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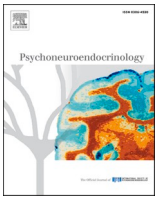

Negative associations between maternal prenatal hair cortisol and child socioemotional problems

Paula Mustonen [a,b,c,*], Susanna Kortesluoma [a,b], Noora M. Scheinin [a,b,d], Laura Perasto [a,b], Eeva-Leena Kataja [a,b,e], Katja Tervahartiala [a,b,f,g], Jetro J. Tuulari [a,b,h,i], Bárbara Coimbra [j], Alice S. Carter [k], Ana João Rodrigues [j,l], Nuno Sousa [j], E. Juulia Paavonen [a,b,m,n], Riikka Korja [a,b,e], Hasse Karlsson [a,b,o], Linnea Karlsson [a,b,p,q]

[a] FinnBrain Birth Cohort Study, Turku Brain and Mind Center, Department of Clinical Medicine, University of Turku, Medisiina A, Kiinamyllynkatu 10, 20520 Turku, Finland
[b] Centre for Population Health Research, University of Turku and Turku University Hospital, Medisiina A, Kiinamyllynkatu 10, 20520 Turku, Finland
[c] Department of Child Psychiatry, University of Turku and Turku University Hospital, Kiinamyllynkatu 4-8, 20520 Turku, Finland
[d] Department of Psychiatry, University of Turku and Satakunta Wellbeing Services County, Sairaalantie 3, 28500 Pori, Finland
[e] Department of Psychology, University of Turku and Turku University Hospital, Assistentinkatu 7, 20014 Turku, Finland
[f] Department of Psychology and Speech-Language Pathology, University of Turku, Assistentinkatu 7, 20014 Turku, Finland
[g] Department of Psychology, University of Jyväskylä, Seminaarinkatu 15, 40014 Jyväskylä, Finland
[h] Department of Psychiatry, University of Turku and Turku University Hospital, Kiinamyllynkatu 4-8, 20520 Turku, Finland
[i] Turku Collegium for Science, Medicine and Technology, University of Turku, 20520 Turku, Finland
[j] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, R. da Universidade, 4710-057 Braga, Portugal
[k] Department of Psychology, University of Massachusetts Boston, 100 Morrissey Blvd, Boston, MA 02125, USA
[l] Clinical Academic Center-Braga (2CA), Hospital de Braga, Lugar de Sete Fontes, S. Victor, 4710-243 Braga, Portugal
[m] Department of Public Health and Welfare, Finnish Institute for Health and Welfare, Mannerheimintie 166, 00271 Helsinki, Finland
[n] Pediatric Research Center, Child Psychiatry, University of Helsinki and Helsinki University Hospital, Haartmaninkatu 4, 00029 Helsinki, Finland
[o] Department of Psychiatry, University of Turku and Turku University Hospital, Kiinamyllynkatu 4-8, 20520 Turku, Finland
[p] Department of Pediatrics and Adolescent Medicine, University of Turku and Turku University Hospital, Savitehtaankatu 5, 20520 Turku, Finland
[q] Department of Clinical Medicine, Unit of Public Health, University of Turku and Turku University Hospital, Savitehtaankatu 5, 20520 Turku, Finland

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ABSTRACT

Maternal prenatal distress can participate in the programming of offspring development, in which exposure to altered maternal long-term cortisol levels as measured by hair cortisol concentrations (HCC) may contribute. Yet, studies investigating whether and how maternal prenatal HCC associates with problems in child socioemotional development are scarce. Furthermore, questions remain regarding the timing and potential sex-specificity of fetal exposure to altered cortisol levels and whether there are interactions with maternal prenatal distress, such as depressive symptoms. The subjects were drawn from those FinnBrain Birth Cohort families that had maternal reports of child socioemotional problems (the Brief Infant-Toddler Social and Emotional Assessment [BITSEA] at 2 years and/or the Strengths and Difficulties Questionnaire [SDQ] at 5 years) as follows: HCC1 population: maternal mid-pregnancy HCC measured at gestational week 24 with 5 cm segments to depict cortisol levels from the previous five months (n = 321); and HCC2 population: end-of-pregnancy HCC measured 1–3 days after childbirth (5 cm segment; n = 121). Stepwise regression models were utilized in the main analyses and a sensitivity analysis was performed to detect potential biases. Negative associations were observed between maternal HCC2 and child BITSEA Total Problems at 2 years but not with SDQ Total difficulties at 5 years, and neither problem score was associated with HCC1. In descriptive analyses, HCC2 was negatively associated with Internalizing problems at 2 years and SDQ Emotional problems at 5 years. A negative association was observed among 5-year-old girls between maternal HCC1 and SDQ Total Difficulties and the subscales of Conduct and Hyperactivity/inattentive problems. When interactions were also considered, inverse associations between HCC2 and BITSEA Internalizing and Dysregulation Problems were observed in subjects with elevated prenatal depressive symptoms. It was somewhat surprising that only negative associations were observed between

* Correspondence to: University of Turku FinnBrain Birth Cohort Study Medisiina A, Kiinamyllynkatu 10, 20520 Turku, Finland.
E-mail address: pmmust@utu.fi (P. Mustonen).

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maternal HCC and child socioemotional problems. However, there are previous observations of elevated end-of-pregnancy cortisol levels associating with better developmental outcomes. The magnitudes of the observed associations were, as expected, mainly modest. Future studies with a focus on the individual changes of maternal cortisol levels throughout pregnancy as well as studies assessing both maternal and child HPA axis functioning together with child socioemotional development are indicated.

1. Introduction

Developmental origins of health and disease (DOHaD) and fetal programming theories (Barker, 1986; O'Donnell and Meaney, 2017) postulate that child development is especially susceptible to environmental factors during the fetal and early postnatal periods because of high plasticity of the developing brain and neuroendocrine regulatory systems. One of the most studied prenatal exposures, maternal prenatal psychological distress (PPD) comprising experiences of stress, anxiety, and/or depression, is associated with long-lasting effects on offspring behavior and mental health and data on accompanied altered brain structure, function and connectivity is accumulating (Lautarescu et al., 2020; van den Bergh et al., 2020, 2018).

Altered fetal exposure to maternal glucocorticoids is considered a major mechanistic pathway underpinning detrimental offspring outcomes (Beijers et al., 2014; Lautarescu et al., 2020; Rakers et al., 2017). In a recent systematic review, elevated maternal prenatal cortisol levels (measured from different biological matrices) were reported to have stronger predictive value for adverse infant outcomes than maternal PPD (Caparros-Gonzalez et al., 2022). In typical pregnancy, maternal cortisol levels rise up to 2–3-fold to prepare both the mother and the fetus to parturition (Jung et al., 2011). While the reactivity of the hypothalamic-pituitary-adrenal (HPA) axis decreases during pregnancy, PPD may increase fetal exposure to cortisol by diminishing the activity of placental 11β-HSD2 in converting cortisol to inactive cortisone (Janssen et al., 2016). Thus, the timing matters; increased amnionic, salivary and hair cortisol levels during the first two trimesters, as opposed to the third, have more consistently been associated with adverse infant outcomes such as impaired neurodevelopment, increased affective problems and related neuroimaging findings (Buss et al., 2012; Caparros-Gonzalez et al., 2019; Davis and Sandman, 2010; Graham et al., 2019; Troller-Renfree et al., 2020). In contrast, elevated maternal salivary and hair cortisol levels during the last trimester and perinatal period reportedly associate also with advantageous neurocognitive and socioemotional outcomes (Caparros-Gonzalez et al., 2019; Davis and Sandman, 2010; Wright et al., 2019).

Hair cortisol concentrations (HCC) provide a means to quantify long-term cortisol levels. In a recent meta-analysis including 29 studies assessing perinatal psychological distress and HCC (Khoury et al., 2023), maternal PPD measures were associated with prenatal HCC but the overall association no longer reached significance when also early postnatal measures were included (Khoury et al., 2023). Correspondingly, more congruent associations between the two were observed in mid-pregnancy rather than perinatally in our systematic review (Mustonen et al., 2018) although they appeared to depict partially different phenomena.

