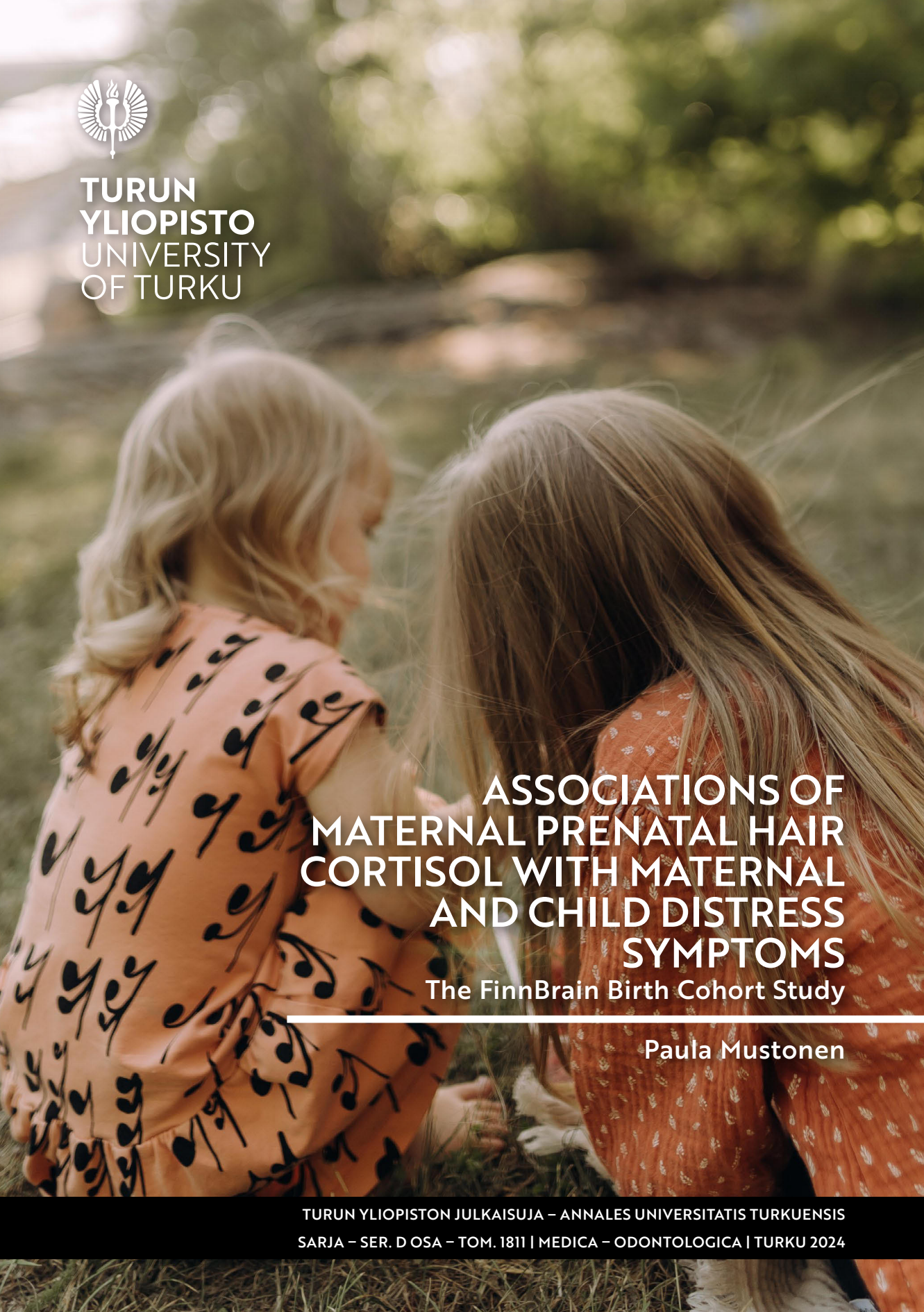

TURUN YLIOPISTO
UNIVERSITY OF TURKU

# ASSOCIATIONS OF MATERNAL PRENATAL HAIR CORTISOL WITH MATERNAL AND CHILD DISTRESS SYMPTOMS

## The FinnBrain Birth Cohort Study

Paula Mustonen

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1811 | MEDICA – ODONTOLOGICA | TURKU 2024

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*Life thus forms a long, unbroken chain of generations, in which the child becomes the mother, and the effect becomes the cause.*
*Rudolph Virchow*

*To Olivia and Mimosa, the ones continuing my chain*

UNIVERSITY OF TURKU
Faculty of Medicine
Department of Clinical Medicine
Psychiatry
PAULA MUSTONEN: Associations of Maternal Prenatal Hair Cortisol with Maternal and Child Distress Symptoms – The FinnBrain Birth Cohort Study
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## ABSTRACT

Maternal prenatal psychological distress (PD) can steer offspring development through adaptations in fetal programming. Maternal systemic cortisol levels are hypothesized to be a major mechanism in transmitting distress signals and hair cortisol concentrations (HCC) can be a proxy measure for the individual's long-term cortisol exposure. The aims of this doctoral thesis were to systematically review the literature on the associations between maternal prenatal PD and HCC (Study I) and to investigate the associations of maternal prenatal HCC with maternal PD (Study II) and with child socioemotional problems (Study III).

Studies II and III were conducted among FinnBrain Birth Cohort Study subjects with maternal HCC measurements from either gestational week 24 (HCC1, n = 467) or 1–3 days after delivery (HCC2, n = 222) and with relevant questionnaire data (a selection of maternal prenatal PD measures for Study II and maternal reports of child socioemotional problems at 2 and 5 years for Study III; n = 321 for HCC1 and n = 121 for HCC2).

Associations between maternal prenatal PD and HCC were inconsistent in the six studies included in the systematic review (Study I). In Study II, concurrent PD was not associated with HCC, but elevated maternal HCC2 associated with trajectories of chronically elevated maternal prenatal depressive symptoms. Lower maternal HCC2 was associated with increased overall child socioemotional problems at a child's age of 2 years and an increase in internalizing symptoms at 2 and 5 years in Study III. HCC1 is only associated with symptoms in girls, and the interaction between maternal PD and HCC2 was associated with child symptoms.

Novel evidence on maternal prenatal HCC presented here advances research assessing concurrent maternal mood and problems in child socioemotional development. Methodology has an impact on explaining whether associations between HCC and PD can be observed and the relevance of also lower maternal prenatal HCC on child outcomes should be acknowledged. Longitudinal and multidisciplinary study designs are essential to elucidate the mechanisms and clinical relevance of the offspring outcomes.

KEYWORDS: cortisol, hair cortisol, HPA axis, prenatal stress, prenatal distress, fetal programming, socioemotional development, internalizing symptoms

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Psykiatria
PAULA MUSTONEN: Yhteydet äidin raskaudenaikaisen hiuskortisolin ja äidin ja lapsen stressioireiden välillä – FinnBrain-syntymäkohorttitutkimus
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## TIIVISTELMÄ

Äidin raskaudenaikainen stressi voi vaikuttaa lapsen tulevaan kehitykseen sikiöaikaisen ohjelmoitumisen kautta. Äidin elimistön kortisolitasojen oletetaan toimivan yhtenä välittävänä mekanismina ja hiuskortisolimittauksella voidaan arvioida yksilön pitkäaikaista kortisolialtistusta. Väitöstutkimuksessa selvitettiin aiemmasta kirjallisuudesta äidin raskaudenaikaisen hiuskortisolin ja stressin välisiä yhteyksiä (tutkimus I) sekä tutkittiin, miten äidin raskaudenaikainen hiuskortisoli on yhteydessä äidin stressioireisiin (tutkimus II) ja lapsen sosioemotionaaliseen kehitykseen FinnBrain-kohorttitutkimusaineistossa (tutkimus III).

Tutkimuksessa II olivat mukana äidit, joilta oli tutkittu hiuskortisolikonsentraatio raskausviikolla 24 (n = 467) tai 1–3 päivää synnytyksen jälkeen (n = 222) ja jotka olivat vastanneet raskaudenaikaisia stressioireita mittaaviin kyselyihin. Tutkimuksessa III olivat mukana ne äiti-lapsiparit, joilta löytyi lisäksi tietoa lapsen sosioemotionaalisista oireista 2- ja 5-vuotiaana (hiuskortisoli keskiraskaudesta n = 321 ja loppuraskaudesta n = 121).

Systemaattisessa katsauksessa (tutkimus I) todettiin raskaudenaikaisen hiuskortisolin ja stressin kuvaavan toisilleen rinnakkaisia, mutta erillisiä ilmiöitä, ja metodologiset erot selittivät osaltaan vaihtelevien tulosten esiintymistä. Äidin stressioireet eivät olleet yhteydessä samanaikaiseen hiuskortisoliin tutkimuksessa II, mutta korkeampia loppuraskauden kortisolikonsentraatioita todettiin niillä, joilla oli pitkäaikaisesti koholla olevia masennusoireita. Tutkimuksessa III matalampi kortisolikonsentraatio loppuraskaudessa oli yhteydessä voimakkaampiin lapsen oireisiin ja yhteys korostui masennusoireisilla äideillä. Keskiraskauden hiuskortisoli oli yhteydessä lapsen oireiluun vain tytöillä.

Löydökset äidin raskaudenaikaisesta hiuskortisolista edistävät alan tutkimusta äidin samanaikaisia mielialaoireita ja lapsen sosioemotionaalisen kehityksen pulmia tutkittaessa. Metodologia selittää osaltaan kortisolitasojen ja oireiden välisen yhteyden havaitsemista ja matalatkin raskaudenaikaiset kortisolitasot voivat liittyä lapsen oireiluun. Pitkittäisillä ja tutkimusaloja yhdistelevillä tutkimuksilla voidaan saada lisätietoa välittävistä mekanismeista ja tulosten kliinisestä merkityksestä.

AVAINSANAT: kortisoli, hiuskortisoli, HPA-akseli, raskaudenaikainen stressi, sikiöaikainen ohjelmoituminen, sosioemotionaalinen kehitys, sisään päin suuntautuvat oireet

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# Abbreviations

| | |
|---|---|
| 11β-HSD2 | 11β-hydroxysteroid dehydrogenase type 2 |
| ACTH | adrenocorticotropic hormone |
| BITSEA | Brief Infant-Toddler Socioemotional Assessment |
| BMI | body mass index |
| CBCL | Child Behavior Checklist |
| CLIA | chemiluminescent immunoassay |
| CRH | corticotropic-releasing hormone |
| DAG | directed acyclic graph |
| DHEA | dehydroepiandrosterone |
| DNA | deoxyribonucleic acid |
| DOHaD | Developmental Origins of Health and Disease |
| ELISA | enzyme-linked immunosorbent assay |
| EPDS | Edinburgh Postnatal Depression Scale |
| gw | gestational week |
| HCC | hair cortisol concentrations |
| HPA | hypothalamic-pituitary-adrenal |
| IBQ-R | Infant Behavior Questionnaire, revised |
| LC-MS/MS | liquid chromatograph – mass spectrometry |
| MRI | magnetic resonance imaging |
| pCRH | placental corticotropic-releasing hormone |
| PAPA | Preschool Age Psychiatric Assessment |
| PD | psychological distress |
| PRAQ-R2 | Pregnancy-specific Anxiety Questionnaire, revised |
| SCL-90 | Symptom Checklist-90 |
| SDQ | Strengths and Difficulties Questionnaire |
| SSRI | selective serotonin reuptake inhibitor |
| SNRI | selective serotonin and noradrenalin reuptake inhibitor |

# List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Mustonen P, Karlsson L, Scheinin NM, Kortesluoma S, Coimbra B, Rodrigues AJ, Karlsson H. Hair Cortisol Concentration (HCC) as a Measure for Prenatal Psychological Distress – a Systematic Review. *Psychoneuroendocrinology*, 2018; 92: 21–28. doi: 10.1016/j.psyneuen.2018.03.019.

II Mustonen P, Karlsson L, Kataja EL, Scheinin NM, Kortesluoma S, Coimbra B, Rodrigues AJ, Sousa N, Karlsson H. Maternal prenatal hair cortisol is associated with prenatal depressive symptom trajectories. *Psychoneuroendocrinology*, 2019; 109: 104383. doi: 10.1016/j.psyneuen.2019.104383.

III Mustonen P, Kortesluoma S, Scheinin NM, Perasto L, Kataja EL, Tervahartiala K, Tuulari JJ, Coimbra B, Carter AS, Rodrigues AJ, Sousa N, Paavonen EJ, Korja R, Karlsson H, Karlsson L. Negative associations between maternal prenatal hair cortisol and child socioemotional problems. *Psychoneuroendocrinology*, 2024; 162: 106955. doi: 10.1016/j.psyneuen.2023.106955.

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# 1 Introduction

During one's lifetime, the fetal period has the most rapid development. The plasticity of all organ systems peaks prenatally, and thus, these systems are especially vulnerable to environmental cues. Conceptualized as the developmental origins of health and disease (DOHaD, Barker, 2007) and fetal programming (Godfrey, 1998; Seckl et al., 1999), maternal prenatal health and well-being can steer the developmental trajectories of the offspring to a wide range of future health outcomes (Moisiadis and Matthews, 2014a; Monk et al., 2019; Risnes et al., 2011; Sacchi et al., 2020; Su et al., 2021). Maternal prenatal psychological distress (PD) has been associated with offspring mental and somatic health outcomes and alterations in child brain structure, function, and connectivity (Burgueño et al., 2020; Entringer et al., 2015; Flanigan et al., 2018; Lafortune et al., 2021; Lautarescu et al., 2020a; Luoma et al., 2001; van den Bergh et al., 2018, 2020). While obstetric care has improved significantly during the past decades, the same cannot be stated for the emotional support and psychiatric treatment of pregnant women; it has been estimated that maternal prenatal anxiety and depression can contribute to up to 10–15% of the attributable load of child behavioral and emotional outcomes (Glover, 2014).

Cortisol, the hormonal end-product of the hypothalamic-pituitary-adrenal (HPA) axis, has been hypothesized to act as one of the major mediators of these detrimental offspring outcomes (Moisiadis and Matthews, 2014b; Rakers et al., 2017; Seckl and Meaney, 2004). Cortisol has several acute and long-term effects on all organ systems. In the short term, it activates the body to "fight or flight" by increasing alertness, focus and preparedness to function, increasing heart rate, blood flow and blood pressure, releasing energy, decelerating gastrointestinal functioning, and suppressing immune function. The secretion of cortisol fluctuates according to circadian rhythms in a partly bidirectionally regulated manner (de Weerth et al., 2003; Walker et al., 2020). When the recovery from stress response is inadequate or the exposure to cortisol levels is otherwise altered, this can lead to maladaptive outcomes such as anxiety and other psychiatric symptoms, problems in sleep, metabolic and cardiovascular problems and deficits in cognitive processing (Adam et al., 2017).

Since the 1970s, the associations between maternal prenatal cortisol levels and maternal prenatal mood have been studied (Handley et al., 1977). Early on, however, cortisol could only be measured momentarily from saliva or blood and the methodological demands for studies were variably met (Seth et al., 2016). During the past 20 years, measuring long-term systemic cortisol concentrations from hair samples has been universalized and has become a golden standard when assessing chronic levels of cortisol as opposed to the acute stress reactivity or diurnal cortisol output where momentary measures are needed (Russell et al., 2012; Stalder et al., 2017). In meta-analyses and systematic reviews, hair cortisol concentrations (HCC) are associated with life situations related to chronic stress, psychiatric conditions, adversity, hypertension, and metabolic markers (Khoury et al., 2019; Koumantarou Malisiova et al., 2021; Ling et al., 2020; Pageau et al., 2023; Stalder et al., 2017) and are widely assessed during pregnancy (Marceau et al., 2020). The association between chronic stress and HCC has also been observed in a meta-analysis concerning children (Li et al., 2023). Despite accumulating studies, discrepancies regarding the links between maternal prenatal PD and HCC persist (Khoury et al., 2023).

Problems in socioemotional development of small children are common and relatively stable; albeit there is significant heterogeneity in the trajectories of psychiatric symptom prevalence during childhood and adolescence, yet up to 50% of toddler-aged children with psychiatric diagnoses continue to present with some psychiatric symptoms above the diagnostic threshold at school-age (Lavigne et al., 1998). The risk for socioemotional problems and child psychiatric conditions are known to be increased after exposure to maternal prenatal PD (Monk et al., 2019). Yet, longitudinal studies assessing the associations between maternal prenatal HCC and child socioemotional outcomes are scarce. Some studies have suggested that the interplay between maternal prenatal HCC and maternal well-being or parenting attributes is associated with child temperament, self-regulation, or anxiety disorders (Bosquet Enlow et al., 2017; Galbally et al., 2022, 2023b), however overall associations between the two have not been included as a main study question in any previous studies. In addition, the potential sex-specificity of the outcomes and maternal prenatal HCC interactions with prenatal PD symptoms need to be further assessed.

As the demands for mental health services for children and adolescents continue to increase, a further understanding of the risk and protective factors for child psychosocial development is crucial (Polanczyk et al., 2015). Adding emphasis on the potential long-lasting effects of maternal prenatal well-being is essential in order to make recommendations for public policies (Glover et al., 2023). Investments directed to pregnancy or early childhood are associated with the greatest returns in human capital (García et al., 2016). With interventions that promote maternal

prenatal mental health, not only can we give a more secure basis for the postnatal environment of the child but also positively impact the early life programming of the offspring (Lewis et al., 2014). As the treatment of maternal prenatal depression associates with a decreased psychopathology of the offspring (Goodman et al., 2018), a need for effective and widely utilizable interventions prenatally is warranted.

# 2 Review of the Literature

## 2.1 Fetal Programming

### 2.1.1 Developmental Origins of Health and Disease

Beginning in the 1980s with the work by David Barker (Barker, 1986), the significant effects of the fetal environment in steering child development have been recognized and increasingly studied. The concept of the developmental origins of health and disease (DOHaD; Barker, 2007, 2003) also known as the Barker's hypothesis and the fetal programming theory, first described increased risks for ischemic heart diseases in individuals exposed to poor nutrition prenatally (Barker, 1986). Convincing evidence of the long-term consequences of the prenatal period has also been gained from the work of The Dutch Famine Birth Cohort, a historical birth cohort examining children of mothers having been exposed to extreme prenatal food deprivation during the winter of 1944 to 1945 (De Rooij et al., 2022; Lumey et al., 1993).

The basis for these theories is that the fetal period programs the offspring to adapt to the expected postnatal environment. Thus, in cases of food deprivation, it is beneficial for the individual to be able to absorb efficiently all available nutrition. However, in cases where the postnatal environment does not match with what was prenatally predicted and the prenatally food deprived child grows up in a milieu in which nutrition is abundantly available, the risks for obesity and metabolic conditions are increased (Barker, 2007b; Ravelli Gian-Paolo et al., 1976).

In addition to the metabolic outcomes, prenatal food restrictions were observed to be associated with an increased prevalence of depressive and anxiety disorders (Brown et al., 1995; Thompson et al., 2001). This was discussed to potentially relate to the role of the hypothalamic-pituitary-adrenal (HPA) axis functioning in mediating the effects of maternal prenatal undernutrition to the fetus or to be explained by other prenatal factors, such as maternal prenatal distress that could have influenced fetal programming.

Based on the results of rodent models starting from the 1960s, human studies focusing specifically on prenatal stress emerged (reviewed in Weinstock et al., 1988). Already in 1973, the effects of severe and continuing personal tensions,

especially marital discord, were observed to be associated with worse child somatic health outcomes, increased neurological dysfunction, developmental lag, and alterations in behavior (Stott, 1973).

### 2.1.2 Fetal Development

While the complex, carefully regulated and multifaceted process of fetal development cannot be comprehensively described within the scope of this thesis, it is important to briefly introduce it here. The development of the HPA axis and other key factors that affect the programming of the child's socioemotional well-being are described in more detail in the next two chapters.

After the implantation of a blastocyst into the wall of the uterus at approximately day six after fertilization, its cells divide to form the embryo, the amniotic sac and the fetal component of the placenta (Herrick and Bordoni, 2023). The placenta is the means of communication between the fetus and the mother by not only allowing the bidirectional exchange of substances through the placental membranes but also by functioning as an active immunological and endocrine organ. The placenta produces many hormones and growth factors that are vital for the development of the fetus and the progression of a healthy pregnancy. In addition, the placenta plays a key role in regulating fetal exposure to substances from maternal blood flow. One the most important hormones in transferring developmental cues, and, thus, steering fetal development and programming, is cortisol (Constantinof et al., 2015).

By the end of gestational week (gw) eight, a basis for all organ systems has been formed. Currently, a fetus can be viable if born preterm after gw 24 although regardless of the time of the delivery, the rapid development all but discontinues after birth. While the fetus gains its nutrients and oxygen via the placenta, the maturation of lungs with surfactant is needed to adapt to the postnatal environment (Hillman et al., 2012). In normal pregnancies, the end-of-pregnancy surge of cortisol activates the maturation of lungs and other organs but in cases where preterm birth is suspected, antenatal glucocorticoids injections are administered to help prevent preterm infant respiratory distress syndrome (McGoldrick et al., 2020).

### 2.1.3 The Programming of the HPA Axis

The HPA axis involving the hypothalamus, anterior pituitary gland, and adrenal glands is a major component in inducing and regulating the human physiological stress response. Briefly, the paraventricular nucleus in the hypothalamus synthesizes corticotropic-releasing hormone (CRH), which stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which in turn induces the production and release of cortisol from the adrenal cortices. Increasing

cortisol levels inhibit the secretion of CRH and ACTH by a negative feedback loop (Miller, 2018).

Based on substantial data from animal studies and some human studies, the fetal HPA axis is well developed and capable to produce hormones and substantially contribute to cortisol levels in the fetal blood stream from approximately gw 20 onwards (Mastorakos and Ilias, 2003; Moisiadis and Matthews, 2014a; Wood and Keller-Wood, 2016). For instance, in a controlled study, increased levels of ACTH were measured in the fetal bloodstream after a vena puncture of an innervated intrahepatic vein causing stress to the fetus in comparison to a puncture to the umbilical vein that is not innervated (Kosinska-Kaczynska et al., 2012).

