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Maternal depression and adverse neighbourhood conditions during pregnancy are associated with gestational epigenetic age deceleration

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ABSTRACT

Gestational epigenetic age (GEA) acceleration and deceleration can indicate developmental risk and may help elucidate how prenatal exposures lead to offspring outcomes. Depression and neighbourhood conditions during pregnancy are well-established determinants of birth and child outcomes. Emerging research suggests that maternal depression may contribute to GEA deceleration. It is unknown whether prenatal neighbourhood adversity would likewise influence GEA deceleration. This study examined whether maternal depression and neighbourhood conditions independently or jointly contributed to GEA deceleration, and which social and environmental neighbourhood conditions were associated with GEA. Participants were from the Albany Infant and Mother Study (n = 204), a prospective non-probability sampled cohort of higher risk racial/ethnic diverse mother/infant dyads. GEA was estimated from cord blood. Depressive symptoms and census-tract level neighbourhood conditions were assessed during pregnancy. Maternal depression (β = −0.03, SE = 0.01, p = 0.008) and neighbourhood adversity (β = −0.32, SE = 0.14, p = 0.02) were independently associated with GEA deceleration, controlling for all covariates including antidepressant use and cell type proportions. Neighbourhood adversity did not modify the association of maternal depression and GEA (β = 0.003, SE = 0.03, p = 0.92). igher levels of neighbourhood poverty, public assistance, and lack of healthy food access were each associated with GEA deceleration; higher elementary school test scores (an indicator of community tax base) were associated with GEA acceleration (all p < 0.001). The results of this study indicated that maternal depression and neighbourhood conditions were independently and cumulatively associated GEA in this diverse population.

KEYWORDS

Gestational epigenetic age acceleration; developmental origins of health and disease; maternal depression; neighbourhood conditions

Introduction

Gestation is a critical period of development when a range of exposures can alter developing body systems and influence health risk across the life course for the foetus [1,2]. Epigenetic alterations may be key mechanisms linking intrauterine exposures to phenotypic variation in health and developmental outcomes for children [3], as epigenetic factors like DNA methylation regulate gene expression and are malleable during foetal development. Epigenetic age acceleration, which reflects the difference between chronological age and DNA methylation estimated age, has been useful in studies of adults in discriminating health risk according to exposures, with epigenetic ageing signatures often being more sensitive indicators of health risk than chronological age [4–6]. Among adults, depression [7] and range of stressors [8] have been linked with accelerated epigenetic ageing, suggesting psychosocial adversity in adulthood may contribute to premature ageing and health risk. Emerging evidence suggests that epigenetic age in the gestational context may likewise forecast risk for child outcomes and may be a mechanism linking prenatal exposure to phenotype [9–11]. Thus, research focused on exogenous and modifiable prenatal exposures that could influence gestational epigenetic ageing signatures is warranted.

Gestational epigenetic age acceleration or deceleration reflects the difference between epigenetic age estimated from DNA methylation (typically measured in cord blood or placenta) and chronological gestational age estimated clinically via ultrasound [9]. The direction of change in

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epigenetic age during gestation according to exposure is just beginning to be characterized. Gestational epigenetic age acceleration and deceleration have been linked to adverse prenatal exposures [11,12], with decelerated ageing associated with maternal depression during pregnancy [10,13]. Decelerated epigenetic ageing *in utero* might suggest a more premature physiology than chronological gestational age would indicate. Specifically, from a Developmental Origins of Health and Disease (DOHaD) perspective [1], depression during pregnancy may contribute to an adverse intrauterine environment via overexposure of glucocorticoids during foetal development and through poor maternal health behaviours like smoking [14,15]. In turn, this adverse intrauterine environment might inhibit or disrupt developmental processes *in utero*, perhaps resulting in a dysregulated or premature physiology at birth despite the neonate's chronological age.

The first study focused on maternal depression and gestational epigenetic ageing was a prospective cohort of 694 pregnant women in Finland; authors found that maternal depression was associated with gestational epigenetic age deceleration, which in turn partially mediated associations with internalizing behaviour problems for boys at age 3.7 years [10]. The association between maternal depression and gestational epigenetic age deceleration was replicated by McKenna et al. in a different sample of 303 pregnant women and their infants, though associations were explained by SSRI use during pregnancy [13]. Given this is a new area of research, replication is needed in independent samples to determine whether and how gestational epigenetic ageing and maternal depression during pregnancy may be related. In addition, as the extant work to date has been conducted among largely white and higher educated study populations, examination of maternal depression and gestational epigenetic ageing research question within diverse groups is warranted.

In addition to maternal depression, social and environmental neighbourhood conditions are well-established determinants of birth outcomes [16–18], and a few studies have considered neighbourhood conditions during pregnancy in relation to gestational epigenetic alterations. For example, in two separate epigenome wide analyses in the Project Viva cohort, residential proximity to major highways and roadways [19], as well as socioeconomic context during pregnancy [20], were associated with DNA methylation levels at several CpG sites at birth in cord blood. While indicative of the plasticity of the epigenome according to neighbourhood level exposures during pregnancy, researchers have yet to consider neighbourhood conditions in relation to gestational epigenetic age acceleration. Among non-pregnant adults, emergent research suggests that living in adverse neighbourhood conditions is associated with epigenetic age acceleration, which may indicate health risk. For example, one study of 2,630 women found neighbourhood deprivation (assessed with a census-block level socioeconomic composite indicator) was associated with accelerated epigenetic ageing according to four clock measures, controlling for individual level socioeconomic status, health behaviours, and body mass index [21]. Another study among 158 adults also found that neighbourhood poverty was associated with accelerated epigenetic ageing, with some evidence that neighbourhood social cohesion buffered the effects [22].