Problems in early socioemotional development are predictive of later mental health problems (Essex et al., 2006; Nielsen et al., 2019). Socioemotional problems are typically divided into internalizing/emotional problems and externalizing/conduct problems, although some measures also include dysregulatory problems such as hyperactivity and inattention, or difficulties in social interaction (peer relationship problems or autism-like behaviors; Briggs-Gowan and Carter, 2006; Goodman, 1997).

Some longitudinal studies on associations between maternal perinatal HCC and offspring socioemotional outcomes exist, all of them with no findings of direct associations (Bosquet Enlow et al., 2017; Bruinhof et al., 2022; Galbally et al., 2023, 2022). However, interactions between maternal perinatal HCC and psychosocial factors (e.g. lifetime trauma exposure and parenting) have been associated with infant affectivity and childhood anxiety (Bosquet Enlow et al., 2017; Galbally et al., 2023, 2022). The mechanism of the interplay is theoretically consolidated as fetal exposure to maternal cortisol depends on the activity of 11β-HSD2 directly linked to maternal PPD (Janssen et al., 2016). Evidence exists of placental HPA-axis-related epigenetic changes mediating the association between maternal PPD and altered child hair glucocorticoids and reported aggression and anxiety symptoms at 3–4 years (Nomura et al., 2021). Thus, it seems essential to assess both of these parallel phenomena with partially overlapping mechanisms (Caparros-Gonzalez et al., 2022).

Associations between maternal prenatal HCC and a range of infant outcomes also frequently depend on or vary by infant sex (Bosquet Enlow et al., 2019; Cowell et al., 2021; Freedman et al., 2021; Stoye et al., 2020). Importantly, the developmental psychopathological outcome or phenotype presentation varies by child's biological sex: e.g., externalizing problems are more common in male offspring (Martel, 2013). To the best of our knowledge, the potential sex-specificity of socioemotional outcomes has not, thus far, been studied in a similar context.

This study aimed to assess prenatal maternal long-term cortisol levels as a biomarker for risk to offspring socioemotional development in early childhood. Associations between maternal HCC measured prenatally at gestational week (gwk) 24 and at 1–3 days after childbirth and maternal reports of child socioemotional problems at (i) 2 and (ii) 5 years were studied using total problem scores as primary outcomes. Secondary descriptive assessments included problem subscales of internalizing/emotional, externalizing/conduct, dysregulatory/hyperactivity and inattention, and peer relationship problems/autism-like behaviors. Furthermore, potential (iii) interactions between maternal prenatal depressive symptoms and HCC were assessed. Finally, we evaluated if (iv) the direct associations were sex-specific. We hypothesized that there are associations between maternal prenatal HCC and child's socioemotional problems and expected the associations to be especially pronounced with mid-pregnancy HCC. We further wanted to explore whether differential associations per the type of socioemotional problems would arise. Based on the complex pregnancy-related HPA-axis alterations and previous diverse findings on the associations between long-term cortisol levels and different symptoms, both positive and negative associations were considered possible. We hypothesized to see interactions between maternal symptoms and HCC on child socioemotional problems, and expected some of the observed associations to present differentially by child's sex.

2. Methods

2.1. Study population

The FinnBrain Birth Cohort Study is a population-based longitudinal pregnancy cohort investigating the significance of different environmental and genetic factors on child development (www.finnbrain.fi; Karlsson et al., 2018). The recruitment took place following a normal screening result of a routine ultrasound at gwk 12 between December 2011 and April 2015 at three sites in Turku, Finland and the Åland Islands, Finland and consecutive pregnant women giving their written informed consent were recruited. Inclusion criteria were sufficient knowledge of Finnish or Swedish and there were no exclusion criteria.

The reference numbers for approvals of the Ethics Committee of the Hospital District of Southwest Finland were ETMK57/180/2011 and ETMK12/180/2013. 3808 women were recruited in the whole cohort. The participation rate was 66% of those informed about the project. The attrition rate prenatally was 8.1% (Karlsson et al., 2018).

This study included two separate sub-populations of subjects with relevant measures available among cohort participants (see Fig. 1). HCC1 population (n = 321) comprised families of mothers donating a hair sample at a second trimester gwk 24 study visit (HCC1) and filling out questionnaires on child socio-emotional problems at 2 years and/or at 5 years. Consecutive Cohort subjects from March 2013 onwards were invited to the gwk 24 study visit and a hair sample was collected from those who consented and had adequate hair for sample collection. The participation rate for the study visit was 44% (n = 1671) of the Cohort mothers and 57% (n = 953) of the participants donated a hair sample (Karlsson et al., 2018). The final number of HCC1 samples considered for this study was reduced to n = 474 due to various reasons (see Fig. 1). HCC2 population (n = 121) comprised women donating hair samples at the delivery ward 1–3 days after giving birth (HCC2; samples taken n = 270, considered for this study n = 230, for details see Fig. 1) and filling out questionnaires on child socio-emotional problems at two years and/or at five years (Fig. 1). The collection of HCC2 hair samples took place between December 2014 and December 2015. 65 women were included in both HCC1 and HCC2 populations, thus limiting the potential to longitudinally assess the effects of fetal cortisol exposure and leading to the decision to include two separate subpopulations.

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies v4 was followed in reporting.

2.2. Hair cortisol concentration

Maternal hair samples were collected at gwk 24 (HCC1, n = 953) and 1–3 days after delivery (HCC2, n = 270). Hair samples were cut from a standardized area of the posterior vertex region of the head most proximal to the scalp. A minimum of 5 mg hair and at least 5 cm long sample of each hair was cut as close to the scalp as possible. A 5 cm segment was used to depict the accumulated systemic cortisol levels of the past 5 months of pregnancy. Albeit many prenatal HCC studies utilize 3 cm segments to assess specific trimesters (D'Anna-Hernandez et al., 2011), a slightly longer segment length was selected to ensure adequate hair weight for reliable analyses for more samples also enabling the coverage of the entire duration of pregnancy by two samples. Hair cortisol extraction was performed in the University of Minho, Portugal according to a protocol adapted from Davenport (Davenport et al., 2006; Mustonen et al., 2019): After washing with isopropanol three times, hair segments were finely minced using surgical scissors and 5–15 mg of hair was transferred to a cryovial. 1.5 ml of methanol was