The development and programming of the HPA axis are susceptible to maternal signals such as maternal cortisol and placental CRH levels (see Chapter 2.2.2 for a more detailed description of the functioning and alterations of maternal HPA axis during pregnancy). In addition, several other prenatal factors may contribute to a child's later HPA axis activity with maternal prenatal alcohol use being one of the most potent predictors (Pearson et al., 2015). The first three months of pregnancy, i.e., the time corresponding with the majority of HPA axis development and maturation, have been proposed to impose a distinctively sensitive window for HPA axis-related epigenetic adaptations in response to maternal signals (Montenegro et al., 2019). Nevertheless, programming occurs throughout the pregnancy even though, given the complexity of the phenomena, the findings between measures of maternal prenatal and offspring HPA axis functioning are varied (reviewed in Wood and Keller-Wood, 2016).

Importantly, if synthetic glucocorticoids are administered to accelerate the maturation of fetal organs when at risk for premature birth, they can freely pass through the placenta. While necessary and potentially life-saving for the neonates that are born early preterm, accumulating evidence indicates that there are disadvantageous long-term outcomes for both the HPA axis functioning and other health outcomes for those receiving it without experiencing prematurity (Ninan et al., 2023).

### 2.1.4 The Programming of Socioemotional Development

Child socioemotional development describes a child's social and psychological well-being or distress and it comprises the child's emotional reactivity and self-regulatory capacities as a combination of innate features and adaptation to the environment (see also Chapter 2.6 regarding the child socioemotional problems; Campbell et al., 2016). If simplified, socioemotional problems in early childhood can be divided into emotional/internalizing symptoms, such as mood and anxiety symptoms and fears; conduct/externalizing symptoms, such as oppositional or aggressive behaviors;

dysregulation, e.g., problems in attention, activity, feeding and sleeping; and problems in social interactions, e.g., difficulties in peer relations, communication and reciprocal social interactions (Briggs-Gowan et al., 2001; Goodman, 1997). It is, however, typical that multiple underlying causes can lead to similar behavioral phenotypes especially in small children. The biological sex assigned at birth and gender also shape the prevalence and appearance of the emotional and regulative difficulties in childhood (Chaplin and Aldao, 2013).

Factors that affect toddlers' socioemotional problems and competencies are multiple, for instance, a child's individual characteristics, such as cognitive profile and temperament traits, genetic and epigenetic factors, prenatal exposures, parenting, parental well-being, family socioeconomic factors, and other environmental factors. As the child grows, the importance of factors outside of the family unit, such as out-of-home day care, school, and peer relationships, increase. Importantly, the individual susceptibility to be influenced by different pre- and postnatal exposures varies markedly as described by the differential susceptibility theory (Belsky and Pluess, 2013; Boyce and Ellis, 2005); the developmental trajectories of some children are more robust whereas others are both more vulnerable to risk factors as well as can benefit more from advantageous environmental factors.

Regarding the prenatal factors that may dispose to the development of socioemotional problems, fetal brain development and factors that contribute to it play a key role. Brain development begins during the first month following fertilization and continues throughout the lifespan (Bethlehem et al., 2022). The modifying effect of fetal sex in the developing brain has been identified beginning from the prenatal period and sex-specific brain growth curves have been formulated (Bethlehem et al., 2022).

At the embryonic phase, i.e., the first eight weeks of gestation, disruptions in the developmental process tend to lead to either abortion of the pregnancy or to severe malformations such as spina bifida or anencephaly. By gw 20, the fetal brain has the structures needed for mature functioning, and, beginning from gw 27, sensory signaling in the fetal brain has been reported in imaging studies (Draganova et al., 2007). The proliferation and migration of neuronal cells are rapid in early- and mid-gestation with the amount of neurons throughout life peaking at gw 28 (Anderson and Thomason, 2013; Lautarescu et al., 2020a). During the second half of pregnancy, the connectivity, myelination, and specialization of the central nervous system develop rapidly and pruning of unnecessary neurons begins (Monk et al., 2019).

This delicate process is sensitive to different external and internal influences such as chemical exposures and maternal health characteristics (Pulli et al., 2019). The detrimental effects of prenatal alcohol exposure have long been understood (Donald et al., 2015) and exposure to tobacco products and illicit drugs have also

been repeatedly shown to associate with alterations in neonatal brain structure and functional maturation (Pulli et al., 2019). Out of the pharmaceutically utilized drugs, one of the most studied groups is selective serotonin (and noradrenalin) reuptake inhibitors (SSRI/SNRI) used as antidepressants. This field of research is complicated, as it is difficult to disentangle the effects of shared genetic risk factors, exposure to maternal pre- and/or postnatal depression, potentially affecting the offspring in several direct and indirect pathways as discussed later in the thesis, and prenatal SSRI medication *per se* (Malm et al., 2016). In addition to depressive symptoms, also other types of psychological distress are known to be associated with a variety of child outcomes as discussed in detail in Chapter 2.2 Prenatal Psychological Distress. Furthermore, maternal health related factors such as maternal obesity or malnutrition, proinflammatory states and parental socioeconomic factors have been associated with altered neuroimaging findings in neonates (Pulli et al., 2019).

## 2.2 Maternal Prenatal Psychological Distress

Maternal prenatal PD is often defined as symptoms of depression, anxiety, perceived stress, pregnancy-specific anxiety, stress related to daily life, or previous or concurrent negative life events. In the literature, the terms stress and distress are both used in describing the phenomena, and occasionally, they may be considered nearly interchangeable. However, as by definition, the term stress depicts situations that can be expected to directly generate a stress response, it should be utilized in markedly specific occasions, whereas psychological distress covers a wider range of experiences of discomfort and states that challenge psychological well-being ("APA Dictionary of Psychology," n.d.; Phua et al., 2023). Distress more often depicts agony rather than discomfort, however, in this context, it does not define the severity of the indicated negative emotional state.

Generally, the most consistent associations with adverse offspring outcomes are observed in the context of the most objective stress experiences, specifically natural disasters and armed conflicts (Keasley et al., 2017; Lafortune et al., 2021). Yet, also lower levels of PD and subjective self-reports of maternal prenatal PD are known to be associated with a wide range of clinically relevant and long-lasting effects on child development (Burgueño et al., 2020; Entringer et al., 2015; Flanigan et al., 2018; Lautarescu et al., 2020a; van den Bergh et al., 2018, 2020). The prevalence of prenatal depression has varied between 7% and 20% in high-income countries and has been higher in low- and middle-income countries (Biaggi et al., 2016; Glover, 2014). In addition, even subclinical symptom levels of depression or other experiences of PD may be highly relevant for offspring outcomes (Glover, 2014)

### 2.2.1 Measuring Maternal Prenatal PD

The most common means of measuring maternal prenatal PD is by self-report questionnaires. A variety of validated questionnaires is widely utilized in measuring symptoms of depression, such as the Edinburgh Postnatal Depressive Scale (EPDS; Cox et al., 1987) and the Hospital Anxiety and Depression Scale (HADS-D; Zigmond and Snaith, 1983); anxiety, for instance, the Symptom Checklist-90, (SCL-90; Derogatis et al., 1973) and the Hospital Anxiety and Depression Scale (HADS-A; Zigmond and Snaith, 1983); pregnancy-related anxiety, such as the Pregnancy-specific Anxiety Questionnaire, revised (PRAQ-R; Huizink et al., 2016); perceived stress, such as the Perceived Stress Scale (PSS; Cohen et al., 1983); stress related to daily life, such as the Daily Hassles (Korpela et al., 2008); or negative/traumatic life events, such as the Life Events Checklist (LEC; Gray et al., 2004). In addition, some studies use diagnostic interviews, such as the Structured Clinical Interview for DSM5 (SCID-5; First et al., 2016), and others extract data of prior psychiatric disorders from national registries. In addition, exposure to natural disasters, pandemics, or armed conflicts can be assessed, for instance, based on the timing or proximity of the center of events or with questionnaires on the consequences of being exposed to the stressor (Howells et al., 2023).

The timeline that the questionnaires depict most typically varies between a week and a month, however some questionnaires assess the experiences of even shorter periods or request the subject to assess the overall level of symptoms during longer periods. The timelines are especially relevant in the context of HCC studies, as the cortisol measured from hair depicts the systematic cortisol levels of the time the hair segment has grown; thus, the timelines need to correspond to even theoretically assume that the two measures are interrelated. When assessing PD symptoms during longer periods of time, the risk for reporting bias increases, and, because of the potential fluctuation in symptom prevalence, it may not be possible to describe the trajectories of PD experiences with only one assessment. Thus, repetitive measures are generally regarded essential in assessing symptom prevalence during longer periods of time.

### 2.2.2 Maternal Hypothalamic-Pituitary-Adrenal Axis Functioning During Pregnancy

During normal pregnancy, significant alterations occur in the functioning of nearly all maternal systems to allow the growth and development of the fetus and to prepare for the delivery (reviewed in Tan and Tan, 2013). While a comprehensive description of these physiological changes is beyond the scope of this thesis, it can be briefly mentioned that the efficacies of maternal cardiovascular and respiratory systems increase as maternal cardiac output increases up to 30–50% and oxygen consumption

up to 20%, while the functional residual capacity decreases by 10–25% (Tan and Tan, 2013). Hematological changes occur as the maternal immune system encounters paternal antigens, and procoagulant activity increases. The anatomy of the organs in the abdominal cavity adjusts allowing for the growing uterus. The metabolic demands of the mother and the fetus increase and several endocrine adaptations are therefore needed. Here, we focus on the alterations related to the HPA axis.

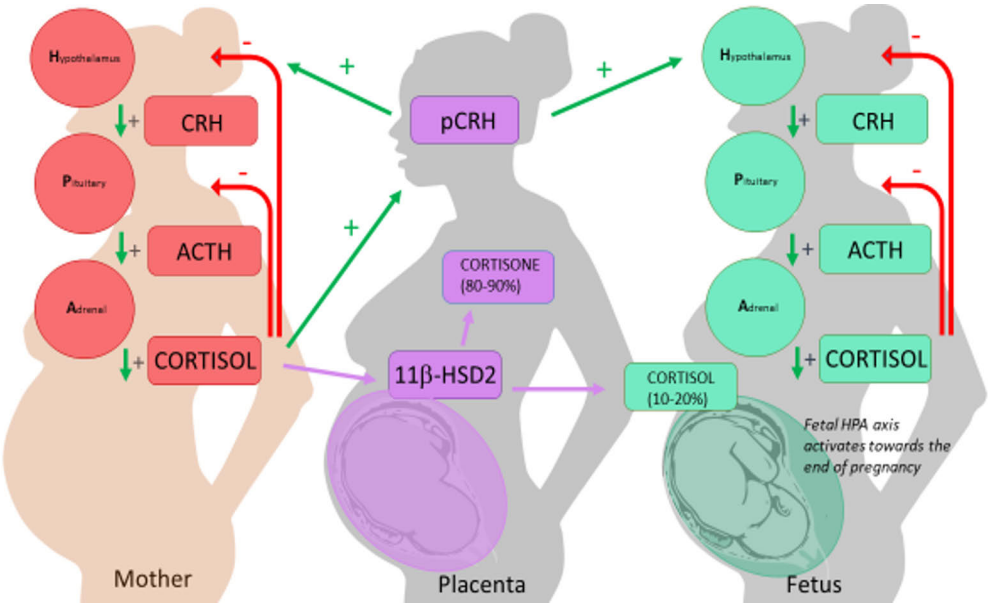


**Figure 1.** Functioning of the HPA axis during pregnancy. Maternal HPA axis (left) continues to function similarly as in non-pregnant states, but placental secretion of pCRH (middle) and its positive feedback to the HPA axis increase the levels of circulating CRH and cortisol. The fetus (right) is influenced both by maternal pCRH (activating the fetal HPA axis towards the end of pregnancy) and the unconverted proportion of maternal cortisol passing through the placenta. Abbreviations: HPA = hypothalamic-pituitary-adrenal; CRH = corticotropin releasing hormone; ACTH = adrenocorticotrophic hormone; pCRH = placental CRH; 11β-HSD2 = 11β-hydroxysteroid dehydrogenase type 2. (Figure is from Original Publication I with the permission of the publisher)

The alterations in maternal HPA axis functioning during pregnancy and their effects on the cortisol exposure of the fetus are depicted in **Figure 1**. During pregnancy, the levels of CRH, ACTH and cortisol increase towards the end of the pregnancy (de Weerth and Buitelaar, 2005). This is needed for fetal organ maturation and to induce labor (Li et al., 2014). In addition to the CRH secreted from the hypothalamus, the placenta also secretes placental CRH (pCRH) throughout gestation (Riley and Challis, 1991). Instead of the negative feedback loop that

usually downregulates systemic cortisol levels, a positive feedback loop related to pCRH increases circulating cortisol levels up to 2–3-fold towards the end of pregnancy (Jung et al., 2011). Simultaneously, the stress reactivity of the HPA axis is attenuated (Entringer et al., 2010). Fetal exposure to circulating maternal cortisol levels is regulated by the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) that converts cortisol into its inactive metabolite cortisone (Seckl, 2004).

### 2.2.3 Mechanisms Mediating the Effects of Maternal Prenatal PD to the Fetus

Altered fetal exposure to maternal glucocorticoids is considered one of the major mechanistic pathways underpinning detrimental offspring outcomes (Rakers et al., 2017). As the key hormone of the stress regulating HPA axis, cortisol presents as an axiomatic mediator between maternal stressful experiences and fetal programming for an anticipatedly high-stress environment. Indeed, a systematic review has concluded that when assessing the associations between offspring outcomes with regards to both maternal prenatal PD and cortisol measures, cortisol measures were stronger in predicting adverse offspring outcomes than were self-reports of PD (Caparros-Gonzalez et al., 2022).

The importance of the glucocorticoid pathway is highlighted by there being multiple parallel mechanisms that link maternal prenatal PD to the fetal exposure of maternal cortisol levels. In addition to the potential direct increase in circulating cortisol levels, maternal prenatal PD can also diminish the activity of placental 11β-HSD2 metabolizing cortisol into inactive cortisone (O'Donnell et al., 2009). In addition, maternal PD has been observed to induce epigenetic alterations contributing to the placental expression of 11β-HSD2 and glucocorticoid receptors (Cao-Lei et al., 2017). Prenatal-stress-related placental transcriptomic reorganization is also linked to offspring stress regulation and behavior; in a study assessing the effects of stress caused by Hurricane Sandy during pregnancy (Nomura et al., 2021), placental expression of several immune, vascular, and endocrine gene sets (including reduced expression of HSD11B2, HSD3B1, and GH2, which are critical for cortisol metabolism, progesterone synthesis, and growth hormone synthesis, respectively) was altered. Many of these alterations mediated the association between maternal prenatal stress and offspring outcomes at 3–4 years, specifically, altered HPA axis functioning as measured by child hair corticosteroids and reported aggression and anxiety symptoms (Nomura et al., 2021).

The disadvantageous offspring outcomes associated with the administration of antenatal glucocorticoids given to accelerate fetal maturation in the risk of preterm birth support the role of glucocorticoid exposure as a mechanistic pathway. The

interpretation of the findings is difficult, as previously, there always have been signals of risk that may have affected the fetal programming even though the risk of preterm birth would not have actualized. However, it seems the risk for adverse outcomes is significant in the cases with early antenatal glucocorticoid exposure but with the pregnancy continuing into late preterm/term (Ninan et al., 2023).

As a parallel stress regulation system, the activity of the sympatho-adrenomedullary system, and thus, the maternal catecholamine concentrations and the homeostasis of the maternal autonomic nervous system (sympathetic or parasympathetic activity) are suggested to be potential mechanisms (Beijers et al., 2014; Doyle et al., 2015). It is mechanistically challenging to measure the long-term levels of catecholamines and studies assessing them are scarce (Petraglia et al., 2001). However, assessing both maternal and fetal autonomic nervous system activity is achievable by heart rate variability assessments, which have been associated with maternal prenatal PD (Doyle et al., 2015). One additional mechanism that has some support from earlier literature is impairment of the uterine artery blood flow in association with maternal prenatal anxiety (Van den Bergh et al., 2005).

In addition to the associations related to fetal exposure of maternal cortisol, there are also direct associations between maternal experiences of PD and placental functioning (Glover et al., 2009; Seth et al., 2015). Notably, the placenta is an active organ in secreting hormones and thus, it may also contribute reciprocally to maternal mood (Janssen et al., 2016).

Recently, one major focus of studies in the field of prenatal programming has lied in assessing telomere lengths, a biological marker for cellular aging (Bosquet Enlow et al., 2019; Naudé et al., 2023; Rinne et al., 2023; Stout-Oswald et al., 2022). Both maternal prenatal PD symptoms (Rinne et al., 2023; Stout-Oswald et al., 2022) and maternal prenatal HCC (Bosquet Enlow et al., 2019) have been associated with shorter telomeres in the offspring with the HCC finding being sex-specific.

The maternal immunological state, measured by the cytokine levels, and the gut microbiome of the mother and the infant have also been associated with the prevalence of maternal PD thus also potentially playing a role (Hameete et al., 2021; Mepham et al., 2023). In addition, some interventions, such as maternal prenatal SSRI medication, may directly contribute to fetal development albeit discovering any causalities in the effects has proved difficult (Leshem et al., 2021). One explanation for the observed associations may, of course, be the shared genetic background of the mother and the offspring.

## 2.3 Hair Cortisol Concentrations

The measurement of hair cortisol concentrations (HCC) is a relatively novel means to assess systemic long-term cortisol levels and thus, indirectly, the functioning of

the HPA axis. The method of HCC analysis from human hair was introduced only two decades ago (Raul et al., 2004), and the amount of HCC studies has rapidly accumulated in the recent years with only nine studies published before the year 2010 and now with more than 1200 studies that assess hair cortisol. The use of HCC in research has been validated in several systematic reviews and meta-analyses within different age groups and biological sexes, healthy subjects and subjects with psychiatric or somatic illnesses, and in a variety of life situations such as pregnancy (Khoury et al., 2023, 2019; Kuckuck et al., 2024; Li et al., 2023; Ling et al., 2020; Marceau et al., 2020; Pageau et al., 2023; Stalder et al., 2017; Wang et al., 2024). Some steps have been taken to be able to use HCC also in the clinical setting especially for diagnosing Cushing's syndrome that is defined by severe hypercortisolemia, however the practice is yet to be established (Savas et al., 2022; Stalder and Kirschbaum, 2012). The intercorrelation of HCC assessments performed in different laboratories has been observed to be high when the methods of HCC analyses are the same, however differences in absolute HCC values are observed when varying methods are used (Russell et al., 2015).

Cortisol, as well as other lipophilic substances, is continuously incorporated into the hair shaft from the capillaries and sebaceous and sweat glands of the hair follicles thus forming a retrospective "calendar" chronicling systemic cortisol levels during the time the analyzed hair was grown (Kirschbaum et al., 2009). Hair growth rate depends on the individual, and, for instance, their ethnic background, age, sex, and being pregnant are affecting factors (Loussouarn et al., 2016; Marceau et al., 2020). However, in HCC studies, the approximated hair growth rate of 1 cm per month can be used (Greff et al., 2018). Thus, by choosing how many scalp-near centimeters from a hair strand will be analyzed, one can roughly decide the retrospective time period during which the cortisol has accumulated in hair.

### 2.3.1 Cortisol Measured from Other Substrates

Prior to the universalization of HCC as the method of assessing chronic levels of cortisol, salivary cortisol was the prevalent means to attempt to measure also long-term cortisol levels. In addition, cortisol concentrations have traditionally been measured from blood and urinary samples and occasionally from amniotic fluid. Notably, salivary and blood cortisol concentrations report the momentary level of cortisol. However, systemic cortisol levels fluctuate considerably based not only on the stress reactivity and the circadian rhythm but also on physical activity, food consumption, and sleep. Further, external factors, such as noise or the purity of respiratory air can contribute to varying cortisol levels. Thus, methodological factors related to the sampling procedure, e.g., time/times of day when a sample is taken and number of days with sampling, and modeling of the data, for instance, the utilization

of cortisol awakening response, area under the curve, or the slope of diurnal decline, can markedly affect the observed results. With a 24-hour urinary sample collection, the accumulated cortisol levels of the past day can be measured, however the process requires dedication from the study subjects and longer periods of sample collection are seldom achievable. Both blood and especially amniotic fluid sampling are invasive, which restricts their use in academic settings.

According to systematic reviews assessing the associations between maternal prenatal cortisol and either prenatal PD or offspring outcomes that mostly comprised studies having momentary cortisol measures, the varying procedures and methodological challenges in measuring cortisol have contributed to the inconsistent results (Seth et al., 2016; Zijlmans et al., 2015).

### 2.3.2 Hair Cortisol Concentration Analyses

For HCC analysis, a hair strand of approximately 150 single hairs with a diameter of approximately 0.5mm needs to be cut as close to the scalp as possible. This is important for the hair segment to represent accurately the time period of interest. Hair growth rate and the ratio of hair follicles in the phase of active growth alters in different areas of the scalp with the most uniform growth rates measured from the posterior vertex of the scalp (Pragst and Balikova, 2006). Thus, it is univocally accepted that HCC samples should be cut from that area.

As stated before, it is acceptable to estimate the study subjects' hair growth rate to be approximately on average 1 cm/month. Based on that, the segment length utilized in the study needs to be specified in the study protocol. The most used segment length is 3 cm depicting the cortisol exposure during the previous three months, but a variation between 1 to 6 cm is standard. Some studies include several segments of the same hair samples, however the role of wash-out effects, i.e., the degradation of cortisol molecules due to hair washing, hair treatments, exposure to sun light, and time, needs to then be considered (Hamel et al., 2011; Stalder et al., 2017; Stalder and Kirschbaum, 2012). Physiological concentrations of cortisol can reliably be measured six months retrospectively, and pathologically high cortisol levels measured from patients with Cushing's syndrome have been observable for up to 18 retrospective months (Kirschbaum et al., 2009; Thomson et al., 2010).