Taken together, these studies suggest that an association between gestational epigenetic age signatures and neighbourhood conditions is plausible but has not yet been studied. Moreover, as the neighbourhood and epigenetic ageing work has heretofore been conducted among adults, it is not known whether exposure to adverse neighbourhood conditions during pregnancy would be associated with accelerated or decelerated ageing. Similar to maternal depression, a DOHaD perspective might suggest that stress attributable to living in adverse neighbourhood conditions during pregnancy may influence the quality of the intrauterine environment for the foetus, disrupt developmental processes, and possibly result in decelerated epigenetic age at birth. This hypothesis has not yet been considered. Also, as social and environmental factors tend to co-occur and can cumulatively impact birth outcomes and epigenetic signatures [23,24], a broad examination of a range of neighbourhood level social factors (e.g., poverty rate) *and*

environmental exposures (e.g., access to greenspace) in relation to gestational epigenetic age acceleration is warranted.

The present study had three aims. First, we endeavoured to replicate the association between maternal depression and gestational epigenetic age deceleration in a racial/ethnic diverse and lower socioeconomic status study population, while controlling for important confounding variables, including antidepressant use during pregnancy. We hypothesized that higher levels of maternal depressive symptoms would be associated with decelerated gestational epigenetic ageing. Second, we examined the association between neighbourhood conditions during pregnancy and gestational epigenetic ageing. We considered both a broad summary indicator of adverse neighbourhood conditions, as well as individual indicators of neighbourhood social, environmental, and educational contexts during pregnancy. We hypothesized that adverse neighbourhood conditions during pregnancy would be associated with gestational epigenetic age deceleration, and that some domains of neighbourhood conditions would be more predictive of gestational epigenetic ageing signatures than others. Finally, as depression and poor neighbourhood conditions tend to co-occur [25], we tested whether these factors would jointly influence gestational epigenetic age deceleration. Given that our prior work in this cohort has shown the effect of maternal depression on epigenetic alterations at birth were modified by an environmental risk common in low-income neighbourhoods (e.g., lead) [23], we hypothesized that the effect of maternal depression on gestational epigenetic age deceleration would be exacerbated by adverse neighbourhood conditions. To our knowledge, this study is the first to consider prenatal neighbourhood level exposures in relation to gestational epigenetic age acceleration/deceleration, as well as the first to study whether maternal depression and neighbourhood context can cumulatively impact gestational epigenetic ageing.

Methods

Study population

Participants were part of the Albany Infant and Mother Study (AIMS), a prospective observational cohort study of racially and ethnically diverse and socioeconomically disadvantaged pregnant women and their infants born at the Albany Medical Center (Albany, New York, USA). Accrual and characteristics of the study population have been described elsewhere [16,23,26,27]. Briefly, using convenience sampling, English-speaking women, 18–40 years old, with singleton pregnancies were eligible to participate and enrolled on average at 27 weeks gestation at an outpatient obstetrics clinic. At the prenatal enrolment visit, participants provided current residential addresses, completed questionnaires on depressive symptoms and covariates, and provided biospecimens. At birth, umbilical cord blood samples were collected and assayed for epigenetic information. Following the birth, study physicians conducted a structured medical record review to abstract information on maternal health, birth, and infant outcomes. Three-hundred mother-infant pairs enrolled, with 272 eligible participants completing the prenatal and birth assessments, of whom 204 provided an umbilical cord blood sample, and in turn had available epigenetic information [23]. Three outliers (i.e., those whose DNA methylation (DNAm) predicted gestational age was greater than three standard deviations from the mean of actual gestational age) were removed from the sample. The present analysis includes participants with complete data on maternal depression, neighbourhood conditions, covariates, and epigenetic data (n = 200). Protocols and informed consent documents were approved by Institutional Review Boards at Albany Medical Center and the University at Albany State University of New York.

Measures

Depression

Maternal depression during pregnancy was measured with the Edinburgh Postnatal Depression Scale (EPDS; $\alpha = 0.87$), a self-report assessment that has been validated for use among pregnant and postpartum populations that focuses on the cognitive and affective features of depression rather than somatic complaints [28]. The EPDS assesses the intensity of depressive symptoms in the past week. Select items were reverse scored and

responses were summed to obtain a total depressive symptoms score, with higher scores indicating more depressive symptoms. Total EPDS scores were examined in analysis.

