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Prenatal stress, epigenetically-assessed glucocorticoid exposure at birth, and child psychiatric symptoms: A prospective, multi-cohort study

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Abstract

Background. Recent work suggests that DNA methylation can be used as a proxy of fetal glucocorticoid exposure (MPS-GC), showing associations with maternal psychopathology during pregnancy. However, it is unknown whether the MPS-GC may act as a marker for broader prenatal stress and whether it partially mediates associations of prenatal stress with child internalizing and externalizing symptoms.

Methods. Using harmonized data from three prospective birth cohorts (N_{pooled} = 6086), we examined whether a cumulative measure of prenatal stress, and its individual stress domains, associate with the MPS-GC in cord blood at birth. Next, we examined (i) whether the MPS-GC at birth associates with child psychiatric symptoms, (ii) whether this association is moderated by postnatal stress, and (iii) whether the effect of prenatal stress on child psychiatric symptoms is partially mediated by the MPS-GC at birth.

Results. Our meta-analysis revealed no significant associations between the MPS-GC at birth and prenatal stress or the individual stress domains. Moreover, the MPS-GC did not significantly associate with later child internalizing or externalizing symptoms, and there were no moderating effects of postnatal stress. Additionally, while prenatal stress significantly associated with child psychiatric symptoms, we found no partial mediation via the MPS-GC at birth.

Conclusions. We did not find support that the MPS-GC in cord blood reliably proxies prenatal stress, associates with child psychiatric risk, or partially mediates the associations between prenatal stress and psychiatric risk.

Keywords: ALSPAC, MoBA, DNA methylation, glucocorticoid, internalizing, externalizing

Prenatal stress, epigenetically-assessed glucocorticoid exposure at birth, and child psychiatric symptoms: A prospective, multi-cohort study

1. Introduction

Exposure to maternal stress during pregnancy, or 'prenatal stress', has been shown to increase the risk for child psychiatric symptoms (1–4) and thus psychopathology later in life (5). Prenatal stress includes a diverse array of psychosocial stressors (e.g., maternal psychopathology, socioeconomic difficulties, stressful life events, and family conflict), which often co-occur (6) and can have a cumulative effect on child outcomes (4,7–9). Importantly, the association between prenatal stress and child psychiatric symptoms is not fully explained by postnatal exposures or genetic confounding(10–13), supporting a direct link with the in-utero environment. However, as yet, the biological mechanisms that link prenatal stress with child psychiatric symptoms are not fully understood and there are no robust markers of risk associated with exposure to prenatal stress (14,15). Addressing these gaps has the potential to inform strategies for improving early risk detection and preventing the development of psychopathology.

Epigenetic profiling is emerging as a promising tool to assess early life exposures and health risk (16,17). The most studied epigenetic modification is DNA methylation (DNAm), which typically involves the addition of a methyl group to a cytosine-guanine dinucleotide in the DNA and can functionally regulate gene expression (18). There is some evidence linking specific prenatal stressors to differential patterns of DNA methylation (19–21). However, cumulative prenatal stress does not show a strong signal at the epigenome-wide level from which to build a methylation profile score (22). As such, rather than taking an exploratory epigenome-wide approach to develop a proxy for prenatal stress, an alternative is to test DNA methylation-based markers that represent theorized biological pathways as potential proxies for cumulative prenatal stress. One of several potential pathways by which prenatal stress may influence child psychiatric symptoms is via the long-term impact of in-utero glucocorticoid (GC) exposure on the epigenetic programming of the offspring's stress response system during fetal development. Although the mechanisms are still under contention, it has been posited that prenatal stress increases fetal GC exposure due to increased maternal cortisol levels and altered placental metabolism of cortisol (23–25, see 11 for a broader overview of biological mechanisms). In turn, enhanced GC signaling in

utero could program future stress responsivity by inducing changes in DNAm of stress-related genes in the offspring (11,15,23). DNAm can influence gene expression and thereby provides a potential mechanism by which prenatal stress could impact long-term development and health. By extension, DNAm patterns related to in-utero GC exposure may have utility as a marker for prenatal stress exposure.

Recently, a DNA methylation profile score has been developed to index in-utero glucocorticoid exposure in cord blood (MPS-GC)(26). To develop the score, Provençal et al. first identified lasting changes in DNAm in response to synthetic GC exposure within a human hippocampal progenitor cell (HPC) line, which is used to study fetal neurogenesis. Next, DNAm changes in HPCs were compared to those observed in response to synthetic GC exposure in whole blood. Based on sites showing differential methylation across the two tissues, a weighted MPS-GC was computed with an elastic net regression that selected 24 loci, and was then applied to DNAm measured in cord blood in the PREDO birth cohort (n = 817). The MPS-GC showed small, negative associations with cumulative maternal anxiety and depression in pregnancy, providing some preliminary indication for its use as a biologically informed proxy for prenatal stress associated with maternal psychopathology. However, it has not been established whether other types of prenatal stressors (e.g., stressful life events and financial worries) are associated with the MPS-GC at birth, and whether associations are cumulative or driven by specific types of stressors.