The procedure for extracting cortisol from the hair segments has gone through some alterations throughout the years and between-laboratory variation in results remains (Russell et al., 2015). In the analytical process, the hair samples are first measured, and the selected segment lengths are cut and weighed. The accuracy of how precisely the analyzed segment consists of hair that has grown during the time of interest depends on the quality of sample taking; the scalp-near end of the segment can be uneven if cut from too wide of an area. The suggested sample weight has

varied in time as previously more hair mass was assumed to be needed (Russell et al., 2015); however it seems to notably affect the results if the hair mass is either too low or too high in comparison to the volume of the solvent and the limits of detection of the quantifying method. Therefore, currently, many laboratories standardize the sample weight or limit it to between approximately 5 to 15 mg (Gao et al., 2013). Some laboratories have discovered special techniques to allow reliable HCC analyses from extremely low mass samples from preterm infant's hair (Hoffman et al., 2017; Nist et al., 2020).

The samples are then washed 1–3 times with isopropanol and allowed to dry under a fume. Previously, it was assumed the hair segments needed to be finely minced with scissors or ground with a ball mill to ensure stable extraction of the cortisol molecules from inside the hair. The mincing with scissors is, however, time consuming and difficult to be standardized, while the ball mill technique may result in sample carryover or chemical degradation of cortisol by the induced heat. Simultaneously, a reliable and reproducible analysis is achievable using whole hair, thus this method has become the standard (Gao et al., 2013). The extraction is performed by incubating the hair in a solvent such as methanol, and after evaporation of the solvent, the solvent is reconstituted by adding phosphate buffer or distilled water. Finally, the concentration of cortisol is quantified by either enzyme-linked immunosorbent assays (ELISA), chemiluminescent immunoassays (CLIA), or liquid chromatograph – mass spectrometry (LC-MS/MS).

Prior, immunoassays were the most utilized analysis methods. There is, however, significant interassay variation between laboratories and commercially available immunoassays. With immunoassays, there is a greater risk for cross-reactivity, which has been observed as lower between-laboratory correlations for immunoassays than for LS-MS/MS in studies where the same hair samples were analyzed at various laboratories with different methods (Russell et al., 2015; Slominski et al., 2015). In addition, when utilizing LC-MS/MS, hair glucocorticoids other than cortisol, such as cortisone and dehydroepiandrosterone (DHEA) (Chen et al., 2013; Noppe et al., 2015) which complement the picture of biological responses to stress, can be measured (de Mendonça Filho et al., 2023). Because cortisone is a non-active metabolite of cortisol and DHEA and its more abundant sulfite-form DHEA-S work as partial antagonists of cortisol, including assessments of their ratios provides more precise indices of HPA axis functioning (Kamin and Kertes, 2017).

### 2.3.3 Determinants of HCC

A meta-analysis assessing the basic determinants and anthropometric factors related to HCC has identified the subject's age, sex, BMI, waist-to-hip ratio, systolic blood pressure, and frequency of hair washing to be the potential covariates that should be

considered to be accounted for in HCC studies (Stalder et al., 2017). Hair treatments and oral contraceptives were also related on a trend level. A systematic methodologically focused review on maternal prenatal HCC studies emphasized the need to explore the covariates essential for the sample utilized, i.e., to establish the associations related to HCC in the sample in question while paying attention to the number of covariates compared to the statistical power of the study (Marceau et al., 2020). Besides the more frequently included covariates, the authors highlighted the role of seasonality and batch effect, which were observed to associate with HCC with considerable effect sizes in some of the studies reporting on their associations. The batch effect may result from interassay variation, variations in the technical procedure, and cortisol degradation due to time or freezing of the samples (Russell et al., 2015; Slominski et al., 2015). As it may be difficult to statistically correct for this type of variation, it is always advisable to analyze the entire sample with the same methods and time.

## 2.4 Maternal Prenatal HCC and Maternal Prenatal PD

Previously, the methodological variation in momentary cortisol measurements was likely affecting the results of their potential associations with maternal prenatal PD, and the fact that the results varied was assumed to at least partially derive from that inconsistency (Seth et al., 2016; Zijlmans et al., 2015). The measurement of HCC yields one figure to depict the chronic exposure to cortisol during the past months, which was anticipated to diminish the methodology-related difficulties and to clarify the interpretation of the findings.

From any HCC studies, the ones including maternal prenatal assessments were among the first (Kalra et al., 2007; Kirschbaum et al., 2009) even though a pronounced advancement of studies in this field started in the late 2010s (Khoury et al., 2023). The systematic review that is a part of this thesis was published in 2018 with six papers fulfilling the inclusion criteria (Mustonen et al., 2018). Since, the amount of publications has markedly increased, and a recent meta-analysis included as many as 29 studies (Khoury et al., 2023). In this thesis, the aim was to provide an update on the current state of research by presenting studies fulfilling the same inclusion criteria that were utilized in the 2018 systematic review (see Chapter 4.1 and **Table 1**). The literature search then found 69 articles in PubMed, while the same search on January 16, 2024 resulted in 267 articles. However, as this thesis does not aim to provide a new systematic review on such an extensive area of research along with a recently published meta-analysis with slightly different inclusion and exclusion criteria, the review presented here is based on a descriptive and qualitative assessment and may not be fully comprehensive.

Thirty studies assessing associations between maternal prenatal PD and maternal prenatal HCC were now identified (Abdul Jafar et al., 2023; Bowers et al., 2018; Braig et al., 2015; Broeks et al., 2021; Bruinhof et al., 2022; Budnik-Przybylska et al., 2020; Caparros-Gonzalez et al., 2019a; Conradt et al., 2020; Dobernecker et al., 2023; Duffy et al., 2018; Freedman et al., 2021; Galbally et al., 2019; Hoffman et al., 2016; Howells et al., 2023; Hunter et al., 2021; Jahangard et al., 2019; Kalra et al., 2007; Khoury et al., 2020; King et al., 2022; Kramer et al., 2009; Madigan et al., 2023; Musana et al., 2020; Mustonen et al., 2019a; Orta et al., 2018; Robertson et al., 2023; Scharlau et al., 2017; Swales et al., 2018; van der Voorn et al., 2018; Viitaniemi et al., 2021; Wikenius et al., 2016). In cases where data on the associations between PD and HCC were strictly provided, the papers were included even when the main aim would differ from purely assessing these associations. The number of pregnant individuals included in the studies varied between 23 (Hunter et al., 2021) and 768 (Braig et al., 2015). Most studies had primarily questionnaires in assessing PD symptoms, but, in some, diagnoses or diagnostic interviews of mental health disorders were included (Broeks et al., 2021; Galbally et al., 2019). While some studies reported associations between HCC and PD measured at different timepoints from one another, many studies aimed to assess HCC and PD symptoms with corresponding timelines. Yet, a measure of long-term PD exposure was rarely chosen (Mustonen et al., 2019a; Orta et al., 2018). Two of the studies assessed both between-subject and within-subject associations between PD and HCC (King et al., 2022; Robertson et al., 2023). Corticosteroids other than cortisol were included in several studies (Freedman et al., 2021; Hunter et al., 2021; Jahangard et al., 2019; Musana et al., 2020; Robertson et al., 2023; Scharlau et al., 2017; van der Voorn et al., 2018). However, a detailed analysis of their role is beyond the scope of this thesis.

When summarizing the observations (**Table 1**), most studies (16/30) did not observe overall associations between maternal prenatal HCC and the pregnant subjects' experiences of any of the measured types of PD. However, positive associations between HCC and either all or some of the measured types of PD were reported in nearly as many studies (14/30, **Table 1**) when including both overall associations and those only observed within certain subgroups. Specifically, positive associations between prenatal HCC and PD were observed in mothers with higher socioeconomical status, with two or more adverse childhood events, and in those carrying female fetuses (Bowers et al., 2018; Bruinhof et al., 2022; and Freedman et al., 2021, respectively). In six studies (**Table 1**), some aspects of prenatal PD and HCC were negatively associated. Both negative and positive associations were reported in three studies (**Table 1**). Here, only the associations related to HCC are described even though some studies did observe differential associations between prenatal PD and hair cortisone or DHEA concentrations or with ratios of different corticosteroids to those they observed with HCC.

One study found maternal prenatal HCC to be positively associated with all types of PD they measured (Hoffman et al., 2016), while others observed more diverse findings or only null findings. Findings regarding the type of PD that would associate with HCC varied; however, general anxiety was the most replicated type of PD reported to be positively associated with prenatal HCC (Hoffman et al., 2016; Madigan et al., 2023; Orta et al., 2018; van der Voorn et al., 2018). Two studies observed a positive association between HCC and either depressive symptoms (Hoffman et al., 2016; Mustonen et al., 2019a) or perceived stress (Hoffman et al., 2016; Kalra et al., 2007). Additionally, single findings of positive associations between HCC and emotion dysregulation (Conradt et al., 2020), experiences of stress regarding relationships (Mustonen et al., 2019a), and natural disaster -related stress measures (Howells et al., 2023) were found. Three studies found prenatal HCC to negatively associate with depressive symptoms (Jahangard et al., 2019; Khoury et al., 2020; Robertson et al., 2023) and two with perceived stress (Orta et al., 2018; Robertson et al., 2023). In addition, one study found a negative association between prenatal HCC and pregnancy-related anxiety (Bowers et al., 2018) and another with dental anxiety (Viitaniemi et al., 2021).

When summarized, more studies observed positive associations between maternal prenatal HCC and PD when they had measures in later stages of pregnancy or perinatally, meaning that the hair sample was obtained soon after delivery in the perinatal period, whereas the negative associations were observed more often in early- or mid-pregnancy. On the other hand, the overall proportion of studies with end-of-pregnancy assessments was high, and when addressed in more detail, trimester-specific assessments were overall more likely to yield significant associations than those with either perinatal measures or those reporting assessments imprecisely throughout pregnancy. Studies with smaller study populations seemed to be slightly more likely to report observing either negative or positive associations, whereas studies with larger populations more often reported null findings. The study population of one study was significantly larger than that of others (n = 768 in Braig et al., 2015). This study reported null findings with HCC and several types of PD measured with questionnaires, and both HCC as well as PD assessments were measured soon after delivery. The two studies that modeled PD symptoms both momentarily and over the longer term, reported associations only when modeling the long-term prevalence of PD symptoms. The studies assessing within-person associations found the subjects' HCC to increase together with experiences of PD (Robertson et al., 2023) and to be positively associated with recent adversities (King et al., 2022).

In the meta-analysis including 29 studies (Khoury et al., 2023), maternal prenatal HCC was weakly associated with maternal prenatal PD measures (z = 0.06, 95% CI [0.02, 0.10]**), but the overall association including also the early

postnatal measures yielded non-significant results (z = 0.04, 95% CI [-0.01, 0.08], p = 0.06; Khoury et al., 2023). When compared to the updated review presented here, they share the main findings of heterogenic studies with both non-significant and significant associations. In the meta-analysis, the order of PD and HCC measures was found to be a major mediator of the potential relationship such that associations were only observed when PD measures were assessed before, not after or concurrently, with HCC (z = 0.08, 95% CI [0.03, 0.13]**). This finding further affirms the notion that, even within the same data, the means of modeling the PD symptoms are essential as to whether associations between the two measures will be observed. One key difference between the results of the meta-analysis (Khoury et al., 2023) and what is described here is that based on more visual and qualitative assessment, several studies observe positive associations between maternal prenatal HCC and PD at the end of pregnancy or with perinatal measures, whereas the meta-analysis observed associations occur only during pregnancy and not when both pre- and perinatal assessments were included. It may be that the large study with null findings significantly contributes to this meta-analysis result (Khoury et al., 2023).

**Table 1.** Overview of articles assessing the association between maternal prenatal HCC and PD organized according to the time of publication beginning with the most recent ones. Articles included in Study I are marked with an asterisk.

| | AUTHOR | STUDY POPULATION | TIMING OF HAIR SAMPLE | PD TYPES | OVERALL ASSOCIATIONS | DIRECTION OF ASSOCIATIONS | DESCRIPTION OF THE ASSOCIATIONS BETWEEN PD AND HCC |
|---|---|---|---|---|---|---|---|
| 1 | Madigan et al., 2023 | 53 | 3rd trim | stress, anxiety, depression | yes | + | + for anxiety |
| 2 | Robertson et al., 2023 | 34 (34 pregnant + 34 non-pregnant) | gw 12,26, 38 | perceived stress, depression, anxiety | yes | both + and - | - for perceived stress and depression (positive within-person coupling for HCC and PD) |
| 3 | Abdul Jafar et al., 2023 | 266 | preconception T0 + T1–T3 prenatally | depression, anxiety, sleep | no | | none (only preconception HCC associated with prenatal PD) |
| 4 | Doberdecker et al., 2023 | 149 | varying gw | depression and anxiety | no | | none |
| 5 | Howells et al., 2023 | 39 | 2nd or 3rd trimester | natural disaster-related stress measures | yes | + | + for distal segments |
| 6 | Bruinhof et al., 2022 | 97 | varying gw | pandemic-related stress measures | no | + | none (positive association observed only in subjects with high socioeconomic status) |
| 7 | King et al., 2022 | 82 | between gw 12–37 and perinatal | depressive symptoms and recent adversity | no | + | none for depression and between-person adversity measures, + for within-person adversities |
| 8 | Hunter et al., 2021 | 23 | gw 28–33 | depression | no | | none, - for the ratio of HCC/total corticosteroids and depression |
| 9 | Viitaniemi et al., 2021 | 442+176 | gw24 and perinatal | dental anxiety | yes | - | - in mid-pregnancy |

| | AUTHOR | STUDY POPULATION | TIMING OF HAIR SAMPLE | PD TYPES | OVERALL ASSOCIATIONS | DIRECTION OF ASSOCIATIONS | DESCRIPTION OF THE ASSOCIATIONS BETWEEN PD AND HCC |
|---|---|---|---|---|---|---|---|
| 10 | Broeks et al., 2021 | 45 | 6 weeks postpartum | mental health diagnosis | yes | + | + (HCC higher in patients vs controls) |
| 11 | Budnik-Przybylska et al., 2020 | 29 | na | perceived stress | no | | none |
| 12 | Freedman et al., 2020 | 181 | after gw 16 | perceived stress, depression, anxiety | no | | none; gw16 PD positively associated with 2nd trimester HCC with female fetuses |
| 13 | Khoury et al., 2020 | 51 | 3rd trim | depression | yes | - | - |
| 14 | Conradt et al., 2020 | 137 | gw 33 (26–40) | emotion dysregulation, chronic and episodic stress | yes | + | + for emotion dysregulation, none for stress |
| 15 | Caparros-Gonzales et al., 2019 | 60 | perinatal | perceived stress, perinatal distress | no | | none |
| 16 | Musana et al., 2019 | 150 | gw 22–28 | perceived stress | no | + | none (distinct cluster of stress symptoms positively associated) |
| 17 | Jahangard et al., 2019 | 48 | gw 28 | depression | yes | - | - for HCC and cortisone, + for DHEA |
| 18 | Mustonen et al., 2019 | 476 + 222 | gw 24 and perinatal | depression, anxiety, pregnancy-related anxiety, daily hassles | yes | + | + for depressive trajectories and perinatal HCC, + for relationship-worries and gw24 HCC |

| | AUTHOR | STUDY POPULATION | TIMING OF HAIR SAMPLE | PD TYPES | OVERALL ASSOCIATIONS | DIRECTION OF ASSOCIATIONS | DESCRIPTION OF THE ASSOCIATIONS BETWEEN PD AND HCC |
|---|---|---|---|---|---|---|---|
| 19 | Galbally et al., 2019 | 241 | perinatal | psychiatric diagnoses, depression, anxiety, stressful life events | no | | none |
| 20 | Orta et al., 2019 | 97 | preconception T0 + T1–T3 prenatally | perceived stress, depression, anxiety, PTSD | yes | both + and - | - for stress trajectories, + for anxiety trajectories, none for momentary assessments |
| 21 | Van der Voorn et al., 2018 | 172 | perinatal | depression, anxiety | yes | + | + for anxiety (both HCC and hair cortisone), none for depression |
| 22 | Swales et al., 2018 | 90 | gw 28 | previous and prenatal traumatic events | no | | none for prenatal events |
| 23 | Bowers et al., 2018 | 30 | n.a. | perceived stress, depression, pregnancy-related anxiety | yes | both + and - | - for pregnancy-related anxiety, none for others (positive association in mothers with > 2 ACEs) |
| 24 | Duffy et al., 2018 | 52 | 1st, 2nd, and 3rd trim | perceived stress | no | | none |
| 25 | Scharlau et al., 2017* | 45 | gw 25 and 37 | depression | no | | none for HCC, - for 2nd trim cortisone and HCC/cortisone ratio |
| 26 | Wikenius et al., 2016* | 181 | gw 25 | depression | no | | none |
| 27 | Hoffman et al., 2016b* | 90 | 16, 28 and 40 gw | perceived stress, depression, anxiety | yes | + | + for all |

| | AUTHOR | STUDY POPULATION | TIMING OF HAIR SAMPLE | PD TYPES | OVERALL ASSOCIATIONS | DIRECTION OF ASSOCIATIONS | DESCRIPTION OF THE ASSOCIATIONS BETWEEN PD AND HCC |
|---|---|---|---|---|---|---|---|
| 28 | Braig et al., 2015b* | 768 | perinatal | depression, anxiety, pregnancy-related anxiety, chronic stress | no | | none |
| 29 | Kramer et al., 2009* | 117 | perinatal | depression, anxiety, pregnancy-related anxiety | no | | none |
| 30 | Kalra et al., 2007* | 25 | 1st trim | perceived stress | yes | + | + |

Abbreviations: HCC = hair cortisol concentrations; PD = psychological distress; gw = gestational week; trim = trimester, PTSD = post-traumatic stress disorder; DHEA = dehydroepiandrosterone; and ACE = adverse childhood experiences

## 2.5 Maternal Prenatal HCC and Child Outcomes

Studies on various child outcomes related to maternal prenatal HCC are accumulating. The majority of the research is focused on the development of child hormonal and parasympathetic stress regulation (Caparros-Gonzalez et al., 2019c, 2019b; Cowell et al., 2021; Galbally et al., 2019; Hollanders et al., 2017; Hunter et al., 2021; Karlén et al., 2013; Koenig et al., 2018; Romero-Gonzalez et al., 2018; Schury et al., 2017). In addition, some studies have assessed maternal prenatal HCC together with child brain and neurocognitive outcomes (Caparros-Gonzalez et al., 2019b; Freedman et al., 2021; Puertas-Gonzalez et al., 2023; Stoye et al., 2020), some have included epigenetic and telomere outcomes (Bosquet Enlow et al., 2019; Sharma et al., 2022), birth outcomes (Caparros-Gonzalez et al., 2019c; Conradt et al., 2020; Flom et al., 2018), autoimmune conditions (Braig et al., 2017; Scherman et al., 2021), and infant gut microbiota composition (Aatsinki et al., 2020).

Studies assessing prenatal HCC in the context of child socioemotional development, temperament, and parenting (Bosquet Enlow et al., 2017; Bruinhof et al., 2022; Galbally et al., 2023b, 2023a, 2022, 2019; Khoury et al., 2020; Nyström-Hansen et al., 2019) will be presented and discussed in more detail in Chapter 2.6. Some of the observed associations depend on infant sex assigned at birth, which will be further discussed in Chapter 2.6.5.

### 2.5.1 Stress Regulation Outcomes

Some studies on maternal pre- or perinatal HCC and child HCC have assessed them cross-sectionally within two weeks after delivery (Hollanders et al., 2017; Koenig et al., 2018; Schury et al., 2017), but also longitudinal studies exist where one or more maternal prenatal hair samples were collected and their relation to offspring HCC was examined (Caparros-Gonzalez et al., 2019c, 2019b; Galbally et al., 2019; Karlén et al., 2013; Romero-Gonzalez et al., 2018). Some studies have assessed neonatal HCC (Caparros-Gonzalez et al., 2019c, 2019b; Romero-Gonzalez et al., 2018); one continued follow-up until 12 months with both hair and saliva measurements from the children (Galbally et al., 2019); and one until the child's age of eight years with HCC measurements at one, three, five, and eight years (Karlén et al., 2013). The subjects in the studies by Caparros-Gonzalez et al. (2019b, 2019a) were drawn from the same population, thus their findings are discussed here as one independent study.

Three out of seven independent studies reported positive associations between maternal prenatal and child postnatal HCC (Hollanders et al., 2017; Karlén et al., 2013; Koenig et al., 2018). The study by Karlén et al (2013) observed maternal prenatal HCC to be associated with child HCC at a child's ages of one and three years, but not at five or at eight years. However, a negative association between maternal early pregnancy HCC and neonatal HCC has also been reported (Romero-

Gonzalez et al., 2018). Three out of seven independent studies did not observe an association between maternal pre- or perinatal HCC and infant HCC (Caparros-Gonzalez et al., 2019c, 2019b; Galbally et al., 2019; Schury et al., 2017). Associations were more likely to be observed in cross-sectional than in longitudinal studies. However, although no association was observed between maternal prenatal and child HCC at 12 months (Galbally et al., 2019), elevated maternal third trimester HCC was associated with blunted infant salivary cortisol reactivity in response to a parent separation and reunion test. In addition, two studies (Koenig et al., 2018; Schury et al., 2017) included measures of steroids other than cortisol with contrasting findings; one only found an association between maternal and child HCC and not between cortisone or DHEA concentrations (Koenig et al., 2018), whereas only DHEA measures, as opposed to cortisol, were associated within the dyads in the other study (Schury et al., 2017).

Two studies assessed non-hormonal stress reactivity of the offspring in relation to maternal prenatal HCC (Cowell et al., 2021; Hunter et al., 2021) with the Cowell et al. study showing more adaptive outcomes in relation to lower and the Hunter et al. study with higher long-term steroid levels. In these studies, elevated end-of-pregnancy cortisol concentrations were associated with dampened parasympathetic responsivity as measured by infant respiratory and cardiac responses during the still-face paradigm in girls at 6 months (Cowell et al., 2021). However, elevated maternal mid-pregnancy steroid hormone ratios were observed to be associated with higher fetal heart rate variability which is considered to be related to more maturation of the fetus (Hunter et al., 2021).

