather than genetics.**
5. Elo IT, Vang Z, Culhane JF. Variation in birth outcomes by mother's country of birth among non-hispanic Black women in the United States. *Matern. Child Health J.* 18(10), 2371–2381 (2014).
6. York TP, Strauss JF, Neale MC, Eaves LJ. Racial differences in genetic and environmental risk to preterm birth. *PLoS ONE* 5(8), 5 (2010).
- **Estimates the contribution both genes and environment contribute to the disparity in preterm births between White and Black women.**
7. Burris HH, Hacker MR. Birth outcome racial disparities: a result of intersecting social and environmental factors. *Semin. Perinatol.* 41(6), 360–366 (2017).
8. Cushing L, Faust J, August LM, Cendak R, Wieland W, Alexeeff G. Racial/ethnic disparities in cumulative environmental health impacts in California: evidence from a statewide environmental justice screening tool (CalEnviroScreen 1.1). *Am. J. Public Health* 105(11), 2341–2348 (2015).
9. Morello-Frosch R, Jesdale BM. Separate and unequal: residential segregation and estimated cancer risks associated with ambient air toxins in U.S. metropolitan areas. *Environ. Health Perspect.* 114(3), 386–393 (2006).
10. Mohai P, Saha R. Racial inequality in the distribution of hazardous waste: a national-level reassessment. *Soc. Probl.* 54(3), 343–370 (2007).
11. Pratt GC, Vadali ML, Kvale DL, Ellickson KM. Traffic, air pollution, minority and socio-economic status: addressing inequities in exposure and risk. *Int. J. Environ. Res. Public Health* 12(5), 5355–5372 (2015).
12. Mohai P, Lantz PM, Morenoff J, House JS, Mero RP. Racial and socioeconomic disparities in residential proximity to polluting industrial facilities: evidence from the Americans' Changing Lives Study. *Am. J. Public Health* 99(Suppl. 3), S649–S656 (2009).
13. Collins JW, David RJ, Handler A, Wall S, Andes S. Very low birthweight in African American infants: the role of maternal exposure to interpersonal racial discrimination. *Am. J. Public Health* 94(12), 2132–2138 (2004).
14. Mendez DD, Hogan VK, Culhane JF. Institutional racism, neighborhood factors, stress, and preterm birth. *Ethn. Heal.* 19(5), 479–499 (2014).
15. Krivo LJ, Vélez MB, Lyons CJ, Phillips JB, Sabbath E. Race, crime, and the changing fortunes of urban neighborhoods, 1999–2013. *Du Bois Rev.* 15(1), 47–68 (2018).
16. Krivo LJ, Peterson RD, Kuhl DC. Segregation, racial structure, and neighborhood violent crime. *Am. J. Sociol.* 114(6), 1765–1802 (2009).
17. Bellavia A, Urch B, Speck *Met al.* DNA hypomethylation, ambient particulate matter, and increased blood pressure: findings from controlled human exposure experiments. *J. Am. Heart Assoc.* 2(3), e000212 (2013).
18. Weaver ICG, Cervoni N, Champagne FA *et al.* Epigenetic programming by maternal behavior. *Nat. Neurosci.* 7, 847–854 (2004).
19. Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics* 3(2), 97–106 (2008).
20. Murthy VH, Krumholz HM, Gross CP. Participation in cancer clinical trials: race-, sex-, and age-based disparities. *J. Am. Med. Assoc.* 291(22), 2720–2726 (2004).
- **Shows the lack of racial and ethnic representation in clinical trials.**
21. Newman LA, Roff NK, Weinberg AD. Cancer clinical trials accrual: missed opportunities to address disparities and missed opportunities to improve outcomes for all. *Ann. Surg. Oncol.* 15(7), 1818–1819 (2008).

22. Stewart JH, Bertoni AG, Staten JL, Levine EA, Gross CP. Participation in surgical oncology clinical trials: gender-, race/ethnicity-, and age-based disparities. *Ann. Surg. Oncol.* 14(12), 3328–3334 (2007).