Interestingly, Provençal et al. found that the lasting DNAm changes seen in HPCs after GC exposure altered the set-point for cellular responses to future stressors. They suggested these alterations could lead to dysregulated responses to stressors after birth, thus increasing the likelihood of developing psychiatric symptoms later in life (27). That said, an extension of the work in the same cohort (n = 814) revealed that the MPS-GC did not associate with total child behavior problems, which comprises both internalizing and externalizing symptoms, but did associate with length of psychiatric treatment (28). However, the null results could be due to a lack of statistical power to detect effects given the relatively small sample and therefore requires testing in a larger sample. Moreover, stress-related

pathways to child internalizing versus externalizing symptoms may differ and thus warrant separate consideration in terms of their association with the MPS-GC (29). Furthermore, it was not tested whether an association between the MPS-GC and psychiatric symptoms is dependent on postnatal stress exposure, which we might expect if prenatal GC exposure indeed primes an exaggerated response to future stressors and may explain the lack of a main effect of the MPS-GC on child psychiatric symptoms (11).

In the current preregistered study (https://osf.io/49cn7), we used cord-blood DNAm data from three of the largest epigenetic birth cohorts in the world (N_{pooled} = 6086: the Generation R Study, GenR; the Avon Longitudinal Study of Parents and Children, ALSPAC; and the Norwegian Mother, Father and Child Cohort Study, MoBA) to investigate prospective associations between prenatal stress, the MPS-GC, and child psychiatric symptoms (see Figure 1 for a visual overview of the study aims). As our primary aim, we evaluated if the MPS-GC in cord blood is a proxy for prenatal stress. Specifically, we examined whether a cumulative measure of prenatal stress, and the individual stress domains comprising the measure, were associated with the MPS-GC at birth. For our second aim, we examined whether the MPS-GC at birth associates with child internalizing and externalizing symptoms. Building on this, for our third aim, we examined whether associations between the MPS-GC and psychiatric symptoms are moderated by postnatal stress. Finally, for our fourth aim, we examined whether the MPS-GC partially mediates an effect of prenatal stress on child internalizing and externalizing symptoms, as a potential biological pathway underlying these associations. Given reported sex differences in responses to prenatal stress (30), we also repeated the analyses stratified by sex. Additionally, we conducted sensitivity analyses controlling for birthweight and gestational age as both have been shown to be associated with prenatal stress, DNA methylation, and child psychiatric symptoms (31–34).

<FIGURE 1>

2. Materials and Methods

2.1. Study population

Data were drawn from three independent population-based early-life cohorts: 1) GenR (Rotterdam, the Netherlands), an ongoing population-based prospective cohort study of parents and children from fetal life onwards (35,36); 2) ALSPAC (Avon, UK), a transgenerational prospective observational study investigating influences on health and development across the life course (37,38), and 3) MoBA (Norway), a pregnancy cohort initiated by the Norwegian Institute of Public Health, with a family design that aims to understand the etiology of complex diseases (39). Further information about the cohorts, including recruitment, consent, and ethical approval, can be found in the supplementary methods. We included participants (N_{pooled} = 6086) with available cord blood DNAm data that passed quality control and, for sibling pairs, included one sibling based on data availability, or if equal, at random. The GenR cohort comprised two subcohorts based on the timing and beadchip used for DNAm analysis ($GenR_{450k}$ and $GenR_{EPIC}$). For the MoBA cohort, we retained four subcohorts (MoBa1, MoBa2, MoBa4, MoBa8) drawn from the general population and excluded subcohorts that were selected based on specific criteria (e.g., in vitro fertilization). All participants were of European ancestry and the sample characteristics are summarized in Table 1. The sample size exceeded that indicated in the power analysis, which was conducted during preregistration (https://osf.io/49cn7).

Measures

1.2 Prenatal and Postnatal Stress.

A cumulative prenatal stress score and a cumulative postnatal stress score were computed based on previous work for each cohort (4,40). Full details of the variables contained in the scores and measurement timing for the GenR and ALSPAC cohorts are available here: https://github.com/SereDef/cumulative-ELS-score. Comparable scores were developed in the MoBa cohort as described in earlier work (40). In short, across cohorts, the cumulative prenatal stress score included measurements covering all three trimesters, while the cumulative postnatal stress score included measurements covering the whole period from 0-10 years. Multi-informant data (i.e., reports from both parents, or mother and teacher) were

used where available to reduce the risk of bias. Each cumulative pre- or postnatal stress score consisted of the following domains: (i) life events (e.g., death of a parent or pregnancy complications), (ii) contextual risk (e.g., financial difficulties or neighborhood problems), (iii) parental risk (e.g., parental criminal record or parental psychopathology), and (iv) interpersonal risk (e.g., family conflicts or loss of a friend), and, for the postnatal stress score only, also (v) direct victimization (e.g., child bullied by peers or physically hurt). Approximately 100 items per cohort were dichotomized into 'risk' (1) or 'no risk' (0) and mean averaged to form the scores for each domain. Cumulative scores for prenatal stress and postnatal stress were computed by summing their respective domain scores, with higher scores representing more stressors. The scores have been shown to statistically predict child internalizing and externalizing symptoms in previous work in the study cohorts (4,40).

2.2. DNA Methylation

In each subcohort, 500ng genomic DNA was extracted from cord blood samples taken at birth and bisulfite converted using the EZ-96 DNA Methylation kit (Shallow) (Zymo Research Corporation, Irvine, USA). Samples were then processed using the Infinium HumanMethylation450 BeadChip (450K) or Infinium MethylationEPIC Beadchip v1.0 (EPIC; Illumina Inc., San Diego, USA) before undergoing quality control and normalization (see supplementary methods), which resulted in DNAm β-values (0-1) representing the ratio of methylation signal to overall signal at each CpG locus.

