



OPEN Profiling placental DNA methylation associated with maternal SSRI treatment during pregnancy

Amy M. Inkster^{1,2}, Chaini Konwar^{1,3}, Maria S. Peñaherrera^{1,2}, Ursula Brain¹, Almas Khan^{1,5}, E. Magda Price^{1,2,6}, Johanna M. Schuetz^{1,2}, Élodie Portales-Casamar^{1,5}, Amber Burt⁷, Carmen J. Marsit⁷, Cathy Vaillancourt⁸, Tim F. Oberlander^{1,4,9}✉ & Wendy P. Robinson^{1,2,9}✉

Selective serotonin reuptake inhibitors (SSRIs) for treatment of prenatal maternal depression have been associated with neonatal neurobehavioral disturbances, though the molecular mechanisms remain poorly understood. In utero exposure to SSRIs may affect DNA methylation (DNAm) in the human placenta, an epigenetic mark that is established during development and is associated with gene expression. Chorionic villus samples from 64 human placentas were profiled with the Illumina MethylationEPIC BeadChip; clinical assessments of maternal mood and SSRI treatment records were collected at multiple time points during pregnancy. Case distribution was 20 SSRI-exposed cases and 44 SSRI non-exposed cases. Maternal depression was defined using a mean maternal Hamilton Depression score > 8 to indicate symptomatic depressed mood (“maternally-depressed”), and we further classified cases into SSRI-exposed, maternally-depressed ($n = 14$); SSRI-exposed, not maternally-depressed ($n = 6$); SSRI non-exposed, maternally-depressed ($n = 20$); and SSRI non-exposed, not maternally-depressed ($n = 24$). For replication, Illumina 450K DNAm profiles were obtained from 34 additional cases from an independent cohort ($n = 17$ SSRI-exposed, $n = 17$ SSRI non-exposed). No CpGs were differentially methylated at $FDR < 0.05$ comparing SSRI-exposed to non-exposed placentas, in a model adjusted for mean maternal Hamilton Depression score, or in a model restricted to maternally-depressed cases with and without SSRI exposure. However, at a relaxed threshold of $FDR < 0.25$, five CpGs were differentially methylated ($|\Delta\beta| > 0.03$) by SSRI exposure status. Four were covered by the replication cohort measured by the 450K array, but none replicated. No CpGs were differentially methylated ($FDR < 0.25$) comparing maternally depressed to not depressed cases. In sex-stratified analyses for SSRI-exposed versus non-exposed cases (females $n = 31$; males $n = 33$), three additional CpGs in females, but none in males, were differentially methylated at the relaxed $FDR < 0.25$ cut-off. We did not observe large-scale alterations of DNAm in placentas exposed to maternal SSRI treatment, as compared to placentas with no SSRI exposure. We also found no evidence for altered DNAm in maternal depression-exposed versus depression non-exposed placentas. This novel work in a prospectively-recruited cohort with clinician-ascertained SSRI exposure and mood assessments would benefit from future replication.

As many as 20% of pregnant individuals are affected by mood disorders such as depression during pregnancy, and up to 6% of all pregnant individuals are treated with antidepressants such as selective serotonin reuptake

¹BC Children’s Hospital Research Institute (BCCHR), 950 W 28th Ave, Vancouver, BC V5Z 4H4, Canada. ²Department of Medical Genetics, University of British Columbia, Vancouver, BC V6T 1Z3, Canada. ³Centre for Molecular Medicine and Therapeutics, Vancouver, BC V6H 0B3, Canada. ⁴School of Population and Public Health, University of British Columbia, Vancouver, BC V6T 1Z3, Canada. ⁵Department of Pediatrics, University of British Columbia, Vancouver, BC V6T 1Z4, Canada. ⁶Children’s Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, ON K1H 5B2, Canada. ⁷Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA 30322, USA. ⁸INRS-Centre Armand Frappier and Réseau intersectoriel de recherche en santé de l’Université du Québec, Laval, QC H7V 1B7, Canada. ⁹These authors jointly supervised this work: Tim F. Oberlander and Wendy P. Robinson. ✉email: toberlander@bcchr.ca; wrobinson@bcchr.ca

inhibitors (SSRIs), based on estimates from several North American and European studies^{1–4}. Clinical management decisions for treatment of perinatal depression are complex, and in each case the clinician must balance the risks of treatment with the severity of depressive symptoms⁵. Given the prevalence of maternal depression and SSRI treatment during gestation, much research has been conducted focusing on the fetal effects of both^{6,7}.

Maternal prenatal depressed mood has been associated with preterm birth (< 37 weeks of gestation), growth and developmental delays, and increased postnatal infant stress^{8,9}. Additionally, the literature suggests that the presence of maternal depressive symptoms independent of SSRI therapy is associated with increases in offspring childhood internalizing behaviours and affective disorders, though these exposures are hard to separate in human studies¹⁰. Animal studies have permitted further investigation into the effect of maternal depressed mood on offspring outcomes by enabling the randomization of SSRI treatment. Rodent models have confirmed that associations identified between maternal depressed mood and offspring anxiety disorders are not mediated by SSRI exposure, and that SSRI treatment does not protect offspring from these outcomes; this topic is reviewed in¹⁰.

Regarding the impacts of SSRI treatment on the fetoplacental unit, Oberlander et al. previously found that maternal SSRI treatment was associated with altered blood flow to the fetus, increased rates of fetal hypoxemia, altered early neurodevelopment, lower arousal index throughout the newborn period, and altered psychomotor test scores^{6,7,11–13}. Prenatal SSRI exposure also tended to be associated with lower fetal-placental weight ratios and poorer fetal vascular perfusion, indicative of less efficient fetal blood flow to or from the placenta¹⁴. Both of these factors indicate less efficient placental function in association with prenatal SSRI exposure, which may be related to downstream neonatal cognitive outcomes¹⁴. Exposure to maternal SSRI treatment has previously been reported to affect syncytialization of placental trophoblasts and extravillous trophoblast function, and to affect placental serotonin levels^{15–17}. Taken together, the effect of SSRI exposure on pregnancy outcomes suggests an interplay between the placenta and SSRI pharmacobiology, which could occur by a variety of mechanisms. It has, however, been postulated that both depression and SSRI exposure may impact fetal outcomes via altered serotonin signalling and downstream effects.

Serotonin (5-hydroxytryptamine, or 5-HT) is a central neurotransmitter and a critical signaling factor in many contexts, including during prenatal neurodevelopment of the fetus¹⁸. Low levels of serotonin in the central nervous system have historically been associated with depressive symptoms in adults, in the 5-HT theory of depression¹⁹. SSRI therapeutics, which increase extracellular levels of 5-HT, have been associated with alleviation of depressive symptoms²⁰. In vivo, serotonin is synthesized from the essential amino acid L-tryptophan, by tryptophan hydroxylase enzymes 1 and 2 (TPH-1, TPH-2), and is degraded by monoamine oxidases A and B (MAOA, MAOB)^{21,22}. SSRIs function to block the serotonin transporter (SERT, also known as 5-HTT (5-hydroxytryptamine transporter)), inhibiting the reabsorption of 5-HT by the presynaptic neurons and thereby increasing extracellular serotonin levels in the central nervous system¹³. Several aspects of serotonin and SSRI biology are relevant to prenatal development, and both maternal depression and SSRI exposure have been associated with altered fetal outcomes. Notably, serotonin is first expressed early in gestation, when placental TPH-1 and TPH-2 convert maternal L-tryptophan to serotonin de novo^{22,23}. Over the course of gestation, serotonin (5-HT) is essential to many processes of prenatal development including embryogenesis, placentation, and neurodevelopment^{21,24,25}. SSRIs readily cross the placenta, leading to fetal drug exposure and altered fetal cardiac autonomic activity, presumably via altered serotonin signaling in the placenta and/or fetus^{26,27}.

One plausible mechanism by which SSRI exposure may impact pregnancy outcomes is via DNA methylation (DNAm). DNAm is an epigenetic mark involving the addition of a methyl group to the 5' carbon of a cytosine molecule, which usually occurs in the context of cytosine-guanine dinucleotides (CpGs). In human tissues, DNAm signatures are associated with gene expression patterns²⁸, and are altered in association with certain environmental and chemical exposures, such as air pollution, arsenic, and tobacco smoke; for a review of this topic see²⁹.

DNAm patterns in the human placenta vary in association with certain maternal exposures and environments, but are relatively stable once established, and serve as a possible record of gene expression patterns and exposures during gestation^{30,31}. The placenta exists at the interface of the maternal environment and the developing fetus, and environmental factors can gain access to the fetus in many cases only via the placenta^{32,33}. As SSRIs readily cross the placenta³⁴, it is possible that SSRI species alter placental DNAm patterns directly via association with epigenetic readers and writers (DNMT or TET enzymes), or through altering gene expression patterns in the serotonin signalling pathway, or other related pathways²⁹.

In this study, we hypothesized that prenatal SSRI treatment would be associated with distinct placental DNAm signatures. In a prospective cohort of 64 placentas with extensive maternal mood assessments during gestation, both with and without SSRI exposure, we investigated the effects of maternal SSRI treatment and maternal depressed mood on placental DNA methylation profiles. We considered the effect of SSRI exposure on placental DNAm as our primary outcome. The secondary outcome considered was the impact of maternal depression, which was investigated only in participants not treated with SSRIs. Additionally, given previously reported sex differences in neurodevelopmental and placental outcomes related to maternal stress exposure^{35–40}, we also assessed placental DNAm associated with maternal SSRI treatment in separate sex-stratified models.