### 2.5.2 The Brain and Neurocognitive Outcomes

Based on the current literature, fetal vulnerability to long-term exposure to altered levels of maternal prenatal cortisol seems to vary according to the timing of the exposure and the sex of the fetus. Increased maternal end-of-pregnancy HCC were associated with neonatal amygdala microstructure alterations in boys and structural connectivity changes in girls at a term-equivalent age (Stoye et al., 2020). In addition, elevated maternal mid-pregnancy HCC were associated with decreased cerebral inhibitory neurocircuits only in girls at one month of age (Freedman et al., 2021).

Two studies have assessed the associations between maternal prenatal HCC and child neurodevelopment during the first year of life with both of them observing them to be related (Caparros-Gonzalez et al., 2019b; Mariño-Narvaez et al., 2023). In a recent study by Mariño-Narvaez et al. (2023), increased maternal HCC throughout the prenatal period was associated with accelerated motor and language development of the offspring at 12 months. In addition, both maternal and infant

HCC were reported to associate with infant cognitive and motor development at six months (Caparros-Gonzalez et al., 2019b). Of note, in this study, the alterations in cortisol levels at different trimesters seemed to have opposite effects on child development. This study showed elevated HCC at early- and mid-pregnancy to be associated with decelerated motor development, whereas elevated end-of-pregnancy HCC associated with better motor and cognitive development. Elevated infant HCC at six months associated with decelerated motor development. As mentioned previously, no association was observed between infant HCC and maternal HCC throughout pregnancy (Caparros-Gonzalez et al., 2019b).

In a randomized controlled study including 24+24 pregnant subjects, a prenatal cognitive behavioral therapy intervention that reduced both maternal PD and maternal HCC was associated with both decreased infant HCC as well as improved cognitive and motor development at six months (Puertas-Gonzalez et al., 2023). However, null findings have also been reported between maternal prenatal HCC and neonatal neurodevelopmental measures of attention and arousal (Conradt et al., 2020).

### 2.5.3 Epigenetic and Telomere Outcomes

An epigenome-wide association study found biomarkers such as maternal end-of-pregnancy HCC to obtain stronger associations with newborn DNA methylation in areas related to neuronal, immune and endocrine homeostasis in comparison to the associations observed related to maternal prenatal psychosocial stress measures (Sharma et al., 2022). In addition, maternal end-of-pregnancy HCC were associated with increased infant telomere length in girls (Bosquet Enlow et al., 2019).

### 2.5.4 Birth Outcomes

Unlike maternal prenatal PD, maternal prenatal HCC are not as consistently associated with different birth outcomes (Ding et al., 2021). Two out of three studies did not observe associations between maternal prenatal HCC and measures of neonatal growth or gestational age at birth when all subjects were included in the assessments (Conradt et al., 2020; Flom et al., 2018). The third study did not report overall associations but observed varying associations between maternal prenatal HCC and child birth length and head circumference depending on whether the pregnancy was conceived naturally or with assisted reproductive technologies (Caparros-Gonzalez et al., 2019c). Overall, it suggested higher HCC to mainly associate with slower neonatal growth in either group (Caparros-Gonzalez et al., 2019c). Only in boys, one study observed maternal prenatal HCC to mediate the

association between maternal lifetime trauma and the child's birth weight (Flom et al., 2018).

### 2.5.5 Other Child Health Outcomes

Studies assessing child somatic health in relation to maternal prenatal HCC are few, and, for instance, no studies have assessed the associations between maternal prenatal HCC and child postnatal growth despite metabolic factors being one of the first outcomes to be associated with the fetal environment (Barker et al., 1989). Some studies have assessed maternal prenatal HCC' associations to child autoimmune diseases. Maternal end-of-pregnancy HCC were not associated with atopic dermatitis before the age of two years (Braig et al., 2017), however elevated maternal mid-pregnancy HCC were associated with an increased prevalence of childhood wheeze in a study population exposed to socioeconomical adversity and maternal smoking (Scherman et al., 2021). In addition, maternal end-of-pregnancy HCC have been associated with 2.5-month-old infants' gut microbiota composition, and elevated maternal HCC associated with decreased abundancies of potentially health promoting bacteria (Aatsinki et al., 2020).

## 2.6 Child Socioemotional Problems

Child socioemotional development is a concept that defines social and emotional capacity, behavioral problems, emotional expression and self-regulation of the child (Campbell et al., 2016). Both socioemotional problems and competencies are conceptualized and can be measured (Campbell et al., 2016). Socioemotional problems can be dichotomized into internalizing/emotional problems and externalizing/conduct problems, however, dysregulatory problems such as hyperactivity and inattention, and difficulties in social interaction such as peer relationship problems or autism-like behaviors are also important to acknowledge (Briggs-Gowan and Carter, 2006; Goodman, 1997). The competencies include, for instance, prosocial behavior, such as a willingness to share and help others, and functional means of interacting with peers and caregivers (Eisenberg et al., 2015). In this study, the focus is on the problems in socioemotional development and thus, further discussion of the competencies is beyond the scope of this thesis.

Socioemotional problems are shaped by the combination of the child's innate temperament and individual capacities for self-regulation and self-expression together with the contribution of the environment in modifying these abilities and reactions. Temperament describes, according to the conceptualization by Mary K. Rothbart (Rothbart, 1981), the child's tendencies regarding emotional reactivity and self-regulation. The smaller the baby, the stronger the role that temperament plays

in their behavioral reactions. Nevertheless, adaptation to environmental cues begins already during the first months of life (Jansen et al., 2009). Thus, assessing socioemotional development or socioemotional problems instead of purely temperamental traits from early on gives a wider perspective of the child's risks for future psychopathologies.

The range of normal behavior in small children is wide. It is common and not a sign of psychopathology if a two-year-old is having tantrums or hits sometimes. Two-year-old children can be shy of strangers, and changes in the environment can markedly alter their behavior. Individually predicting the trajectories and persistence of problems in regulation or behavior in toddlerhood remains challenging, however, especially the children whose difficulties exceed diagnostic thresholds at the age of 1.5 to 3 years are up to 37–50% likely to persist having significant difficulties in follow-up during the preschool years (Lavigne et al., 1998; Mathiesen and Sanson, 2000). In addition, there is moderate longitudinal stability in parent-rated questionnaires of toddler-aged children's socioemotional development (Briggs-Gowan and Carter, 1998; Keenan et al., 1998). The hyperactivity-inattentive problems seem to persist also over a longer follow-up until the age of 12 years, whereas the predictive value for emotional symptoms was lower (Nielsen et al., 2019). This is expected, as the course of neuropsychiatric disorders is developmental, and difficulties are typically observed already during the early years in contrast to the incidence of affective problems peaking at adolescence (Khanal et al., 2022). In addition, the development of emotional, in comparison to neuropsychiatric, symptoms are more often heterotypic. Externalizing symptoms during toddlerhood may present as depressive symptoms during later childhood and adolescence, and childhood fears or depressive symptoms may later translate into anxiety. Comorbidities across different symptom modalities are common across age groups and increase the risk for the persistence of problems (Lavigne et al., 1998).

One factor that significantly contributes to the incidence of socioemotional problems during childhood and adolescence is the biological sex assigned at birth. Externalizing problems are observed more often in male offspring and internalizing in females (Martel, 2013). Overall psychopathology in childhood is more prevalent among males than in females, however the opposite is true in adolescence when the prevalence of anxiety disorders and depression sharply increase in girls (Healy et al., 2022; Khanal et al., 2022).

### 2.6.1 Measuring Child Socioemotional Problems

Child socioemotional problems are often assessed by validated parent-rated questionnaires that assess a range of behavioral symptoms, such as the (Brief) Infant-Toddler Socioemotional Assessment ([B]ITSEA [Briggs-Gowan and Carter, 2006,

1998], the Strengths and Difficulties Questionnaire [SDQ; Goodman, 1997], and Child Behavior Checklist [CBCL; Achenbach and Edelbrock, 1983]). Some studies use diagnostic interviews such as the Preschool Age Psychiatric Assessment (PAPA; Egger et al., 2006). As the studies showing associations between maternal prenatal HCC and child socioemotional problems are scarce, the assessment of infant temperament is included as an outcome measure in this part of the thesis. That can be assessed with parent-rated questionnaires such as the revised Infant Behavior Questionnaire (IBQ-R; Gartstein and Rothbart, 2003).

As the behavioral phenotypes in small children are versatile and fluctuating, the psychometric properties of most of these questionnaires are affected. In addition, it is necessary to note the potential bias related to parental observations and interpretations of their child's behavior (Najman et al., 2001). Nevertheless, to learn more about the factors contributing to the perseverance or discontinuation of the symptoms throughout childhood and later in life and assessing socioemotional problems longitudinally beginning from an early age is essential.

### 2.6.2 Maternal Prenatal PD and Child Socioemotional Problems

Meta-analyses have shown that maternal prenatal PD associates with worse child socioemotional and behavioral outcomes including social and emotional competence, temperament, internalizing and externalizing behavioral problems, attention, sleep problems, crying, colic, and autistic-like symptoms or traits (Lafortune et al., 2021; Madigan et al., 2018; Phua et al., 2023). In a meta-analysis related to natural disasters during pregnancy, detrimental socio-emotional and behavioral outcomes were consistently observed regardless of the type of maternal prenatal natural-disaster-related stress (Lafortune et al., 2021), and both the overall prenatal PD symptoms as well as prenatal depressive and anxiety symptoms assessed separately were positively associated with adverse child socioemotional outcomes (Madigan et al., 2018; Phua et al., 2023). The trajectories of increased maternal anxiety and depressive symptoms have also been associated with more child socioemotional problems in the FinnBrain Cohort (Korja et al., 2024). In addition, maternal PD inversely associated with positive child outcomes, whereas better maternal prenatal mental health only associated with more advantageous child outcomes and not with the amount of problems in child socioemotional development (Phua et al., 2023). Ten studies included in the most recent meta-analysis had reported gender-specific effects of maternal PD on adverse child outcomes, however the total effects between sexes did not differ (Phua et al., 2023).

When compared to previous studies on offspring outcomes related to exposure to altered maternal prenatal long-term cortisol levels, data on the associations

between maternal prenatal PD and altered offspring brain structure, function, and connectivity are more convincing (for a meta-analysis and reviews, see Entringer et al., 2015; Lafortune et al., 2021; Lautarescu et al., 2020a; van den Bergh et al., 2020, 2018). This is significant also in the context of observed behavioral outcomes, as it is the fetal brain and stress regulation systems that are potentially affected during the prenatal period. The most consistently replicated neuroimaging findings after prenatal PD exposure are cortical thinning and decreased grey matter volume in frontal and temporal lobes (e.g., Davis et al., 2020; El Marroun et al., 2016; Lebel et al., 2016; Sandman et al., 2015). Some of the studies also link their findings to alterations in child behavior (Davis et al., 2020; Sandman et al., 2015). In addition to volume changes, diffusion tensor imaging and functional magnetic resonance imaging (MRI) studies have reported changes in white matter circuitry in limbic and frontal regions (Dean et al., 2018; Demers et al., 2021; Lautarescu et al., 2020b; Lean et al., 2022) and altered functional connectivity in limbic regions (e.g., Posner et al., 2016; Soe et al., 2018) after exposure to maternal prenatal stress.

Recently, evidence on the significance of prenatal-stress-related fetal brain alterations has increased as studies have used fetal brain imaging and connected the observed findings to infant behavioral measures. One longitudinal study utilizing fetal MRI and infant neurodevelopmental assessments at 18 months showed that fetal left hippocampal volume mediated the observed negative association between maternal prenatal stress and infant cognitive performance (Wu et al., 2022). Moreover, in the same study, infant socioemotional behavior was observed to be linked with changes in fetal cortical folding (Wu et al., 2022). In another recent study, maternal PD symptoms and salivary cortisol levels were shown to be associated with fetal hippocampal connectivity (Hendrix et al., 2022).

### 2.6.3 Maternal Prenatal HCC and Child Socioemotional Problems

Associations between maternal prenatal HCC and features of child socioemotional development, symptoms, or the incidence of child psychiatric disorders and temperamental traits have emerged during the past five years (Bosquet Enlow et al., 2017; Bruinhof et al., 2022; Galbally et al., 2023b, 2022, 2019). In addition, some studies have assessed the associations of maternal prenatal HCC with maternal parenting styles during infancy (Galbally et al., 2023a; Khoury et al., 2020; Nyström-Hansen et al., 2019). Even though the number of studies including these measures is robust, none of the studies have focused specifically on the associations between maternal prenatal HCC and child socioemotional problems. Half of these studies are based on an Australian selected prospective birth cohort study, The Mercy Pregnancy and Emotional Well-being Study, that is enriched with individuals having

maternal depression. The study populations for different study questions described in this Chapter vary between 170 and 241 mother-child-dyads. HCC was analyzed from a 3-cm segment of maternal hair donated on the first day post-delivery. Child socioemotional development at twelve months was measured with BITSEA and childhood anxiety disorders at four years were assessed with PAPA and CBCL.

Neither the incidence of one or more childhood anxiety disorders with 41.8% of the children in the cohort nor the anxiety symptoms reported in CBCL were directly correlated with maternal prenatal HCC (Galbally et al., 2023b, 2022). The observations related to the mediating role of maternal prenatal cortisol levels, as indicated by HCC, are further described in Chapter 2.6.4.

In addition, a study focusing on transgenerational associations in HPA axis functioning provided a tentative indication of potential links between maternal and offspring HPA axis functioning and child socioemotional development (Galbally et al., 2019). Increased maternal end-of-pregnancy HCC were associated with dampened salivary cortisol reactivity in the parental separation and reunion test, but not child HCC, at 12 months (see also Chapter 2.5.1). Lower infant salivary reactivity was observed to associate with concurrent elevated externalizing symptoms as measured with BITSEA. No reports of potential direct associations between maternal prenatal HCC and infant socioemotional problems were included. Nevertheless, the observation that maternal perinatal HCC associates with dampened infant cortisol reactivity, which is further associated with child externalizing symptoms does suggest there to be a mechanistic pathway warranting further studies. In a later publication by the same group, however, the salivary cortisol reactivity did not mediate the association between maternal prenatal PD or HCC measurements and observed anxiety disorders at four years of age (Galbally et al., 2023b).

In addition to the studies conducted in The Mercy Pregnancy and Emotional Well-being Study cohort, two other studies have included both maternal prenatal HCC and infant temperament measures (Bosquet Enlow et al., 2017; Bruinhof et al., 2022). The Bosquet Enlow et al. study focused primarily on the associations between maternal history of childhood trauma and infant temperament and included maternal prenatal HCC donated within a week after delivery as a moderating factor (n = 194 mother-infant dyads) and the Bruinhof et al. study on the effects of the Covid-19-pandemic with 97 women at different stages of the perinatal period during the home lockdowns of the Covid-19-pandemic. Although only referred to in a table and not separately discussed in the paper, there seemed to be a direct unadjusted negative correlation between maternal prenatal HCC and the infant temperamental trait of Falling Reactivity indicating that, according to maternal observations, children of mothers with increased cortisol levels at the end-of-pregnancy were slower to recover from stress (Bosquet Enlow et al., 2017). In contrast, maternal HCC were not associated with maternal reports of infant negative temperament or with

regulative and orienting behavior (IBQ-R short assessed at six months; Bruinhof et al., 2022).

Some studies investigated whether maternal prenatal HCC is related to maternal parenting in infancy reporting associations between maternal prenatal HCC and disrupted maternal interaction styles observed with the Still-Face Paradigm (Khoury et al., 2020; Nyström-Hansen et al., 2019) and the Strange Situation procedure (Galbally et al., 2023a). In these studies, no measures of child behavior were included, thus for the context of this thesis, this field of research provides an additional understanding of the postnatal factors that potentially modify the possible effects of maternal prenatal cortisol levels on child outcomes (see Chapters 2.6.4 and 2.6.6).

**Table 2.** Overview of articles on the associations between maternal prenatal HCC and child socioemotional problems.

| | AUTHOR | STUDY POPULATION | HAIR SAMPLE TAKEN | CHILD OUTCOME | DIRECT ASSOCIATIONS | INTERACTION BETWEEN HCC x PD ON CHILD OUTCOME | DESCRIPTION OF THE ASSOCIATIONS BETWEEN MATERNAL HCC AND CHILD SOCIOEMOTIONAL PROBLEM |
|---|---|---|---|---|---|---|---|
| 1 | Galbally et al., 2023b | 190 dyads | perinatal | PAPA, CBCL | no | yes | Attachment style moderated the association between prenatal HCC and offspring anxiety disorders; elevated HCC + organized attachment = increased risk, whereas elevated HCC + disorganized attachment = decreased risk |
| 2 | Bruinhof et al., 2022 | 97 dyads | different stages of the perinatal period | IBQ-R short | no | n.a. | Maternal HCC not associated with infant negative temperament nor with regulative and orienting behavior |
| 3 | Galbally et al., 2022 | 170 dyads | perinatal | PAPA, CBCL | no | yes | More childhood anxiety symptoms with elevated end-of-pregnancy HCC and increased parenting stress at 6 months post-delivery |
| 4 | Galbally et al., 2019 | 241 dyads | perinatal | BITSEA | n.a. | n.a. | Elevated maternal HCC associated with blunted infant salivary cortisol reactivity at 12months, which was further associated with increased concurrent BITSEA Externalizing symptoms |
| 5 | Bosquet Enlow et al., 2017 | 194 dyads | perinatal | IBQ-R | yes | yes | Increased maternal HCC associated with slower recovery from stress. Increased infant negative affectivity when maternal HCC was elevated and a maternal history of childhood trauma was present. |

Abbreviations: HCC = hair cortisol concentrations; PAPA = Preschool Age Psychiatric Assessment; CBCL = Child Behavior Checklist; IBQ-R = Infant Behavioral Questionnaire -Revised; and BITSEA = Brief Infant-Toddler Socio-Emotional Assessment

### 2.6.4 The Interplay between Maternal Prenatal HCC and PD

As noted, only few studies have assessed the associations between maternal prenatal HCC and child socioemotional outcomes and, apart from the side notion of a negative association between maternal prenatal HCC and the infant temperamental trait Falling Reactivity related to the pace of recovering from stress, only null findings have been reported (Bosquet Enlow et al., 2017; Bruinhof et al., 2022; Galbally et al., 2023b, 2022, 2019). However, the results change slightly when other aspects of maternal well-being and parenting are considered. Maternal history of childhood trauma was observed to be associated with higher infant negative affectivity, i.e., IBQ-R assessed at six months, only when the fetus was also exposed to higher levels of maternal cortisol levels (Bosquet Enlow et al., 2017). In addition, the children of mothers with increased prenatal HCC were more likely to experience anxiety symptoms when maternal parenting stress at six months was also increased (Galbally et al., 2022). Within the same cohort, a positive association between maternal end-of-pregnancy HCC and the prevalence of childhood anxiety disorders at four years was observed in children with organized attachment, whereas children with disorganized attachment styles had a higher risk of anxiety disorders when maternal prenatal HCC was lower (Galbally et al., 2023b).

With regards to maternal attachment styles, an interaction between maternal mid-to-late pregnancy HCC measured at gw 34 and maternal prenatal depressive symptoms was observed, as when both depressive symptoms and HCC were increased, maternal attachment style at four months postnatally was more likely to be withdrawing, and more inappropriate/intrusive attachment behaviors were observed with elevated HCC and decreased depressive symptoms (Khoury et al., 2020). In addition, maternal perinatal HCC has been reported to mediate the association between maternal psychopathology and a disrupted interaction with four-month-old infants (Nyström-Hansen et al., 2019).

When addressing the joint effects of maternal prenatal HPA axis functioning and PD on child socioemotional symptoms, placental HPA axis-related epigenetic changes seem to mediate the associations between maternal prenatal PD and reported child aggression and anxiety symptoms as well as altered child hair glucocorticoids at 3–4 years (Nomura et al., 2021). This study sheds light on the understanding of the mechanisms potentially transmitting the effects of maternal PD to the fetus and suggests that there may be significant HPA axis-related factors involved even without observations of altered maternal prenatal HCC.

### 2.6.5 Sex Differences

An increasing number of studies have reported sex-specific associations of maternal prenatal PD x child sex – interactions on a variety of offspring outcomes, and these

suggest that the role of a child's biological sex should always be considered in studies assessing the programming effects of maternal prenatal PD (Glover and Hill, 2012; Sutherland and Brunwasser, 2018). Yet, the findings are inconsistent. It seems the results on sex-specific associations between maternal prenatal PD and offspring neuroimaging findings as well as infant temperament have been most robust (Sutherland and Brunwasser, 2018).

Similarly, considering the consensus on offspring outcomes that relate to maternal prenatal HCC, few conclusions can be drawn on the sex-specific vulnerability to altered maternal prenatal cortisol levels. There are, however, some recent studies that shed light on the subject. Increased early- and mid-pregnancy HCC have been associated with better performance in neurocognitive assessments in 12-month-old girls (Mariño-Narvaez et al., 2023). On the other hand, increased maternal mid-pregnancy HCC were also associated with decreased cerebral neurocircuits in girls, a finding that was discussed to increase risk for later psychopathology (Freedman et al., 2021). Elevated maternal end-of-pregnancy HCC have been associated with neonatal amygdala microstructure alterations in boys and structural connectivity changes in girls (Stoye et al., 2020) and with dampened parasympathetic responsivity to stress in girls at 6 months (Cowell et al., 2021).

### 2.6.6 Postnatal Factors Affecting Child Socioemotional Problems

The rapid development and maturation of the offspring continues after delivery and the critical importance of the first 1000 days, from conception until the second birthday, is widely recognized and affects policies, for example, through the work by UNICEF (Cusick and Georgieff, 2013). In addition, significant developmental windows with increased plasticity recur during childhood and adolescence (Bundy et al., 2018).