2.3 Methylation Profile Score for Fetal Glucocorticoid Exposure (MPS-GC)

A weighted DNAm profile score was computed based on DNAm β-values at 24 CpG loci previously reported as showing lasting DNAm changes in response to synthetic glucocorticoid exposure in (i) a HPC line and, (ii) the whole blood of individuals in the Max Planck Institute of Psychiatry (MPIP) cohort (26). In line with earlier work, coefficients from the MPIP cohort analysis were used as weights (26,28), included in Table S1 alongside the availability of loci across subcohorts. The score for each participant was calculated by

multiplying the DNAm β-value with the corresponding weight for each CpG, adding these values together to form a single score, then z-scoring the score to ensure standardization across subcohorts.

2.4. Child Psychiatric Symptoms

Symptoms were reported by mothers at child age 5, age 9, or age 10 in the MoBA, ALSPAC, GenR cohorts, respectively. Separate scales for internalizing and externalizing symptoms were formed with items from the Child Behavior Checklist for ages 6-18 (41), Strengths and Difficulties Questionnaire (42), or a short-form version of the Child Behavior Checklist for ages 1.5-5 (43,44). Higher scores represent a higher frequency of symptoms.

2.5. Covariates

In all analyses, we controlled for child sex, maternal smoking status during pregnancy, cell proportions (CD4+ T-lymphocytes, CD8+ T-lymphocytes, natural killer cells, B-lymphocytes, monocytes, granulocytes, with nRBCs not included to avoid multi-collinearity)) estimated using a cord-blood reference set (45), four European-specific genetic principal components to account for population stratification, and either sample plate or surrogate variables to adjust for batch effects (see supplementary methods for details).

2.6. Statistical Analyses

All analyses were performed in the software package R version 4.3.1 (46). An alpha level of .05 was used to assess statistical significance. To reduce bias and improve power, missing data were imputed by fully conditional multiple imputation (47) with 60 iterations and 30 imputed datasets and estimates were pooled with Rubin's rule (see supplementary methods for details). All continuous variables were z-scored to standardize across cohorts.

2.6.1. Prenatal Stress and the MPS-GC

In each subcohort, we used robust linear regression using M-estimation with the MASS package (48) to test whether cumulative prenatal stress statistically predicted the MPS-GC.

We repeated the analyses using the four individual stress domains as separate predictors in a single model to assess their independent associations with the MPS-GC.

2.6.2. The MPS-GC, Child Psychiatric Symptoms, and Moderation by Postnatal stress

We used two separate robust linear regression models using M-estimation in each subcohort to test whether the MPS-GC statistically predicted 1) child internalizing symptoms and 2) child externalizing symptoms. We repeated each model adding an interaction term of the MPS-GC by postnatal stress (i.e., an additional variable of the z-scored MPS-GC score × z-scored postnatal stress score for each participant) to test whether associations were moderated by levels of postnatal stress.

2.6.3. Mediation Analyses

Partial mediation was tested with a regression-based approach (49) performed in the *Lavaan* package (50). In each subcohort, we estimated the indirect effect of cumulative prenatal stress (predictor) on 1) child internalizing symptoms (outcome model 1) and 2) child externalizing symptoms (outcome model 2) via the MPS-GC (partial mediator) with two separate mediation analyses. Each mediation analysis tested a total effect model of the relationship between cumulative prenatal stress and child internalizing/externalizing symptoms, and a direct effect model of the same relationship while additionally controlling for MPS-GC scores. The indirect effect was calculated with the difference-in-coefficients methods, representing the reduction in the effect of cumulative prenatal stress on child internalizing/externalizing symptoms when MPS-GC scores were included in the model (for an example of the model specification see https://lavaan.ugent.be/tutorial/mediation.html). A significant indirect effect was taken to indicate the presence of partial mediation via the MPS-GC.

2.6.4. Meta-analyses

Variance-weighted random-effects models were used to pool the subcohort-specific results with a random effect of subcohort using the *metafor* package (51). Where applicable, array

type and/or child age at symptom measurement were tested with meta-regression as potential moderators of the subcohort estimates. Where the moderation effect was significant the pooled estimates were adjusted based on the average for array type (dummy coded) or average age at outcome, otherwise unadjusted pooled estimates based on random-effects models were interpreted. Given that the predictors and outcome were standardized in all models across cohorts, the pooled estimates represent the change in standard deviations of the given outcome (i.e., the MPS-GC or child psychiatric symptoms) with each standard deviation change in the predictor (i.e., prenatal stress scores or MPS-GC).

2.6.5. Sensitivity Analyses. In the sensitivity analyses, we repeated all models (i) stratified by sex in all cohorts and (ii) controlling for child birthweight and gestational age.

3. Results

3.1. Maternal Prenatal Stress and the MPS-GC at Birth

In the meta-analysis, cumulative prenatal stress did not significantly predict the MPS-GC (β = -0.01, 95% CI [-0.02, 0.005], p = .220), although the estimated effect was in the expected direction (Table 2). Coefficients were inconsistent in direction across cohorts (ranging -0.03 to 0.02) with significant negative associations in two cohorts (Table S2).

Additionally, the meta-analysis revealed no significant independent associations between individual stress domains and the MPS-GC (see Table 2). At the cohort level, the directions of the coefficients were inconsistent across subcohorts and domains (Table S2).