Methods

Discovery cohort. Participants were recruited as part of a prospective, longitudinal cohort approved by the University of British Columbia/Children's and Women's Health Centre of British Columbia research ethics board: H12-00733⁴⁰. Written informed consent was obtained from all participants, and all procedures complied with the ethical standards on human experimentation and with the Helsinki Declaration of 1975 (revised in 2008). This specific study was additionally approved under certificate H16-02280.

Pregnant individuals with and without prenatal diagnoses of clinical depression, and with/without SSRI treatment plans were recruited at the British Columbia Women's Hospital (BCWH) in the 20th week of gestation ($n = 64$). At recruitment, all pregnant individuals received the MINI assessment to screen for DSM-IV Axis I Depression. SSRI use was reported by the treating clinicians, and was defined as treatment for at least 75 days including the 3rd trimester with one of the following: fluoxetine (Prozac), paroxetine (Paxil), sertraline (Zoloft), citalopram (Celexa), escitalopram (Lexapro), or venlafaxine (Effexor). Compliance with SSRI treatment was assessed and recorded at each prenatal study visit. While placental drug concentrations were not available at the time of this study, previous work on this cohort indicated that maternal plasma drug concentrations during gestation were consistent with self-report of SSRI use²⁶. Maternal mood was assessed at recruitment, 36 weeks of gestation, postnatal day 6, and at 24 months postnatally. The mood assessment included multiple clinician and patient-rated measures, of which the Hamilton Depression score, a 17-item clinician implemented assessment of the severity of depressive symptoms⁴¹, and the Edinburgh Postnatal Depression score (EPDS), a 10-item self-assessment screening tool for depression⁴², were analyzed.

Exclusion criteria were: bipolar illnesses, hypertension, any diabetes, current substance abuse, placental insufficiency, multi-fetal pregnancies, infants with congenital brain malformations, intrauterine growth restriction, or preterm birth. For all participants, measures of obstetric history, prenatal medication use, and sociodemographic variables were obtained. At birth, additional clinical information was collected for each infant including gestational age at delivery, infant sex assigned at birth, and infant birth weight; placentas were collected for DNAm studies.

After delivery, chorionic villus samples were obtained from 4 distinct cotyledonary sites ($1.5\text{--}2\text{ cm}^3$) below the surface of the fetal-facing side of each placenta, as previously described⁴³. DNA was extracted from each of the 4 sites and pooled in equimolar amounts to provide a representative sample of the whole placenta prior to obtaining DNAm profiles. DNAm data for these 64 cases was collected in a single processing batch using the Illumina MethylationEPIC ("EPIC") array platform (Illumina, San Diego, CA, USA), which profiles $> 850,000$ CpGs genome-wide.

Case distribution was as follows: 20 SSRI-exposed and 44 SSRI non-exposed samples (Table 1). Cases were additionally sub-categorized into four groups for analysis based on both SSRI exposure and maternal depression status using a mean maternal Hamilton Depression score of > 8 to indicate symptomatic depressed mood ("maternally depressed") as follows: SSRI-exposed, maternally-depressed ($n = 14$); SSRI-exposed without maternal depression ($n = 6$); SSRI non-exposed, maternally depressed ($n = 20$); and SSRI non-exposed without maternal depression ($n = 24$), see Table 2 and Fig. 1.

Replication cohort. An independent cohort of 34 cases with (i) known SSRI-exposure status and (ii) pre-collected placental DNAm data were obtained from a subset of the Rhode Island Child Healthy Study (RICHS)⁴⁴. Cohort participants were treated with one of the following SSRIs: fluoxetine, paroxetine (Paxil), sertraline (Zoloft), citalopram (Celexa), or escitalopram (Lexapro). Assessments of maternal mood were not available for these cases. DNAm data for these 34 cases were previously collected with the Illumina HumanMethylation450 ("450K") array platform, and are available on the Gene Expression Omnibus (GEO) repository under accession number GSE75248⁴⁴.

Data processing. Processing and normalization of the Illumina EPIC DNAm data for the discovery cohort is described in full in the Supplementary Methods. First, sample sex (XY chromosome complement) and genetic uniqueness were assessed and confirmed to match the annotated metadata. Next, polymorphic and cross-hybridizing probes were removed from this dataset⁴⁷, as were probes with a detection p value > 0.01 in $> 5\%$ of cases. Subsequently, CpGs with non-variable DNAm in this cohort ($n = 87,572$) were removed from the dataset to decrease multiple test correction burden. Non-variable CpGs were defined as in⁴⁸ as CpGs that met both of the following conditions: (i) $< 5\%$ range in DNAm beta (β) values between the 10th–90th centile, and (ii) previously reported as placenta-non-variable in⁴⁹. After processing and dasen + noob normalization⁵⁰ 659,036 autosomal CpGs in 64 cases, plus 8 pairs of technical replicates, remained for analysis. The technical replicates were used to estimate an absolute delta beta ($|\Delta\beta|$) cut-off for subsequent analyses, based on the average root mean squared error of the technical replicate pairs (0.026) and the average standard error of all CpGs in all cases (0.0048). The highest of these values was selected and rounded up, to establish a $|\Delta\beta|$ cut-off of > 0.03 between groups; anything less than $|\Delta\beta| = 0.03$ could represent technical noise and was unlikely to be biologically meaningful in this cohort. After processing, the replicate pairs were removed from the cohort and excluded from downstream analysis.

Batch correction was performed after identifying an effect of EPIC array row on DNAm data using Principal Components Analysis, see Supplementary Fig. S1. Prior to batch correction, the distribution of cases across EPIC array rows was confirmed to be independent of our primary outcomes of interest: SSRI status (Fisher test $p > 0.05$) and mean maternal Hamilton depression score (ANOVA $p > 0.05$), see Supplementary Fig. S2. ComBat was used to correct the remaining categorical effect of the "row" variable, with SSRI exposure (yes/no) and mean maternal Hamilton Depression score (continuous) included as variables of interest in the ComBat model matrix as recommended by the *sva* package authors⁵¹. Results for batch-corrected analyses were confirmed in non-batch-corrected data to ensure that false signal was not introduced during batch correction, in accordance with the recommendations outlined in⁵², see Supplementary Fig. S1.

Replication cohort. The raw data for the full 335-sample RICHS cohort was downloaded from GEO (GSE75248) and processed analogously to the discovery cohort: first sample sex and genetic uniqueness were checked, data were dasen + noob normalized, and polymorphic and cross-hybridizing probes were removed⁴⁷,

Discovery cohort			
	SSRI-exposed	Non-SSRI-exposed	<i>p</i> value [†]
n	20	44	
Maternal Hamilton Depression Score (mean (SD))			
26 weeks	10.6 (5.4)	6.6 (3.6)	0.001**
36 weeks	10.8 (4.2)	8.3 (3.6)	0.021*
Mean	10.6 (4.5)	7.5 (3.5)	0.004**
Length SSRI exposure (days, mean (SD))	264.4 (29.9)	–	–
Type of SSRI exposure (n (%))			
Citalopram	7 (35.0)	–	–
Escitalopram	7 (35.0)	–	
Fluoxetine	1 (5.0)	–	
Paroxetine	1 (5.0)	–	
Sertraline	4 (20.0)	–	
Delivery type (n vaginal (%))	10 (50.0)	29 (65.9)	0.351
Gestational age at birth (weeks, mean (SD))	39.3 (1.4)	39.6 (1.3)	0.438
Gestational diabetes (n, mean (SD))	0 (0)	0 (0)	–
Infant sex (n male (%))	8 (40.0%)	25 (56.8%)	0.328
Infant birthweight Z-score (mean (SD))	– 0.01 (1.00)	– 0.03 (0.73)	0.915
PlaNET ancestry[§] (mean (SD))			
Coordinate 1	0.00 (0.00)	0.00 (0.01)	0.354
Coordinate 2	0.05 (0.21)	0.13 (0.31)	0.300
Coordinate 3	0.95 (0.21)	0.86 (0.31)	0.286
Replication cohort			
	SSRI-exposed	Non-SSRI-exposed	<i>p</i> value [†]
n	17	17	
Type of SSRI exposure (n (%))			
Citalopram	3 (17.6)	–	–
Escitalopram	2 (11.8)	–	
Fluoxetine	2 (11.8)	–	
Paroxetine	1 (5.9)	–	
Sertraline	9 (52.9)	–	
Delivery type (n vaginal (%))	4 (23.5)	12 (70.6)	0.016*
Gestational age at birth (weeks, mean (SD))	39.0 (0.9)	39.0 (0.9)	0.999
Infant sex (n male (%))	7 (41.2)	10 (58.8)	0.493
Gestational diabetes (n mean (SD))	5 (29.4)	3 (18.8)	0.758
PlaNET ancestry[§]			
Coordinate 1	0.00 (0.01)	0.08 (0.25)	0.212
Coordinate 2	0.01 (0.04)	0.12 (0.32)	0.174
Coordinate 3	0.98 (0.05)	0.80 (0.38)	0.057

Table 1. Demographics of the discovery and replication cohorts by SSRI exposure status. SSRI refers to selective-serotonin reuptake inhibitor treatment during pregnancy. Birthweight Z-scores were calculated using gestational-age and sex-adjusted birthweight curves⁴⁵. [†]*p* values are from chi-squared tests for categorical variables and t-tests for continuous variables, comparing SSRI-exposed to SSRI non-exposed, * indicates <0.05, ** indicates <0.005. [§]The PlaNET algorithm outputs DName-based ancestry probability values ranging from 0 to 1, which sum to 1 for each sample. Coordinate 1 is associated with probability of African ancestry, coordinate 2 with East Asian ancestry, and coordinate 3 with European ancestry⁴⁶.

as were probes with a detection *p* value > 0.01 in > 5% of cases. For a complete description of data processing see the Supplementary Methods. The processed data were subsetted to 34 cases for which gestational SSRI treatment status was recorded in the medical chart. Replication cohort demographics are presented in Table 1.