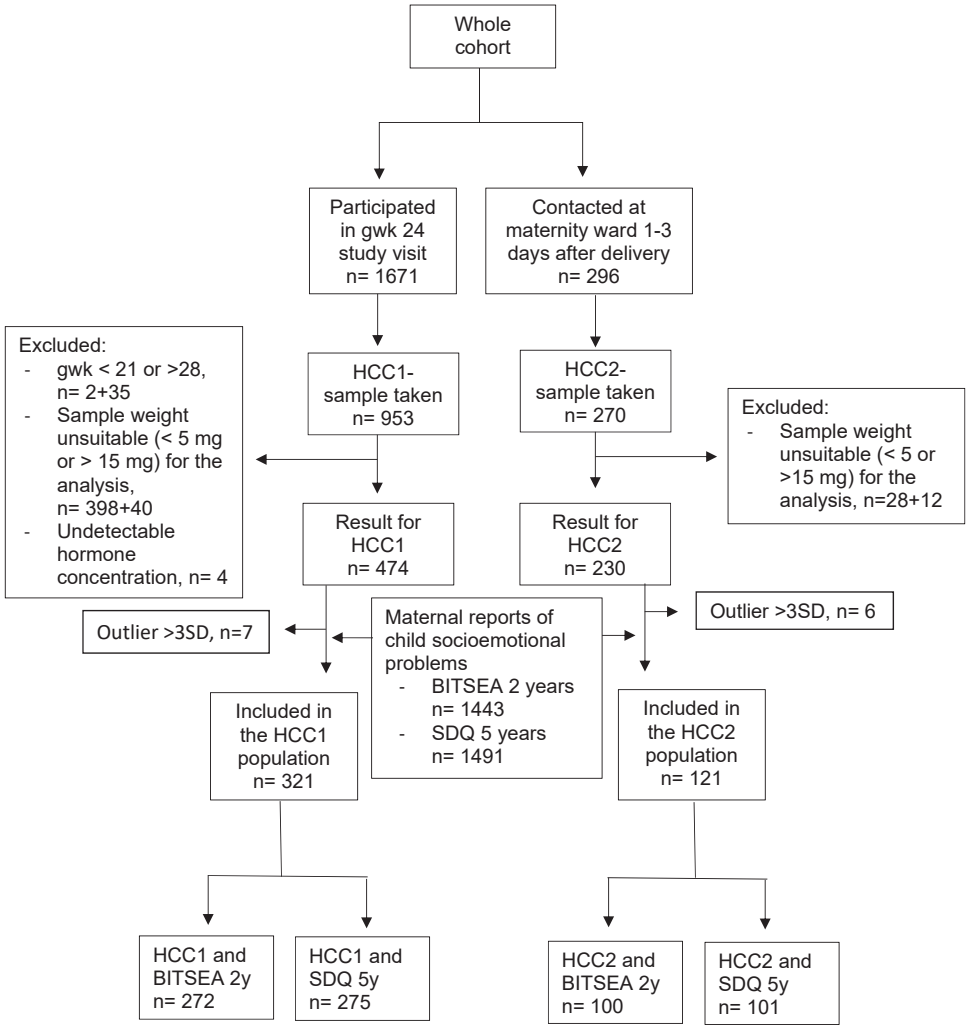

Fig. 1. Flow chart of the study design. Notes of abbreviations: gwk = gestational week; HCC = hair cortisol concentrations; HCC1 = HCC measured at gwk 24; HCC2 = HCC measured 1–3 days after delivery; BITSEA = Brief Infant-Toddler Social and Emotional Assessment; SDQ = Strengths and Difficulties Questionnaire; and y = years.

added to each sample and incubated at 55ºC for 24 h. After centrifuging, the supernatant was transferred to a new vial and methanol was evaporated at 60ºC under a constant stream of nitrogen until samples were dried completely. Finally, 0.15 ml of phosphate buffer was added. 50 µl of each sample was measured in an ELISA kit following the manufacturer's procedure (IBL International Cortisol Saliva ELISA).

After exclusions (see Fig. 1), the total amounts HCC samples considered to be included in the final analyses before examining for outliers were n = 474 for HCC1 and n = 230 for HCC2.

2.3. Socioemotional problems of children

The assessment of child socioemotional problems in the whole cohort was based on parent-rated questionnaires. The research questionnaires or an online link were sent to participants.

2.3.1. BITSEA at 2 years

At 2 years, the BITSEA –screening instrument (Brief Infant-Toddler Social and Emotional Assessment; Briggs-Gowan and Carter, 2006) assessing traits in child socio-emotional behavior was utilized. BITSEA includes subscales Externalizing problems (difficulties in activity/impulsivity, aggression/defiance, and peer aggression), Internalizing problems (for instance fearfulness, worry, nervousness, and distress upon separation), Dysregulation problems (difficulties in regulating negative emotionality, sleep, or eating, and sensory sensitivities), Competence items (socioemotional competencies), Autism spectrum disorder items (behaviors and deficits often observed related to autism spectrum disorders), and Red flag items (clinically relevant items, which may endanger the child, for instance "runs away in public places", "hurts self in purpose" and "gags and chokes on food") with 42 different items to be rated as 0 = Not true / Rarely, 1 = Somewhat true / Sometimes, or 2 = Very true / Often. Some of the items belong to more than one subscale. The sums of all problem-related subscales were calculated (ranges from 0–12 to 0–24) with missing values imputed as the mean of other items in the same subscale (the maximum of missing values depending on the number of items included in the subscale). In this study, the focus was on the problems in socio-emotional development and thus, the Competence items subscale was not included as it depicts a partly different phenomenon. The Red flag items subscale is more targeted for clinical use and its questions are versatile. Therefore, this subscale was excluded from the study. A Problem Total score (range 0–62) was calculated as a sum of all problem items, i.e., the 31 problem-related items not related to the Competence items -subscale and similarly, the missing values were imputed as the mean of other included items. The total and individual problem sum scores were included in the statistical models as continuous variables. Alphas for subscales and Problem Total score were in between 0.469 and 0.665.

2.3.2. SDQ at 5 years

At 5 years, a widely used 25-item behavioral screening instrument for 4–17 year old children, The Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997), was utilized. The SDQ includes 25 items to be rated on the scale of 0 = Not true, 1 = Somewhat true, and 2 = Certainly true. The items are divided to five-item subscales (ranges 0–10); Emotional symptoms (similar to Internalizing problems in BITSEA), Conduct problems (mostly similar to Externalizing problems with a focus slightly more shifted to conduct disorder symptoms), Hyperactivity/inattention problems (dysregulation of attention, activity, and impulsivity), Peer relationship problems (for instance, prefers adults or solitude, and is not well liked by peers), and Prosocial behavior (socioemotional competencies). All subscale sums were calculated and missing values were imputed as the mean of other items in the same subscale with a maximum of two missing items. A Total Difficulties score was calculated as the sum of four beforementioned problem item sums (range 0–40). Similarly as in BITSEA, the Prosocial behavior subscale was left out of the study as we focused on socioemotional problems. The total and individual problem sum scores were utilized as continuous variables with alphas ranging from 0.532 to 0.832.

2.4. Maternal depressive symptoms

As a measure of maternal PPD, we included maternal prenatal depressive symptoms based on our previous findings (Mustonen et al., 2019). Depressive symptoms were assessed at gwks 14, 24, and 34 with Edinburgh Postnatal Depressive Scale (EPDS; Cox et al., 1987) which covers symptoms during the last week with ten items to be rated with a four-step verbal scale from 0–3 (range for total sum scores 0–40, alphas 0.823 - 0.834). For this study, a sum of two consecutive sum scores was calculated separately early-to mid-pregnancy (gwks 14 and 24) and mid- to end-of-pregnancy (gwks 24 and 34) to be comparable with the five-month-period assessed by hair cortisol measurements. In regards to missing data in one assessment (n = 6–15; 2.5–8% varying between study questions), the missing value was imputed with the median sum score of all cohort subjects at that time point. Postnatal depressive symptoms were assessed with EPDS at child ages of 2 and 5 years.

2.5. Covariates

Data on potential confounding factors were available from the Cohort questionnaires at gwks 14 and 34 and upon hair sample taking (Table 1) and/or the Medical Birth Register administered by the Finnish Institute of Health and Welfare (Table 1). Register data on pregnancy and birth complications (yes/no) included common maternal risk factors and complications such as gestational diabetes or pre-eclampsia and neonatal health outcomes. All subjects were of Caucasian origin thus not indicating significant differences in hair steroids based on differences in genetic background.

The selection of covariates was based on known determinants for HCC (age, sex, BMI, waist-to-hip ratio, systolic blood pressure, and frequency of hair washing; Stalder et al., 2017) and prenatal factors associated with child neurodevelopment as measured by brain metrics (maternal mental health, SSRI or substance use, BMI, infections and inflammatory states, family SES and ethnicity, and child sex, gestational age at birth and birth weight; Pulli et al., 2019).