<TABLE 2 HERE>

3.2. The MPS-GC at Birth and Child Psychiatric Symptoms

As shown in Table 3, meta-analysis revealed that the MPS-GC at birth was not significantly associated with child internalizing symptoms (β = -0.03, 95% CI [-0.09, -0.03], p = .397) or externalizing symptoms (β = -0.01, 95% CI [-0.01, 0.02], p = .727), although coefficients were in the hypothesized direction. Associations were also not significant at the cohort-level with coefficients showing an inconsistent direction across subcohorts for both internalizing

symptoms (ranging -0.15 to 0.04) and externalizing symptoms (ranging -0.08 to 0.08; Table S2).

3.3. Postnatal Stress as a Moderator of Associations Between the MPS-GC and Child Psychiatric Symptoms

As shown in Table 3, in the moderation analyses, the MPS-GC did not predict child internalizing symptoms (β = -0.02, 95% CI [-0.08, 0.04], *p* =.553) and externalizing symptoms while controlling for postnatal stress (β = -0.002, 95% CI [-0.08, 0.07], *p* =.957). In contrast, postnatal stress predicted both child internalizing symptoms (β = 0.23, 95% CI [0.18, 0.29], *p* = <.001) and externalizing symptoms (β = 0.23, 95% CI [0.17, 0.29], *p* = <.001) while controlling for the MPS-GC. The interaction term (MPS-GC × postnatal stress) was not significant in the model for child internalizing symptoms (β = -0.001, 95% CI [-0.03, 0.02], *p* =.881) and externalizing symptoms (β = -0.001, 95% CI [-0.02, 0.02], *p* = .948), indicating no moderating effect of postnatal stress on associations between the MPS-GC and child psychiatric symptoms. At the cohort level, the interaction terms were non-significant in all subcohorts (Table S2).

<TABLE 3 HERE>

3.4. Indirect Effects of Maternal Prenatal Stress on Child Psychiatric Symptoms via the MPS-GC

Cumulative prenatal stress significantly predicted child internalizing and externalizing symptoms in the pooled total effect models and pooled direct effect models (Figure 2). This indicates that exposure to higher cumulative prenatal stress associates with higher child internalizing and externalizing symptoms (with or without controlling for MPS-GC). However, the pooled indirect effects were not significant for internalizing symptoms (indirect effect = 0.0009, *p* =.873) and externalizing symptoms (indirect effect = 0.002, *p* =.634), indicating no evidence of partial mediation by the MPS-GC at birth. Results were consistent at the subcohort level (Table S3).

< FIGURE 2 HERE >

All four prenatal individual stress domains significantly statistically predicted child internalizing symptoms in the pooled total effect models and pooled direct effect models; while life events, contextual risk, and parental risk significantly statistically predicted child externalizing symptoms (Table 4). However, the pooled estimates for the indirect effects were non-significant for all domains, indicating no partial mediation by the MPS-GC. At the cohort-specific level, the total and direct effects differed in direction and significance across cohorts but the indirect effects were consistently non-significant (Table S3).

<TABLE 4 HERE>

3.5. Sensitivity Analyses

3.5.1. Sex Stratification

There was one change to the significance of the results when the analyses were stratified by sex (Tables S4 and S5). Specifically, prenatal interpersonal risk was significantly associated with a lower MPS-GC in girls (β = -0.02, p = .007), but not boys (β = -0.01, p = .383).

3.5.2. Birthweight and Gestational Age as Additional Covariates

There were significant differences between subcohorts in child birthweight (F(6, 6079) = 11.86, p < .001) and gestational age (F(6,6079) = 32.24, p = < .001). Sensitivity analyses revealed no meaningful changes to the pooled results when birthweight and gestational age were controlled for in the models (Tables S6 and S7).

4.1. Discussion

In this study, using data from three large population-based cohorts, we observed that a DNA methylation-based proxy for glucocorticoid exposure at birth (i.e., the MPS-GC) was not significantly associated with exposure to prenatal stress, either measured cumulatively or in relation to individual stress domains (e.g., life events and parental risk). Moreover, the MPS-GC, which was measured in cord blood at birth, did not associate with parent-reported internalizing and externalizing symptoms in the same children measured later in development (ages 5-10yrs). Further, given previous findings suggesting that glucocorticoid exposure may prime cells to exaggerated responses to future stressors via DNA methylation changes (26), we hypothesized that relations between the MPS-GC and child psychiatric

symptoms may be accentuated in children who are exposed to greater postnatal stress. However, we found no interaction effects between the MPS-GC and a cumulative measure of postnatal stress on child internalizing or externalizing symptoms. Finally, we showed that although cumulative prenatal stress, and several of the individual stress domains, significantly statistically predicted child internalizing and externalizing symptoms, the MPS-GC did not partially mediate these effects. While we focus our interpretation of the study findings on the meta-analytic results, notably the direction of coefficients was largely inconsistent across the subcohort results prior to meta-analysis. This could be related to the unreliable signal of the MPS-GC but may also reflect study heterogeneity and sample size differences. Hence, we strongly recommend interpretation is focused on the meta-analytic results, which employed random-effects models to allow between study heterogeneity in the true effect alongside adjustments for effect differences related to array type and outcome age.