Neighbourhood conditions

Participant residential addresses during pregnancy were geocoded in ArcMap (version 10; ESRI, Redlands CA) using the building geocoding function and the *US Address-Single House* address locator. These geocoded addresses were linked to 2015 census tracts using QGIS [29]. Neighbourhood conditions were assessed with the 2015 Childhood Opportunity Index (COI) 2.0, a multidimensional composite indicator that reflects a range of census-tract level community risks and resources that can contribute to health [30,31]. Table 1 lists the component parts of the COI according to its health and environmental, social and economic, and educational domains. Complete information on COI component parts, derivation and validation, is available at www.diversitydatakids.org. National distributions of COI scores were categorized as quintiles indicating gradations in neighbourhood conditions: very low, low, moderate, high, and very high opportunity [30,31]. In analysis, we examined neighbourhood conditions in two ways. First, we considered a dichotomous summary indicator of adverse neighbourhood conditions (very low/low versus moderate/high/very high neighbourhood opportunity). Second, we examined the continuous raw scores of each of the health and environmental, social and economic, and educational COI domains as separate predictors in analysis. All neighbourhood condition variables were from 2015, the year in which the majority of AIMS participants were pregnant and/or the immediate time preceding the pregnancy.

Gestational epigenetic ageing

DNA was extracted from umbilical cord blood samples, and was processed and sequenced using the Illumina EPIC Infinium array (Illumina, San Diego, CA) [32] at the USC Molecular Genomics Data Production Core. Methylation data were preprocessed in R for quality control, background correction, normalization, type 1 and II probe scaling, and batch adjustment following established protocols [33]. DNA methylation was extracted from raw files using the *minfi* R package [34]. Functional normalization was conducted to remove poor quality probes that fell below the detection limit ($p < 0.01$) and data were adjusted for type 1 and type 2 probe variation via the Beta Mixture Quantile Dilation (BMIQ) function of the wateRmelon package [35], and batch effects were adjusted with the ComBAT function of the sva package [36,37]. β values (representing a ratio of methylated versus unmethylated DNA at each CpG site) were used in analysis. Cell type proportions (B-cells, $CD4^+$ T-cells, $CD8^+$ T-cells, granulocytes, monocytes, natural killer cells and nucleated red blood cells) for each sample were estimated from the β value matrix using the *estimateCellCounts* function and the *FlowSorted.CordBloodCombined.450k* reference in the ENmix R package [38].

To facilitate comparisons with prior studies in this area [10,13], we calculated the Knight clock [9] to quantify epigenetic ageing at birth. Specifically, gestational epigenetic age was calculated according to the procedures developed by Horvath [4] and adapted by Knight et al. [9]. The Knight clock was developed specifically for use in umbilical cord blood and reflects a weighted average of methylation beta values at 148 CpG sites. Seven of the 148 Knight clock probes are not assayed on the Illumina EPIC Infinium array. We, therefore, calculated Knight epigenetic age with the remaining 141 probes. Previous research has shown that DNAm age estimates derived with the EPIC array were robust to missing probes compared to other platforms [39]. Moreover, in sensitivity analyses, we likewise found that calculated ages were robust to the removal of the seven probes not included on the EPIC array using the example data provided by Knight et al. (data not shown). In the present study, gestational epigenetic age acceleration and deceleration reflected the arithmetic difference between DNAm estimated gestational age and chronological gestational age estimated clinically from ultrasound. We used this difference variable in analysis. This difference variable was also used in the initial study of maternal depression and gestational epigenetic age deceleration [10]; we use it here to facilitate comparisons of results

Table 1 Domains and indicators of neighbourhood conditions.

Domain	Indicator	Description (source)
Health & environment		
	Access to healthy food	Percent households without a car located further than a half-mile from the nearest supermarket, reversed (USDA)
	Access to green space	Percent impenetrable surface areas such as rooftops, roads, or parking lots (CDC)
	Extreme heat exposure	Summer days with maximum temperature above 90°F (CDC)
	Health insurance coverage	Percent individuals ages 0–64 with health insurance coverage (ACS)
	Ozone concentration	Mean estimated 8-hour average ozone concentration (EPA)
	Particulate matter ($PM_{2.5}$) concentration	Mean estimated $PM_{2.5}$ concentration (CDC)
	Industrial pollutants in air, water or soil	Index of toxic chemical released by industrial facilities (EPA)
	Hazardous waste dump sites	Average number of Superfund sites within a 2-mile radius (EPA)
	Housing vacancy rate	Percent housing units that are vacant (ACS)
	Walkability	EPA Walkability Index (EPA)
Social & economic		
	Poverty rate	Percent individuals living in households with incomes below 100% of the federal poverty threshold (ACS)
	Public Assistance	Percentage receiving cash public assistance or food stamps/Supplemental Nutrition Assistance Program (ACS)
	Homeownership rate	Percent owner-occupied housing units (ACS)
	High-skill employment	Percent individuals ages 16 and over employed in management, business, financial, computer, engineering, science, education, legal, community service, health care practitioner, health technology, arts and media occupations (ACS)
	Median household income	Median income of all households (ACS)
	Employment rate	Percent adults ages 25–54 who are employed (ACS)
	Single-headed households	Percent family households that are single-parent headed (ACS)
	Commute duration	Percent workers commuting more than one hour one way (ACS)
Education		
	Adult educational attainment	Percent adults ages 25 and over with a college degree or higher (ACS)
	High school graduation rate	Percent ninth graders graduating from high school on time (EDFacts and GS)
	ECE enrolment	Percent 3- and 4-year-olds enrolled in nursery school, preschool or kindergarten (ACS)
	Early childhood education (ECE) centres	Number of ECE centres within a 5-mile radius (data collection from state and federal sources)
	High-quality ECE centres	Number of NAEYC accredited centres within a 5-mile radius (data collection from state and federal sources)
	School poverty	Percent students in elementary schools eligible for free or reduced-price lunches, reversed (NCES CCD)
	Teacher experience	Percent teachers in their first and second year (CRDC)
	Third grade math proficiency	Percent third graders scoring proficient on standardized math tests, converted to NAEP scale score points (EDFacts, GS and SEDA)
	Third grade reading proficiency	Percent third graders scoring proficient on standardized reading tests, converted to NAEP scale score points (EDFacts, GS and SEDA)
	Advanced Placement (AP) course enrolment	Ratio of students enrolled in at least one AP course to the number of 11^{th} and 12^{th} graders (CRDC)
	College enrolment in nearby institutions	Percent 18–24 year-olds enrolled in college within 25-mile radius (ACS)