These HCC- or child neurodevelopment -related factors were evaluated by directed acyclic graph (DAG) to determine the necessary covariates by using the DAGitty web application (See Supplementary Fig. S1; dagitty.net; Textor et al., 2016). The potential confounders included in the final models, i.e. factors that were associated with both the exposures and the outcomes, were child's sex assigned at birth and maternal pre-pregnancy BMI, education, prenatal depressive symptoms, and prenatal SSRI use. The number of subjects reporting the use of prenatal SSRI was, however, too low for reliable statistical testing (n = 12 for HCC1 and n = 0 for HCC2). In exploratory analyses for the HCC1 population, subjects with SSRI use did not differ from others in terms of HCC or child socioemotional symptoms (see Supplementary Fig. S2 and Table S1) and excluding these subjects did not significantly alter the results. Thus, these subjects were not excluded from the final analyses.

Associations between potential covariates and the exposures and the outcomes were also examined within our data (see Table S1). As there were preterm births and significantly obese individuals included in the study populations, these factors were also assessed categorically to observe potential nonlinear associations related to high-risk individuals. This examination identified maternal BMI, child's sex and maternal prenatal EPDS as essential confounders, thus consolidating the literature-driven covariate selection (Table S1).

Although maternal postnatal depressive symptoms were not suggested to be included in the final models by the DAG (Fig. S1), they were considered to potentially cause maternal reporting bias and to affect child socioemotional problems. Thus, maternal postnatal depressive symptoms (EPDS measured simultaneously with child socioemotional problems, i.e., at 2 and 5 years, respectively) were decided to be

Table 1
Population characteristics of the FinnBrain Birth Cohort and the subpopulations included in this study (HCC1 and HCC2 populations), and attrition analysis.

	Whole cohort		HCC1 population		HCC2 population	
	mean (SD, range) / n (%)	n	mean (SD, range) / n (%)	n	mean (SD, range) / n (%)	n
Maternal age[a,b]	**30.2 (4.7, 17-46)**	**3839**	**31.4 (4.1, 22-41)** * **	**321**	**31.4 (4.0, 23-42)** * *	121
Maternal education[a]			* **		* *	
-high school/ vocational (<12 years)	**1173 (37.8)**		**63 (20.5)**		**27 (23.1)**	
-polytechnics	**899 (29.0)**		**109 (35.5)**		**34 (29.1)**	
-university	**1031 (33.2)**		**135 (44.0)**		**56 (47.9)**	
Maternal BMI before pregnancy (kg/m^2)[a,b]	24.6 (4.9, 15.6-60.6)	3686	24.6 (4.6, 17.7-45.6)	317	24.3 (4.8, 17.2-44.1)	119
Marital status[a]						
-married/domestic partnership	2805 (93.0)		286 (94.1)		109 (93.9)	
-others	210 (7.0)		18 (5.9)		6 (6.1)	
Parity[a]			* **		* **	
-nullipara	**1594 (51.5)**		**188 (61.4)**		**81 (69.6)**	
-multipara	**1501 (48.5)**		**118 (38.6)**		**37 (31.4)**	
Ethnicity[a]						
-Finnish	2993 (96.8)		301 (97.7)		116 (99.1)	
-other	98 (3.2)		7 (2.3)		1 (0.9)	
Gestational weeks at delivery[a]	39.7 (1.8, 24.1-42.6)	3764	39.8 (1.6, 30.6-42.3)	321	39.9 (1.4, 34.4-42.3)	121
Child's sex assigned at birth[a,b]						
-boy	1965 (52.2)		156 (48.6)		63 (52.1)	
-girl	1797 (47.8)		165 (51.4)		58 (47.9)	
Maternal smoking during pregnancy[a,b]			* **		* **	
-no	**3201 (86.9)**		**296 (93.7)**		**113 (95.0)**	
-only 1st trimester	**275 (7.5)**		**17 (5.4)**		**6 (5.9)**	
-after 1st trimester	**207 (5.6)**		**3 (0.9)**		**0 (0)**	
Maternal use on alcohol during pregnancy[a]						
-no	2416 (79.4)		231 (76.2)		89 (79.5)	
-yes, before knowing of pregnancy	454 (14.9)		51 (16.8)		19 (17.0)	
-yes, after knowing of pregnancy	157 (5.2)		19 (6.3)		4 (3.6)	
Maternal use of SSRI during pregnancy[a]						
- gwk14 - no	2959 (96.7)		290 (96.0)			
- yes	100 (3.3)		12 (4.0)			
- gwk34 - no	2498 (97.2)				114 (100)	
- yes	73 (2.8)				0 (0)	
Maternal use of glucocorticoids during pregnancy[a]						
- gwk14 - no	2939 (96.1)		290 (96.0)			
- local	117 (3.8)		11 (3.6)			
- systemic	3 (0.1)		1 (0.3)			
- gwk34 - no	2471 (96.1)				110 (96.5)	
- local	92 (3.6)				3 (2.6)	
- systemic	8 (0.3)				1 (0.9)	
- synthetic glucocorticoids when at risk of preterm birth[b]						
- no	3557 (96.3)		306 (95.3)		115 (96.6)	
- yes	135 (3.7)		15 (4.7)		4 (3.4)	
Pregnancy or birth complications[b]						
- no	2719 (70.8)		226 (69.5)		92 (76.0)	
- yes	1120 (29.2)		99 (30.5)		29 (26.0)	
Maternal EPDS sum[a]						
- gwk14	5.2 (4.0, 0-27)	3051	4.8 (3.8, 0-18)	308	4.4 (3.9, 0-15)	113
- gwk24	5.0 (4.1, 0-25)	2770	4.6 (4.0, 0-25)	320	4.3 (3.6, 0-18)	117
- gwk34	4.9 (4.1, 0-26)	2602	4.7 (4.3, 0-19)	314	4.3 (3.8, 0-19)	116
- child's age of 2 years	4.6 (4.3, 0-27)	1369	4.5 (4.4, 0-21)	264	4.9 (4.0, 0-16)	96
- child's age of 5 years	5.1 (4.6, 0-26)	1482	5.0 (4.4, 0.20)	278	5.1 (4.5, 0-20)	100
Child BITSEA Problem Total score at 2 years[a]	7.5 (4.3, 0-34)	1437	7.8 (4.1, 0-22)	272	7.8 (4.2, 0-22)	99
Child SDQ Total Difficulties score at 5 years[a]	8.9 (5.0, 0-29)	1490	8.7 (5.0, 0-25)	275	8.1 (4.8, 1-24)	101
Maternal hair washing[a]						
-HCC1 - 4 or more times a week	156 (28.3)		79 (25.2)			
- less than 4 times a week	395 (71.7)		234 (74.8)			
-HCC2 - 4 or more times a week	61 (25.3)				31 (25.6)	
- less than 4 times a week	180 (74.7)				90 (74.4)	
Season of which hair sample was taken[a]						
-HCC1 - spring	161 (29.2)		107 (33.3)			
- summer	145 (26.3)		88 (27.4)			
- autumn	147 (26.7)		74 (23.1)			
- winter	98 (17.8)		52 (16.2)			
-HCC2 - spring	65 (26.2)				36 (30.0)	
- summer	59 (23.8)				24 (20.0)	
- autumn	23 (9.3)				19 (15.8)	
- winter	101 (40.7)				41 (34.2)	
Hair cortisol concentration HCC1 (pg/mg)						

(continued on next page)

Table 1 (*continued*)

	Whole cohort		HCC1 population		HCC2 population	
- outliers included	28.7 (88.2, 0.3-1174)	474	28.4 (86.1, 0.3-1174)	325		
- outliers excluded	19.8 (34.6, 0.3-270)	467	20.3 (33.4, 0.3-270)	321		
Hair cortisol concentration HCC2 (pg/mg)						
- outliers included	27.6 (48.3, 1.0-357)	230			25.1 (58.9, 1.0-357)	124
- outliers excluded	20.5 (19.9, 1.0-117)	224			18.0 (17.0, 1.0-117)	121

Abbreviations: HCC1 population = subjects with hair cortisol concentrations measured at gestational week (gwk) 24 and a relevant child outcome measure; HCC2 population = subjects with hair cortisol concentrations measured 1-3 days after delivery and a relevant child outcome measure; BMI = body mass index; SSRI = selective serotonin reuptake inhibitors; gwk = gestational weeks; BITSEA = Brief Infant-Toddler Social and Emotional Assessment; SDQ = Strengths and Difficulties Questionnaire;; EPDS = Edinburgh Postnatal Depressive Scale. Significant differences between the whole cohort and either of the two subpopulations are bolded and marked with asterisks (*, when $p < 0.05$, **, when $p < 0.01$, and ***, when $p < 0.001$; t-test for equality of means, two-sided p, equal variances assumed/not assumed based on Levene's test, or Pearson Chi-Square)

[a] Data gathered from self-report questionnaires

[b] Data gathered from Medical Birth Register

included as a supplementary sensitivity analysis.