23. Hamel LM, Penner LA, Albrecht TL, Heath E, Gwede CK, Eggly S. Barriers to clinical trial enrollment in racial and ethnic minority patients with cancer. *Cancer Control* 23(4), 327–337 (2016).
24. Brown SD, Lee K, Schoffman DE, King AC, Crawley LM, Kiernan M. Minority recruitment into clinical trials: experimental findings and practical implications. *Contemp. Clin. Trials* 33(4), 620–623 (2012).
25. Webster GM, Teschke K, Janssen PA. Recruitment of healthy first-trimester pregnant women: lessons from the chemicals, health & pregnancy study (CHirP). *Matern. Child Health J.* 16(2), 430–438 (2012).
26. Smith YR, Johnson AM, Newman LA, Greene A, Johnson TRB, Rogers JL. Perceptions of clinical research participation among African American women. *J. Women's Heal.* 16(3), 423–428 (2007).
27. Moher D, Liberati A, Tetzlaff J, Altman DG. PRISMA 2009 Flow Diagram. The PRISMA statement. *PLoS Med.* 6(7), e1000097 (2009).
28. Ledyard R, Burris HH. Disparities in racial and ethnic representation in perinatal epigenomic studies. PROSPERO: international prospective register of systematic reviews (2019). www.crd.yourk.ac.uk/prospero/
29. Bhavnani SK, Dang B, Kilaru V *et al.* Methylation differences reveal heterogeneity in preterm pathophysiology: results from bipartite network analyses. *J. Perinat. Med.* 46, 509–521 (2018).
30. Parets SE, Conneely KN, Kilaru V *et al.* Fetal DNA methylation associates with early spontaneous preterm birth and gestational age. *PLoS ONE* 8(6), e67489 (2013).
31. Wu Y, Lin X, Lim IY *et al.* Analysis of two birth tissues provides new insights into the epigenetic landscape of neonates born preterm. *Clin. Epigenetics* 11(1), 26 (2019).
32. Herzog EM, Eggink AJ, Willemsen SP *et al.* Early- and late-onset preeclampsia and the tissue-specific epigenome of the placenta and newborn. *Placenta* 58, 122–132 (2017).
33. Lee H, Jaffe AE, Feinberg JI *et al.* DNA methylation shows genome-wide association of NFIX, RAPGEF2 and MSRB3 with gestational age at birth. *Int. J. Epidemiol.* 41(1), 188–199 (2012).
34. Manuck TA, Smeester L, Martin EM *et al.* Epigenetic regulation of the nitric oxide pathway, 17- α hydroxyprogesterone caproate, and recurrent preterm birth. *Am. J. Perinatol.* 35(8), 721–728 (2018).
35. Mitsuya K, Singh N, Sooranna SR, Johnson MR, Myatt L. Epigenetics of human myometrium: DNA methylation of genes encoding contraction-associated proteins in term and preterm labor. 90(5), 1–8 *Biol. Reprod.* (2014).
36. Cruickshank MN, Oshlack A, Theda C *et al.* Analysis of epigenetic changes in survivors of preterm birth reveals the effect of gestational age and evidence for a long term legacy. *Genome Med* 5(10), 96 (2013).
37. Parets SE, Conneely KN, Kilaru V, Menon R, Smith AK. DNA methylation provides insight into intergenerational risk for preterm birth in African Americans. *Epigenetics* 10(9), 784–792 (2015).
38. Maccani JZJ, Koestler DC, Houseman EA, Marsit CJ, Kelsey KT. Placental DNA methylation alterations associated with maternal tobacco smoking at the RUNX3 gene are also associated with gestational age. *Epigenomics* 5(6), 619–630 (2013).
39. Schuster J, Uzun A, Stablia J, Schorl C, Mori M, Padbury JF. Effect of prematurity on genome wide methylation in the placenta. *BMC Med Genet* 20(1), 116 (2019).
40. Schroeder JW, Conneely KN, Cubells JC *et al.* Neonatal DNA methylation patterns associate with gestational age. *Epigenetics* 6(12), 1498–1504 (2011).