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Abbreviations: ACS = American Community Survey; CDC = Centers for Disease Control and Prevention; CRDC = Civil Rights Data Collection; EPA = Environmental Protection Agency; EDFacts = U.S. Department of Education EDFacts Data; GS = GreatSchools; NCES CCD = National Center for Health Statistics Common Core of Data; SEDA = Stanford Education Data Archive; USDA = United States Department of Agriculture.

across study populations. Analyses were repeated using a residualized gestational epigenetic age acceleration variable [13]. As results were essentially the same (data not shown), we report on findings with the difference score.

Covariates

Covariates were selected *a priori* based on their potential to confound the associations of interest [13,40,41] and included demographic, maternal health and delivery factors, as well as infant attributes. Demographics included self-reported maternal age, race/ethnicity (white not-Hispanic versus Black/Hispanic/other) and education attainment (high school degree or less versus more than high school). Maternal health and delivery factors included pre-pregnancy body mass index (weight in kilograms/height in metres [2]; calculated from self-reported information provided at the enrolment visit), self-reported smoking during pregnancy (yes/no), antidepressant use during pregnancy (abstracted from medical records; yes/no), delivery mode, pregnancy complications, and self-reported diet. Diet was assessed in pregnancy with a 25-item Food Frequency Questionnaire [42,43], which assessed how often (on average) women consumed different foods during pregnancy. A 9-point Likert scale provided response options ranging from consuming a given food as 'never' to '6 or more a day.' A western dietary pattern summary score was derived that reflected the frequency of consumption of foods from western categories (e.g., red meats, processed meats, refined grains, high-fat dairy products, potatoes, sugar sweetened beverages); scores for each western food item were added up, with the higher total scores indicating greater consumption of western food types [16,23,26]. Delivery factors abstracted from medical records included parity (nulliparous or not) and mode of delivery (vaginal, c-section). Pregnancy complications were abstracted from medical records and summarized into an index that included pregnancy conditions (gestational diabetes, preeclampsia, eclampsia), placental abnormalities (abruption, previa, accreta, marginal bleed), gynaecologic bacterial infections (Group B Streptococcus, Chorioamnionitis), and preterm premature rupture of the membranes. As most complications were relatively low prevalence (e.g., <10%) which precluded the examination of each condition separately, we combined the information into a summary score. Specifically, we dichotomized each complication as either present or absent, and then added up the number of conditions per person, with higher scores indicating more pregnancy complications (observed range in this sample 0–4 conditions) [16,23]. Infant covariate information was collected from medical records and included sex (male/female) and gestational age at delivery as estimated from ultrasound scans.

Analytic plan

Those with (n = 204) and without (n = 68) DNA methylation information were compared according to maternal age, race/ethnicity, education, depressive symptoms, and neighbourhood conditions. Descriptive statistics were calculated for each study variable. Pearson's correlations assessed the associations between study variables; correlations with DNAm epigenetic age were adjusted for cell-type proportions. Next, we considered the independent and joint contributions of depression and adverse neighbourhood conditions during pregnancy with gestational epigenetic ageing via four multivariable linear regression models. To test our first study aim and replicate findings from prior work [10,13], we examined the main effect of depression on gestational epigenetic age deceleration (adjusted for all covariates, excluding neighbourhood conditions). To test our second study aim, a regression model examined the main effect of neighbourhood conditions on gestational epigenetic age deceleration (adjusted for all covariates, excluding depression). To test our third aim on the joint contribution of depression and neighbourhood conditions, we (a) fit a model that included both depression and neighbourhood conditions simultaneously adjusted for all covariates, and (b) fit a fourth model that additionally included an interaction term for depression and neighbourhood conditions.

Finally, to further contextualize the association between neighbourhood conditions and gestational epigenetic ageing per our second study aim, we examined each of the 29 component parts (Table 1) of the health and environmental,

Table 2. Participant characteristics (n = 200).