As mentioned before, the emotional regulation and behavioral responses of the child develop postnatally by the influence of the child's individual qualities and the environment. The most critical impact for the development and well-being of a child depends on their caregivers, of whom, the infant is dependent (Winnicott, 1964). Evolutionarily, the key to a child surviving is keeping the caregivers in proximity, thus the child is programmed to behave in manners they assume would enhance parents' attachment and attention. That includes increasing the behaviors that get rewarded with most attention, mirroring the ways of expressing emotions, and reflecting on the means of self-regulation from the ones closest to them (Reck et al., 2023).

In both clinical use and research purposes, the attachment organization style is one means to describe the parent-child relationship (Bowlby, 1982). It depicts the predictability and sensitivity of parental reactions in response to the child's needs. Securely attached children can rely on sensitive and timely responses and help from

their parents, which strengthens the child's functional emotion regulation strategies. Insecurely attached children learn to predict their parents' reactions and thus develop strategies to ensure the parents attention and their own safety, however the strategies may be partially maladaptive, i.e., either avoidant or ambivalent patterns. A disorganized attachment style depicts situations where parental reactions are harmful and unpredictable for the child, which prohibits the child from adapting successful strategies for emotion regulation and poses higher risks for later psychopathology.

A parental history of close relationships often shapes intrinsic parenting models. Additively, concurrent mental health and environmental factors affecting the stressfulness of daily life, such as financial instability, employment status, lack of a family's supportive network, health worries, or experiences of discrimination, can significantly affect momentary and long-lasting parenting capacities. The accumulation of risk factors is common. In addition, the society's demands for any individuals, including parents, are high, and the needs for child mental health services have increased (Patel et al., 2007; Polanczyk et al., 2015). On the other hand, parental resilience factors, i.e., their abilities to cope with and adapt to distress, predictable and structured daily routines, a strong supportive network, and other sensitive adults close to the child can markedly contribute to the beneficial socioemotional development of the child. Many of these factors may be received through adequately resourced out-of-home daycare.

## 2.7 Summary and Gaps in the Literature

The consequences of maternal prenatal PD on child socioemotional problems are scientifically and clinically highly relevant. Maternal HCC are increasingly used as a proxy measure for long-term systemic cortisol levels, and the literature on its associations with maternal prenatal PD is extensive. However, as the interrelations between maternal experiences of PD and their biological responses, such as the alterations in the HPA axis activity are all but straightforward, few definitive conclusions can be drawn, and further studies with multidisciplinary approaches and careful methodological considerations are needed.

Studies on child outcomes related to maternal prenatal HCC are scarce and include multiple important gaps for future studies. The overall associations between maternal prenatal HCC and child socioemotional development have not been the main study aim in any previous study, thus far, and the preliminary results found in studies assessing factors related to these uncertainties offer more additional questions than are answered. Specifically, studies assessing the associations between maternal prenatal HCC and child symptoms related to socioemotional development are needed and it is essential to address the roles of maternal prenatal PD symptoms and child sex on the observed results.

# 3 Aims

The main aims of this study were to broaden the knowledge on the prenatal associations between maternal systemic long-term cortisol levels as measured by HCC and symptoms of psychological distress, and to assess whether they are linked to offspring socioemotional development. Specifically, our aims were:

1. To systematically review the current literature on prenatal associations between maternal HCC and PD (Study I).
2. To study the associations between HCC and different types and timelines of PD symptoms in expecting mothers of the FinnBrain Birth Cohort Study (Study II).
3. To assess the associations between maternal prenatal HCC and child socioemotional problems in early childhood (Study III). Further, we aimed to assess whether the potential associations were sex-specific or related to the interaction between concurrent maternal depressive symptoms and HCC.
4. To explore child HCC as a potential mediator between maternal prenatal HCC and child socioemotional problems to further understand the link between maternal and child distress symptoms, and the role of long-term cortisol levels as a mechanistic pathway between them (unpublished data).

Our main hypothesis was that maternal prenatal psychological distress may alter the functioning of maternal stress regulation systems leading to alterations in maternal prenatal systemic long-term cortisol levels. We further hypothesized the fetal exposure to altered maternal cortisol levels to potentially steer the programming of the child's stress regulation systems and to increase the risk of socioemotional problems in early childhood.

# 4 Materials and Methods

## 4.1 Systematic Review (Study I)

Study I was a systematic review of previous research on the associations between maternal prenatal PD and HCC conducted according to the then existing PRISMA 2009 guidelines. A systematic literature search utilizing several databases (PubMed, Ovid MEDLINE®, Embase, PsycINFO, WorldCat and WEB of SCIENCE) was conducted by the author of this thesis to identify all applicable studies. A consensus method together with the second and third author regarding all equivocal studies was applied. All literature searches were last updated on 26.12.2017. The searches were conducted using the following search term combination: (hair cortisol OR (hair AND cortisol)) AND (pregnancy OR prenatal OR antenatal OR gestational). The inclusion criteria were 1. original papers published in peer review journals 2. in human 3. written in English 4. assessing maternal HCC and 5. maternal PD, and 6. being prenatal. Prenatal PD was conceptualized to comprise symptoms of perceived stress, depression, anxiety, pregnancy-related stress or anxiety, and/or exposure to stressful or traumatic events during pregnancy. Studies only assessing maternal childhood or lifetime exposure to adverse events were not included. The aim of the systematic review was to assess the associations between maternal prenatal PD and HCC, and only studies with systematic reports of their associations could be included, thus studies assessing these factors only as mediators or covariates for other main outcomes were excluded. Presentation abstracts and case reports were also excluded.

The identified papers were first screened by titles and abstracts and duplicates were removed. Full-text reviews were conducted to verify whether the papers met the inclusion and exclusion criteria. Further, eligible papers were searched by a reference search of the identified papers.

As the key research questions and methods of the identified papers were heterogeneous, statistical analyses of the data were not feasible. Thus, a critical review was carried out. Data assessing the associations between maternal prenatal HCC and different types of prenatal PD were gathered together with relevant data on study characteristics (i.e., author, year of publication, study population, hair sample characteristics, analysis method, PD measurement, association between PD and HCC, HCC mean and range, and main findings).

## 4.2 Study Design and Participants in Studies II and III

### 4.2.1 The FinnBrain Birth Cohort Study

The FinnBrain Birth Cohort Study (www.finnbrain.fi; Karlsson et al., 2018) is an extensive population-based longitudinal cohort study that aims to identify individual, familial and environmental factors that influence child neurodevelopment and risks for psychiatric and somatic illnesses. Multidisciplinary data have been gathered from pregnancy onwards and data collection will continue throughout the lifespan of the offspring to add to the understanding on the diverse and complex trajectories in child development when affected by maternal prenatal distress.

The study population of the Cohort comprises consecutive pregnant women and their partners recruited following a normal screening result of a routine first trimester ultrasound between December 2011 and April 2015 at three sites in the Turku region and the Åland Islands. An inclusion criterion was sufficient knowledge of Finnish or Swedish, and there were no exclusion criteria. Subjects gave their written informed consent. The number of study subjects agreeing to participate in the Cohort was 3808 mothers, 2623 partners, and 3837 children (including 29 twin pairs) in total (Karlsson et al., 2018). The participation rate was 66% of those informed about the project. The attrition rate during pregnancy was 8.1% (Karlsson et al., 2018). The research questionnaires or online links to the questionnaires were sent to the participants.

### 4.2.2 Study Population of the Studies II and III

The subpopulations included in the present study were drawn from the FinnBrain Birth Cohort subjects (see **Figure 2** for a flow chart of the Study Design). Both Studies II and III used two separate study populations including participants donating an HCC sample either at a mid-pregnancy study visit at gw 24 (HCC1) or at the delivery ward 1 to 3 days after delivery (HCC2). In Study II, participants were included when self-reports of maternal prenatal PD symptoms were also provided (questionnaires from gw 14 and 24 with regards to HCC1, and from 24 and 34 with regards to HCC2, see **Figure 2** and Chapter 4.4 for details). In Study III, mother-child dyads with data on maternal prenatal HCC (HCC1 for study population 1 and HCC2 for study population 2) and maternal reports on child socioemotional development at 2 and/or 5 years were included (**Figure 2** and Chapter 4.5).

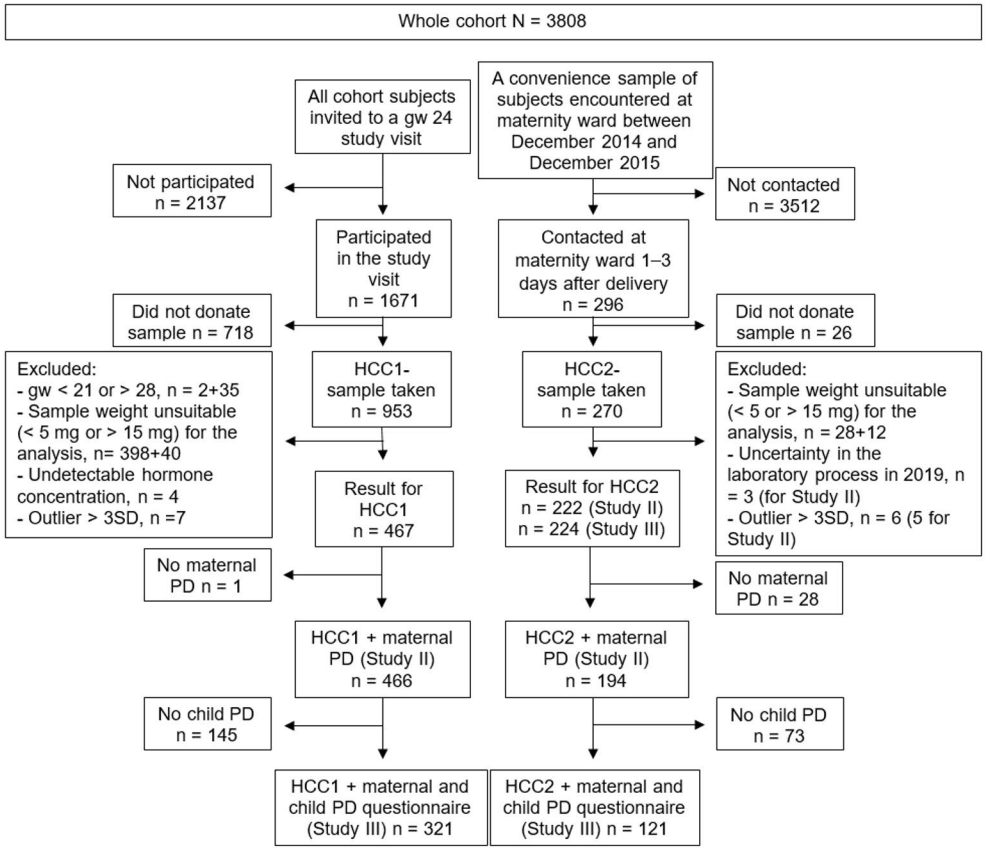


**Figure 2**. The flow chart of the study design. HCC1 and HCC2 sample collections were independent of each other, thus, the same individuals could have donated both, either or neither of them (n = 94 mothers belong to the final HCC1 and HCC2 study populations in Study II and n = 65 in Study III). Abbreviations: BITSEA = Brief Infant-Toddler Social and Emotional Assessment; SDQ = Strengths and Difficulties Questionnaire; PD = psychological distress; EPDS = the Edinburgh Postnatal Depression Scale; SCL-90 = the Symptom Checklist -90; PRAQ-R2 = the Pregnancy-related Anxiety Questionnaire -Revised2; gw = gestational week; HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; HCC2 = HCC measured 1–3 days after delivery.

## 4.3 Hair Cortisol Concentrations

### 4.3.1 Sample Collection

Maternal mid-pregnancy hair samples were collected by a study nurse at a study visit approximately at gw 24. All Cohort parents from March 2013 onwards were invited to the study visit with the aim to collect different biological samples by study nurses, and the participation rate was 44% (n = 1671) of the Cohort mothers (Karlsson et al., 2018). Fifty-seven percent of the subjects (n = 953) participating in the visit agreed

to donate a hair sample. The end-of-pregnancy hair samples were collected at the delivery wards 1 to 3 days after delivery by the author of this thesis between December 2014 and December 2015. All women present at the delivery wards at the times of sample collection were contacted (n = 296), and 270 of them (91%) agreed to donate a hair sample. In addition to donating the sample, the subjects filled in a hair sample-related questionnaire at the time of the sampling including questions for hair color, washing habits, and potential hair dyes or chemical treatments. Other factors potentially affecting systemic cortisol levels, such as medications and body mass index, were gathered from questionnaires and register data (please see Chapter 4.6 for details).

In addition to maternal prenatal hair samples used in the original publications included in the thesis, child hair samples were collected at 30 months ($HCC_{30mo}$, n = 327) and 5 years ($HCC_{5y}$, n = 531) and they were used in a complimentary mediation analyses to comprehensively account for the main study questions of the thesis. Child hair samples were collected at neuropsychological study visits by researchers and study nurses from children following both child and parental consent except for 17 of the $HCC_{5y}$ samples that were collected by their parents at home according to separate instructions related to a Covid-19-pandemic substudy. A total of 474 study visits were carried out at the child age of 30 months and 545 at the child age of 5 years. Thus, hair samples were donated by 69% of the 30-month-olds and 94% of the 5-year-old children participating in the visits. The number of dyads with all relevant maternal and child HCC, and PD data were 71 for HCC1, $HCC_{30mo}$ and BITSEA; 92 for HCC1, $HCC_{5y}$ and SDQ; 33 for HCC2, $HCC_{30mo}$, and BITSEA; and 39 for HCC2, $HCC_{5y}$ and SDQ.

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 of hair and at least a 3–5-cm long sample of each hair was cut as close to the scalp as possible. Hair samples were stored in foil in a dry place protected from light according to good research practice, Finnish legislation and data protection until the analyses. For maternal prenatal samples, a 5-cm segment was used to show the accumulated systemic cortisol levels of the past five months of pregnancy. For postnatal samples, a 3-cm segment was used to cover the past three months.

## 4.3.2 Analysis Protocol

### 4.3.2.1 Maternal Prenatal HCC

Hair cortisol extraction and analysis of the maternal prenatal samples were performed as a research collaboration in the laboratory of Prof. Nuno Sousa at the University of Minho, Portugal according to a protocol adapted from Davenport

(Davenport et al., 2006). Hair strands were measured and the most proximal 5 cm of hair was used as the analyzed hair segment. Five-centimeter segments were selected to be able to show the cortisol levels over the whole prenatal period with two samples and to ensure an adequate sample weight for reliable analyses, as some samples were slightly lighter than expected. Hair segments were then washed in isopropanol for 3 min, and after decanting, this wash cycle was repeated two more times. Then, hair was allowed to dry under a fume in a fume hood. Hair was finely minced using surgical scissors, and 5–15 mg of hair was transferred to a cryovial. 1.5 ml of methanol was added to each sample and incubated at 55ºC for 24 h. The samples were then centrifuged at 10000 rpm for 2 minutes and the supernatant was transferred to a new vial. 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 in duplicate following the manufacturer's procedure (IBL International Cortisol Saliva ELISA).

#### 4.3.2.2 Child HCC

Corticosteroid extraction and analysis of the child hair samples were performed in the Technical University of Dresden, Germany, followed by a protocol described in detail previously by Gao et al. (2013). Briefly, hair strands were washed by 2.5 ml isopropanol for 3 min and allowed to dry in a fume hood for at least 12 h. 7.5 mg of nonpulverized whole hair was weighed out, 1.8 ml of methanol, and 50 µL of internal standard was added. The sample was then incubated for 18 h at room temperature for steroid extraction. After centrifugation, 10 ml of the clear supernatant was taken and the alcohol was evaporated at 65ºC under a constant stream of nitrogen until completely dried. The dry residue was resuspended by adding 250 µL of distilled water, and 200 µL of the suspension was used for LC–MS/MS analysis for cortisol, cortisone, and DHEA concentrations.

Seventy of the child $HCC_{30mo}$ samples were analyzed with ELISA at the University of Minho and with LC-MS/MS at the University of Dresden to enhance the comparability of the results produced by the two different methods. Here, all analyses on child HCC are performed using only the data achieved from the LC-MS/MS analyses apart from the findings on the comparability that are provided in Chapter 5.5.

## 4.4 Maternal Prenatal Psychological Distress

Different subtypes of maternal prenatal PD, including depressive symptoms, anxiety, pregnancy-related anxiety, and stressfulness of daily experiences, were measured by

utilizing several standardized self-report research questionnaires at gw 14, 24, and 34.

Depressive symptoms were assessed by a widely utilized and cross-culturally validated questionnaire for both pre- and postnatal depression, the Edinburgh Postnatal Depressive Scale (EPDS; Cox et al., 1987). It comprises ten questions scored on a 4-point Likert Scale, from 0 to 3 with higher points indicating more symptoms, that assess experienced symptoms during the past week. The continuous total sum score with a potential range from 0 to 30 was used. Missing values of individual items were imputed with a mean of the other item responses of the study subject when a maximum of three responses were missing. In Study II, each timepoint's total sum was used separately as a measure of recent depressive symptoms and a cumulative mean score was calculated as a mean of two consecutive sum scores ([EPDS gw 14 + EPDS gw 24] / 2 = EPDS mean1 to correspond with HCC1 and [EPDS gw 24 + EPDS gw 34] / 2 = EPDS mean2 to correspond with HCC2). The cumulative means were calculated only when data from both questionnaires were available. In addition, the course of maternal depressive symptoms throughout pregnancy was modeled using Latent Growth Mixture Modelling (LGMM; Muthén and Muthén, 2000) in Study II.

In Study III, cumulative sum scores of two consecutive EPDS sum scores were calculated. With missing data from either measure point (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 timepoint. Although not a prenatal PD measure, Study III included sensitivity analyses utilizing continuous sum scores of maternal EPDS postnatally assessed at child ages of 2 and 5 years.

General anxiety symptoms were assessed with the anxiety subscale of the Symptom Checklist -90 (SCL-90; Derogatis et al., 1973) that has also been validated among the Finnish population (Holi et al., 1998). It includes 10 items each scored on a 5-point Likert scale, from 0 to 4 with higher numbers referring to elevated symptom levels, with a potential total sum score ranging from 0 to 40 assessing experienced symptoms during the past month. Missing values with a maximum of three missing responses per questionnaire were imputed with the means of the subjects' other item responses. Continuous total sum scores as a measure of recent anxiety, cumulative means of consecutive measure points calculated similarly as with EPDS, and trajectories of prenatal total sum scores modeled using LGMM were utilized in Study II.

Anxiety symptoms related to pregnancy were measured using the Pregnancy-Related Anxiety Questionnaire -Revised (PRAQ-R2; Huizink et al., 2016, 2004). It comprises ten items each scored from 1 to 5 with a total sum score ranging from 10 to 50. Higher scores depict more pregnancy-related anxiety. The instructions of PRAQ-R2 do not specify the time span but ask the subject to rate, "what best

describes your situation." The items can be divided into three subscales being Fear of Giving Birth, Worries about Bearing a Physically or Mentally Handicapped Child, and Concern about Own Appearance. However, in this study, the subscales were not utilized. We used the continuous total sum scores and cumulative means of sum scores of two consecutive measurement points. The imputation of missing values was based on the same principles as with EPDS and SCL-90. The maternal prenatal trajectories of PRAQ-R2 could not be modeled by LGMM due to the low number of subjects with data from all three measurement points ($N_{PRAQ\ gw14} = 333$, $N_{PRAQ\ gw24} = 615$, and $N_{PRAQ\ gw34} = 573$). This resulted from PRAQ-R2 being included in the gw 14 questionnaires only in June 2014.

The stressfulness of daily experiences was measured with a modified Daily Hassles scale (Korpela et al., 2008). It includes six items with each of which scored on a 4-point Likert scale on both how much concern (from -3 to 0) and content (from 0 to 3) they have incurred in daily life during the past three months. In this study, the concerns related to three of these items (i.e., hassles related to social relationships [DH1], work [DH2], and money [DH3]) were included, and the data from each measure point were used separately to show recent stress and calculated a measure of cumulative stress as the mean of two consecutive responses. The psychometric properties of the Daily Hassles questionnaire did not enable modeling by LGMM.

The Cronbach α values for EPDS, SCL-90, and PRAQ-R2 at gw 14, 24, and 34 were between 0.82–0.86.

## 4.5 Child Socioemotional Problems

The assessment of child socioemotional problems in the whole Cohort was based on parent-rated questionnaires. In this thesis, maternal reports of their child socioemotional development at child ages of 2 and 5 years were utilized. As this study focused on the problems in child socioemotional development, the items assessing child competencies or prosocial behavior were not included as they represent a partially different phenomena.

At 2 years, traits in child socio-emotional behavior were assessed with the BITSEA screening instrument (Brief Infant-Toddler Social and Emotional Assessment; Briggs-Gowan and Carter, 2006). BITSEA includes 42 different items assessing child behavior and expressions of emotions during the past months on a 3-point Likert scale (0 = Not true / Rarely, 1 = Somewhat true / Sometimes, or 2 = Very true / Often). Thirty-one of the items are related to problems in child socioemotional development and were thus included in the study. The problem items are divided into the subscales of Externalizing problems (i.e., difficulties in activity/impulsivity, aggression/defiance, and peer aggression), Internalizing problems (for instance, fearfulness, worry, nervousness, and distress upon

separation), Dysregulation problems (i.e., difficulties in regulating negative emotionality, sleeping, or eating, and sensory sensitivities), Autism spectrum disorder items (i.e., behaviors and deficits often observed related to autism spectrum disorders), and Red flag items (i.e., clinically relevant items that may endanger the child, for instance "runs away in public places," "hurts self on purpose," and "gags and chokes on food"). Some of the items belong to more than one subscale. The missing values were imputed with the mean of other items in the same subscale (the maximum of missing values depending on the number of items included in the subscale). The sums of all problem-related subscales (ranges from 0–12 to 0–24) and a Problem Total score including all 31 problem-related items (range 0–62) were calculated. The Red flag items subscale is more targeted for clinical use and its questions are versatile. Therefore, this subscale was excluded from the study. The Problem Total and individual subscale sum scores were included in the statistical models as continuous variables. Alphas for subscales and Problem Total score were between 0.47 and 0.67.