41. Mani S, Ghosh J, Lan Y *et al.* Epigenetic changes in preterm birth placenta suggest a role for ADAMTS genes in spontaneous preterm birth. *Hum. Mol. Genet.* 28(1), 84–95 (2019).
42. Salihu HM, Das R, Morton L *et al.* Racial differences in DNA-methylation of CpG sites within preterm-promoting genes and gene variants. *Matern. Child Health J.* 20(8), 1680–1687 (2016).
43. Kim J, Pitlick MM, Christine PJ *et al.* Genome-wide analysis of DNA methylation in human amnion. *Sci. World J.* 2013, 678156 (2013).
44. Hong X, Sherwood B, Ladd-Acosta C *et al.* Genome-wide DNA methylation associations with spontaneous preterm birth in US Blacks: findings in maternal and cord blood samples. *Epigenetics* 13(2), 163–172 (2018).
45. Dhobale MV, Pisal HR, Mehendale SS, Joshi SR. Differential expression of human placental neurotrophic factors in preterm and term deliveries. *Int. J. Dev. Neurosci.* 31(8), 719–723 (2013).
46. Dhobale M, Chavan P, Kulkarni A, Mehendale S, Pisal H, Joshi S. Reduced folate, increased vitamin B12 and homocysteine concentrations in women delivering preterm. *Ann. Nutr. Metab.* 61(1), 7–14 (2012).
47. Burris HH, Just AC, Haviland MJ *et al.* Long noncoding RNA expression in the cervix mid-pregnancy is associated with the length of gestation at delivery. *Epigenetics* 13(7), 742–750 (2018).
48. Luo X, Pan J, Wang L *et al.* Epigenetic regulation of lncRNA connects ubiquitin-proteasome system with infection-inflammation in preterm births and preterm premature rupture of membranes. *BMC Pregnancy Childbirth* 15, 35 (2015).

49. Luo X, Shi Q, Gu Y *et al.* LncRNA pathway involved in premature preterm rupture of membrane (PPROM): an epigenomic approach to study the pathogenesis of reproductive disorders. *PLoS ONE* 8(11), e79897 (2013).
50. Sanders AP, Burris HH, Just AC *et al.* MicroRNA expression in the cervix during pregnancy is associated with length of gestation. *Epigenetics* 10(3), 221–228 (2015).
51. Sanders AP, Gennings C, Svensson K *et al.* Bacterial and cytokine mixtures predict the length of gestation and are associated with miRNA expression in the cervix. *Epigenomics* 9(1), 33–45 (2017).
52. Burris HH, Rifas-Shiman SL, Baccarelli A *et al.* Associations of long interspersed nuclear element-1 DNA methylation with preterm birth in a prospective cohort study. *J. Dev. Orig. Heal. Dis.* 3(3), 173–181 (2012).
53. Walsh SW, Chumble AA, Washington SL, Archer KJ, Sahingur SE, Strauss JF. Increased expression of toll-like receptors 2 and 9 is associated with reduced DNA methylation in spontaneous preterm labor. *J. Reprod. Immunol.* 121, 35–41 (2017).
54. Khot VV, Chavan-Gautam P, Mehendale S, Joshi SR. Variable methylation potential in preterm placenta: implication for epigenetic programming of the offspring. *Reprod. Sci.* 24(6), 891–901 (2017).
55. Provenzi L, Carli PD, Fumagalli M *et al.* Very preterm birth is associated with PLAGL1 gene hypomethylation at birth and discharge. *Epigenomics* 10(8), 1121–1130 (2018).
56. Behnia F, Parets SE, Kechichian T *et al.* Fetal DNA methylation of autism spectrum disorders candidate genes: association with spontaneous preterm birth. *Am. J. Obstet. Gynecol.* (212(4), 533.e1–533.e9 (2015).
57. Stükel W, Pan H, Chew SB *et al.* Transcriptome changes affecting Hedgehog and cytokine signalling in the umbilical cord: implications for disease risk. *PLoS ONE* 7(7), e39744, (2012).