Covariate selection. We next sought to identify characteristics that could be associated with SSRI exposure in the discovery and replication cohorts, which should be included as covariates in statistical models. Demographic variables considered include those presented in Tables 1 and 2. The discovery cohort was well-balanced across all demographic variables by both SSRI exposure and maternal depression status. Gestational age, infant sex, and PlaNET ancestry were selected for inclusion as additive covariates in linear modelling analyses as it is known that these factors drive large amounts of DName variation in the placenta^{46,53}. The same variables were selected in the replication cohort (infant sex, gestational age at birth, PlaNET ancestry), with the addition of

	SSRI-exposed, maternally-depressed	SSRI-exposed, not maternally depressed	SSRI non-exposed, maternally-depressed	SSRI non-exposed, not maternally-depressed	SSRI exposed vs non-exposed <i>p</i> value [†] (maternally-depressed)	SSRI exposed vs non-exposed <i>p</i> value [†] (not maternally-depressed)
Depression group	Depressed	Not depressed	Depressed	Not depressed	Depressed vs. depressed	Not depressed vs. not depressed
n	14	6	20	24		
Maternal Hamilton Depression Score (mean (SD))						
26 weeks	13.4 (3.9)	4.5 (2.4)	9.7 (2.6)	4.3 (2.2)	0.003*	0.841
36 weeks	12.8 (2.8)	5.6 (2.6)	11.0 (2.2)	5.8 (2.7)	0.045*	0.869
Mean	13.0 (2.9)	5.2 (1.8)	10.5 (2.2)	5.0 (2.1)	0.009*	0.877
Length SSRI exposure (days, mean (SD))	266.93 (19.28)	258.33 (48.65)	–	–	–	–
Type of SSRI exposure (n (%))[‡]						
Citalopram	5 (35.7)	2 (33.3)	–	–	–	–
Escitalopram	5 (35.7)	2 (33.3)	–	–	–	–
Fluoxetine	0 (0.0)	1 (16.7)	–	–	–	–
Paroxetine	1 (7.1)	0 (0.0)	–	–	–	–
Sertraline	3 (21.4)	1 (16.7)	–	–	–	–
Delivery type (n vaginal (%))	5 (35.7)	5 (83.3)	11 (55.0)	18 (75.0)	0.281	0.99
Gestational age at birth (weeks, mean (SD))	39.1 (1.4)	39.7 (1.4)	39.6 (1.3)	39.5 (1.4)	0.447	0.697
Gestational diabetes (n, mean (SD))	0 (0)	0 (0)	0 (0)	0 (0)	–	–
Infant sex (n male (%))	6 (42.9%)	2 (33.3%)	11 (55.0%)	14 (58.3%)	0.727	0.522
Infant birthweight Z-score (mean (SD))	– 0.01 (1.06)	0.00 (0.94)	– 0.02 (0.62)	– 0.05 (0.82)	0.989	0.906
PlaNET ancestry[§] (mean (SD))						
Coordinate 1	0.00 (0.00)	0.00 (0.00)	0.01 (0.02)	0.00 (0.00)	0.292	0.665
Coordinate 2	0.07 (0.25)	0.01 (0.01)	0.14 (0.32)	0.12 (0.31)	0.488	0.370
Coordinate 3	0.93 (0.25)	0.99 (0.01)	0.85 (0.32)	0.87 (0.31)	0.460	0.369

Table 2. Discovery cohort demographics by SSRI and depression group status. Maternal depression was categorized using mean maternal Hamilton Depression score, ≤ 8 was categorized as not depressed, > 8 as depressed, this is reflected in the “Depression Group” row. SSRI refers to selective-serotonin reuptake inhibitor treatment during pregnancy. Birthweight Z scores were calculated using gestational-age and sex-adjusted birthweight curves⁴⁵. [†]*p* values are from chi-squared tests for categorical variables and t-tests for continuous variables, * indicates < 0.05 , ** indicates < 0.005 . [‡]*p* = 0.59 (chi-squared test) for proportion of SSRI types utilized by SSRI-exposed, maternally-depressed participants versus SSRI-exposed, not maternally-depressed participants. [§]The PlaNET algorithm outputs DName-based ancestry probability values ranging from 0–1, which sum to 1 for each sample. Coordinate 1 is associated with probability of African ancestry, coordinate 2 with East Asian ancestry, and coordinate 3 with European ancestry⁴⁶.

type of delivery (vaginal/other), as delivery type frequency differed by SSRI exposure in the replication cohort only, see Table 1.

Using tools from the *PlaNET* R package^{54,55}, we also estimated additional variables in the discovery cohort from the DName data itself: the proportion of six major placental cell types (cytotrophoblasts, syncytiotrophoblasts, endothelial cells, stromal cells, Hofbauer cells, and nucleated red blood cells), and placental epigenetic age. Cell type proportions were not significantly associated with either SSRI treatment or mean maternal Hamilton Depression score across gestation (t-test for cell type proportions ~ SSRI exposed (yes/no), all *p* values > 0.05 ; Pearson correlation for mean maternal Hamilton Depression score and each cell type proportion, all *p* values > 0.05), see Supplementary Fig. S3. Accordingly, cell type proportions were not included as covariates in subsequent linear models. Epigenetic age acceleration was calculated as the residual of the Control Placental Clock epigenetic age regressed on gestational age at birth, adjusted for sex, ancestry, and EPIC array row. Intrinsic epigenetic age acceleration (cell-type independent) was calculated similarly, but with additional adjustment for numeric cell type proportions. Placental epigenetic age was not considered as a possible covariate in linear models, but was analyzed separately for associations with SSRI exposure as previous studies have reported conflicting relationships between cord blood epigenetic age acceleration and SSRI treatment, so parallel exploration in the placenta was warranted^{56,57}, see “Results”.

Linear modelling to identify placental DName associated with SSRI exposure and maternal depression.

To identify differential DName associated with both SSRI exposure and exposure to maternal depression in the discovery cohort, three linear models were explored, see Fig. 1. Linear modelling on M values was conducted using *limma* in R^{58,59}. In addition to the main effects, all models were adjusted for infant sex,