Our primary aim was to establish whether the MPS-GC could provide a reliable proxy for exposure to cumulative maternal stress during pregnancy, which could be used in research as an alternative to behavioral measures or in a prevention setting to identify risk. Although our results showed a significant albeit small association between cumulative prenatal stress and the MPS-GC in two cohorts, the findings were not consistent across all cohorts or at the meta-analytic level. Thus, we did not find support for the use of the MPS-GC as a reliable methylation-based proxy for fetal exposure to cumulative prenatal stress. Moreover, there were no associations of the individual stress domains with the MPS-GC in the main analysis. However, in the sex stratified analyses, there was a significant albeit very small association between prenatal interpersonal risk across pregnancy (e.g., family conflicts or marital problems) and lower MPS-GC for females only. In terms of predictive utility, direct comparison of the effect size with those of common DNAm-based proxies for other phenotypes (e.g. for prenatal smoking and gestational age) is difficult given heterogeneity in study design, the methods used for calculating effect sizes, and differences in the overall strength of epigenetic correlations across phenotypes (52). With that in mind, larger effects

are reported in other work; for example, in one study a DNA methylation-based maternal smoking score was strongly associated with smoking status during pregnancy (53), while in another study two methylation-based gestational age scores were moderately to strongly correlated with clinic-measured gestational age (54). In terms of biological significance, small differences in DNAm could substantially affect cell function, and the function of its progeny, depending on the nature of the cell (55). However, the MPS-GC was derived from 24 CpG loci selected with elastic-net regression to minimize the number of features for better prediction, rather than based on epigenome-wide significance, and was tested as a cross-tissue marker in cord blood as opposed to the discovery tissues, which means differences in the MPS-GC score may not be biologically meaningful (52).

Interestingly, the MPS-GC was not associated with scores on the parental risk domain, which contained information on the presence of maternal anxiety and depression during pregnancy, alongside partner mental health and both mother and partner criminality and violence. By comparison, in earlier work, cumulative maternal anxiety and depression during pregnancy were associated with the MPS-GC in the PREDO cohort (26). However, measurement timing did differ from the PREDO cohort where symptoms of maternal anxiety and depression were measured at regular intervals within all three trimesters. In the current study, measurements were taken at fewer timepoints and only in the second trimester (ALSPAC, MoBa) and/or third trimester (ALSPAC, MoBa, GenR) for maternal anxiety and depression. Thus, the measurement of cumulative maternal anxiety and depression in the PREDO cohort was more precise, potentially increasing the ability to detect associations with the MPS-GC. Additionally, any effects of maternal mental health during pregnancy on the MPS-GC might have been masked by inclusion of other variables in the score in the current study. Whether the MPS-GC could provide a proxy specifically for maternal anxiety and depression during pregnancy requires specific testing with careful consideration of heterogeneity in measurement timing. This was beyond the scope of the current paper, which focused on establishing a marker for a broader cumulative measure of prenatal stress that was already known to reliably predict child psychiatric symptoms (4,40). While future

research could concentrate on the impact of specific stressors and their timing on DNA methylation at birth, research efforts should also continue towards developing reliable proxies for broader prenatal stress since risk factors typically co-occur (6) and are shown to have cumulative effects on psychiatric risk (4,7–9).

In terms of our second aim, we did not find evidence that the MPS-GC at birth associated with future child internalizing or externalizing symptoms, which are important early indicators of subsequent psychopathology (5). These findings are consistent with earlier work that reported no association between the MPS-GC derived from cord-blood and a combined measure of internalizing and externalizing symptoms (28). We also extended existing work by testing whether postnatal stress may act as a moderator in the association between the MPS-GC and child psychiatric symptoms. The null results did not support the theory that the MPS-GC, at least as measured in cord blood at birth, reflects a diathesis for later psychopathology by altering how individuals biologically respond to future stress. However, it should be noted that we did not directly measure stress reactivity in the current study. Moreover, children may also have been exposed to psychosocial factors during the postnatal period that could buffer any adverse effects of prenatal stress on child stress regulation (56). As such, it will be important in future to track the stability of the MPS-GC over time from birth, how it changes with exposure to various resilience factors, and how the longitudinal trajectories relate to child psychiatric symptoms.

In terms of our fourth and final aim, we did not find evidence to support a potential pathway between prenatal stress and child psychiatric symptoms via the MPS-GC at birth. On the one hand, this could indicate that broad reported measures of prenatal stress are not related to in-utero GC exposure. On the other hand, it could be that the MPS-GC does not specifically capture GC exposure in cord blood since it was initially developed based on DNAm derived from HPCs and adult whole blood. To our knowledge, it has not yet been tested whether the MPS-GC associates with maternal cortisol levels, as opposed to synthetic GCs which are more able to cross the placenta (57), or with fetal cortisol levels. Unfortunately, the measures were not available to do so in the current cohorts. The MPS-GC

would require such validation before stronger conclusions can be made regarding its biological role in linking the prenatal environment and child outcomes.

The study has several strengths. First, the large, pooled sample size increased statistical power to detect small effects and the use of cohort data from three different countries enabled us to test the robustness of the results. Second, the use of richly phenotyped cohorts containing largely corresponding exposure data across early development allowed us to generate comprehensive measures of prenatal and postnatal stress. These measures made it possible to study both the cumulative and independent effect of different stressors on the MPS-GC and downstream psychiatric symptoms. Indeed, we found strong and consistent associations of exposure to prenatal and postnatal stress with child internalizing and externalizing symptoms across cohorts, adding confidence to the validity of our measures (i.e., in capturing psychiatric risk). Third, the prospective design minimized recall bias and allowed us to test associations over a relatively long follow-up period in the same children. Finally, we derived the MPS-GC from cord blood, which precludes the possibility that DNAm was influenced by postnatal factors.