2.6. Statistical analyses

Because of the skewed distribution of HCC, natural logarithm (ln) conversion was utilized for all HCC values. The raw values are reported in Table 1, however all statistical tests (including the assessment of equality of means reported in Table 1) were run for ln-converted values and figures only include ln-converted values. Extreme values of HCC are rather common and can disproportionately influence the results, hence careful exploration for outlier values and reporting the data cleaning practices was deemed necessary (Marceau et al., 2020; Stalder et al., 2016). Here, the data were first examined exploratively and the results were more consistent and the 95% confidence intervals of estimates narrower when excluding > 3 SD HCC values. Thus, HCC values > 3 SD were decided to be excluded. This meant excluding seven subjects for HCC1, four for whom child questionnaire data was also available, and six for HCC2 (three with child data). Distributions of other continuous variables and assumptions of linear models were assessed visually and the role of potential outliers was examined. As there was significant attrition from the whole cohort to the final study populations, attrition analyses were performed to understand potential selection biases (Table 1). Based on a power analysis for linear models, effect sizes from 0.04 to 0.15 upwards could be discovered in our sample sizes.

List of the performed regression models.

STEP 1: child symptom ~ intercept + HCC + child sex + maternal BMI + maternal education (low, mid, high).

STEP 2: child symptom ~ intercept + HCC + child sex + maternal BMI + maternal education + maternal prenatal EPDS.

STEP 3: child symptom ~ intercept + HCC + maternal prenatal EPDS + HCC*maternal prenatal EPDS + child sex + maternal BMI + maternal education.

STEP 4: Step 2 (without child sex) performed separately for boys and girls.

STEP 5, sensitivity analysis: child symptom ~ intercept + HCC + child sex + maternal BMI + maternal education + maternal prenatal EPDS + maternal postnatal EPDS.

Stepwise linear regression analyses were performed separately for HCC1 and HCC2 to assess the associations between maternal prenatal HCC and child outcomes. The covariates included in Step 1 were child's sex assigned at birth, maternal pre-pregnancy BMI and maternal education. As Step 2, maternal prenatal depressive symptoms were added in the models. Next (Step 3), we assessed the role of interactions between maternal prenatal HCC and maternal prenatal depressive symptoms in explaining the child outcomes. For significant interactions, a simple slope analysis was conducted to elucidate the relations and to visualize them by utilizing the interactions -function's default values for low (−1 SD, mean sum score= 1.95), intermediate (0 SD, mean sum score= 8.29), and elevated (+1 SD, mean sum score= 14.63) for the constant prenatal EPDS values, however categorization was not performed. To further account for potential sex-specificity (Step 4), the models were also analysed separately for boys and girls. A sensitivity analysis (Step 5) was conducted to detect potential maternal reporting bias due to own depressive mood.

P-values (two-tailed) smaller than 0.05 were interpreted as statistically significant. The beta coefficients (B) and 95% confidence intervals (CI) for the estimates and adjusted R^2 for the models were calculated. The analyses were performed using R (4.0.5, 2021; R Core Team, 2022) apart from the attrition analyses, which were performed using IBM SPSS Statistics 28.

3. Results

3.1. Population characteristics and attrition analysis

Detailed sociodemographic data, maternal depressive symptoms, child socioemotional problems, maternal health-related factors, and hair-related characteristics of all cohort subjects as well as the subjects included in the study populations are described in Table 1. Attrition was related to younger age, lower educational level, multiparity and more smoking during pregnancy (Table 1). Maternal depressive symptoms, child socioemotional problems or HCC of the subjects of the study populations did not differ from the whole cohort participants.

3.2. Associations between prenatal HCC and child socio-emotional problems at 2 years

According to our hypothesis, there was a negative association between the BITSEA Problem Total score and HCC2 in Step 2 after inclusion of the maternal depressive symptoms (B= −0.85, p = .12 in Step 1 and B= −1.06, p = .04 in Step 2, see Table 2, Fig. 2A for Step 2 results and Supplementary Table S2 for all statistics of the regression models). The association between prenatal depressive symptoms and BITSEA Problem Total score was positive (B= 0.22, p = .001). Contrary to our hypothesis, HCC1 was not associated with BITSEA Problem Total score (Table 2, Fig. 2A, Table S2A).

In descriptive analyses, there were no significant associations between HCC1 and any of the BITSEA problem subscales in the Step 1 or Step 2 models (Fig. 2A, and Table S2A).

For HCC2, the type of socioemotional problem was significant as a negative association between maternal HCC2 and BITSEA Internalizing problems at 2 years was observed in Steps 1 and 2 (B= −0.75, p = .006 and B= −0.83, p = .002, respectively; Fig. 2A, Table S2B), while none of the other problem subscales of BITSEA were associated with HCC2 (Fig. 2A, Table S2B).

3.3. Associations between prenatal HCC and child socio-emotional problems at 5 years

Neither HCC1 nor HCC2 were associated to the Total difficulties score at 5 years in Steps 1 nor 2 (Table 2, Fig. 2B, Tables S2C and S2D),

Table 2
The associations between HCC and child socioemotional problems at 2 years (BITSEA) and at 5 years (SDQ).

		HCC1		HCC2		
Outcome	Variable	B	p	B		p
BITSEA Problem Total score	Step 1	0.068	0.80	-0.85		0.12
	Step 2	-0.087	0.74	-1.06	*	0.043
	Step 3 (for EPDSxHCC interaction)	0.05	0.26	-0.11		0.13
	Step 4 (boys)	-0.23	0.59	-1.07		0.070
	Step 4 (girls)	0.053	0.88	-1.15		0.23
	Step 5	0.076	0.78	-0.86		0.11
SDQ Total Difficulties score	Step 1	-0.25	0.42	0.10		0.86
	Step 2	-0.37	0.19	-0.14		0.80
	Step 3 (for EPDSxHCC interaction)	-0.017	0.71	-0.017		0.85
	Step 4 (boys)	0.16	0.73	0.16		0.83
	Step 4 (girls)	-0.70	* 0.049	-0.75		0.36
	Step 5	-0.25	0.37	0.18		0.74

Abbreviations: HCC = hair cortisol concentration; HCC1 = HCC measured at gestational week 24; HCC2 = HCC measured 1-3 days after delivery; B = beta coefficient; BITSEA = Brief Infant-Toddler Social and Emotional Assessment; SDQ = Strenghts and Difficulties Questionnaire; EPDS = Edinburgh Postnatal Depressive Scale. The estimates of significant associations are marked with asterisks (* for <0.05, ** for <0.01, *** for <0.001)

which was in contrast to our main hypothesis.

HCC1 was not associated with the SDQ subscales (Fig. 2B, Table S2C). There was, however, a negative association between HCC2 and SDQ Emotional symptoms (B= −0.38, =.03) and the association remained in Step 2 including maternal depressive symptoms (B= −0.43, p = .02, see Fig. 2B and Table S2D) supporting our secondary hypothesis. HCC2 did not associate with the other SDQ subscales (Fig. 2B, Table S1D).