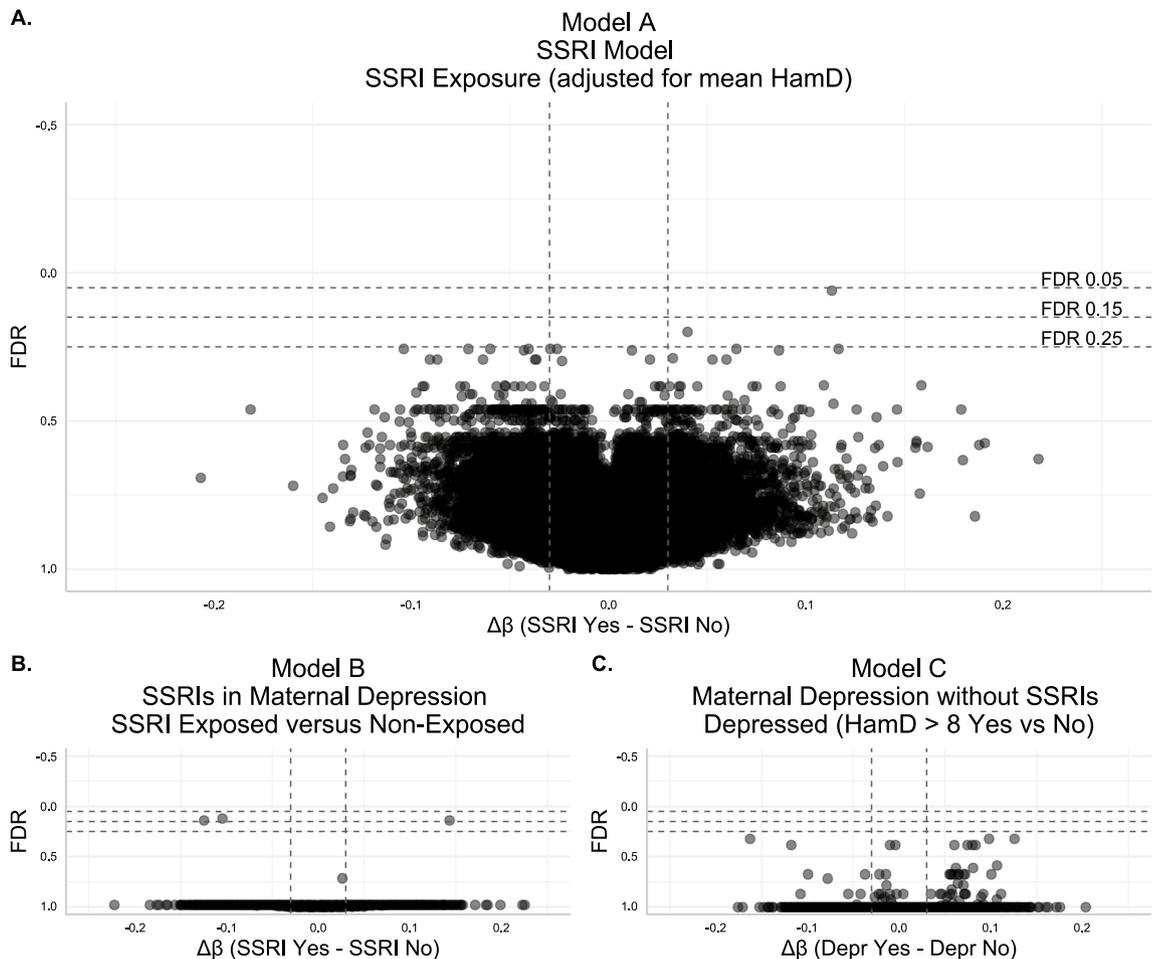


Figure 2. Volcano plots for differential DNAm in association with SSRI exposure and categorical depression. For all plots HamD refers to the mean maternal Hamilton Depression score across gestation for each individual. False discovery rate (FDR) is shown along the Y axis with more significant (lower FDR) values at the top of the plot. Vertical dashed intercepts demarcate $|\Delta\beta| = 0.03$, horizontal dashed intercepts indicate, from top to bottom, FDR = 0.05, FDR = 0.15, and FDR = 0.25. (A) Volcano plot for the SSRI model, investigating differential DNAm associated with SSRI exposure adjusted for mean maternal HamD score, ($n = 64$). Difference in DNAm ($\Delta\beta$) is plotted along the X axis and was calculated as $\Delta\beta = \beta_{\text{SSRI-exposed}} - \beta_{\text{SSRI non-exposed}}$. (B) Volcano plot for the SSRIs in maternal depression model, differential DNAm associated with SSRI exposure in cases with mean maternal HamD score > 8 , ($n = 34$). Difference in DNAm ($\Delta\beta$) is plotted along the X axis and was calculated as $\Delta\beta = \beta_{\text{SSRI-exposed}} - \beta_{\text{SSRI non-exposed}}$. (C) Volcano plot for the maternal depression without SSRIs model, differential DNAm associated HamD score > 8 across gestation, in SSRI non-exposed cases, ($n = 44$). Difference in DNAm ($\Delta\beta$) is plotted along the X axis and was calculated as $\Delta\beta = \beta_{\text{Hamilton} > 8} - \beta_{\text{Hamilton} \leq 8}$, “Depr” indicates “Depressed”, i.e. HamD > 8 .

of +0.11 (higher average DNAm β value in SSRI-exposed placentas). Loosening the threshold to FDR < 0.25 , one additional CpG was differentially methylated by SSRI exposure status (cg20877313), and was also more highly methylated in SSRI-exposed placentas ($\Delta\beta = +0.04$). In the SSRIs in maternal depression model (model B), three CpGs were differentially methylated at the moderate-confidence threshold of FDR < 0.15 (cg12655501, cg26993610, cg14340829). These CpGs all had an average difference in DNAm between SSRI-exposed and SSRI non-exposed cases of $|\Delta\beta| > 0.10$. In the maternal depression without SSRIs model (model C), no CpGs showed evidence of maternal depression-associated differential DNAm at any FDR threshold. For a description of all CpGs that satisfied the moderate and low-confidence FDR thresholds from the SSRI models (A, B) and SSRIs in maternal depression model (C), including test statistics and overlapping genes, see Table 3. Summary statistics of linear modelling from all CpGs tested are presented in Supplementary Tables S2–S4. Boxplots showing average DNAm β values in SSRI-exposed versus SSRI non-exposed cases for the differentially methylated CpGs are shown in Fig. 3.

A differentially methylated region (DMR) analysis was also conducted, to increase power for discovering DNAm-associations by grouping signatures from multiple neighboring CpG sites to reduce the number of statistical comparisons. DMR analysis was only conducted for the SSRI model to enable utilization of the entire cohort of cases and maximize statistical power.

Model	CpG	p value	FDR	FDR threshold	$\Delta\beta$	Chr:position	Gene symbol	Relation to gene	450K locus?
A	cg12900404	9.11e-08	0.06	Moderate confidence	0.11	2: 225811669	<i>DOCK10</i>	1st exon	-
A	cg20877313	6.05e-07	0.20	Low confidence	0.04	12: 56881753	<i>GLS2</i>	1st exon	Yes
B	cg12655501	6.07e-07	0.14	Low confidence	-0.12	1: 115603524	<i>TSPAN2</i>	Body	-
B	cg26993610	6.26e-07	0.14	Low confidence	0.14	1: 115605466	<i>TSPAN2</i>	Body	-
B	cg14340829	1.80e-07	0.12	Low confidence	-0.10	8: 923973	-	-	Yes
DMR	cg14921691, cg06762403	-	1.95e-29	-	0.06	12: 56325797-56325867	<i>DGKA</i>	5'UTR, 1st Exon	Yes

Table 3. Top CpGs with differential DNAm by SSRI-exposure status. Linear modelling p value and false discovery rates (FDR) shown, $\Delta\beta$ is difference in DNAm calculated as $\Delta\beta = \beta_{\text{SSRI-exposed}} - \beta_{\text{SSRI non-exposed}}$. hg19 chromosome (Chr) and coordinates (Position) for each CpG are indicated, as are genes that each CpG overlaps, and whether each CpG is covered by a probe on the 450K DNA methylation array. A and B in the Model Column refer to model A and model B, DMR refers to the differentially-methylated region analysis.

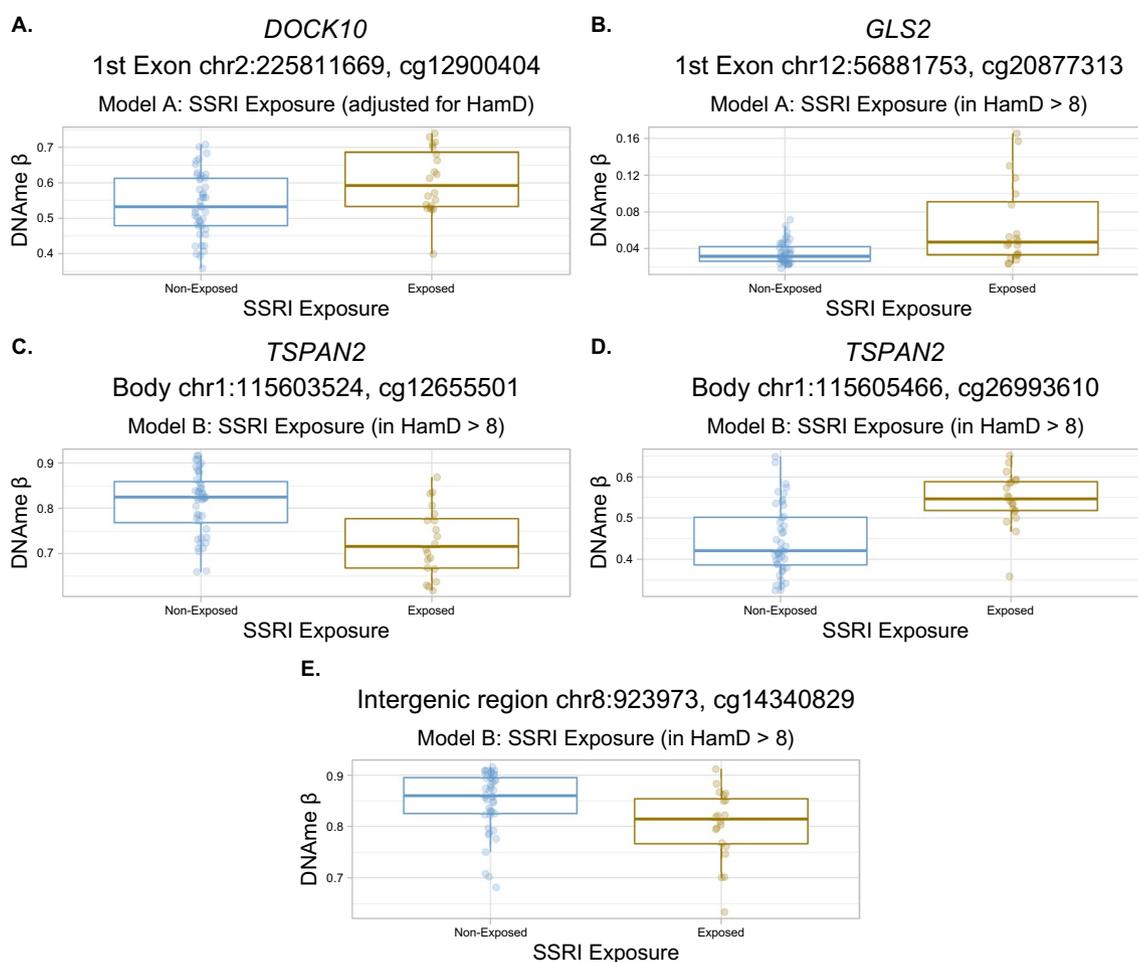


Figure 3. Boxplots of differentially methylated CpGs by SSRI exposure. For all plots, points are colored by SSRI exposure (blue = SSRI non-exposed, dark yellow = SSRI-exposed), and boxplots indicate mean DNAm β value \pm one standard deviation. (A) Boxplot of cg12900404, identified in the SSRI model, model A. (B) Boxplot of cg20877313, identified in the SSRI model, model A. (C) Boxplot of cg12655501, identified in the SSRIs in maternal depression model, model B. (D) Boxplot of cg26993610, identified in the SSRIs in maternal depression model, model B. (E) Boxplot of cg14340829, identified in the SSRIs in maternal depression model, model B.