In terms of limitations, the trade-off of using a more comprehensive measure of prenatal stress was that we were unable to study if the timing and chronicity of different stressors associated with the MPS-GC. This may be particularly important to consider in future research given that the effects of early exposures on DNAm may differ depending on whether they occur during sensitive periods (58,59). Additionally, we relied on parent reports of child psychiatric symptoms, which may be biased (e.g., by parental psychopathology), and we did not have information on psychiatric treatment length to fully replicate earlier work. However, the parent-report instruments used in the current study have been shown to identify the presence and severity of psychiatric diagnoses (60,61). We also note that we did not consider child psychiatric genetic risk in the current study, however, to better understand pathways to psychiatric risk, it will also be important to consider how genetic predisposition interacts with prenatal stress to shape DNAm (15,62), especially given recent finding of interactive effects of cumulative prenatal stress with genotype on DNA methylation at birth in

the ALSPAC and GenR cohorts (63). Importantly, we did not have measures of GC exposure; as such, we were not able to test to what extent the MPS-GC in cord blood captures fetal GC-exposure, or whether fetal GC-exposure (irrespective of the MPS-GC) mediates the effect of prenatal stress on psychiatric symptoms. Finally, the study was based on a sample of European ancestry only, which matches the MPIP cohort that provides the weights for the MPS-GC but limits generalization of the results to other populations.

5. Conclusions

To conclude, we did not find strong evidence that the MPS-GC in cord blood at birth is a reliable 1) proxy of exposure to cumulative or individual prenatal stressors, and 2) predictor of risk for later child internalizing or externalizing symptoms. We also did not find support for postnatal stress as a moderator of MPS-GC associations with these symptoms. Finally, we found no evidence that the MPS-GC partially mediates associations of prenatal stress with child psychiatric symptoms. To better understand the role of DNAm in pathways from prenatal stress to psychiatric risk and to develop reliable biomarkers, future research could incorporate measures of genetic risk, consider the timing and chronicity of prenatal exposures, assess longitudinal change in the MPS-GC, and examine potential buffering effects of postnatal factors.

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CRediT authorship contribution statement.

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Tables

Table 1.

Sample Characteristics

	$GenR_{450K}$	$GenR_{EPIC}$	MoBa1	MoBa2	MoBa4	MoBa8	ALSPAC
Total *N*	1125	706	976	502	908	1100	769
Female, *n* (%)	575(51)	375(53)	461(47)	214(43)	462(51)	573(52)	393(51)
Array type	450k	EPIC	450k	450k	EPIC	EPIC	450k
Mean birthweight in grams *M(SD)*	3559(477.8)	3534(495.6)	3625(539.8)	3661(544.5)	3653(512.6)	3619(554.3)	3494(444.4)
Gestational age in weeks *M(SD)*	40.2(1.3)	40.1(1.3)	39.6(1.6)	39.5(1.6)	39.6(1.6)	39.5(1.9)	39.6(1.5)
Born < 37 weeks (%)	2.4	2.7	3.5	3.8	3.9	4.6	2.2
Maternal smoking during pregnancy, *n* (%)							
None	814(72)	505(72)	754(77)	399(80)	673(74)	846(77)	418(54)
Quit when pregnancy known	100(9)	59(8)	-	-	-	-	197(26)
Continued	119(11)	100(14)	128(13)	52(10)	69(8)	96(9)	78(10)
Missing	92(8)	42(6)	94(10)	51(10)	166(18)	158(14)	76(10)
Maternal education, *n* (%)							
Secondary education or lower	764(68)	224(32)	565(58)	302(60)	570(63)	725(66)	163(21)
Higher education	350(31)	450(64)	350(36)	168(34)	306(34)	326(29)	592(77)
Missing	11(1)	32(45)	61(6)	32(6)	32(3)	49(4)	14(2)

Table 2.

Pooled and Subcohort-Specific Associations of Cumulative Prenatal Stress, and the Individual Stress Domains, with the MPS-GC

	Pooled results					Subcohort results				
				95% CI		450k array				
	β	*SE*	*p*	Lower	Upper	GenR	ALSPAC	MoBa1	MoBa2	MoB
Model 1										
Cumulative prenatal stress	-0.01	0.01	.220	-0.02	0.005	- *	-	-*	+	-
Model 2										
Prenatal life events	-0.004	0.01	.498	-0.01	0.01	-	+	-	-	-
Prenatal interpersonal risk	-0.004	0.01	.508	-0.02	0.01	-	-	-*	+	+
Prenatal contextual risk	-0.01	0.01	.195	-0.03	0.01	+	+	-	+	+
Prenatal parental risk	0.004	0.01	.460	-0.01	0.01	-	+	+	-	-

Note. * p < .05, ** p < .01. *N* = 6086. MPS-GC = methylation profile score for fetal glucocorticoid exposure, β = standardized regression coefficient, CI = confidence interval. Covariates: child sex, maternal smoking in pregnancy, cell types, genetic principal components, sample plate / surrogate variables to adjust for technical variation. For the subcohort-specific results the direction of the coefficient is denoted by + (positive) or - (negative).

Table 3.