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 chosen. The SDQ includes 25 items to be rated on the scale of 0 = Not true, 1 = Somewhat true, and 2 = Certainly true and represents traits in child behavior during the past six months. The five Strengths-related items that comprise the subscale of Prosocial behavior were excluded from this study. The Difficulties-items are divided into four, five-item subscales ranging from 0 to 10 being 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 such as dysregulation of attention, activity, and impulsivity; and Peer relationship problems, for instance, prefers adults or solitude and is not well liked by peers. Missing values were imputed with the mean of other items in the same subscale with a maximum of two missing items. All subscale sums and a Total Difficulties score as the sum of the 20 Difficulties-items (range 0–40) were calculated. The Total Difficulties and individual subscale continuous sum scores were utilized with alphas ranging from 0.53 to 0.83.

## 4.6 Covariates

Data on potential confounding factors were available from the Cohort questionnaires at gw 14 and 34, upon hair-sample taking, and/or the Medical Birth Register administered by the Finnish Institute of Health and Welfare (Table 1). Data on maternal age (years) at delivery, categorized as < 25, 25–35, and > 35 years; pre-pregnancy body mass index (BMI, kg/m$^2$; categorized as ≤ 20, 20,01–25, 25,01–30, and > 30); synthetic glucocorticoids when at risk of preterm birth (yes/no); smoking

during pregnancy (0 = "not at all", 1 = "yes, before knowing about the pregnancy", 2 = "yes, smoking throughout pregnancy"); sex of the child (girl/boy); gestational weeks at delivery; and data on pregnancy and birth complications including common maternal risk factors and complications such as gestational diabetes or pre-eclampsia and neonatal health outcomes (yes/no) were drawn from the Finnish Medical Birth Register.

Self-report questionnaires were used to obtain information on other covariates including maternal educational level (categorized as "Low" [high school/vocational, < 12 years], "Medium" [polytechnics], or "High" [university degree or comparable]), parity (dichotomized as primiparous vs others), marital status (dichotomized as married/domestic partnership vs others), ethnicity (categorized as Finnish/other Caucasian/others), use of medications (assessed at gw 14 for the HCC1 group and gw 34 for the HCC2 group; SSRI/SNRI [yes/no] and glucocorticoids [yes, local/yes, systemic/no]), substance use during the index pregnancy (based on both gw 14 and 34 questionnaires; alcohol [0 = "not at all", 1 = "yes, before knowing about the pregnancy", and 2 = "yes"], and illicit drugs [yes/no]). The hair-related attributes that were considered were hair color (blond/dark), hair dying (yes/no), frequency of hair washing (more or less frequently than 4 times per week), and the season of sample taking ("Winter" from December to February, "Spring" from March to May, "Summer" from June to August, and "Autumn" from September to November).

The selection of covariates for Study II was based on prior literature (Braig et al., 2015; Stalder et al., 2017), and data were explored to identify factors associated with both the exposures and the outcomes. Maternal age, pre-pregnancy BMI, and education as well as maternal use of SSRI/SNRI were determined to be necessary covariates based on the literature, clinical relevance, and data exploration. Maternal prenatal HCC were observed to also associate with seasonality, marital status, use of systemic anti-inflammatory drugs, and use of illicit drugs. However, as these factors were unrelated to PD measures, they were not included in the final models.

Similarly, the selection of covariates in Study III was based on prior literature (Pulli et al., 2019; Stalder et al., 2017) with the help of a directed acyclic graph (DAG, see **Figure 3**) created in the DAGitty web application (dagitty.net; Textor et al., 2016), and the selection was confirmed by data exploration, in which no other background factors were observed to associate with both the exposures and the outcomes. The known determinants for HCC (i.e., 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 in neuroimaging studies (specifically, maternal mental health, SSRI/SNRI or substance use, BMI, infections and inflammatory states, and ethnicity; family socioeconomic status; and child sex, gestational age at birth, and birth weight; Pulli et al., 2019) were inserted in the DAG,

and the factors that were associated with both the exposures and the outcomes were selected as potential confounders. These included child's sex assigned at birth and maternal pre-pregnancy BMI, education, prenatal depressive symptoms, and prenatal SSRI/SNRI use. The number of subjects reporting the use of prenatal SSRI/SNRI 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/SNRI use did not differ from others in terms of HCC or child socioemotional symptoms (data only presented in the supplementary material of Study III) and excluding these subjects did not significantly alter the results. Thus, these subjects were not excluded from the final analyses.

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

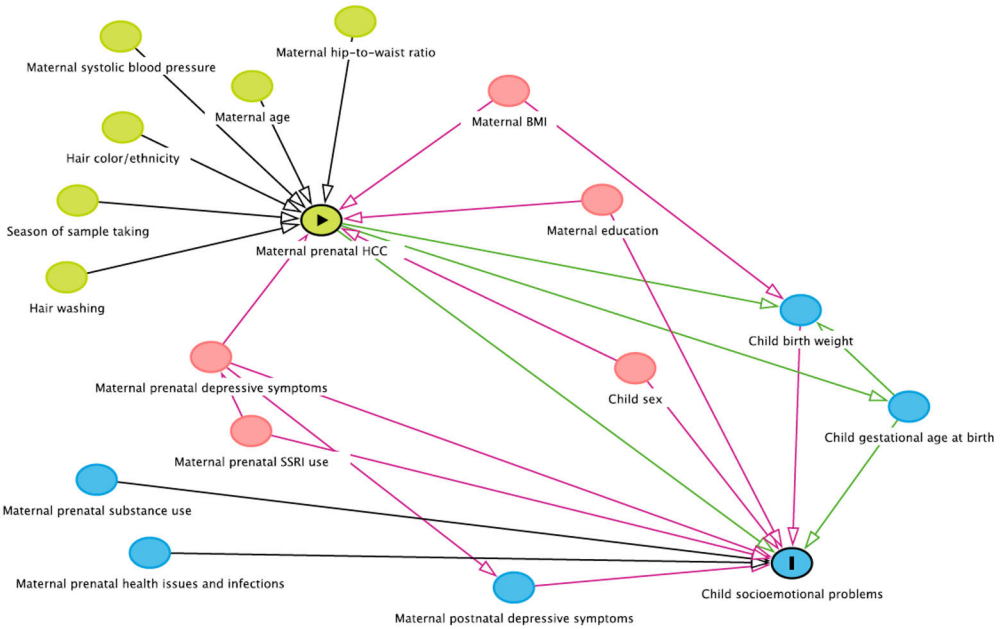


**Figure 3.** A directed acyclic graph (DAG) to determine the essential covariates in Study III (dagitty.net). The arrows depict the known determinants of HCC and child socioemotional problems based on earlier literature. The main exposure is marked with a triangle, and with its ancestors, is illustrated with a green color. The main outcome (marked with a line) with its ancestors is colored blue, and the pink color shows the ancestors of both the exposure and the outcome which are considered as true confounders. Abbreviations: HCC = hair cortisol concentrations; BMI = body mass index; and SSRI = selective serotonin reuptake inhibitors.

## 4.7 Statistical Analyses

Because of the skewed distribution of HCC, natural logarithm (ln) conversion for all HCC values was performed. All statistical tests were run with ln-converted HCC values, and they were also used in the figures. Raw values of HCC are presented in **Table 7** and **Table 8**. As HCC data generally includes extreme values, it was necessary to carefully determine their effect on the results and assess the need for excluding outlier values. For Studies II and III, there were sporadic values exceeding three standard deviations (SD) above the mean and excluding these subjects resulted in narrower 95% confidence intervals (CI). The variance in child HCC, especially in small children, is even greater (see **Table 8**) and exclusion of extreme values > 3SD above the mean was necessary.

As there was significant attrition from the whole Cohort to the final study populations in both Studies II and III, attrition analyses were performed to understand potential selection biases. Differences in any of the assessed covariates, exposures, and outcomes between all Cohort participants, those with applicable HCC1 and/or HCC2 measure, and those with both HCC and relevant child and maternal PD measures were examined (see **Table 3** – **Table 8**).

The trajectories of prenatal depressive and general anxiety symptoms were modeled using LGMM in subjects with both relevant HCC and PD questionnaire data. The factor structures for both symptom categories were examined using structural equation modeling and the longitudinal confirmatory factor analysis of the EPDS and SCL-90 showed good fit with the data (data shown in the supplementary material of Study II). Each subject's symptom growth curves were then estimated separately for both symptom categories using the individual item scores of each timepoint. Then, the identification of prototypic curves for the study populations was conducted to select latent curves, i.e., the developmental patterns in symptoms that most optimally describe the data and are also interpretable. Individuals with missing data on some items were incorporated in the analyses with maximum likelihood under the missing-at-random assumption (Graham, 2009) in order to minimize bias (Nagin, 2005).

The optimal number of selected latent classes for prenatal depressive and general anxiety symptom for both the HCC1 and HCC2 samples were assessed by observing the following statistical indices: Bayesian Information Criterion, where lower value indicates better model fit; Entropy, with values > 0.80 indicating excellent accuracy; and posterior probabilities class membership (i.e., the probability of an individual belonging to a group with a score of 0.80 or above being preferred; Lubke and Muthén, 2007; Nagin, 2005; Nylund et al., 2007). Additionally, theoretical and clinical interpretability of the identified class solutions was assessed.

For EPDS LGMM, all the statistical indices continued to improve and/or were satisfactory up to a 4-group model in both HCC1 and HCC2 samples (data shown in

the Supplements of Study II). The 4-group solution was also clinically interpretable, and retained satisfactory latent group sizes, thus it was selected to model the prenatal trajectories of maternal depressive symptoms in both samples. For SCL-90 LGMM, either a 2- or 4-group solution appeared to best fit the data in the HCC1 sample (see the Supplements of Study II). Statistical analyses were conducted with both solutions with similar unadjusted associations between the group variable and HCC1 were similar regardless of the class solution (data not shown), thus the 2-class variable with larger group sizes was selected. A 3-group solution was the best fit with the data in the HCC2 sample. The selected latent classes with their group labelling and group sizes are described in **Figure 4**. The groups with highest overall level of PD symptoms were selected as reference groups in pairwise comparisons.

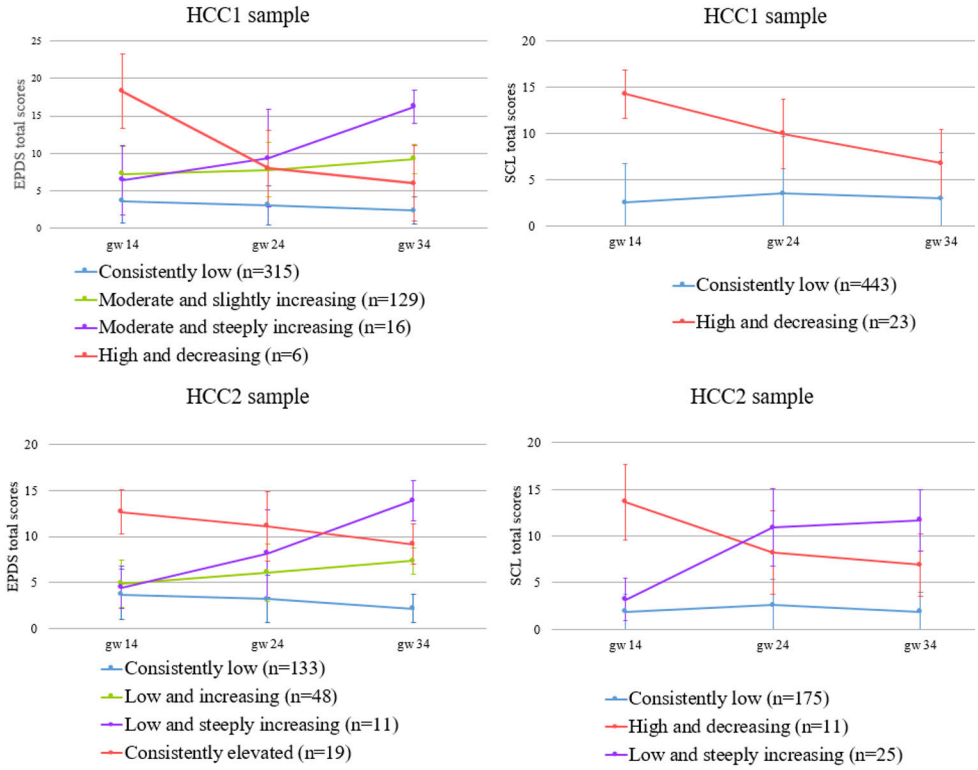


**Figure 4.** The mean levels of depressive and general anxiety symptoms throughout pregnancy for symptom trajectories in the HCC1 and HCC2 samples. Notes of abbreviation: HCC1 = hair cortisol concentrations measured at gw 24; gw = gestational week; HCC2 = hair cortisol concentrations measured 1-3 days after delivery; EPDS = the Edinburgh Postnatal Depression Scale; SCL-90 = the Symptom Checklist -90, anxiety subscale.

Stepwise linear regression models (see lists below) were used for both Study II and III to study the associations between maternal prenatal HCC and maternal and child PD/socioemotional symptoms. In Study II, direct correlations between PD measures and HCC were first tested, and adjusted analyses using Analysis of covariance (ANCOVA) were conducted for those PD subtypes that were suggestive of potential associations ($p < .1$). The covariates included in the adjusted regression models are described below.

In Study III, the covariates included in Steps 1 and 2 are listed below. In Step 3, the role of interactions between maternal prenatal HCC and maternal prenatal depressive symptoms in explaining the child outcomes was assessed. In Step 4, potential sex-specificity was examined, and Step 5 was conducted as a sensitivity analysis to assess potential maternal reporting bias resulting from their concurrent depressive symptoms.

List of the performed regression models in Study II:

STEP 1: maternal PD symptoms ~ maternal prenatal HCC

STEP 2: maternal PD symptoms/trajectory ~ intercept + maternal prenatal HCC + maternal age + maternal BMI + maternal education (low, mid, high).

STEP 3: maternal PD symptoms/trajectory ~ intercept + maternal prenatal HCC + maternal age + maternal BMI + maternal education (low, mid, high) + maternal concurrent use of SSRI/SNRI.

List of the performed regression models in Study III:

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

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

STEP 3: child symptoms ~ intercept + maternal prenatal 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 symptoms ~ intercept + maternal prenatal HCC + child sex + maternal BMI + maternal education + maternal prenatal EPDS + maternal postnatal EPDS.

For significant interactions observed in Step 3 in Study III, a simple slope analysis was conducted to further elucidate the relations of the interactions and to visualize them. Here, the interactions -function's default values were set to a constant for different prenatal EPDS values; low (mean −1 SD = 1.95), intermediate (mean = 8.29), and elevated depressive symptoms (mean +1SD = 14.63). Albeit utilizing

these constant EPDS values in the analyses and in visualizations, categorization of the variable was not performed.

In the supplementary analyses regarding child HCC as a mediator for the association between maternal prenatal HCC and child socioemotional symptoms, the basic assumptions for the mediator analyses were first assessed with direct correlations between maternal HCC1 and HCC2, child $HCC_{30m}$ and $HCC_{5y}$, and the total scores of child socioemotional problems at 2 and 5 years. The mediation analyses were executed with both unadjusted and adjusted linear models by utilizing the Baron and Kenny method (Baron and Kenny, 1986). The utilized covariates were the same that were used in Step 2 in Study III.

P-values (two-tailed) smaller than 0.05 were interpreted as statistically significant. The beta coefficients (β) and 95% confidence intervals (CI) for the estimates and adjusted $R^2$ for the models were calculated. Based on a power analysis for linear models, Cohen's effect sizes from 0.04 to 0.15 upwards could be discovered in the sample sizes.

The statistical analyses for Study II were performed using SPSS v.24 apart from the LGMM, which was performed using Mplus 6 (Muthén and Muthén, 2000). All attrition analyses were performed using IBM SPSS Statistics 28, and other analyses for Study III together with supplemental analyses conducted for the purposes of this thesis were performed using R (4.0.5, 2021; R Core Team, 2022).

## 4.8 Ethical Considerations

The FinnBrain Birth Cohort Study has received approval from the Joint Ethics Committee of Southwestern Hospital District and the University of Turku alongside other relevant research sites for all components of the study. Additionally, approval has been obtained for the specific modifications made for this substudy. The reference numbers for approvals of the Ethics Committee of the Hospital District of Southwest Finland were ETMK57/180/2011 and ETMK12/180/2013. Written informed consent was obtained from each subject participating in the study. In instances involving infants, parental consent was secured on behalf of the child.

Every subject was assigned a unique identification code, and all data handling, storage, and analysis were conducted using these codes. All data were stored in accordance with established research practices, Finnish legislation, and data protection protocols at the University of Turku. Access to the data was restricted to members of the research group, and those with access to the ID codes were limited to individuals requiring personal information to contact study participants. In the event of clinically significant findings, subjects were promptly contacted for referral to appropriate treatment.

Hair cortisol concentration analyses were conducted internationally, and thus, prior informed consent from the subjects was obtained and the Material Transport Agreements were established.

The collection of hair samples is not an invasive procedure and it does not induce discomfort or an observable cosmetic detriment.

# 5 Results

## 5.1 Systematic Review (Study I)

The literature searches originally resulted in 69 articles in PUBMED, 57 in Ovid MEDLINE®, 139 in Embase, 19 in PsycINFO, 360 in WorldCat, and 75 in WEB of Science (see **Figure 5**). Nineteen papers with measures of maternal prenatal HCC were identified. Six papers were excluded, as they did not include any prenatal PD measures, and a further six were removed as they only assessed prenatal PD as a covariate. One case report was excluded. No additional research papers were identified through a reference search.

Six papers meeting the selected inclusion and exclusion criteria were found (Braig et al., 2015; Hoffman et al., 2016; Kalra et al., 2007; Kramer et al., 2009; Scharlau et al., 2017; Wikenius et al., 2016; see **Table 1** where studies included in Study I are marked with an asterisk). In all, 1,226 subjects were included in the studies. The papers were reviewed in detail and relevant study characteristics were extracted. Maternal prenatal exposure to stressful or traumatic life events could not be included as a type of PD as our search did not yield any studies assessing its association with HCC during pregnancy.

Most studies assessed several types of PD with a variety of self-report questionnaires. Out of the six reviewed studies, five included measures of perceived stress related to the mother's life situation (Braig et al., 2015; Hoffman et al., 2016; Kalra et al., 2007; Kramer et al., 2009; Scharlau et al., 2017), five of depressive symptoms (Braig et al., 2015; Hoffman et al., 2016; Kramer et al., 2009; Scharlau et al., 2017; Wikenius et al., 2016), two of anxiety symptoms (Braig et al., 2015; Hoffman et al., 2016), and two of pregnancy-related anxiety symptoms (Braig et al., 2015; Kramer et al., 2009).

Two studies observed positive associations between HCC and all included types of PD; Hoffman et al. (2016) reported HCC to associate with perceived stress, depression, and anxiety in mid-pregnancy and Kalra et al. (2007) with perceived stress also during mid-pregnancy. Other studies yielded null findings on all studied associations. Neither study assessing maternal end-of-pregnancy HCC observed it to associate with concurrent PD measures, while half of the studies with mid-pregnancy HCC did. All studies comprised general population samples, while some reported

that the study population was biased towards elevated socioeconomical statuses. The study with more ethnic and educational diversity and slightly increased number of subjects with elevated PD symptom severity was the one observing positive associations between maternal prenatal HCC and several PD subtypes (Hoffman et al., 2016).

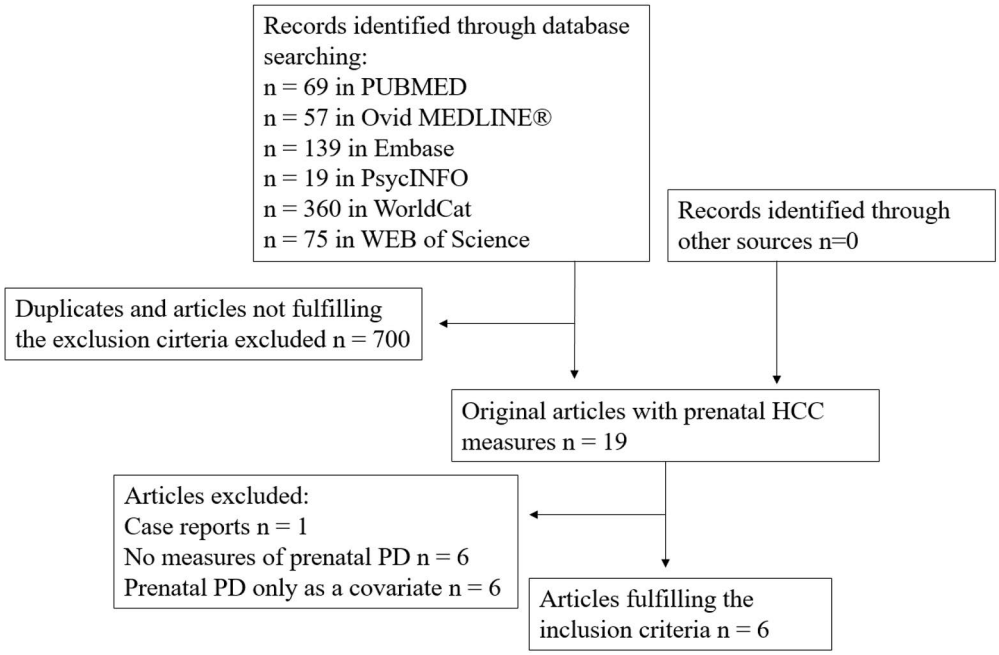


**Figure 5.** Systematic search to identify the papers included in the review (Study I). HCC = hair cortisol concentrations; PD = psychological distress.