	Mean (SD)	*% (n)*
Neighbourhood conditions		
Very low opportunity		32.84 (66)
Low opportunity		13.93 (28)
Moderate opportunity		13.43 (27)
High opportunity		19.90 (40)
Very high opportunity		19.90 (40)
Maternal characteristics		
Depressive symptoms, sum score	8.70 (5.34)	
Age, years	28.66 (5.51)	
Race, white/not Hispanic		59.20 (119)
Race, not white and/or Hispanic		40.80 (82)
Education, high school or less		34.33 (69)
Education, more than high school		65.67 (132)
Antidepressant use during pregnancy, yes		8.46 (17)
Antidepressant use during pregnancy, no		91.54 (184)
Smoked during pregnancy, yes		11.44 (23)
Smoking during pregnancy, no		88.56 (178)
Pre-pregnancy BMI	29.02 (8.51)	
Pregnancy complications, index	0.71 (0.97)	
Western diet, sum score	39.70 (14.57)	
Delivery mode, C-section		30.85 (62)
Delivery mode, vaginal		69.15 (139)
Nulliparous, yes		25.37 (51)
Nulliparous, no		74.63 (150)
Infant characteristics		
Male		49.75 (100)
Female		50.25 (101)
Gestational age at delivery, weeks	39.00 (1.72)	
DNAm gestational age, weeks	39.79 (1.69)	

social and economic, and educational neighbourhood condition domains as separate predictors in adjusted linear regression models. All associations were assessed with generalized estimating equations (GEE) with exchangeable correlation structures. Because observations were not independent due to clustering at the census tract level, we used GEE which provided parameter estimates and p-values that account for this correlated data structure. All models were adjusted for covariates and cell-type proportions. Statistical significance was determined by p-values less than 0.05. To prevent type 1 error due to multiple comparisons, Bonferroni adjusted p-values were also calculated for models considering associations between each of the 29 component parts of the neighbourhood condition variable (p = 0.05/29 tests; Bonferroni adjusted p = 0.002).

Results

There were no significant differences in maternal age, education, depressive symptoms or adverse neighbourhood conditions between those missing epigenetic data and those included in the present analysis (all $p > 0.05$). However, those missing epigenetic information were more likely to be racial/ethnic minorities ($\chi^2 = 15.18$, $p < 0.0001$). Participant characteristics are listed in Table 2. Women were on average 29 years old during pregnancy, 41% were from a racial/ethnic minority group, and 34% reported high school or less as the highest level of education attained. Nearly half of women lived in adverse neighbourhood conditions during pregnancy (areas characterized as having 'low' or 'very low' opportunity). The average depressive symptoms score was 8.70 (SD = 5.34, range 0–27). Most women had given birth previously, and delivered the current pregnancy vaginally. Antidepressant use and smoking during pregnancy were both low prevalence in this sample (8.46% and 11.44% respectively). The average maternal pre-pregnancy BMI was high (M = 29.02, SD = 8.51), and women experienced on average less than 1 pregnancy complication (range 0–4). The sample included an equal proportion of male and female infants. The average DNAm predicted gestational age was

Table 3. Pearson's correlations among study variables.

	DNAm age	Depressive symptoms	Neighbourhood adversity
DNAm age	1.0		
Depressive symptoms	−0.18**	1.0	
Neighbourhood adversity	−0.15*	0.11	1.0
Gestational age	0.65***	−0.07	−0.02
Maternal age	−0.0003	−0.0001	−0.16*
White, not Hispanic	−0.03	0.05	−0.26***
Low education	−0.12	0.01	0.25***
Antidepressant use	−0.06	0.33***	−0.11
Smoker	−0.10	0.15*	−0.06
Pre-pregnancy BMI	−0.02	0.11	0.06
Pregnancy complications	−0.24***	0.15*	0.05
Western diet	−0.11	0.10	0.19**
Delivery mode, vaginal	0.06	−0.08	−0.04
Nulliparous	−0.23**	0.08	−0.003
Infant sex, male	0.06	−0.08	−0.08

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
Correlations with DNAm age are adjusted for cell type proportions.

Table 4. Associations between maternal depression and adverse neighbourhood conditions during pregnancy with gestational epigenetic age deceleration.

	Model 1	Model 2	Model 3	Model 4
		β (SE)		
Depressive symptoms	−0.03 (0.01)		−0.03 (0.01)	−0.03 (0.02)
	0.008		0.02	0.15
Adverse neighbourhood conditions		−0.32 (0.14)	−0.30 (0.14)	−0.32 (0.29)
		0.02	0.03	0.26
Depression x Adverse neighbourhood conditions				0.003 (0.03)
				0.92

All models were adjusted for cell type proportions (Bcell, CD4T, CD8T, Gran, Mono, NK, nRBC), maternal age, race/ethnicity, education, delivery mode, parity, smoking, antidepressant use, pre-pregnancy body mass index, pregnancy complications, diet, infant sex, and gestational age at birth.
Top cell entry is β (SE); bottom cell entry is the p-value

39.79 weeks (SD = 1.61), which was slightly higher than gestational age measured clinically via ultrasound (M = 39.00, SD = 1.72). Predicted DNAm gestational age was positively correlated with clinically assessed gestational age (r = 0.65, p < 0.001).