Pooled and Subcohort-Specific Associations of the MPS-GC, and the Interaction between the MPS-GC and Cumulative Postnatal Stress, with Child Internalizing and Externalizing Symptoms

	Pooled results				Subcohort results						
			95% CI		450k array				EPIC array		
Internalizing symptoms	β	*p*	Lower	Upper	GenR	ALSPAC	MoBa1	MoBa2	MoBa4	MoBa8	GenR
Model 1											
MPS-GC	-0.03	.397	-0.09	0.03	-	-	-	-	-	-	+
Model 2											

MPS-GC	-0.02	.553	-0.08	0.04	+	-	-	-		-	-	+
Postnatal stress[a.]	0.23	1.99E-16	0.18	0.29	+**	+**	+	+		+**	+**	+**
MPS-GC × postnatal stress	0.001	.881	-0.03	0.02	-	-	-	-		+	+	+
Externalizing symptoms												
Model 1												
MPS-GC	-0.01	.727	-0.09	0.06	+	-	-	-		-	+	-
Model 2												
MPS-GC	0.002	.957	-0.08	0.07	+	-	-	-		-	+	-
Postnatal stress	0.23	3.81E-14	0.17	0.29	+**	+**	+**	+**		+**	+**	+**
MPS-GC × postnatal stress	0.001	.948	-0.02	0.02	-	+	+	-		+	+	+

[a.] *Estimate adjusted for array type and age at outcome, QM = 12.28, p = .002*

* p < .05, ** p < .01. *N* = 6086. MPS-GC = methylation profile score for fetal glucocorticoid exposure, β = standardized regression coefficient, CI = 95% confidence interval, QM = estimate for the test of array type and age at outcome as moderators in the meta-analysis (shown only when significant). Covariates: child sex, maternal smoking in pregnancy, cell types, genetic principal components, sample plate / surrogate variables to adjust for technical variation. For the subcohort-specific results the direction of the coefficient is denoted by + (positive) or - (negative).

Table 4.

Pooled Total, Direct, and Indirect Effects of the Cumulative Prenatal Stress and the Individual Stress Domains on Child Internalizing and Externalizing Symptoms via the MPS-GC

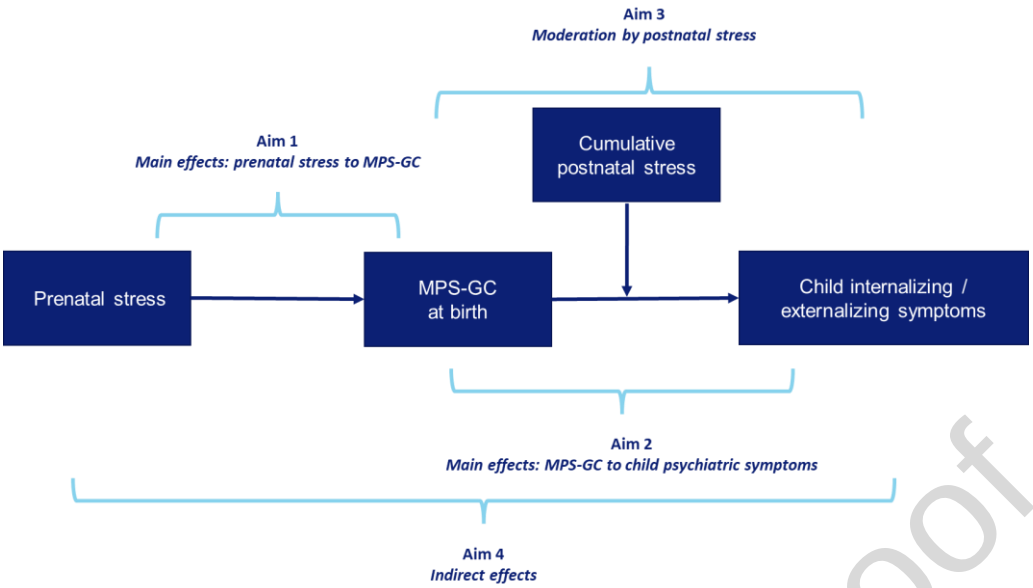
	Internalizing				Externalizing			
			95% CI				95% CI	
	Effect	*p*	Lower	Upper	β	*p*	Lower	Upper
Cumulative prenatal stress								
Total	1.99	.0004	0.89	3.09	1.66	.0001	0.80	2.51
Direct	2.00	.0004	0.89	3.10	1.67	.0001	0.80	2.54
Indirect	-0.001	.873	-0.01	0.01	-0.002	.633	-0.01	0.01
Life events								
Total	3.03	.010	0.74	5.31	1.57	.0001	0.77	2.36
Direct	3.03	.010	0.73	5.33	1.57	.0001	0.77	2.36
Indirect	0.003	.920	-0.05	0.05	0.003	.813	-0.02	0.03
Interpersonal risk								
Total	2.28	.014	0.46	4.10	1.10	.101	-0.22	2.41
Direct	2.29	.014	0.46	4.11	1.13	.096	-0.20	2.46
Indirect	-0.004	.837	-0.05	0.04	-0.01	.577	-0.04	0.02
Contextual risk								
Total	0.91	.0004	0.40	1.42	1.13	.003	0.39	1.87
Direct	0.91	.0004	0.41	1.41	1.13	.003	0.38	1.87
Indirect	0.001	.94	-0.02	0.02	5.61E-05	.996	-0.02	0.02
Parental risk								
Total	1.84	1.58E-06	1.09	2.59	1.53	.006	0.44	2.62
Direct	1.85	1.32E-06	1.10	2.60	1.54	.006	0.45	2.64
Indirect	-0.001	.934	-0.03	0.03	0.001	.94	-0.02	0.02

Note. * p < .05, ** p < .01. *N* = 6086. MPS-GC = methylation profile score for fetal glucocorticoid exposure, CI = 95% confidence interval. Covariates: child sex, maternal smoking in pregnancy, cell types, genetic principal components, sample plate / surrogate variables to adjust for technical variation.