M values were assessed for being part of DMRs for which average DNAm differed by SSRI exposure status using the *DMRcate* R package (FDR < 0.25, $\lambda = 1000$, $C = 2$)⁶¹. Only one DMR was identified with an average DNAm β value that differed by SSRI exposure, this DMR comprised two CpGs 70 base pairs apart (cg06762403 and cg14921691) on chromosome 12, overlapping the *DGKA* gene. The average $\Delta\beta$ across the region was +0.06, indicating higher DNAm in SSRI-exposed placentas, see Table 3 and Supplementary Fig. S4.

Few placental DNAm differences associated with SSRI exposure in sex-stratified models. As the effects of SSRI exposure might differ by sex, we next sought to assess whether unique SSRI-exposure DNAm associations arose in placentas of male versus female infants. Sex-stratified linear models were run in males ($n = 33$, 24% SSRI-exposed) and females ($n = 31$, 39% SSRI-exposed) to test for SSRI-exposure associated DNAm, adjusting for mean maternal Hamilton Depression Score, gestational age at birth, EPIC array row, and PlaNET ancestry. No CpGs were differentially methylated by SSRI exposure in males at any statistical threshold considered. In females, at an FDR < 0.25 and $|\Delta\beta| > 0.03$, three CpGs (cg15849349, cg07481545, and cg03905236) were differentially methylated in association with SSRI exposure (Supplementary Fig. S5).

We previously identified 162 CpGs with robust sex differences in placental DNAm patterns that replicated in an independent dataset⁶². To investigate sex in another context, we tested whether these previously-ascertained sex-differentially-methylated CpGs were also differentially methylated by SSRI exposure status. All 162 replicated CpGs were considered, and were subjected to linear modelling in the discovery cohort for SSRI exposure, adjusting for mean maternal Hamilton Depression Score, gestational age at birth, EPIC array row, and PlaNET ancestry. No CpGs met significance at FDR < 0.05. At the low-confidence FDR < 0.25 threshold, three CpGs (cg22515303, cg27003571, cg26136722) had lower DNAm in SSRI-exposed placentas than non-exposed placentas, in addition to the previously identified sex effect at these loci (Supplementary Fig. S6).

No replication of DNAm signature associated with SSRI exposure in an independent dataset. We next intended to assess the reproducibility of the five CpGs with SSRI-associated differential DNAm identified in the SSRI model and SSRIs in maternal depression model (Table 3), and the two DMR CpGs. As the replication cohort was run on the Illumina 450K array, only four of these seven discovery cohort CpGs could be assessed for replication (cg14340829, cg20877313, cg06762403, cg14921691). A linear model was applied to the M values from these four CpGs in the replication cohort to test for differential DNAm associated with SSRI exposure, adjusting for infant sex, gestational age at birth, mode of delivery, and PlaNET ancestry. None of the four CpGs replicated differential methylation at a nominal p value < 0.05, see Supplementary Fig. S7.

Placental epigenetic age acceleration not associated with SSRI exposure or maternal depression. Epigenetic age is a useful dimension-reduction technique to analyze relationships between global DNAm patterns and a variety of health outcomes. Altered epigenetic age relative to chronological age (“epigenetic age acceleration”) has been associated with several negative health outcomes including overall greater burden of disease and higher rates of all-cause mortality^{63,64}. Specific to depression, major depressive disorder is associated with increased epigenetic age acceleration in adult blood⁶⁵. Umbilical cord blood epigenetic age deceleration was initially reported in maternal depression⁵⁶, but interestingly this association has since been demonstrated to be largely related to the confounding signature of maternal SSRI treatment rather than depression itself⁵⁷.

Using a recently published placental epigenetic clock⁵⁵, we calculated epigenetic age acceleration in the discovery cohort. We then tested for associations between epigenetic age acceleration metrics and either SSRI exposure or mean maternal Hamilton Depression score using linear models. Neither epigenetic age acceleration nor intrinsic epigenetic age acceleration (cell-type independent) were significantly associated with either SSRI exposure or maternal depression, see Supplementary Fig. S8.

Replication of previously reported differential methylation candidates associated with SSRI-exposure and maternal depression in the discovery cohort. A previous study identified 16 CpGs with altered placental DNAm associated with maternal depression (treated as a categorical variable, where depression was maternal EPDS > 10) at FDR < 0.05 (no $|\Delta\beta|$ threshold)⁶⁶. EPDS measurements were also collected for the discovery cohort cases in our study, and were significantly collinear with mean maternal Hamilton Depression scores across gestation, see Supplementary Fig. S9. To assess whether these previously-reported CpGs were also differentially methylated in association with maternal EPDS in the discovery cohort, we ran a linear model on the DNAm M values from these 16 CpGs, adjusting for infant sex, gestational age at birth, ancestry, and EPIC array row. One CpG (cg06670742) satisfied a nominal p value < 0.05 in our cohort, and had higher DNAm in cases with higher mean maternal EPDS scores (FDR < 0.05, $\Delta\beta = +0.003$). Thus, differential DNAm of this CpG did replicate in our study, however the effect size in our cohort was very small and did not exceed the technical threshold we set of $|\Delta\beta| > 0.03$ (Fig. 4).

Three previous studies investigated genome-wide DNAm alterations associated with SSRI treatment in a prenatal tissue (cord blood). An early study by Non et al. found no association between SSRI exposure and cord blood DNAm at any CpG loci⁶⁷. Cardenas et al. reported one differentially methylated CpG in the gene body of *ZNF575* associated with maternal antidepressant treatment in a cohort of cord blood samples⁶⁸. In a different cohort, Kallak et al. reported 13 CpGs differentially methylated by SSRI exposure status in cord blood⁶⁹. Though cord blood DNAm is very distinct from placenta, we sought to evaluate whether any of the 14 CpGs reported by Cardenas et al.⁶⁸ or Kallak et al.⁶⁹ were also differentially methylated by SSRI exposure status in the placenta. At the CpG reported by Cardenas et al. (cg22159528) we found no difference in DNAm by SSRI exposure (nominal p value > 0.05), see Fig. 4. At one of the 13 CpGs reported by Kallak et al., we found nominally significant

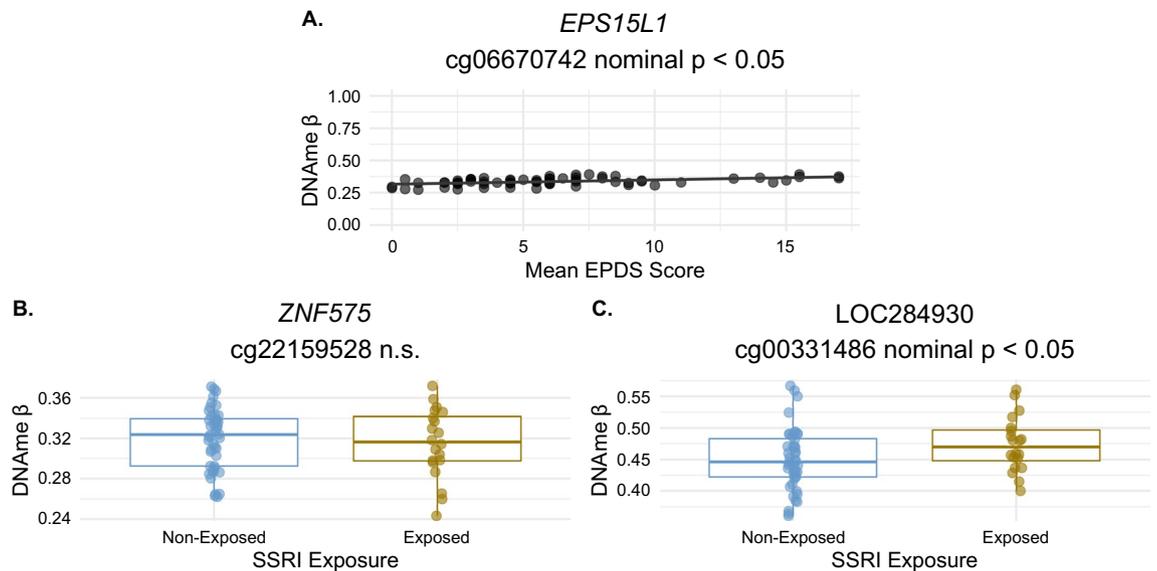


Figure 4. Assessment of literature candidates for SSRI and depression-associated DNAm in the discovery cohort. **(A)** Scatterplot showing the association between DNAm and maternal Edinburgh Postnatal Depression Score (EPDS) at cg06670742 which was previously found to be differentially methylated in association with maternal EPDS score in Tesfaye et al.³⁷ and is differentially methylated by SSRI exposure at $FDR < 0.05$ in our discovery cohort. DNAm β value at this CpG is plotted along the Y axis, mean maternal EPDS score is plotted along the X axis and each point is a case; $\Delta\beta$ per unit increase in EPDS is $\Delta\beta = +0.003$. **(B)** Boxplot showing mean DNAm β value \pm one standard deviation by SSRI exposure status at cg22159628 in *ZNF575*, previously found to be differentially methylated in cord blood with antidepressant exposure. Linear modelling p value = 0.67, points are colored by SSRI exposure (blue = SSRI non-exposed, dark yellow = SSRI-exposed), n.s. = not significant. **(C)** Boxplot showing mean DNAm β value \pm one standard deviation by SSRI exposure status at cg00331486 in *LOC284930* on chromosome 22, previously found to be differentially methylated in cord blood by SSRI exposure status.