Table 3 shows Pearson's correlations with DNAm age, depressive symptoms, neighbourhood adversity and other study variables. Higher maternal depressive symptoms (r = −0.18, p < 0.01) and adverse neighbourhood conditions (r = −0.15, p < 0.05) during pregnancy were significantly correlated with lower DNAm gestational age at birth. Lower DNAm age at birth was also observed according to increasing pregnancy complications (r = −0.24, p < 0.01), and nulliparity (r = −0.23, p < 0.01). Maternal depression was positively correlated with antidepressant use (r = 0.33, p < 0.0001) and smoking (r = 0.15, p < 0.05). Women living in adverse neighbourhood conditions were significantly more likely to be younger (p < 0.05), racial/ethnic minorities (p < 0.001), had lower levels of education (p < 0.001), and higher western diet scores (p < 0.01). There was a non-significant positive trend between depressive symptoms and adverse neighbourhood conditions (r = 0.11, p = 0.11).

Table 4 lists the GEE multivariable linear regression associations for maternal depression, neighbourhood conditions, and gestational epigenetic age deceleration. Higher levels of depressive symptoms during pregnancy were significantly associated with gestational epigenetic age deceleration (Model 1: β = −0.03, SE = 0.01, p = 0.008), controlling for cell-type proportions, demographic, maternal health and delivery factors, as well as infant attributes. Living in adverse neighbourhood conditions during pregnancy was also associated with gestational epigenetic age deceleration (Model 2: β = −0.32, SE = 0.14, p = 0.02),

Table 5. Associations between specific neighbourhood conditions during pregnancy and gestational epigenetic age acceleration.

	β	*SE*	*p*
Health and environmental conditions			
Healthy food inaccessibility	**−0.01**	**0.004**	**0.0008**
Greenspace inaccessibility	−0.01	0.003	0.05
Extreme heat exposure	−0.05	0.12	0.66
Health insurance coverage	0.04	0.02	0.04
Ozone concentration	0.11	0.13	0.41
$PM_{2.5}$ concentration	−0.12	0.17	0.48
Industrial pollutants index	−0.01	0.04	0.89
Proximity to Superfund sites	−0.03	0.02	0.19
Housing vacancy rate	0.0001	0.01	0.98
Walkability	−0.03	0.02	0.04
Social and economic conditions			
Poverty rate	**−0.01**	**0.005**	**0.0008**
Public assistance rate	**−0.01**	**0.004**	**0.001**
Homeownership rate	0.01	0.002	0.02
High-skill employment	0.01	0.01	0.19
Median household income^{+}	0.07	0.03	0.01
Employment rate	0.01	0.01	0.30
Single headed household	−0.002	0.003	0.45
Commute duration	0.01	0.02	0.53
Education conditions			
Adult educational attainment	0.002	0.005	0.65
High school graduation rate	0.02	0.01	0.02
Early childhood education enrolment	−0.0002	0.003	0.93
Proximity to ECE centres	−0.11	0.06	0.07
Proximity to high quality ECE centres	−0.02	0.01	0.10
School poverty	−0.01	0.004	0.07
Teacher experience	−0.002	0.01	0.88
Third grade math proficiency	**0.003**	**0.001**	**0.001**
Third grade reading proficiency	0.002	0.001	0.006
Advanced placement course enrolment	−0.24	0.33	0.46
College enrolment in nearby institutions	−0.02	0.01	0.03

Separate models were fit for each neighbourhood characteristic.
Associations in **bold** were statistically significant after Bonferroni adjustment ($p = 0.002$).
$^{+}$Median household income is expressed in units of $10,000.
All models were adjusted for cell type proportions (Bcell, CD4T, CD8T, Gran, Mono, NK, nRBC), maternal age, race/ethnicity, education, delivery mode, parity, smoking, depressive symptoms, antidepressant use, pre-pregnancy body mass index, pregnancy complications, diet, infant sex, and gestational age at birth.
ECE = Early childhood education.

controlling for all covariates, but not depression. When in the model together (Model 3), depressive symptoms and adverse neighbourhood conditions each maintained their significant associations with gestational epigenetic age deceleration. There was no interaction effect of depression and neighbourhood conditions with epigenetic deceleration (Model 4: $\beta = 0.003$, SE = 0.03, $p = 0.92$).

Tables 5 lists the GEE multivariable linear regression associations for each component part of the neighbourhood conditions composite with gestational epigenetic ageing, adjusted for all study variables including depressive symptoms. Among the neighbourhood health and environmental conditions, lack of access to healthy food, greenspace, and neighbourhood walkability were associated with decelerated epigenetic ageing (all $p \leq 0.05$). Higher levels of insurance coverage in the community was associated with accelerated epigenetic ageing ($p = 0.04$). Toxicant related neighbourhood conditions (e.g., Ozone, $PM_{2.5}$, pollutant sites) were not associated with gestational epigenetic age acceleration. Among the social and economic conditions, higher levels of neighbourhood poverty and public assistance were associated with decelerated epigenetic ageing whereas higher home ownership rates and higher median incomes were associated with accelerated epigenetic age (all $p < 0.02$). Among the neighbourhood educational conditions, higher high school graduation rates and higher 3rd grade proficiency scores were associated with gestational epigenetic age acceleration (all $p < 0.02$). College enrolment nearby was associated with epigenetic age deceleration ($p = 0.03$). Applying the Bonferroni correction, associations between

healthy food inaccessibility, poverty rate, public assistance rate, and third grade math proficiency with gestational epigenetic age remained statistically significant.