## 5.2 Descriptive Results and Attrition Analyses of Studies II and III

Detailed sociodemographic, maternal health-related, and hair sample-related data are presented in **Table 3** and **Table 4**. The descriptives of the whole Cohort as well as those of subpopulations with 1) applicable maternal prenatal HCC results (HCC1 and HCC2) and 2) both HCC1/2 and data on relevant maternal and child PD questionnaires (HCC1/2 and PD) are presented with the results of an attrition analysis. To avoid unnecessary repetition, variables with no differences between the Cohort subjects and any of the subpopulations are only presented in **Table 3** with the exception of variables specifically related to either mid- or end-of-pregnancy, for instance, use of medication and the season of hair sample taking.

**Table 3**. Descriptives and attrition analysis related to HCC1. Significant differences between the whole cohort and the subgroups are in bold (* for p < .05, ** for p < .01, *** for p < .001).

| | **WHOLE COHORT (n = 3808)** | **HCC1 (n = 467)** | **HCC1 + PD (n = 321)** |
|---|---|---|---|
| | mean (SD, range) / n (%) | mean (SD, range) / n (%) | mean (SD, range) / n (%) |
| Maternal age | **30.2 (4.7, 17–46)** | **31.1 (4.4, 19–41)**** | **31.4 (4.1, 22–41)***** |
| Maternal education | | *** | *** |
| - < 12 years | **1173 (37.8)** | **107 (23.9)** | **63 (20.5)** |
| - polytechnics | **899 (29.0)** | **162 (36.3)** | **109 (35.5)** |
| - university | **1031 (33.2)** | **178 (39.8)** | **135 (44.0)** |
| Maternal BMI before pregnancy (kg/m$^2$) | 24.6 (4.9, 16–61) | 24.6 (4.4, 18–46) | 24.6 (4.6, 18–46) |
| Marital status | | | |
| - married/domestic partnership | 2805 (93.0) | 414 (89.5) | 286 (94.1) |
| - others | 210 (7.0) | 26 (5.6) | 18 (5.9) |
| Parity | | ** | *** |
| - nullipara | **1594 (51.5)** | **261 (58.7)** | **188 (61.4)** |
| - multipara | **1501 (48.5)** | **184 (41.3)** | **118 (38.6)** |
| Ethnicity | | | |
| - Finnish | 2993 (96.8) | 433 (98.0) | 301 (97.7) |
| - other Caucasian | 90 (2.9) | 9 (2.0) | 7 ( 2.3) |
| - other | 8 (0.3) | 0 | 0 |
| Gw's at delivery | 39.7 (1.8, 24–43) | 39.7 (1.9, 27–42) | 39.8 (1.6, 31–42) |
| Child's sex assigned at birth | | | |
| - boy | 1965 (52.2) | 231 (49.6) | 156 (48.6) |
| - girl | 1797 (47.8) | 235 (50.4) | 165 (51.4) |
| Maternal smoking | | ** | *** |
| - no | **3201 (86.9)** | **419 (90.9)** | **296 (93.7)** |
| - only 1$^{st}$ trimester | **275 (7.5)** | **33 (7.1)** | **17 (5.4)** |
| - after 1$^{st}$ trimester | **207 (5.6)** | **9 (2.0)** | **3 (0.9)** |
| Maternal use of alcohol | | | |
| - no | 2416 (79.8) | 330 (75.0) | 231 (76.8) |

| | **WHOLE COHORT (n = 3808)** | **HCC1 (n = 467)** | **HCC1 + PD (n = 321)** |
|---|---|---|---|
| | mean (SD, range) / n (%) | mean (SD, range) / n (%) | mean (SD, range) / n (%) |
| - yes, before knowing of pregnancy | 454 (15.0) | 59 (13.4) | 51 (16.9) |
| - yes, after knowing of pregnancy | 157 (5.2) | 51 (11.6) | 19 (6.3) |
| Maternal use of SSRI/SNRI | | | |
| - gw 14 - no | 2959 (96.7) | 421 (95.5) | 290 (96.0) |
| - yes | 100 (3.3) | 20 (4.5) | 12 (4.0) |
| Maternal use of GCs | | | |
| - gw 14 - no | 2939 (96.1) | 400 (95.2) | 290 (96.0) |
| - local | 117 (3.8) | 17 (4.1) | 11 (3.7) |
| - systemic | 3 (0.1) | 3 (0.7) | 1 (0.3) |
| - sGC when at risk of preterm birth | | | |
| - no | 3557 (96.3) | 438 (96.1) | 306 (95.3) |
| - yes | 135 (3.7) | 18 (3.9) | 15 (4.7) |
| Pregnancy or birth complications | | | |
| - no | 2719 (70.8) | 316 (68.5) | 226 (69.5) |
| - yes | 1120 (29.2) | 145 (31.5) | 99 (30.5) |
| Maternal hair washing | | | |
| - ≥ 4 times /week | 156 (28.3) | 127 (27.3) | 79 (25.2) |
| - < 4 times /week | 395 (71.7) | 330 (70.7) | 234 (74.8) |
| Sample taking season | | | |
| - spring | 161 (29.2) | 142 (30.4) | 107 (33.3) |
| - summer | 145 (26.3) | 123 (26.3) | 88 (27.4) |
| - autumn | 147 (26.7) | 119 (25.5) | 74 (23.1) |
| - winter | 98 (17.8) | 83 (17.8) | 52 (16.2) |

Abbreviations: HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; PD = psychological distress; HCC1 + PD = subjects with both HCC1 and child and maternal PD questionnaires available; BMI = body mass index; gw = gestational week; SSRI/SNRI = selective serotonin (and noradrenalin) reuptake inhibitors; GCs = glucocorticoids; sGC = synthetic glucocorticoids

**Table 4.** Descriptions and attrition analysis related to HCC2; only covariates with between-group differences (in bold with asterisks) or specific for end-of-pregnancy are presented here.

| | **WHOLE COHORT (n = 3808)** | **HCC2 (n = 222)** | **HCC2 + PD (n = 121)** |
|---|---|---|---|
| | mean (SD, range) / n(%) | mean (SD, range) / n (%) | mean (SD, range) / n (%) |
| Maternal age | **30.2 (4.7, 17–46)** | 30.6 (4.2, 19–42) | **31.4 (4.0, 23–42)**** |
| Maternal education | | | ** |
| - < 12 years | **1173 (37.8)** | 63 (30.7) | **27 (23.1)** |
| - polytechnics | **899 (29.0)** | 61 (29.8) | **34 (29.0)** |
| - university | **1031 (33.2)** | 81 (39.5) | **56 (47.9)** |
| Parity | | *** | *** |
| - nullipara | **1594 (51.5)** | **134 (65.0)** | **81 (68.6)** |
| - multipara | **1501 (48.5)** | **72 (35.0)** | **37 (31.4)** |
| Maternal smoking | | | *** |
| - no | **3201 (86.9)** | 196 (90.0) | **113 (95.0)** |
| - only 1st trimester | **275 (7.5)** | 15 (6.9) | **6 (5.0)** |
| - after 1st trimester | **207 (5.6)** | 7 (3.2) | **0 (0)** |
| Maternal use of SSRI | | | |
| - gw 34 - no | 2498 (97.2) | 167 (98.2) | 114 (100) |
| - yes | 73 (2.8) | 3 (1.8) | 0 (0) |
| Maternal use of GCs | | | |
| - gw 34 - no | 2471 (96.1) | 163 (95.3) | 110 (96.5) |
| - local | 92 (3.6) | 7 (4.1) | 3 (2.6) |
| - systemic | 8 (0.3) | 1 (0.6) | 1 (0.9) |
| Maternal hair washing | | | |
| - ≥4 / week | 61 (25.3) | 56 (25.4) | 31 (25.6) |
| - < 4 times /week | 180 (74.7) | 165 (74.6) | 90 (74.4) |
| Sample taking season | | | |
| - spring | 65 (26.2) | 58 (26.1) | 36 (30.0) |
| - summer | 59 (23.8) | 57 (25.7) | 24 (20.0) |
| - autumn | 23 (9.3) | 22 (9.9) | 19 (15.8) |
| - winter | 101 (40.7) | 85 (38.3) | 41 (34.2) |

Abbreviations: HCC = hair cortisol concentrations; HCC2 = HCC measured 1–3 days after delivery; PD = psychological distress; HCC2 + PD = subjects with both HCC2 and child and maternal PD-questionnaires available; SSRI = selective serotonin reuptake inhibitors; GCs = glucocorticoids

In brief, the attrition analysis showed selective attrition related to the HCC1 population and the subpopulations that included data on PD questionnaires, whereas the HCC2 population differed from all Cohort subjects only in terms of parity, i.e., subjects with a HCC2 sample were more likely to be nullipara than the subjects in the whole Cohort (**Table 3** and **Table 4**). This most likely results from the sample-taking procedure, as the HCC2 samples were requested from all mothers that could be contacted 1 to 3 days after delivery at the delivery wards. As multiparity is associated with a faster hospital discharge, those women were less likely to have been reached. The sample taking did not require the subject's own initiative, which was the case for HCC1, as those samples were donated at a voluntary study visit with a participation rate of 44%. Additionally, there was attrition related to the postnatal questionnaires on child development with data on BITSEA available for 38% of the Cohort families and data on SDQ for 39%. The attrition related to subpopulations that did require the subject's own initiative was associated with a younger age, lower educational attainment, multiparity, and smoking during pregnancy (**Table 3** and **Table 4**).

Most subjects experienced rather low levels of prenatal PD and the use of SSRI/SNRI medication especially at the end-of-pregnancy was rare. The ethnic background of all subjects was Caucasian thus not indicating significant differences in hair steroids based on differences in ethnic background.

Importantly, the subpopulations did not differ compared to the whole Cohort or with each other in terms of any of the maternal prenatal PD measures (**Table 5**), child socioemotional problems, or maternal postnatal depressive symptoms (**Table 6**).

**Table 5.** Maternal prenatal PD in subjects of the whole Cohort and study subjects of Study II. No significant differences between the subgroups were observed.

| | WHOLE COHORT | | | HCC1 | | | HCC2 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean score | SD | N | Mean score | SD | N | Mean score | SD | N |
| EPDS T1 | 5.2 | 4.0 | 3051 | 5.0 | 3.9 | 442 | 4.8 | 3.6 | 199 |
| EPDS T2 | 5.0 | 4.1 | 2770 | 4.7 | 3.9 | 457 | 4.8 | 3.8 | 191 |
| EPDS T3 | 4.9 | 4.1 | 2602 | 4.8 | 4.2 | 428 | 4.6 | 3.8 | 172 |
| SCL-90 T1 | 3.3 | 3.9 | 3053 | 3.2 | 3.6 | 442 | 3.2 | 3.7 | 199 |
| SCL-90 T2 | 3.9 | 4.2 | 2767 | 3.8 | 4.2 | 455 | 3.8 | 4.2 | 190 |
| SCL-90 T3 | 3.2 | 4.0 | 2598 | 3.2 | 3.9 | 427 | 3.1 | 3.9 | 172 |
| PRAQ-R2 T1 | 22.3 | 6.6 | 601 | 22.4 | 6.3 | 199 | 22.8 | 6.4 | 190 |
| PRAQ-R2 T2 | 22.9 | 6.7 | 2767 | 22.7 | 6.4 | 457 | 23.5 | 6.2 | 191 |
| PRAQ-R2 T3 | 23.2 | 6.8 | 2595 | 22.7 | 6.7 | 427 | 23.8 | 6.4 | 172 |
| DH1 T1 | 1.3 | 0.7 | 2980 | 1.2 | 0.7 | 429 | 1.2 | 0.7 | 193 |
| DH1 T2 | 1.2 | 0.7 | 2698 | 1.2 | 0.7 | 442 | 1.2 | 0.7 | 184 |
| DH1 T3 | 1.1 | 0.7 | 2540 | 1.1 | 0.7 | 419 | 1.1 | 0.6 | 170 |
| DH2 T1 | 1.4 | 0.9 | 2967 | 1.5 | 0.8 | 433 | 1.4 | 0.8 | 194 |
| DH2 T2 | 1.3 | 0.8 | 2705 | 1.3 | 0.8 | 444 | 1.3 | 0.8 | 187 |
| DH2 T3 | 1.0 | 0.8 | 2530 | 1.1 | 0.9 | 421 | 1.1 | 0.8 | 170 |
| DH3 T1 | 1.2 | 0.9 | 3014 | 1.2 | 0.9 | 437 | 1.1 | 0.8 | 194 |
| DH3 T2 | 1.2 | 0.8 | 2730 | 1.1 | 0.8 | 446 | 1.0 | 0.8 | 187 |
| DH3 T3 | 1.1 | 0.8 | 2559 | 1.1 | 0.8 | 427 | 1.1 | 0.7 | 170 |

Abbreviations: HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; HCC2 = HCC measured 1–3 days after delivery; PD = psychological distress; T1 = questionnaires from gw 14, T2 = questionnaires from gw 24; T3 = questionnaires from gw 34; EPDS = the Edinburgh Postnatal Depression Scale; SCL-90 = the Symptom Checklist -90; PRAQ-R2 = the Pregnancy-related Anxiety Questionnaire -Revised2; DH = Daily Hassles Questionnaire

**Table 6.** The prevalence of child socioemotional problems and maternal postnatal depressive symptoms in different subpopulations.

| | **CHILD BITSEA PROBLEM TOTAL** | | **CHILD SDQ TOTAL DIFFICULTIES** | |
|---|---|---|---|---|
| | **at 2 years** | | **at 5 years** | |
| | mean (SD, range) | n | mean (SD, range) | n |
| Whole Cohort | 7.5 (4.3, 0–34) | 1437 | 8.9 (5.0, 0–29) | 1490 |
| HCC1 | 7.8 (4.1, 0–22) | 272 | 8.7 (5.0, 0–25) | 275 |
| HCC1 + PD | 7.8 (4.1, 0–22) | 272 | 8.7 (5.0, 0–25) | 275 |
| HCC2 | 7.8 (4.2, 0–22) | 99 | 8.1 (4.8, 1–24) | 101 |
| HCC2 + PD | 7.8 (4.2, 0–22) | 99 | 8.1 (4.8, 1–24) | 101 |
| | **MATERNAL EPDS** | | **MATERNAL EPDS** | |
| | **at child's age of 2 years** | | **at child's age of 5 years** | |
| | mean (SD, range) | n | mean (SD, range) | n |
| Whole Cohort | 4.6 (4.3, 0–27) | 1369 | 5.1 (4.6, 0–26) | 1482 |
| HCC1 | 4.5 (4.4, 0–21) | 261 | 5.0 (4.3, 0–20) | 272 |
| HCC1 + PD | 4.5 (4.4, 0–21) | 264 | 5.0 (4.4, 0–20) | 278 |
| HCC2 | 4.9 (4.1, 0–16) | 95 | 5.0 (4.5, 0–20) | 99 |
| HCC2 + PD | 4.9 (4.0, 0–16) | 96 | 5.1 (4.5, 0–20) | 100 |

Abbreviations: HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; HCC2 = HCC measured 1–3 days after delivery; PD = psychological distress; HCC1/2 + PD = subjects with both the relevant HCC and child and maternal PD questionnaires available; BITSEA = Brief Infant-Toddler Socioemotional Assessment; SDQ = Strengths and Difficulties Questionnaire; EPDS = the Edinburgh Postnatal Depressive Scale

In addition to the comparable maternal and child PD measures, the subjects of the whole Cohort did not differ from the subjects included in the final subpopulations of Study II or III in terms of maternal or child HCC (**Table 7** and **Table 8**). As expected, the observed HCC was higher in the end-of-pregnancy samples (mean HCC2 = 20.5 [SD 19.9] pg/mg vs. mean HCC1 = 19.8 [SD 34.6] pg/mg; p = .002). This difference was not, however, observed in the subpopulation with PD measures.

**Table 7.** The descriptives of maternal HCC in mid- and end-of-pregnancy in different subpopulations before and after the exclusion of outliers (> mean + 3SD). Data of the whole Cohort are omitted as HCC1 and HCC2 comprise all subjects with hair samples.

| | **HCC (pg/mg)** | | | |
|---|---|---|---|---|
| | mean | SD | range | n |
| HCC1 | 28.7 | 88.2 | 0.3–1174 | 474 |
| - without outliers | 19.8 | 34.6 | 0.3–270 | 467 |
| HCC1 + PD | 28.4 | 86.1 | 0.3–1174 | 325 |
| - without outliers | 20.3 | 33.4 | 0.3–270 | 321 |
| HCC2 | 27.6 | 48.3 | 1.0–357 | 230 |
| - without outliers | 20.5 | 19.9 | 1.0–117 | 224 |
| HCC2 + PD | 25.1 | 58.9 | 1.0–357 | 124 |
| - without outliers | 18.0 | 17.0 | 1.0–117 | 121 |

Abbreviations: HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; HCC2 = HCC measured at 1–3 days after delivery; PD = psychological distress; HCC1/2 + PD = subjects with both the relevant HCC and child and maternal PD questionnaires available

**Table 8.** The descriptives of child HCC at 30 months and 5 years in different subpopulations before and after the exclusion of outliers (> mean + 3SD).

| | **$HCC_{30mo}$ (pg/mg)** | | | | **$HCC_{5y}$ (pg/mg)** | | | |
|---|---|---|---|---|---|---|---|---|
| | mean | SD | range | n | mean | SD | range | n |
| Whole Cohort | 385 | 1395 | 1.2–15158 | 291 | 152 | 900 | 0.3–16969 | 530 |
| - without outliers | 225 | 549 | 1.2–3370 | 286 | 79.1 | 209 | 0.3–1577 | 525 |
| HCC1 | 460 | 1778 | 1.6–15158 | 89 | 123 | 417 | 0.9–3880 | 114 |
| - without outliers | 223 | 520 | 1.6–2930 | 87 | 90.1 | 221 | 0.9–1354 | 113 |
| HCC1 + PD | 472 | 1799 | 1.6–15158 | 87 | 119 | 419 | 0.9–3880 | 104 |
| - without outliers | 231 | 531 | 1.6–2930 | 85 | 82.5 | 194 | 0.9–1051 | 103 |
| HCC2 | 408 | 1047 | 3.2–5851 | 43 | 37.7 | 79.7 | 1.3–502 | 50 |
| - without outliers | 278 | 619 | 3.2–2932 | 42 | 28.2 | 43.6 | 1.3–205 | 49 |
| HCC2 + PD | 443 | 1109 | 3.2–5851 | 38 | 45.5 | 86.7 | 1.3–502 | 45 |
| - without outliers | 298 | 655 | 3.2–2932 | 37 | 35.1 | 52.3 | 1.3–205 | 44 |

Abbreviations: HCC = hair cortisol concentrations; $HCC_{30mo}$ = child HCC measured at 30 months; $HCC_{5y}$ = child HCC measured at 5 years; HCC1 = maternal HCC measured at gw 24; HCC2 = maternal HCC measured at 1–3 days after delivery; PD = psychological distress; HCC1/2 + PD = subjects with both the relevant HCC and child and maternal PD questionnaires available

## 5.3 Associations between Maternal Prenatal HCC and Maternal Prenatal PD (Study II)

### 5.3.1 Recent PD

In Step 1 for recent PD measures in cross-sectional analyses with the concurrent maternal prenatal HCC measure, no associations were observed between any type of recent PD and HCC at either assessment point (β's between −0.075 and 0.12, p-values between .11 and .94, **Table 9**). Thus, no Step 2 and Step 3 analyses were performed.

### 5.3.2 Cumulative PD

Some unadjusted associations were observed between the cumulative PD symptoms as measured by the mean of two consecutive timepoints from cross-sectional analysis for cumulative PD and HCC1 (**Table 9**). HCC1 was negatively associated with PRAQ-R2 mean1 (β = −0.03, p = .03) and positively with DH1 mean1 (β = 0.22, p = .01). The other measures, EPDS, SCL-90, DH2, and DH3, were not associated with HCC1 (β´s between −0.02 and 0.02, p-values between .27 and .83). There were no associations between HCC2 and PD mean2 variables, however as the analysis plan stated adjusted analyses would be performed with all associations suggestive of statistical significance (p < .1), the association between HCC2 and EPDS mean2 (β = 0.04, p = .09) was included in the adjusted models.

The positive association between HCC1 and mean1 of DH1 remained in the adjusted models in Step 2 and Step 3 (β = 0.25 in both, p-values < .005, see **Table 9**). Albeit staying negative, the association between PRAQ-R2 mean1 and HCC1 no longer reached significance in Step 2 or 3 (**Table 9**). EPDS mean2 and HCC2 were not associated in the adjusted models (β's 0.03, p-values between .12 and .18).

**Table 9.** Unadjusted cross-sectional associations between recent (gw 24 related to HCC1 and gw 34 related to HCC2) and cumulative (mean of gw 14 and 24 related to HCC1 and mean of gw 24 and 34 related to HCC2) PD and HCC and adjusted Step 2 and 3 associations for all observed associations. (* for p < .05, ** for p < .01, *** for p < .001)

Abbreviations: PD = psychological distress; HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; HCC2 = HCC measured 1–3 days after delivery; EPDS = the Edinburgh Postnatal Depression Scale; SCL-90 = the Symptom Checklist -90; PRAQ-R2 = the Pregnancy-related Anxiety Questionnaire -Revised2; DH = Daily Hassles Questionnaire

| | HCC1 | | | HCC2 | | |
|---|---|---|---|---|---|---|
| | β | p | N | β | p | N |
| **EPDS** - Recent | 0.01 | .24 | 457 | 0.03 | .15 | 172 |
| - Cumulative | 0.02 | .27 | 433 | 0.04 | .09 | 169 |
| - Step 2 | | | | 0.03 | .12 | 162 |
| - Step 3 | | | | 0.03 | .18 | 160 |
| **SCL-90** - Recent | 0.02 | .17 | 455 | 0.003 | .89 | 172 |
| - Cumulative | 0.02 | .27 | 431 | 0.003 | .89 | 168 |
| **PRAQ-R2** - Recent | 0.001 | .88 | 457 | -0.002 | .85 | 172 |
| - Cumulative | **-0.03*** | **.03** | 196 | -0.006 | .65 | 169 |
| - Step 2 | -0.03† | .08 | 192 | | | |
| - Step 3 | -0.02† | .09 | 190 | | | |
| **Daily Hassles** | | | | | | |
| Worries about relationships (DH1) - Recent | 0.12 | .11 | 436 | -0.009 | .94 | 169 |
| - Cumulative | **0.22*** | **.01** | 399 | -0.11 | .46 | 160 |
| - Step 2 | **0.25**** | **.005** | 394 | | | |
| - Step 3 | **0.25**** | **.004** | 388 | | | |
| Worries about work (DH2) - Recent | 0.02 | .76 | 436 | -0.08 | .39 | 169 |
| - Cumulative | -0.02 | .73 | 399 | 0.005 | .97 | 160 |
| Worries about money (DH3) - Recent | -0.02 | .72 | 436 | 0.06 | .57 | 169 |
| - Cumulative | 0.02 | .83 | 399 | 0.06 | .64 | 160 |

### 5.3.3 Trajectories of PD

In Step 1 examinations, HCC2 was found to be elevated in relation to continuously higher levels of depressive symptoms (Adj $R^2 = 0.03$, $p = .04$, see **Table 11**). The mothers in the "Consistently elevated" group presented with higher HCC than mothers in both the "Consistently low" ($\beta = -0.54$, $p = .02$ for the contrast) and the "Low and increasing" ($\beta = -0.55$, $p = .03$ for the contrast) groups. There was a non-

significant trend-level association between the EPDS trajectories and HCC1 (Adj $R^2 = 0.008$, $p = .08$) and thus, adjusted Step 2 and 3 models were conducted with trajectories of prenatal depressive symptoms in relation to both HCC1 and HCC2.