58. Tomlinson MS, Bommarito PA, Martin EM *et al.* Microorganisms in the human placenta are associated with altered CpG methylation of immune and inflammation-related genes. *PLoS ONE* 12(12), e0188664, (2).
59. Tilley SK, Martin EM, Smeester L *et al.* Placental CpG methylation of infants born extremely preterm predicts cognitive impairment later in life. *PLoS ONE* 13(3), e0193271, (2018).
60. Santos HP Jr, Bhattacharya A, Martin EM *et al.* Epigenome-wide DNA methylation in placentas from preterm infants: association with maternal socioeconomic status. *Epigenetics* 14(8), 751–765 (2019).
61. Knight AK, Smith AK, Conneely KN *et al.* Relationship between epigenetic maturity and respiratory morbidity in preterm infants. *J. Pediatr.* 198, 168–173.e2 (2018).
62. Konwar C, Price EM, Wang LQ, Wilson SL, Terry J, Robinson WP. DNA methylation profiling of acute chorioamnionitis-associated placentas and fetal membranes: insights into epigenetic variation in spontaneous preterm births. *Epigenet. Chromatin* 11(1), 63 (2018).
63. de Goede OM, Lavoie PM, Robinson WP. Cord blood hematopoietic cells from preterm infants display altered DNA methylation patterns. *Clin. Epigenetics* 9, 39 (2017).
64. Hannon E, Schendel D, Ladd-Acosta C *et al.* Variable DNA methylation in neonates mediates the association between prenatal smoking and birth weight. *Philos. Trans. R. Soc. B Biol. Sci.* 374, 20180120 (2019).
65. Bohlin J, Haberg SE, Magnus P *et al.* Prediction of gestational age based on genome-wide differentially methylated regions. *Genome Biol.* 17(1), 207 (2016).
66. Simpkin AJ, Suderman M, Gaunt TR *et al.* Longitudinal analysis of DNA methylation associated with birth weight and gestational age. *Hum. Mol. Genet.* 24(13), 3752–3763 (2015).
67. Wang XM, Tian FY, Fan LJ, Xie CB, Niu ZZ, Chen WQ. Comparison of DNA methylation profiles associated with spontaneous preterm birth in placenta and cord blood. *BMC Med Genomics* 12(1), 1 (2019).
68. Fernando F, Keijser R, Henneman P *et al.* The idiopathic preterm delivery methylation profile in umbilical cord blood DNA. *BMC Genomics* 16, 736 (2015).
69. Yoo JY, You YA, Kwon EJ, Park MH, Shim S, Kim YJ. Differential expression and methylation of integrin subunit alpha 11 and thrombospondin in the amnion of preterm birth. *Obs. Gynecol Sci* 61(5), 565–574 (2018).
70. Ching T, Song MA, Tiirikainen M *et al.* Genome-wide hypermethylation coupled with promoter hypomethylation in the chorioamniotic membranes of early onset pre-eclampsia. *Mol. Hum. Reprod.* 20(9), 885–904 (2014).
71. Goff SL, Youssef Y, Pekow PS *et al.* Successful strategies for practice-based recruitment of racial and ethnic minority pregnant women in a randomized controlled trial: the IDEAS for a healthy baby study. *J. Racial Ethn. Heal. Disparities* 3(4), 731–737 (2016).
72. Yudell M, Roberts D, DeSalle R, Tishkoff S. Science and society: taking race out of human genetics. *Science* 351(6273), 564–565 (2016).
73. Barcelona de Mendoza V, Wright ML, Agaba C *et al.* A systematic review of DNA methylation and preterm birth in African American women. *Biol. Res. Nurs.* 19(3), 308–317 (2017).
- **A previously conducted systematic review that shows similar results to what we found in this review.**
74. World Population Review. (2019). <http://worldpopulationreview.com/>
75. Office for National Statistics. 2011 Census: ethnic group, local authorities in the United Kingdom (2013). <https://webarchive.nationalarchives.gov.uk/20160105170152/>