Discussion

The results of this study indicated that maternal depression as well as several social-environmental neighbourhood conditions experienced during pregnancy were independently associated with gestational epigenetic ageing. We replicated findings from prior work conducted in other samples [10,13] showing that maternal depression during pregnancy was associated with gestational epigenetic age deceleration, and showed for the first time that neighbourhood conditions during pregnancy may also be associated with gestational epigenetic ageing. These findings are noteworthy because we demonstrated these associations among a racial/ethnic and socioeconomically diverse study population, and associations were maintained when controlling for several important demographic, maternal health (including antidepressant use), delivery, and infant confounding factors, as well as cell-type proportions. Effect sizes were small: for each additional depressive symptom, DNAm age was 0.03 weeks (or about 0.21 days) lower than actual gestational age at birth; living in neighbourhood adversity during pregnancy showed a 0.32 week (or about 2.25 days) lower DNAm age at birth compared to actual gestational age. Nevertheless, the study results were consistent with a developmental origin of health and disease framework and suggested that maternal depression and neighbourhood context during pregnancy were each associated with epigenetic ageing signatures *in utero*.

In recent years, several DNA methylation candidate gene studies (e.g., *NR3C1*) and epigenome wide association studies have shown that maternal depression during pregnancy is associated with epigenetic signatures at birth [44–48]. Our study is consistent with this evidence base, and also replicates recent research showing maternal depression during pregnancy is associated with epigenetic age deceleration at birth [10,13]. However, contrary to previous findings by McKenna et al. that SSRI use confounded the depression and epigenetic age association [13], we found the association between maternal depression on gestational epigenetic age deceleration was maintained when controlling for antidepressant use. This difference could reflect, in part, the different prevalence of antidepressant use and distributions of race/ethnicity across the two samples. For example, in AIMS, 41% of participants were racial/ethnic minorities and 8% had antidepressant use recorded in their medical records whereas in the McKenna et al. study, 17% were racial ethnic minorities and 50% self-reported any SSRI use during pregnancy. In the US, substantial disparities in antidepressant use exist, with racial/ethnic minorities taking SSRIs and other antidepressant medications at dramatically lower rates than white individuals [49]. As our study population was racially and ethnically diverse and had concomitant low levels of antidepressant use, it is unlikely the associations presented here were confounded by SSRI use. Indeed, the correlation between antidepressant use and epigenetic age acceleration in this sample was null ($r = -0.06$, $p = 0.44$). Other study populations with higher prevalence of white participants with SSRI use may be more prone to this confounding effect. Thus, sociodemographic characteristics may be key in understanding whether and how prenatal depression and SSRI use influence gestational epigenetic ageing. As maternal depression and SSRI use during pregnancy are of significant clinical importance, we encourage future work to continue to study this point among diverse populations.

We found support for our hypothesis that adverse neighbourhood conditions during pregnancy would be associated with gestational epigenetic age deceleration. We did not find support for our hypothesis that adverse neighbourhood conditions would modify the association between maternal depression and gestational epigenetic age deceleration. There was a non-significant positive trend between depressive symptoms and adverse neighbourhood conditions, and in adjusted models, we found that depressive symptoms and neighbourhood adversity were each associated with gestational epigenetic age deceleration. This finding is congruent with the life course epidemiology 'accumulation of risk' model whereby different types of exposures (e.g.,

environmental conditions, behaviours, psychosocial factors; either correlated or independent) can exert cumulative damage to biologic systems [50]. In the context of pregnancy when the developing foetus is particularly sensitive to exogenous exposures, these results suggest that an accumulation of both individual-level and area-level adversities, many of which are amenable to intervention in clinical and public health settings, may influence molecular signatures during gestation.

Several different health and environmental, social and economic, and educational neighbourhood conditions experienced during pregnancy were associated with gestational epigenetic age acceleration and deceleration. After applying the Bonferroni correction, neighbourhood access to healthy food, poverty rate, public assistance, and third grade math proficiency scores (an indicator of tax base in the community) remained significantly associated with gestational epigenetic ageing. These findings suggest that community-level deprivation and assets might impact gestational epigenetic ageing, net of individual-level risks like maternal depression, diet, smoking, pregnancy morbidity, and education level. It is noteworthy that these associations were maintained when controlling for maternal education, a robust indicator of individual level socioeconomic status [51]. This means that in our analysis, neighbourhood conditions were independently associated with gestational epigenetic ageing signatures and were not simply functioning as a marker for individual level socioeconomic status.

Neighbourhood level toxicant and pollutant-related exposures (e.g., $PM_{2.5}$, Ozone, proximity to Superfund sites) were not associated with gestational epigenetic age signatures in this study. This was somewhat surprising given some other studies have found that prenatal exposure to metals, air pollutants, and other environmental risks are associated with DNA methylation at birth [52–55], though studies of gestational epigenetic age acceleration and prenatal toxicant exposure are fewer and have not been as consistent [56,57]. In our study, we relied on the COI to index area-based toxicant exposure. While indicative of overall community risk, these secondary measures may not accurately reflect an individual's toxicant exposure during pregnancy. Moreover, unlike area-based social-contextual exposures that might affect health through a range of psychological and behavioural pathways (e.g., neighbourhood poverty can lead to increased psychosocial stress, decreased physical activity, poor diet) [58], environmental chemicals often need direct contact with the individual in order to affect health (e.g., inhalation of $PM_{2.5}$, ingestion of contaminated water), particularly during developmentally sensitive times in the life course [59]. Therefore, we suggest that future work utilize direct monitoring and individual level biomarker-based assessments of prenatal pollutant exposure in order to more precisely characterize the association between prenatal toxicant exposure and gestational epigenetic age acceleration.