3.4. Interactions between maternal prenatal depressive symptoms and maternal prenatal HCC

In Step 3, contradictory to our third hypothesis, the interaction between HCC1 nor HCC2 and maternal prenatal depressive symptoms associated with none of the addressed main outcomes (Table 2, Fig. 3A). However, the interaction of HCC2 and maternal prenatal depressive symptoms was negatively associated with BITSEA Internalizing problems (B= −0.09, p = .01) and BITSEA Dysregulation problems (B= −0.068, p = .031) at 2 years (Table S2B). No interactions were observed related to HCC1 or any of the 5-year SDQ scales (Tables S2A, S2C and S2D).

In the simple slope analysis, no association between HCC2 and BITSEA Internalizing symptoms was observed for subjects with low levels of depressive symptoms (B= −0.24, p = .48), but significant associations between HCC2 and Internalizing symptoms occurred in subjects with intermediate (B= −0.84, p = .001) and elevated (B= −1.43, p < .0001) levels of prenatal depressive symptoms (Fig. 3B). For BITSEA Dysregulation problems, the association between HCC2 and child symptoms occurred for mothers with elevated level of depressive symptoms (B= −0.76, p = .01, Fig. 3C). A similar pattern seemed to occur with BITSEA Problem Total score (Fig. 3A) albeit the association for the interaction failed to reach statistical significance (B= −0.11, p = .13).

3.5. Sex-specific associations

When addressing the above-mentioned study questions separately for boys and girls (Step 4), a novel sex-specific negative association was observed between HCC1 and SDQ Total difficulties score in 5-year-old girls which supported our fourth hypothesis of potential sex-specific findings (B= −0.70, p = .05, Table 2). Other main outcomes were not associated with HCC1 or HCC2 in analyses stratified by sex (Table 2). In descriptive analyses, both Conduct (B= −0.32, p = .03) and Hyperactivity/inattentive problem subscales (B= −0.38, p = .03) were negatively associated with maternal HCC1 in girls (Fig. 4).

The other descriptive analyses indicated that the negative association between HCC2 and BITSEA Internalizing problems at 2 years was observed in boys (B= −0.38, p = .03) and the negative association between HCC2 and SDQ Emotional symptoms at 5 years in girls (B=

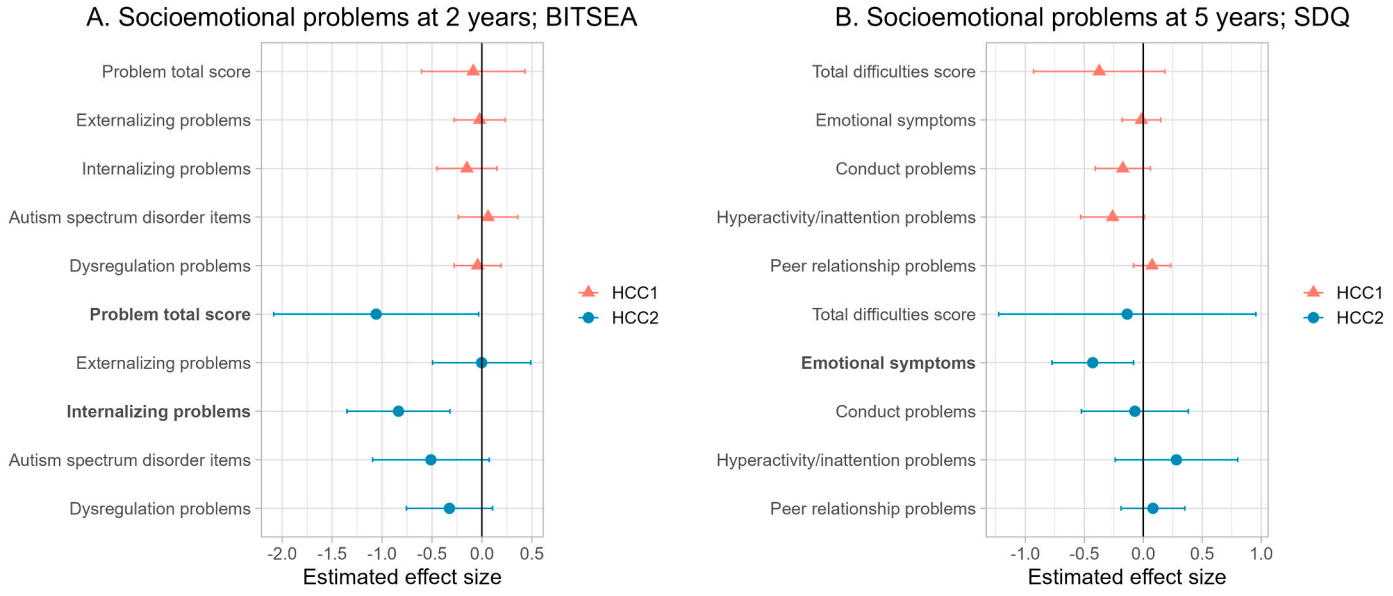

Fig. 2. Forest plots of associations between prenatal HCC and child socio-emotional problems at 2 years (2A) and 5 years (2B) based on Step 2 regression models. Notes of abbreviations: HCC = hair cortisol concentrations; HCC1 = HCC measured at gwk 24; HCC2 = HCC measured 1–3 days after delivery; BITSEA = Brief Infant-Toddler Social and Emotional Assessment; and SDQ = Strengths and Difficulties Questionnaire.

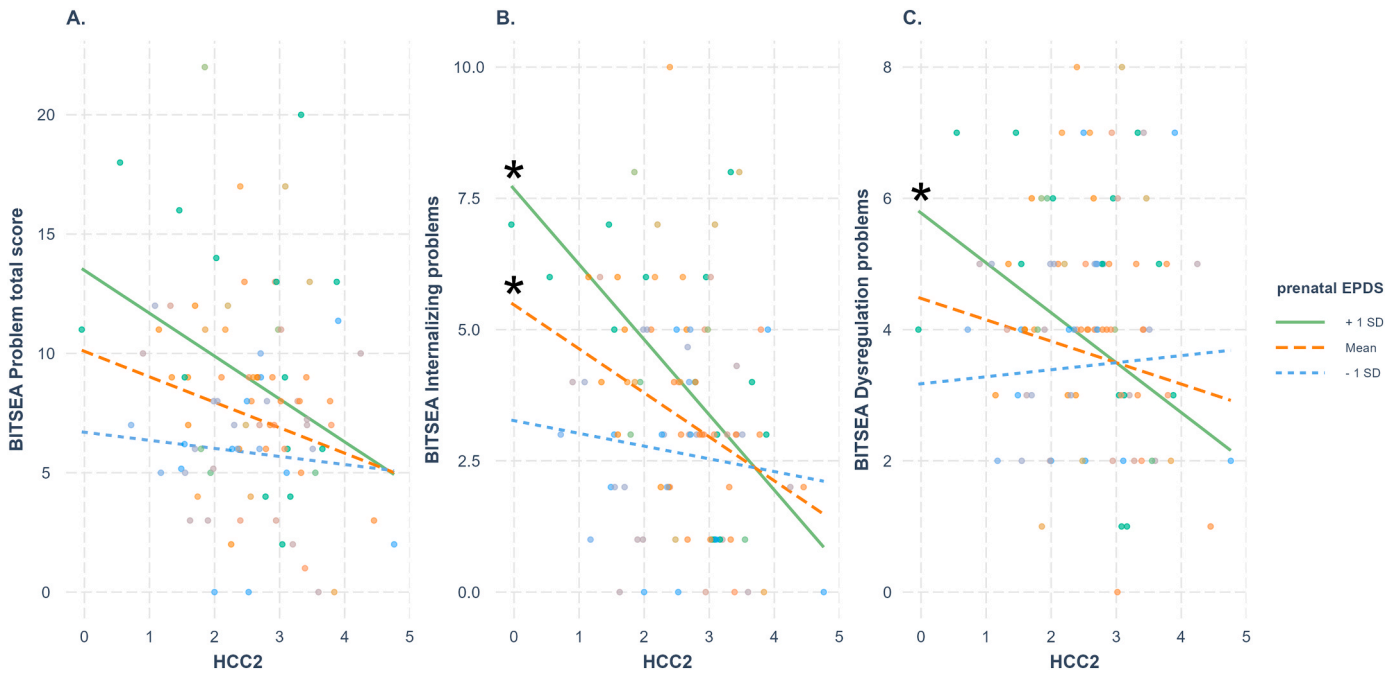

Fig. 3. Scatter plots with simple slope analysis curves depicting interactions between maternal prenatal depressive symptoms and HCC for observed interactions. Significant correlation curves are marked with asterisks (*).Notes of abbreviations: HCC = hair cortisol concentrations; HCC2 = HCC measured 1–3 days after delivery; BITSEA = Brief Infant-Toddler Social and Emotional Assessment; and SDQ = Strengths and Difficulties Questionnaire.