($p < 0.05$) differential DNAm associated with SSRI exposure status in the placenta in the same direction as the original cord blood association: higher DNAm in SSRI-exposed cases. This was cg00331486 in *LOC284930* on chromosome 22, which encodes a MYC-interacting long noncoding RNA species⁶⁹.

Discussion

In this study, we examined genome-wide placental DNAm patterns associated with SSRI exposure or maternal depression during gestation. Taken together, our results suggest the absence of a major impact of SSRI antidepressant exposure on the placental DNA methylome. Specifically, we found no differentially methylated CpGs associated with either SSRI exposure or maternal depression that met the standard significance threshold of $FDR < 0.05$. These results hold despite known alterations elicited by SSRIs to maternal gestational serotonin signalling and infant outcomes, suggesting that the effects of SSRI exposure on infant outcomes are not associated with a coincident DNAm signature in the placenta.

At more relaxed statistical thresholds, differential DNAm analysis highlighted five CpGs associated with SSRI exposure status. Three of the five differentially methylated CpGs overlapped the following genes, respectively: *DOCK10* (CpG not present in replication cohort), *GLS2* (CpG did not replicate), and *TSPAN2* (CpG not present in replication cohort). The *DOCK10* gene encodes the Dopamine Receptor Interacting Protein⁷⁰, and was recently found to be one of the twelve most predictive mRNA biomarkers of depression in a gene expression study of adult whole blood⁷¹. These researchers identified that *DOCK10* expression was higher in association with a more positive, less depressed mood⁷¹. *DOCK10* is also a target of increased expression by the action of the antidepressant ketamine⁷¹. *TSPAN2* encodes Tetraspanin-2, which mediates signal transduction in processes related to cellular growth and development⁷². *Tspan2* has been reported to be upregulated in the rat hippocampus after chronic exposure to the SSRI fluoxetine⁷³. We also identified one differentially methylated region in the 5' untranslated region of *DGKA*, with higher DNAm in SSRI exposed placentas. *DGKA* encodes a diacylglycerol kinase that is involved in intracellular signalling⁷⁴, and *DGKA* activity was recently reported to be inhibited by Ritanserin, a pharmaceutical serotonin receptor type 2 (5-HT₂) antagonist that is not currently in clinical use⁷⁵.

Inherent to studying the impact of SSRI exposure are the concurrent, and potentially confounding, effects of maternal mood disturbances. As such, studies must be carefully designed to interrogate the effects of either SSRI treatment or depression exposure while accounting for the other factor. Only a limited placental DNAm signature has so far been associated with maternal mood disorders⁶⁶, and to our knowledge no work has yet been conducted interrogating genome-wide placental DNAm signatures associated with SSRI exposure. Tesfaye et al.⁶⁶ investigated placental DNAm in association with maternal depression, though SSRI treatment or antidepressant use was not reported on or investigated and could underlie some of these associations. Of the 16 CpGs

they identified as differentially methylated by maternal depression status across six gestational time points, only one CpG in the *EPS15L1* gene was also differentially methylated in our study by mean maternal EPDS score across gestation (FDR < 0.05), and although the effect was in the same direction, the effect size ($\Delta\beta = +0.003$ with each unit increase in mean EPDS) was well below our biological $|\Delta\beta|$ threshold of > 0.03, and therefore may not be a biologically meaningful change in DNAm.

In non-placental tissue, Kallak et al.⁶⁹ identified 13 CpGs that were differentially methylated in cord blood in association with SSRI exposure, one of which replicated at nominal significance in the same direction (higher DNAm in SSRI-exposed cases) in our cohort. Cardenas et al. also identified one CpG in the gene body of *ZNF575* (cg22159528) with lower DNAm in antidepressant-exposed cord blood samples from Project Viva, which they replicated in an external cohort (Generation R Study)⁶⁸. In our cohort, placental DNAm at this CpG was not associated with SSRI exposure. Although cord blood and placenta are both conceptus-derived tissues relevant to prenatal development and early life, they have unique origins (embryonic versus extraembryonic lineages, respectively) and thus distinct DNAm profiles are expected. Our finding that cord blood differential DNAm does not replicate at high rates in the placenta may suggest a cord-blood-specific DNAm signature at these CpGs in association with maternal SSRI treatment.

In sex-stratified analyses, three CpGs were differentially methylated by SSRI exposure in females: two in intergenic regions on chromosomes 4 and 6 respectively, and one in the 5' untranslated region of the *SH3GL3* gene. *SH3GL3* encodes the Endophilin-A3 protein, which interacts selectively with the Huntingtin protein to promote the formation of polyglutamine-containing protein aggregates⁷⁶. Promoter DNA methylation of the *SH3GL3* gene was previously inversely correlated with its expression in human colon cancer cell lines versus matched double knockout cell lines for DNMT1 and DNMT3b⁷⁷. Lower DNAm upstream of the promoter of the *SH3GL3* gene in SSRI-exposed female placentas may indicate higher expression of this gene relative to the placentas from SSRI non-exposed females, which should be followed up in future work. In a second sex-focused analysis we tested whether previously reported sex-biased CpGs⁶² were differentially methylated by SSRI status, three CpGs met the low-confidence FDR < 0.25 threshold (two in the 5' untranslated region of *GTDC1*, one in the gene body of *C14orf132*). Together, these analyses suggest that there may be a sex-biased signature of SSRI treatment on the placental DNA methylome or transcriptome, and future studies in this field should be designed to analyze sex as a variable of interest.

The major strength of our study lies in the curation of both SSRI treatment and maternal depression information per participant. This prospective design enabled us to investigate the potential confounding effects of these two variables in controlled contexts (i.e. studying SSRI exposure while adjusting for depression scores, and studying the effect of maternal depression in SSRI non-exposed samples). Additionally, in this study we measured genome-wide DNAm in an effort to reduce bias that arises from candidate gene or CpG pyrosequencing studies, and utilized the most modern Illumina EPIC array platform to capture the highest resolution data currently afforded by a DNAm microarray. Further strengths of our study include strict effect size and significance thresholds, the presence of an external replication cohort, the ability to account for covariates such as genetic ancestry and cell type proportions, and the ability to stratify analyses by sex.

Limitations of this work include SSRI exposure being defined as maternal treatment with any of six different SSRIs during gestation. It is possible that SSRI subtypes have different effects on placental DNAm, though this study was not able to undertake sub-analysis by SSRI type, and future work should consider this. The extent to which SSRI treatment improves maternal depression scores (i.e. remittance of symptoms) may also be associated with placental DNAm patterns and should be investigated in future longitudinal cohorts. Lastly, although sample size was relatively small, our cohort is among one of a handful of prospectively recruited cohorts that we are aware of with placental tissue collected alongside maternal depression and SSRI treatment information, including the BASIC and Generation R Studies^{78,79}. Cord blood DNAm data exists for both of these cohorts and has previously been studied in association with maternal SSRI exposure^{68,69}. Cohorts such as these, with extensive clinical records of maternal mood and medication use across gestation, are incredibly valuable resources to investigate how depression status and antidepressant medications may interact with fetal outcomes.

In conclusion, our work demonstrates that limited placental DNAm alterations are associated with maternal SSRI exposure; this work agrees with earlier findings of a similarly limited placental DNAm signature of maternal depression. Future work in larger cohorts will determine whether other molecular features are altered in association with maternal mood disorders or SSRI treatment. As larger cohorts with clinical characterization of maternal SSRI treatment and prenatal depressed mood become available, genetic sequence variation, gene expression patterns, DNA methylation alterations, and alterations in other epigenetic marks should all be considered, as well as their interactions.

Data availability

The raw and preprocessed EPIC array data and corresponding sample metadata supporting the conclusions of this article are available on the Gene Expression Omnibus (GEO), under the accession number GSE203396.

Received: 15 June 2022; Accepted: 8 December 2022

Published online: 30 December 2022

References

1. Andersson, L. et al. Point prevalence of psychiatric disorders during the second trimester of pregnancy: A population-based study. *Am. J. Obstet. Gynecol.* **189**(1), 148–154 (2003).
2. Gorman, L. L. et al. Adaptation of the structured clinical interview for DSM-IV disorders for assessing depression in women during pregnancy and post-partum across countries and cultures. *Br. J. Psychiatry Suppl.* **46**, s17–23 (2004).