Figures

Figure 1.

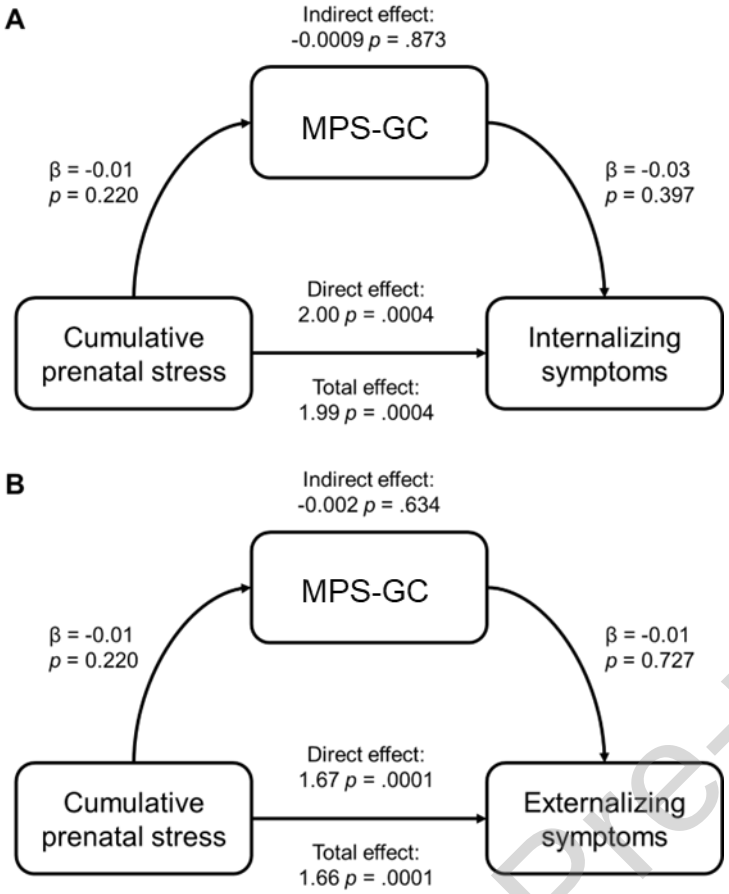
Overview of the study aims embedded in the theoretical framework



Note. The figure describes the study aims within the theoretical model whereby prenatal stress predicts a lower methylation profile score for fetal glucocorticoid exposure (MPS-GC) at birth (aim 1), which in turn increases risk for child psychiatric symptoms (aims 2), thereby partially mediating the effect of MPS-CG on psychiatric symptoms (aim 4). Additionally, given that a lower MPS-GC is posited to reflect a priming of the stress response, we predict that the pathway from the MPS-GC to child psychiatric symptoms would be accentuated in the presence of higher postnatal stress (aim 3). In the current study, prenatal stress is measured as a cumulative score, reflecting multiple types of stressors across pregnancy, and as stress-specific domains that aggregate specific types of stressors (i.e., stressful life events, interpersonal risk, contextual risk, and parental risk).

Figure 2.

Model Testing the Indirect Effects of Cumulative Prenatal Stress on Child Internalizing and Externalizing via the MPS-GC



Note. The models show the total and direct effects of cumulative prenatal stress during pregnancy on child internalizing symptoms (panel A) and externalizing symptoms (panel B), and the indirect effects via the methylation profile score for fetal glucocorticoid exposure (MPS-GC) measured in cord-blood at birth. Estimates were derived by pooling path model results from four cohorts using random-effects meta-analyses (N_{total} =6086). Covariates in the total and direct effects models were child sex, maternal smoking in pregnancy, cell types, genetic principal components, sample plate / surrogate variables to adjust for technical variation. In the direct effects model, the MPS-GC was also added as a covariate. The indirect effect represents the difference between the total effect and direct effect and, when significant, indicates partial mediation. Robust regression coefficients are shown for the pathway from cumulative prenatal stress to the MPS-GC, and from the MPS-GC to child internalizing and externalizing symptoms, which were measured at age 5, 9 or 10 years

depending on cohort (mixed-effects meta-analyses showed no moderating effects of outcome age).

Declaration of Interest

The authors declare no conflicts of interest. Below is list of the funding sources for each of the cohort studies and authors.

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Highlights

- In this study, we pooled longitudinal data from three prospective birth cohorts (N = 6086) to improve our understanding of biological markers and mechanisms linking cumulative prenatal stress and child mental health.

- Cumulative prenatal stress across pregnancy was associated with parent-reported child internalizing and externalizing symptoms.
- We found no association between cumulative prenatal stress and a methylation profile score intended to proxy in-utero glucocorticoid exposure (MPS-GC) at birth.
- The MPS-GC at birth did not associate prospectively with child psychiatric symptoms, and we identified no interaction with postnatal stress.
- There was no partial mediation by the MPS-GC of the association between cumulative prenatal stress and child psychiatric symptoms.
- We concluded that the MPS-GC is not a reliable biological marker for cumulative prenatal stress and child psychiatric symptoms.