Neighbourhood risks and favourable features tended to contribute to divergent epigenetic signatures. For example, higher neighbourhood poverty was negatively associated with gestational epigenetic age whereas higher homeownership rate was positively associated epigenetic age. While some of these findings did not meet the Bonferroni threshold for significance and therefore might be artefacts due to multiple testing, the overall trend of the associations suggests that in addition to neighbourhood adversity and epigenetic risk associations, there might also be molecular signatures indicative of positive and health promoting environments. These findings are consistent with prior work among non-pregnant adults that has found neighbourhood social cohesion [22], as well as positive individual-level factors like social support to buffer the effects of adversity on epigenetic ageing[60]. During pregnancy, living in more resourced neighbourhoods characterized by factors such as higher household incomes, higher home ownership rates, greater insurance coverage, more greenspace, and access to healthy foods could work to enhance access to prenatal care, as well as promote favourable behaviours like physical activity and healthy eating. These factors are known to promote foetal growth and birth outcomes [16], and this research suggests contextual-level positive factors may also leave their mark on gestational epigenetic age as well. However, we acknowledge that due to multiple testing, we cannot rule out that some of these

associations were due to chance. Thus, we encourage future work to consider the novel hypothesis that area-based social and environmental assets may influence epigenetic alterations *in utero*.

This study had a number of strengths. First, where prior work in this area has focused on relatively homogenous populations [9],[10] [11,13], the AIMS population is racially, ethnically, and socioeconomically diverse. Also, we used a multimodal prospective design that integrated information from biologic samples, validated questionnaires, novel area-based measures, and hospital medical records. Moreover, we rigorously controlled for confounding by considering several demographic, maternal health, dietary, delivery, and infant characteristics in the multivariate models. Also, we applied a Bonferroni correction to mitigate type 1 error where appropriate. Finally, this study builds the evidence base by replicating prenatal depression and gestational epigenetic age deceleration associations in an independent sample, and also as the first to consider neighbourhood conditions experienced during pregnancy and gestational epigenetic age acceleration.

This study also has some limitations. First, our analyses focused on cord-blood derived epigenetic age using the Knight [9] clock. As DNA methylation patterns are tissue specific [61], it is not known whether patterns observed here would be found in other tissues. Moreover, the Knight clock has been critiqued for showing lower accuracy in estimating gestational age compared to other clocks [62], including a newer metric that was derived specifically for EPIC array data [63]. As we relied on the Knight clock in this study, the estimates we present here may be imprecise. We used the Knight clock in order to replicate findings from prior work that also used this measure [10,13], and facilitate comparisons across studies and samples. We encourage future work to incorporate additional and newer measures of gestational epigenetic ageing in order to quantify gestational exposure and epigenetic ageing associations more precisely. In addition, while we controlled for several important confounding variables, we included a composite antidepressant variable in the models and could not control specifically for SSRI use as in prior work [13]. Though antidepressant use was low prevalence in this sample, some residual confounding may remain. Also, participants missing DNA methylation information were more likely to be racial and ethnic minorities compared to those included in analysis, which could have constrained variability in the exposure and outcomes and led to underestimated associations. Our team implemented a number of strategies to enhance retention, though some racial/ethnic minority participants were lost to follow-up, resulting in missing cord blood samples/missing DNA methylation data. While our study population has more racial/ethnic and socioeconomic diversity than other studies focused on gestational epigenetic ageing, we encourage future work to allocate significant resources and implement strategies [64] to enhance retention of racial/ethnic minorities in gestational epigenetic ageing research, particularly when considering adversity-related exposures prevalent in health disparity groups. Also, as we had one wave of data collection during pregnancy, information on preconception or trimester-specific timing of depression and SSRI use during pregnancy was unavailable; we encourage future work to consider exposure chronicity and developmental-window-specific associations in future work. Finally, we did not test whether the variation in gestational epigenetic ageing was associated with infant or child outcomes; it is not known whether the signatures observed here would signify child health or developmental risk. As our sample size was moderate and this study was the first to consider neighbourhood conditions and possible joint associations with depression in relation to gestational epigenetic ageing, we encourage future work to replicate these findings and further consider relations with infant and child outcomes.

In our study, we found that maternal depression and adverse neighbourhood conditions were each associated with gestational epigenetic decelerated ageing. Researchers are increasingly considering the accumulated impacts of psychological, social, and environmental exposures to explain health outcomes, disparities, and associated biologic mechanisms across the life span [65]. Gestational epigenetic ageing is a new area of research. As the field continues to mature, researchers should consider how prenatal psychosocial and contextual exposures may be jointly modulating gestational

DNA methylation and downstream child health and development. Doing so may yield new insights on the molecular and developmental origins of health and disease, and also offer novel perspectives for interdisciplinary intervention efforts for pregnant women and infants.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability

Due to the nature of this research, participants of this study did not agree for their data to be shared publicly, so supporting data is not available online. Data sharing requests should be sent to the study investigators directly.

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