3. Melville, J. L., Gavin, A., Guo, Y., Fan, M. Y. & Katon, W. J. Depressive disorders during pregnancy: Prevalence and risk factors in a large urban sample. *Obstet. Gynecol.* **116**(5), 1064–1070 (2010).
4. Tran, H. & Robb, A. S. SSRI use during pregnancy. *Semin. Perinatol.* **39**(7), 545–547 (2015).
5. Kim, D. R., O'Reardon, J. P. & Epperson, C. N. Guidelines for the management of depression during pregnancy. *Curr. Psychiatry Rep.* **12**(4), 279–281 (2010).
6. Hanley, G. E. & Oberlander, T. F. The effect of perinatal exposures on the infant: Antidepressants and depression. *Best Pract. Res. Clin. Obstet. Gynaecol.* **28**(1), 37–48 (2014).
7. Hanley, G. E., Brain, U. & Oberlander, T. F. Prenatal exposure to serotonin reuptake inhibitor antidepressants and childhood behavior. *Pediatr. Res.* **78**(2), 174–180 (2015).
8. Goodman, S. H. & Halperin, M. S. Perinatal depression as an early stress: Risk for the development of psychopathology in children. In *The Oxford Handbook of Stress and Mental Health (Oxford Library of Psychology)* 287–312 (Oxford University Press, 2020).
9. Talge, N. M., Neal, C. & Glover, V. Early Stress, Translational Research and Prevention Science Network: Fetal and Neonatal Experience on Child and Adolescent Mental Health. Antenatal maternal stress and long-term effects on child neurodevelopment: How and why?. *J. Child Psychol. Psychiatry.* **48**(3–4), 245–261 (2007).
10. Hutchison, S. M., Mässe, L. C., Pawluski, J. L. & Oberlander, T. F. Perinatal selective serotonin reuptake inhibitor (SSRI) and other antidepressant exposure effects on anxiety and depressive behaviors in offspring: A review of findings in humans and rodent models. *Reprod. Toxicol. Elmsford N.* **99**, 80–95 (2021).
11. Oberlander, T., Gingrich, J. & Ansorge, M. Sustained neurobehavioral effects of exposure to SSRI antidepressants during development: Molecular to clinical evidence. *Clin. Pharmacol. Ther.* **86**(6), 672–677 (2009).
12. Rurak, D. *et al.* Third trimester fetal heart rate and doppler middle cerebral artery blood flow velocity characteristics during prenatal selective serotonin reuptake inhibitor exposure. *Pediatr. Res.* **70**(1), 96–101 (2011).
13. Rotem-Kohavi, N. & Oberlander, T. F. Variations in neurodevelopmental outcomes in children with prenatal SSRI antidepressant exposure. *Birth Defects Res.* **109**(12), 909–923 (2017).
14. Levy, M. *et al.* Maternal use of selective serotonin reuptake inhibitors (SSRI) during pregnancy—neonatal outcomes in correlation with placental histopathology. *J. Perinatol.* **40**(7), 1017–1024 (2020).
15. Clabault, H. *et al.* Effects of selective serotonin-reuptake inhibitors (SSRIs) on human villous trophoblasts syncytialization. *Toxicol. Appl. Pharmacol.* **349**, 8–20 (2018).
16. Clabault, H., Cohen, M., Vaillancourt, C. & Sanderson, J. T. Effects of selective serotonin-reuptake inhibitors (SSRIs) in JEG-3 and H1PEC cell models of the extravillous trophoblast. *Placenta* **72–73**, 62–73 (2018).
17. Thibeault, A. A. H., de Los Santos, Y. L., Doucet, N., Sanderson, J. T. & Vaillancourt, C. Serotonin and serotonin reuptake inhibitors alter placental aromatase. *J. Steroid Biochem. Mol. Biol.* **195**, 105470 (2019).
18. Velasquez, J., Goeden, N. & Bonnin, A. Placental serotonin: Implications for the developmental effects of SSRIs and maternal depression. *Front. Cell Neurosci.* **7**, 47 (2013).
19. Albert, P. R., Benkelfat, C. & Descarries, L. The neurobiology of depression—revisiting the serotonin hypothesis. I. Cellular and molecular mechanisms. *Philos. Trans. R. Soc. B Biol. Sci.* **367**(1601), 2378–2381 (2012).
20. Moncrieff, J. *et al.* The serotonin theory of depression: A systematic umbrella review of the evidence. *Mol. Psychiatry.* <https://doi.org/10.1038/s41380-022-01661-0> (2022).
21. St-Pierre, J., Laurent, L., King, S. & Vaillancourt, C. Effects of prenatal maternal stress on serotonin and fetal development. *Placenta* **48**(Suppl 1), S66–71 (2016).
22. Bonnin, A. *et al.* A transient placental source of serotonin for the fetal forebrain. *Nature* **472**(7343), 347–350 (2011).
23. Laurent, L. *et al.* Human placenta expresses both peripheral and neuronal isoform of tryptophan hydroxylase. *Biochimie* **140**, 159–165 (2017).
24. Karahoda, R. *et al.* Dynamics of tryptophan metabolic pathways in human placenta and placental-derived cells: Effect of gestation age and trophoblast differentiation. *Front. Cell Dev. Biol.* **8**, 574034 (2020).
25. Karahoda, R. *et al.* Prenatal inflammation as a link between placental expression signature of tryptophan metabolism and preterm birth. *Hum. Mol. Genet.* **30**(22), 2053–2067 (2021).
26. Campbell, K. S. J. *et al.* Maternal serotonin reuptake inhibitor antidepressants have acute effects on fetal heart rate variability in late gestation. *Front. Psychiatry.* **12**, 680177 (2021).
27. Laurent, L. *et al.* In utero exposure to venlafaxine, a serotonin-norepinephrine reuptake inhibitor, increases cardiac anomalies and alters placental and heart serotonin signaling in the rat. *Birth Defects Res. A Clin. Mol. Teratol.* **106**(12), 1044–1055 (2016).
28. Dhar, G. A., Saha, S., Mitra, P. & Nag, C. R. DNA methylation and regulation of gene expression: Guardian of our health. *Nucleus* **64**(3), 259–270 (2021).
29. Martin, E. M. & Fry, R. C. Environmental influences on the epigenome: Exposure-associated DNA methylation in human populations. *Annu. Rev. Public Health.* **39**, 309–333 (2018).
30. Chatterjee, S., Ouidir, M. & Tekola-Ayele, F. Genetic and in utero environmental contributions to DNA methylation variation in placenta. *Hum. Mol. Genet.* **30**(21), 1968–1976 (2021).
31. Vlahos, A., Mansell, T., Saffery, R. & Novakovic, B. Human placental methylome in the interplay of adverse placental health, environmental exposure, and pregnancy outcome. *PLoS Genet.* **15**(8), e1008236 (2019).
32. Burton, G. J. & Fowden, A. L. The placenta: A multifaceted, transient organ. *Philos. Trans. R. Soc. B Biol. Sci.* **370**(1663), 20140066 (2015).
33. Pemathilaka, R. L., Reynolds, D. E. & Hashemi, N. N. Drug transport across the human placenta: Review of placenta-on-a-chip and previous approaches. *Interface Focus.* **9**(5), 20190031 (2019).
34. Ewing, G., Tatarchuk, Y., Appleby, D. & Kim, D. Placental transfer of antidepressant medications: Implications for postnatal adaptation syndrome. *Clin. Pharmacokinet.* **54**(4), 359–370 (2015).
35. Clifton, V. L. Review: Sex and the human placenta: Mediating differential strategies of fetal growth and survival. *Placenta* **31**, S33–S39 (2010).
36. Bale, T. L. Sex differences in prenatal epigenetic programming of stress pathways. *Stress.* **14**(4), 348–356 (2011).
37. Bale, T. L. The placenta and neurodevelopment: Sex differences in prenatal vulnerability. *Dialogues Clin. Neurosci.* **18**(4), 459–464 (2016).
38. Gobinath, A. R., Workman, J. L., Chow, C., Lieblich, S. E. & Galea, L. A. M. Sex-dependent effects of maternal corticosterone and SSRI treatment on hippocampal neurogenesis across development. *Biol. Sex Differ.* **8**, 20 (2017).
39. Sutherland, S. & Brunwasser, S. M. Sex differences in vulnerability to prenatal stress: A review of the recent literature. *Curr. Psychiatry Rep.* <https://doi.org/10.1007/s11920-018-0961-4> (2018).
40. Campbell, K. S. J. *et al.* Prenatal antidepressant exposure and sex differences in neonatal corpus callosum microstructure. *Dev. Psychobiol.* **63**(6), e22125 (2021).
41. Rohan, K. J. *et al.* A protocol for the Hamilton rating scale for depression: Item scoring rules, rater training, and outcome accuracy with data on its application in a clinical trial. *J. Affect. Disord.* **200**, 111–118 (2016).
42. Murray, D. & Cox, J. L. Screening for depression during pregnancy with the Edinburgh depression scale (EDDS). *J. Reprod. Infant Psychol.* **8**(2), 99–107 (1990).
43. Blair, J. D. *et al.* Widespread DNA hypomethylation at gene enhancer regions in placentas associated with early-onset pre-eclampsia. *Mol. Hum. Reprod.* **19**(10), 697–708 (2013).