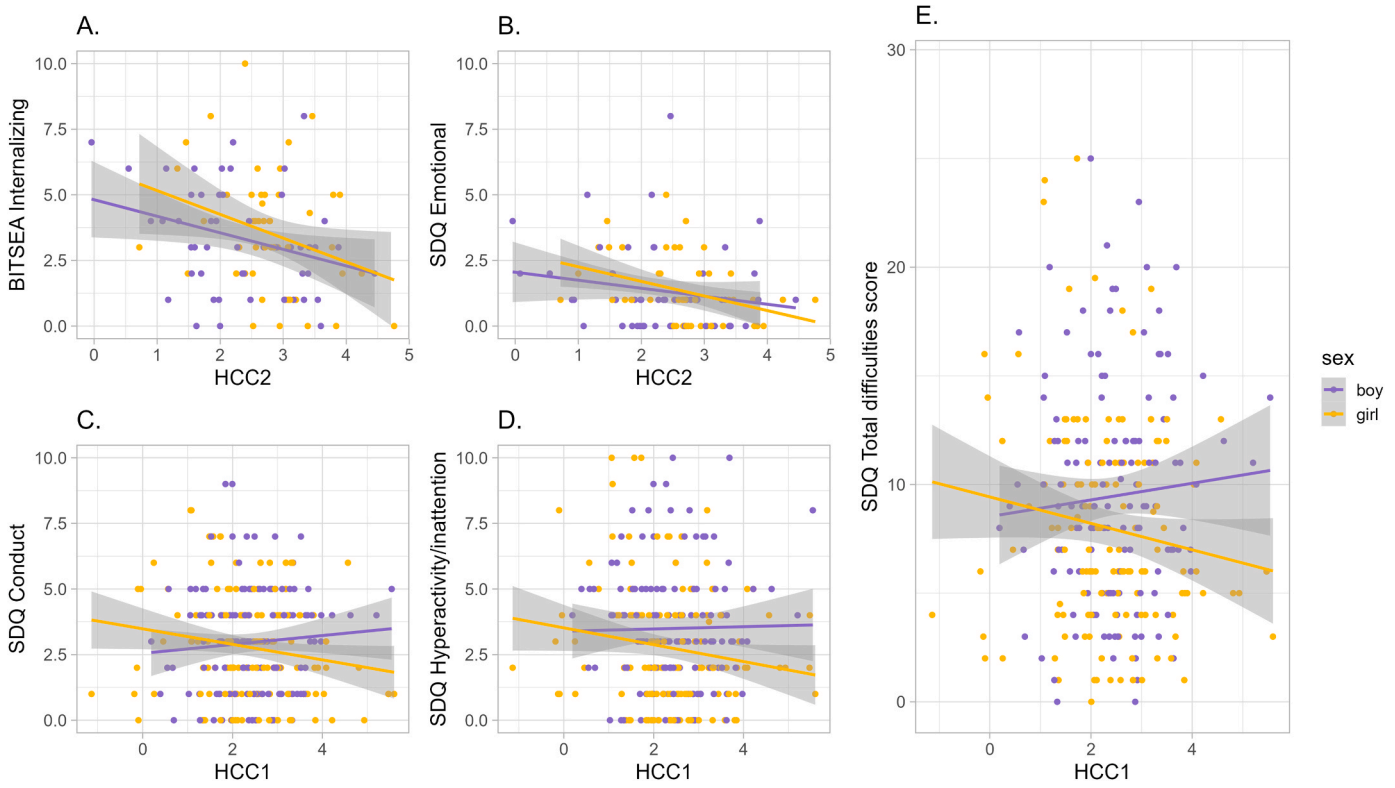

Fig. 4. Scatter plots of sex-specific associations between prenatal HCC and child socioemotional problems.Notes of abbreviations: HCC = hair cortisol concentrations; HCC1 = HCC measured at gwk 24; HCC2 = HCC measured 1–3 days after delivery; BITSEA = Brief Infant-Toddler Social and Emotional Assessment; and SDQ = Strengths and Difficulties Questionnaire.

−0.38, p = .03; Fig. 4, Tables S2C and S2D). No other sex-specific associations were observed.

3.6. Sensitivity analysis

Introducing maternal postnatal depressive symptoms in Step 5 mostly reduced the adjusted R^2 of the models implying worse model fit,

especially in the analyses with HCC2 (Tables S2A-D). The negative association between HCC2 and BITSEA Internalizing problems remained significant (B= −0.75, p = .0061), while the associations with BITSEA Problem Total (B= −0.86, p = .11) and SDQ Emotional symptoms (B= −0.33, CI [−0.68 - 0.016], p = .061) no longer reached statistical significance (Tables S2B and S2D). In the larger HCC1 population, associations between both pre- and postnatal depressive symptoms and child socioemotional problems appeared more often (Tables S2A and S2C).

4. Discussion

Higher maternal end-of-pregnancy HCC measured 1–3 days after childbirth (HCC2) depicting increased systemic hormone levels during the last five months of pregnancy were associated with lower levels of BITSEA Problem Total score at 2 years supporting our main hypothesis. Contrary to our hypothesis, the associations were not more pronounced in mid-pregnancy. The secondary level descriptive analyses suggested negative associations with both BITSEA Internalizing problems at 2 years and SDQ Emotional symptoms at 5 years of age. In interaction analyses, the association between maternal HCC and BITSEA Internalizing and Dysregulation problems at 2 years was observed only with increased maternal depressive symptoms. Regarding the sex-specific associations, higher HCC1 were associated with decreased SDQ Total difficulties in 5-year-old girls; specifically, the amount of Conduct problems and Hyperactivity/inattentive behaviors were lower. While both positive and negative associations were hypothesized to potentially occur, it was unexpected to only observe negative findings.

When interpreting the results, it is important to recognize that the magnitudes of most of the observed associations were modest. This was expected and in line with previous studies, as both the effects of prenatal environment on the offspring and the manifestation of problems in socioemotional development in children are complex and multifactorial. Nevertheless, some associations were stronger as the beta coefficient was − 1.06 for the association between HCC2 and BITSEA Problem Total score and − 1.43 for the association between HCC2 and BITSEA Internalizing problems in subjects with elevated levels of depressive symptoms. The clinical relevance of the observed associations is, especially related to a logarithm-converted predictor, difficult to interpret. The means of BITSEA (2.9–4.1 [SD 1.7–2.4] for different subscales and 7.8 [SD 4.2] for Problem Total score) in HCC2 population indicate, however, that a decrease of more than one unit per an increase of one ln-converted HCC-unit would be relevant.

Additionally, the reliability of the associations was supported by corresponding observations in both measuring points (child ages 2 and 5 years). The observed findings are novel as the area is scarcely studied and no previous reports of associations between prenatal HCC and child socioemotional development exist. Previous studies on the interplay between maternal well-being and prenatal HCC with regards to child socioemotional development have not included prenatal measures of maternal distress, thus the results on the interaction also offer new information to the research field.