44. Paquette, A. G. *et al.* Regions of variable DNA methylation in human placenta associated with newborn neurobehavior. *Epigenetics* **11**(8), 603–613 (2016).
45. Kramer, M. S. *et al.* A new and improved population-based Canadian reference for birth weight for gestational age. *Pediatrics* **108**(2), E35 (2001).
46. Yuan, V. *et al.* Accurate ethnicity prediction from placental DNA methylation data. *Epigenet. Chromatin*. **12**(1), 51 (2019).
47. Zhou, W., Laird, P. W. & Shen, H. Comprehensive characterization, annotation and innovative use of Infinium DNA methylation BeadChip probes. *Nucleic Acids Res.* **45**(4), e22 (2017).
48. Edgar, R. D., Jones, M. J., Robinson, W. P. & Kobor, M. S. An empirically driven data reduction method on the human 450K methylation array to remove tissue specific non-variable CpGs. *Clin. Epigenet.* **9**(1), 11 (2017).
49. Dieckmann, L. *et al.* Reference-based versus reference-free cell type estimation in DNA methylation studies using human placental tissue. in Review (2021) <https://www.researchsquare.com/article/rs-848651/v1> (Accessed 13 Dec 2021).
50. Pidsley, R. *et al.* A data-driven approach to preprocessing Illumina 450K methylation array data. *BMC Genomics* **14**, 293 (2013).
51. Leek, J. T., Johnson, W. E., Parker, H. S., Jaffe, A. E. & Storey, J. D. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* **28**(6), 882–883 (2012).
52. Nygaard, V., Rødland, E. A. & Hovig, E. Methods that remove batch effects while retaining group differences may lead to exaggerated confidence in downstream analyses. *Biostatistics* **17**(1), 29–39 (2016).
53. Konwar, C., Del Gobbo, G., Yuan, V. & Robinson, W. P. Considerations when processing and interpreting genomics data of the placenta. *Placenta* **84**, 57–62 (2019).
54. Yuan, V. *et al.* Cell-specific characterization of the placental methylome. *BMC Genomics* **22**(1), 6 (2021).
55. Lee, Y. *et al.* Placental epigenetic clocks: Estimating gestational age using placental DNA methylation levels. *Aging* **11**(12), 4238–4253 (2019).
56. Suarez, A. *et al.* The epigenetic clock at birth: Associations with maternal antenatal depression and child psychiatric problems. *J. Am. Acad. Child Adolesc. Psychiatry*. **57**(5), 321–328.e2 (2018).
57. McKenna, B. G. *et al.* Maternal prenatal depression and epigenetic age deceleration: Testing potentially confounding effects of prenatal stress and SSRI use. *Epigenetics* **16**(3), 327–337 (2021).
58. Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* **43**(7), e47–e47 (2015).
59. Bass, A. *et al.* biobroom: Turn Bioconductor objects into tidy data frames. <https://github.com/StoreyLab/biobroom>.
60. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B Methodol.* **57**(1), 289–300 (1995).
61. Peters, T. J. *et al.* De novo identification of differentially methylated regions in the human genome. *Epigenet. Chromatin* **8**, 16 (2015).
62. Inkster, A. M. *et al.* A cross-cohort analysis of autosomal DNA methylation sex differences in the term placenta. *Biol. Sex Differ.* **12**(1), 38 (2021).
63. Gale, C. R., Marioni, R. E., Harris, S. E., Starr, J. M. & Deary, I. J. DNA methylation and the epigenetic clock in relation to physical frailty in older people: The Lothian Birth Cohort 1936. *Clin. Epigenet.* **10**(1), 101 (2018).
64. Horvath, S. DNA methylation age of human tissues and cell types. *Genome Biol.* **14**(10), R115 (2013).
65. Han, L. K. M. *et al.* Epigenetic aging in major depressive disorder. *Am. J. Psychiatry*. **175**(8), 774–782 (2018).
66. Tesfaye, M., Chatterjee, S., Zeng, X., Joseph, P. & Tekola-Ayele, F. Impact of depression and stress on placental DNA methylation in ethnically diverse pregnant women. *Epigenomics* **13**(18), 1485–1496 (2021).
67. Non, A. L., Binder, A. M., Kubzansky, L. D. & Michels, K. B. Genome-wide DNA methylation in neonates exposed to maternal depression, anxiety, or SSRI medication during pregnancy. *Epigenetics* **9**(7), 964–972 (2014).
68. Cardenas, A. *et al.* Prenatal maternal antidepressants, anxiety, and depression and offspring DNA methylation: Epigenome-wide associations at birth and persistence into early childhood. *Clin. Epigenet.* **11**(1), 56 (2019).
69. Kallak, T. K. *et al.* DNA methylation in cord blood in association with prenatal depressive symptoms. *Clin. Epigenet.* **13**(1), 1–14 (2021).
70. DOCK10. (National Library of Medicine (US), National Center for Biotechnology Information, 2004) <https://www.ncbi.nlm.nih.gov/gene/55619> (Accessed 25 Mar 2022).
71. Le-Niculescu, H. *et al.* Precision medicine for mood disorders: Objective assessment, risk prediction, pharmacogenomics, and repurposed drugs. *Mol. Psychiatry*. **26**(7), 2776–2804 (2021).
72. TSPAN2. (National Library of Medicine (US), National Center for Biotechnology Information, 2004) <https://www.ncbi.nlm.nih.gov/gene/10100> (Accessed 25 Mar 2022).
73. Kroeze, Y. *et al.* Long-term consequences of chronic fluoxetine exposure on the expression of myelination-related genes in the rat hippocampus. *Transl. Psychiatry*. **5**(9), e642–e642 (2015).
74. DGKA. (National Library of Medicine (US), National Center for Biotechnology Information, 2004) <https://www.ncbi.nlm.nih.gov/gene/1606> (Accessed 25 Mar 2022).
75. Boroda, S., Niccum, M., Raju, V., Purohit, B. W. & Harris, T. E. Dual activities of ritanserin and R59022 as DGKA inhibitors and serotonin receptor antagonists. *Biochem. Pharmacol.* **123**, 29–39 (2017).
76. Sittler, A. *et al.* SH3GL3 associates with the Huntingtin exon 1 protein and promotes the formation of polyglutamine-containing protein aggregates. *Mol. Cell.* **2**(4), 427–436 (1998).
77. Kang, J. Y. *et al.* Identification of long-range epigenetic silencing on chromosome 15q25 and its clinical implication in gastric cancer. *Am. J. Pathol.* **185**(3), 666–678 (2015).
78. Axfors, C. *et al.* Cohort profile: The Biology, Affect, Stress, Imaging and Cognition (BASIC) study on perinatal depression in a population-based Swedish cohort. *BMJ Open* **9**(10), e031514 (2019).
79. Jaddoe, V. W. V. *et al.* The generation R study: Design and cohort profile. *Eur. J. Epidemiol.* **21**(6), 475–484 (2006).

Acknowledgements

We acknowledge all cohort owners and collaborators on the “Using ‘omics to build an atlas of placental development and function across pregnancy” (NIH Project). Thank you to Dr. Michael S. Kobor and the BCCHRI Microarray Core Facilities for support with Illumina EPIC DName runs. We thank Robinson lab members Ruby Jiang, Giulia F. Del Gobbo, and Desmond Hui for their work in sample processing, quality control, and testing, thank you to Victor Yuan and Ziqi Liu for pilot data processing for cases from this cohort. Finally, we sincerely thank the pregnant individuals and families that contributed placental samples to this study.

Author contributions

A.M.I. conducted the analyses and wrote the manuscript, and contributed to sample processing, quality control, and testing. C.K. conducted a preliminary analysis of the effect of SSRI exposure on an earlier version of this data, and contributed to sample processing, quality control, and testing. M.S.P. is the NIH project manager, curated

and organized clinical data, contributed to sample processing, quality control, and testing, and manuscript editing. U.B. conducted clinical data collection. A.K. processed and normalized the discovery cohort EPIC array data. E.M.P. and J.M.S. were involved in data management, sample metadata collation and establishment of the RedCap database, and participated in randomization. É.P.C. oversaw project administration and data curation of this cohort. A.B. performed data curation for the RICHS cohort cases, C.J.M. is cohort owner of the RICHS data and organized data curation for this set of cases. C.V. contributed to the conceptualization and design of this project, and also contributed to funding acquisition. T.F.O. and W.P.R. were responsible for project administration, funding acquisition, and supervision of the SSRI cohort recruitment and the DNAm data generation aims of the project, respectively, and were major contributors in writing and revising the manuscript. T.O. designed the original study. A.M.I., C.K., M.S.P., U.B., A.K., E.M.P., J.M.S., É.P.C., C.V., T.F.O., and W.P.R. all participated in experimental design. All authors read, revised, and approved the final version of the manuscript.

Funding

This work was supported by a grant from the National Institutes of Health (1R01HD089713-01). Collection of the Discovery Cohort was funded by a Canadian Institutes of Health Research grant (MOP-5783). WPR receives salary support through an investigatorship award from the BC Children's Hospital Research Institute. AMI is funded by a Frederick Banting and Charles Best Canada Doctoral Scholarship from the Canadian Institutes of Health Research.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-022-26071-8>.

Correspondence and requests for materials should be addressed to T.F.O. or W.P.R.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2022