The negative associations between maternal prenatal end-of-pregnancy HCC and child internalizing and emotional symptoms were somewhat unexpected but not contradictory to previous literature. Cortisol levels are physiologically known to increase towards the end of pregnancy, thus a less pronounced increase during the last trimester could lead to suboptimal developmental trajectories. This could also explain previous observations where elevated end-of-pregnancy cortisol levels have been associated with improved motor and cognitive development (Caparros-Gonzalez et al., 2019; Davis and Sandman, 2010). Hypothetically, diminished exposure to maternal prenatal cortisol levels especially simultaneously with other signals of increased maternal depressive symptoms could lead to dampened offspring stress reactivity, which, in turn, could result in internalizing symptoms being emphasized in child behavioral phenotype. However, this interpretation is merely speculative and replication of the negative associations with internalizing symptoms is called for. In addition, this study did not include genetic measures, which could also play a role in the finding of the negative association, hence further studies assessing the role of genetic factors is indicated. As we focused on the effects of altered HPA axis functioning regardless the causes, subjects with prenatal medications and pregnancy or birth complications were also included. However, our explorative analyses indicated that these conditions were not related to the exposure nor the outcomes.

It should be noted that we used 5 cm segments instead of the more typical 3 cm segments, which may have diluted some of the pregnancy-specific alterations in HCC. The choice of this segment length was partially based on practical reasons to ensure adequate sample weight for reliable analyses. However, this allowed us to cover the entire pregnancy with two samples. In all, it could be assumed the risk of Type II (false negative) rather than Type I (false positive) error would be increased.

Problems in the regulation of emotions, behavior and activity in toddlerhood are predictive of difficulties in later socioemotional development, however the sensitivity of especially emotional problems in preschool-aged children is modest (Nielsen et al., 2019). Naturally, it is not possible to determine the roots of child socioemotional problems with parent-rated questionnaires only and similar behavioral phenotypes can result from a wide variety of genotypes (Gidziela et al., 2022). Here, for instance, with most girls only being reported to have few symptoms of Conduct problems or Hyperactivity (for girls in HCC1 population, mean sum scores 2.81 [SD 2.04] and 2.82 [SD 2.35], respectively), the results could partly relate to the girls' lower prevalence of these symptoms. The subscales include questions regarding irritability, compliance, restlessness, and distractibility, thus some of the parent-reported behaviors could also derive from mood or anxiety problems. Thus, further studies utilizing observational measures of child socioemotional development and additional informants such as child care early educators are warranted.

For two-year-old's, the association to BITSEA Problem Total score could only be noted when including the effects of both maternal depressive symptoms and HCC. The interaction between HCC2 and prenatal depressive symptoms associated negatively with BITSEA Internalizing and Dysregulation problems. Interestingly, all these effects are bidirectional; lower systemic cortisol levels and higher depressive symptoms were associated with more child socioemotional problems. The interaction models give more insight to this as the correlation between decreased maternal cortisol levels and increased child socioemotional problems seemed to be gradually strengthened with higher levels of maternal prenatal depressive symptoms, while there was no association for mothers with low levels of prenatal depressive symptoms.

This further supports the hypothesis that experienced psychological distress and altered HPA axis activity are interrelated but distinct phenomena and that PPD affects the programming of the fetus through multiple pathways, only some of them directly linked to maternal prenatal cortisol levels (Beijers et al., 2014; Rakers et al., 2017). Specifically, maternal PPD has been associated with downregulation of placental 11β-HSD2 activity in metabolizing cortisol into inactive cortisone (O'Donnell et al., 2012), and with epigenetic alterations such as an elevated methylation of the 11β-HSD2 and NRC31, a glucocorticoid receptor gene (Monk et al., 2016; Oberlander et al., 2008). All these findings highlight the complexity of cortisol regulative system during pregnancy and early development. It is critical to assess the HPA axis functioning alongside with other measures of psychosocial stress as both elevated and decreased steroid levels can be of importance.

Here, both maternal pre- and postnatal depressive symptoms were decided to be included in the analyses for more comprehensive perspective, however the stepwise analytical plan was utilized to avoid overcorrection and misinterpretation of the results. Further studies on the effects of postnatal environment including maternal depression are warranted. Furthermore, it is likely there are non-linear correlations

between the experienced PPD and HPA axis activity, and between them and the offspring development.

In addition to the sex-specific association regarding girls' externalizing and inattentive behaviors at 5 years, HCC1 depicting the early- to mid-pregnancy cortisol levels was not associated with child socioemotional development at two or five years in our data. It could be hypothesized that factors potentially altering early fetal development were more related to cruder metrics such as head circumference (Dancause et al., 2011) and more subtle developmental cues would derive from later fetal period. Interestingly, however, the indicated results that altered maternal cortisol levels in mid-pregnancy would only associate with girls' symptom prevalence were in line with previous literature (Sandman et al., 2013). When assessed visually, the associations between HCC2 and internalizing symptoms at 2 and 5 years seemed rather similar in both boys and girls even though the association reached the level of significancy only for boys at 2 years and girls at 5 years. We performed sex-stratified analyses as we expected there to be differential patterns of associations as child socioemotional problems manifest differently in boys and girls in the general population. Importantly, these analyses do not test for sex differences in the associations or the role of child sex as a moderator.

Strengths of this study include its longitudinal design reaching into pre-school age, multidisciplinary and comprehensive data collection and relatively high number of subjects. Although attrition took place, we were able to collect research material revealing subtle and novel links between decreased or prenatally less increasing long-term cortisol levels and child outcomes. Out of the potential covariates considered for the study, literature and data-driven assessments confirmed the same few essential confounders.

There are also some limitations to consider. There was some selective attrition in terms of sociodemographics as the final study populations were ethnically and socioeconomically relatively homogenous, which limits the generalization of the results. However, the level of reported maternal prenatal and child symptoms were well in line with subjects of the whole FinnBrain cohort and there was significant variation in HCC, thus suggesting the attrition did not cause significant bias to the results. In addition, as the associations emerged regardless of the rather low number of subjects with high levels of PPD (Mustonen et al., 2019) or child socioemotional problems, it could be assumed that similar phenomena would also take place in populations with higher risk. It would have strengthened the study if the number of subjects with both HCC1 and HCC2 samples would have been larger allowing within-person assessments on the subjective change in HCC throughout pregnancy. This study did not include other hormones than cortisol, which might have offered a wider picture on the homeostasis of HPA axis functioning. The means to measure child socioemotional development are widely utilized and validated, however the alphas of BITSEA in our data were somewhat lower than typical ($\alpha = 0.665$ vs $\alpha > 0.700$ for Total problems, respectively). As there are several postnatal environmental factors that could affect child socioemotional wellbeing, it would not have been possible within the scope of this study to comprehensively account for them.

Besides of the complexity of the phenomena itself, there are several methodological factors potentially affecting the varying results in this field of research. Although the latest systematic review suggested maternal prenatal cortisol levels to have significant associations with child outcomes that are more pronounced than those regarding self-reports of maternal PPD, quite contradictory conclusions were made only seven years earlier, when most reviewed studies failed to observe any associations between cortisol levels and offspring outcomes (Caparros-Gonzalez et al., 2022; Zijlmans et al., 2015). Increased utilization of hair steroid measurements most likely has accounted for a part of the added clarity.

5. Conclusions

We observed a negative association between maternal prenatal end-of-pregnancy hair cortisol concentration and child socioemotional total problem score at 2 years of age. Further, higher HCC was associated with decreased internalizing problems at 2 and emotional symptoms at 5 years. Mid-pregnancy HCC only associated with child socioemotional problems in girls at 5 years. Even though the observed effect magnitudes were mostly modest, they were internally consistent, in line with the physiologic alterations taking place prenatally and with previous literature. This study offers new insight on the importance of lower range cortisol levels during pregnancy and future research should include assessments of individual changes in prenatal steroid hormone levels. Assessing HCC together with prenatal depressive symptoms is essential and further studies integrating different aspects of prenatal distress in shaping child development are needed.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2023.106955.

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