The SCL-90 trajectories were associated with neither HCC1 nor HCC2 in the Step 1 analyses, thus no adjusted models were conducted related to anxiety symptom trajectories (see **Table 11**).

The association between HCC1 and EPDS trajectories was not significant in adjusted models. In contrast, HCC2 was associated with the EPDS trajectories also after adjusting for maternal education, age, and BMI in Step 2 (p-value for the EPDS trajectory variable = .04, Adj $R^2 = 0.05$ for the whole model, $p = .03$; see **Table 11**). The association between the EPDS trajectory variable and HCC2 was influenced by the inclusion of maternal use of SSRI/SNRI's (Step 3), and no longer reached significance ($p = 0.07$), but the distinct trajectory groups still predicted HCC2 as HCC2 was higher in the "Consistently elevated" group in comparison to the "Consistently low" ($\beta = -0.71$, $p = .02$) and the "Low and increasing" ($\beta = -0.82$, $p = .01$) groups. The Step 3 model for EPDS trajectories explained 6.4% of the variance in HCC2 ($p = .03$).

**Table 10.** Category descriptions including mean HCC levels of each category and Step 1 associations between trajectories of maternal prenatal PD and HCC1 and HCC2 (* for p < .05, ** for p < .01, *** for p < .001).

| **Trajectories explaining HCC1** | **mean (SD) HCC1 (pg/mg)** | **N** | **Adj $R^2$** | **β** | **p** |
|---|---|---|---|---|---|
| **EPDS trajectories** | | 466 | 0.008 | | .08 |
| - Consistently low | 19.12 (36.82) | 315 | | -0.62 | .14 |
| - Moderate and slightly increasing | 20.52 (30.08) | 129 | | -0.41 | .33 |
| - Moderate and steeply increasing | 18.89 (13.53) | 16 | | -0.32 | .52 |
| - High and decreasing | 24.71 (16.61) | 6 | | 0 | |
| **SCL-90 trajectories** | | 466 | -0.001 | | .41 |
| - Consistently low | 19.62 (34.96) | 443 | | -0.18 | .41 |
| - High and decreasing | 18.83 (15.83) | 23 | | 0 | |
| **Trajectories explaining HCC2** | **mean (SD) HCC2 (pg/mg)** | **N** | **Adj $R^2$** | **β** | **p** |
| **EPDS trajectories** | | 211 | **0.03*** | | **.04** |
| - Consistently low | 18.62 (19.84) | 133 | | **-0.54*** | **.02** |
| - Low and increasing | 19.52 (17.82) | 48 | | **-0.55*** | **.03** |
| - Low and steeply increasing | 25.91 (16.46) | 11 | | -0.08 | .81 |
| - Consistently elevated | 29.16 (25.77) | 19 | | 0 | |
| **SCL-90 trajectories** | | 211 | -0.008 | | .83 |
| - Consistently low | 19.71 (19.16) | 175 | | 0.17 | .56 |
| - Low and steeply increasing | 24.70 (26.77) | 25 | | 0.19 | .57 |

Abbreviations: PD = psychological distress; HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; HCC2 = HCC measured 1–3 days after delivery; EPDS = Edinburgh Postnatal Depressive Scale; SCL-90 = Symptom Checklist 90

**Table 11.** Adjusted models for the association between maternal prenatal HCC and the trajectories of depressive symptoms (* for p < .05, ** for p < .01, *** for p < .001).

| | Step 2 | | | Step 3 | | |
|---|---|---|---|---|---|---|
| | Adj $R^2$ | β | p | Adj $R^2$ | β | p |
| **Adjusted EPDS trajectory model explaining HCC1** | 0.01 | | .15 | 0.02 | | .081 |
| **EPDS trajectory variable** | | | .077 | | | .13 |
| - Consistently low | | -0.67 | .11 | | -0.61 | .14 |
| - Moderate and slightly increasing | | -0.44 | .30 | | -0.40 | .34 |
| - Moderate and steeply increasing | | -0.45 | .36 | | -0.43 | .38 |
| - High and decreasing | | 0 | | | 0 | |
| **Adjusted EPDS trajectory model explaining HCC2** | **0.05*** | | **.03** | **0.06*** | | **.03** |
| **EPDS trajectory variable** | | | **.04** | | | .07 |
| **- Consistently low** | | **-0.53*** | **.02** | | **-0.71*** | **.02** |
| **- Low and increasing** | | **-0.6*** | **.012** | | **-0.82*** | **.01** |
| - Low and steeply increasing | | -0.06 | .86 | | -0.37 | .41 |
| **- Consistently elevated** | | **0** | | | **0** | |

Abbreviations: HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; HCC2 = HCC measured 1–3 days after delivery; EPDS = Edinburgh Postnatal Depressive Scale; SCL-90 = Symptom Checklist 90

## 5.4 Associations between Maternal Prenatal HCC and Child Socioemotional Problems (Study III)

### 5.4.1 Child Socioemotional Problems at 2 years

In Study III, HCC1 was not observed to associate with either BITSEA Problems Total score or with any of the subtypes of problems in the descriptive analyses in the Step 1 or 2 models. In contrast, a negative association between HCC2 and the BITSEA Problem Total score was observed in Step 2 analyses after inclusion of the maternal depressive symptoms as measured by EPDS (β = −0.85, p = .12 and β= −1.06, p = .04 for Steps 1 and 2, respectively; see **Figure 6**, **Table 12**, and **Table 13**).

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 (β = −0.75, p = .006 and β= −0.83, p = .002,

respectively; **Figure 6**, **Table 13**), while none of the other problem subscales of BITSEA were associated with HCC2.

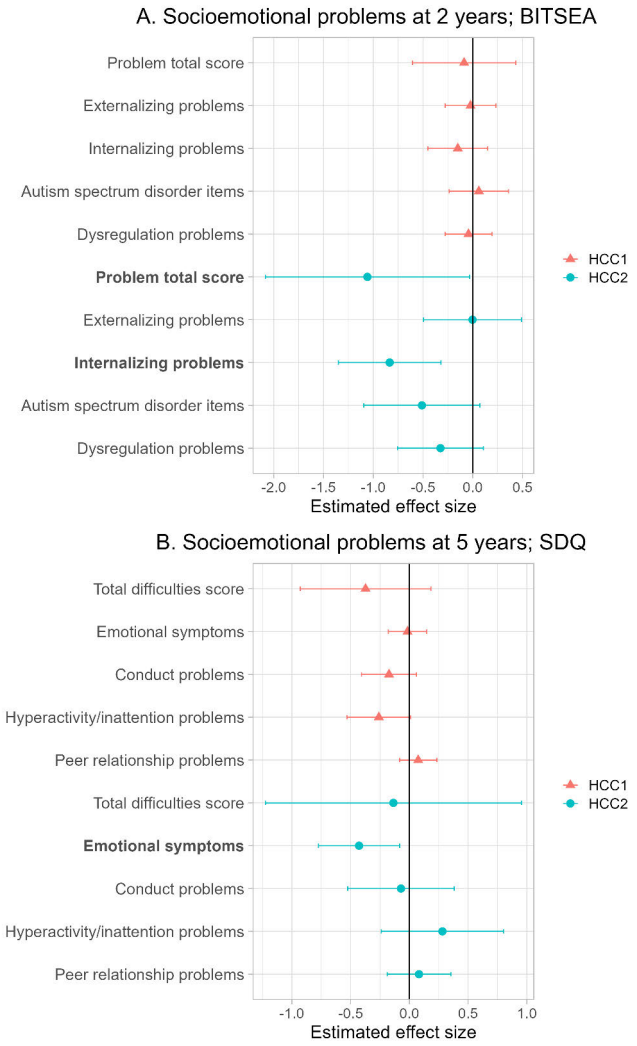


**Figure 6.** Associations between maternal HCC1 and HCC2 and child socioemotional problems at 2 (6A) and 5 (6B) years based on Step 2 models. BITSEA = Brief Infant Toddler Socioemotional Assessment; SDQ = Strengths and Difficulties Questionnaire; HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; HCC2 = HCC measured 1–3 days after delivery.

**Table 12.** Beta coefficients for associations between HCC1 and BITSEA at 2 years in stepwise analyses (presented with one decimal only for readability; * for p < .05, ** for p < .01, *** for p < .001).

| | | **STEP 1** | **STEP 2** | | **STEP 3** | **STEP 4** | | | | **STEP 5** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | BOYS | | GIRLS | | | |
| Outcome | Variable | β | β | | β | β | | β | | β | |
| BITSEA Problem Total score | HCC1 | 0.1 | -0.1 | | -0.3 | -0.2 | | 0.1 | | 0.1 | |
| | Prenatal EPDS | | 0.2 | *** | -0.5 | 0.2 | * | 0.2 | *** | 0.1 | |
| | Postnatal EPDS | | | | | | | | | 0.3 | *** |
| | HCC1* EPDS | | | | 0.1 | | | | | | |
| BITSEA Externalizing problems | HCC1 | 0.0 | -0.0 | | -0.4 | 0.0 | | -0.0 | | 0.0 | |
| | Prenatal EPDS | | 0.1 | ** | -0.4 | 0.0 | | 0.1 | ** | -0.0 | |
| | Postnatal EPDS | | | | | | | | | 0.1 | *** |
| | HCC1* EPDS | | | | 0.0 | | | | | | |
| BITSEA Internalizing problems | HCC1 | -0.1 | -0.2 | | 0.2 | -0.4 | | -0.0 | | -0.1 | |
| | Prenatal EPDS | | 0.1 | *** | 0.0 | 0.1 | ** | 0.1 | ** | 0.1 | ** |
| | Postnatal EPDS | | | | | | | | | 0.1 | * |
| | HCC1* EPDS | | | | -0.0 | | | | | | |
| BITSEA Autism spectrum disorder items | HCC1 | 0.1 | 0.1 | | -0.6 | 0.1 | | 0.1 | | 0.1 | |
| | Prenatal EPDS | | 0.0 | | -0.0 | 0.0 | | 0.0 | | -0.0 | |
| | Postnatal EPDS | | | | | | | | | 0.1 | |
| | HCC1* EPDS | | | | 0.0 | | | | | | |
| BITSEA Dys-regulation problems | HCC1 | -0.0 | -0.0 | | 0.2 | -0.2 | | 0.1 | | 0.0 | |
| | Prenatal EPDS | | 0.0 | * | 0.2 | 0.0 | | 0.1 | * | 0.0 | |
| | Postnatal EPDS | | | | | | | | | 0.1 | ** |
| | HCC1* EPDS | | | | -0.0 | | | | | | |

Abbreviations**:** HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; BITSEA = Brief Infant-Toddler Socioemotional Questionnaire; EPDS = Edinburgh Postnatal Depressive Scale

**Table 13.** Beta coefficients for associations between HCC2 and BITSEA at 2 years in stepwise analyses (presented with one decimal only for readability; * for p < .05, ** for p < .01, *** for p < .001).

| | | **STEP 1** | | **STEP 2** | | **STEP 3** | | **STEP 4** | | | **STEP 5** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | BOYS | | GIRLS | | |
| Outcome | Variable | β | | β | | β | | β | | β | β | |
| BITSEA Problem Total score | HCC2 | -0.9 | | -1.1 | * | -0.1 | | -1.1 | | -1.2 | -0.9 | |
| | Prenatal EPDS | | | 0.2 | ** | 0.5 | * | 0.3 | *** | 0.2 | 0.2 | * |
| | Postnatal EPDS | | | | | | | | | | 0.1 | |
| | HCC2* EPDS | | | | | -0.1 | | | | | | |
| BITSEA Externalizing problems | HCC2 | 0.1 | | 0.0 | | -0.5 | | 0.0 | | 0.0 | 0.1 | |
| | Prenatal EPDS | | | 0.1 | * | 0.0 | | 0.1 | | 0.1 | 0.1 | |
| | Postnatal EPDS | | | | | | | | | | 0.1 | |
| | HCC2* EPDS | | | | | 0.0 | | | | | | |
| BITSEA Internalizing problems | HCC2 | -0.7 | ** | -0.8 | ** | -0.1 | | -0.8 | ** | -0.9 | -0.8 | ** |
| | Prenatal EPDS | | | 0.1 | ** | 0.3 | ** | 0.1 | ** | 0.1 | 0.1 | |
| | Postnatal EPDS | | | | | | | | | | 0.0 | |
| | HCC2* EPDS | | | | | -0.1 | * | | | | | |
| BITSEA Autism spectrum disorder items | HCC2 | -0.4 | | -0.5 | | -0.8 | | -0.3 | | -0.9 | -0.5 | |
| | Prenatal EPDS | | | 0.1 | * | -0.5 | | 0.1 | | 0.1 | 0.1 | |
| | Postnatal EPDS | | | | | | | | | | 0.1 | |
| | HCC2* EPDS | | | | | 0.0 | | | | | | |
| BITSEA Dys-regulation problems | HCC2 | -0.3 | | -0.3 | | 0.6 | | -0.4 | | -0.2 | -0.2 | |
| | Prenatal EPDS | | | 0.0 | | 0.2 | | 0.1 | | 0.0 | 0.0 | |
| | Postnatal EPDS | | | | | | | | | | 0.1 | |
| | HCC2* EPDS | | | | | -0.1 | * | | | | | |

Abbreviations: HCC = hair cortisol concentrations; HCC2 = HCC measured at 1–3 days after delivery; BITSEA = Brief Infant-Toddler Socioemotional Questionnaire; EPDS = Edinburgh Postnatal Depressive Scale

### 5.4.2 Child Socioemotional Problems at 5 years

Neither HCC1 nor HCC2 were associated to the Total difficulties score at 5 years in Steps 1 or 2 (**Figure 6**, **Table 14**, and **Table 15**). Neither was HCC1 associated with any of the SDQ subscales in descriptive analyses. There was, however, a negative

association between HCC2 and SDQ Emotional symptoms in both the Step 1 and 2 models (β = −0.38, p =.03 and β = −0.43, p = .02, respectively; see **Figure 6** and **Table 15**).

**Table 14.** Beta coefficients for associations between HCC1 and SDQ at 5 years in stepwise analyses (presented with one decimal only for readability; * for p < .05, ** for p < .01, *** for p < .001).

| | | **STEP 1** | **STEP 2** | | **STEP 3** | **STEP 4** | | | | **STEP 5** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | BOYS | | GIRLS | | | |
| Outcome | Variable | β | β | | β | β | | β | | β | |
| SDQ Total Difficulties score | HCC1 | -0.2 | -0.4 | | -1.3 | 0.2 | | -0.7 | * | -0.3 | |
| | Prenatal EPDS | | 0.3 | *** | -0.2 | 0.3 | *** | 0.2 | *** | 0.2 | *** |
| | Postnatal EPDS | | | | | | | | | 0.3 | *** |
| | HCC1* EPDS | | | | 0.0 | | | | | | |
| SDQ Emotional symptoms | HCC1 | 0.0 | 0.0 | | -0.1 | 0.0 | | 0.0 | | 0.0 | |
| | Prenatal EPDS | | 0.1 | *** | 0.0 | 0.1 | *** | 0.0 | * | 0.1 | *** |
| | Postnatal EPDS | | | | | | | | | 0.1 | ** |
| | HCC1* EPDS | | | | 0.0 | | | | | | |
| SDQ Conduct problems | HCC1 | -0.1 | -0.2 | | -0.1 | 0.1 | | -0.3 | * | -0.1 | |
| | Prenatal EPDS | | 0.1 | *** | -0.1 | 0.1 | ** | 0.1 | ** | 0.0 | * |
| | Postnatal EPDS | | | | | | | | | 0.1 | *** |
| | HCC1* EPDS | | | | 0.0 | | | | | | |
| SDQ Hyperactive /inattentive problems | HCC1 | -0.2 | -0.3 | | -0.7 | * 0.0 | | -0.4 | * | -0.2 | |
| | Prenatal EPDS | | 0.1 | ** | -0.1 | 0.0 | | 0.1 | ** | 0.1 | * |
| | Postnatal EPDS | | | | | | | | | 0.0 | |
| | HCC1* EPDS | | | | 0.0 | | | | | | |
| SDQ Peer problems | HCC1 | 0.1 | 0.1 | | -0.4 | * 0.1 | | 0.0 | | 0.1 | |
| | Prenatal EPDS | | 0.1 | *** | 0.0 | 0.1 | *** | 0.0 | * | 0.0 | * |
| | Postnatal EPDS | | | | | | | | | 0.1 | ** |
| | HCC1* EPDS | | | | 0.0 | | | | | | |

Abbreviations: HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; SDQ = Strengths and Difficulties Questionnaire; EPDS = Edinburgh Postnatal Depressive Scale

**Table 15.** Beta coefficients for associations between HCC2 and SDQ at 5 years in stepwise analyses (presented with one decimal only for readability; * for p < .05, ** for p < .01, *** for p < .001).

| | | **STEP 1** | **STEP 2** | **STEP 3** | **STEP 4** | | **STEP 5** |
|---|---|---|---|---|---|---|---|
| | | | | | BOYS | GIRLS | |
| Outcome | Variable | β | β | β | β | β | β |
| SDQ Total Difficulties score | HCC2 | 0.1 | -0.1 | -1.3 | 0.2 | -0.8 | 0.2 |
| | Prenatal EPDS | | 0.3 ** | 0.0 | 0.3 * | 0.2 | 0.2 * |
| | Postnatal EPDS | | | | | | 0.3 * |
| | HCC2* EPDS | | | 0.0 | | | |
| SDQ Emotional symptoms | HCC2 | -0.4 * | -0.4 * | -0.3 | -0.4 | -0.5 * | -0.3 |
| | Prenatal EPDS | | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 |
| | Postnatal EPDS | | | | | | 0.1 * |
| | HCC2* EPDS | | | 0.0 | | | |
| SDQ Conduct problems | HCC2 | 0.0 | -0.1 | 0.0 | 0.0 | -0.3 | 0.0 |
| | Prenatal EPDS | | 0.1 ** | -0.4 | 0.1 * | 0.1 | 0.1 * |
| | Postnatal EPDS | | | | | | 0.0 |
| | HCC2* EPDS | | | 0.0 | | | |
| SDQ Hyperactive /inattentive problems | HCC2 | 0.3 | 0.3 | -1.2 * | 0.4 | 0.2 | 0.4 |
| | Prenatal EPDS | | 0.1 | 0.4 | 0.1 | 0.1 | 0.0 |
| | Postnatal EPDS | | | | | | 0.1 |
| | HCC2* EPDS | | | 0.0 | | | |
| SDQ Peer problems | HCC2 | 0.1 | 0.1 | -0.3 | 0.2 | -0.1 | 0.1 |
| | Prenatal EPDS | | 0.0 | 0.4 | 0.0 | 0.0 | 0.0 |
| | Postnatal EPDS | | | | | | 0.0 |
| | HCC2* EPDS | | | 0.0 | | | |

Abbreviations: HCC = hair cortisol concentrations; HCC2 = HCC measured at 1–3 days after delivery; SDQ = Strengths and Difficulties Questionnaire; EPDS = Edinburgh Postnatal Depressive Scale

### 5.4.3 Interactions between Maternal Prenatal Depressive Symptoms and Maternal Prenatal HCC

Overall, while inverse associations consistently occurred between maternal HCC and child socioemotional problems, the associations between depressive symptoms and child outcomes in the same models were positive (data shown in the supplementary material of Study III). This remained for, not only maternal prenatal depressive symptoms, but also maternal postnatal depressive symptoms that were introduced in Step 5 as sensitivity analyses. Positive associations between depressive symptoms and child socioemotional problems were more frequently observed in the mid-pregnancy models in comparison to the end-of-pregnancy models that possessed more associations related to HCC.

In the interaction analyses in Step 3, the interaction between HCC1 or HCC2 and maternal prenatal depressive symptoms associated with none of the addressed total sum scores of child socioemotional problems (**Table 12** – **Table 15**, **Figure 7**). However, the interaction of HCC2 and maternal prenatal depressive symptoms was negatively associated with BITSEA Internalizing problems ($\beta = -0.09$, $p = .01$) and BITSEA Dysregulation problems ($\beta = -0.07$, $p = .03$) at 2 years (**Table 13, Figure 7**). No interactions were observed related to HCC1 or any of the 5-year SDQ scales (**Table 12**, **Table 14**, and **Table 15**).

In the simple slope analysis, no association between HCC2 and BITSEA Internalizing symptoms was observed for subjects with low levels of depressive symptoms ($\beta = -0.24$, $p = .48$), but significant associations between HCC2 and Internalizing symptoms occurred in subjects with intermediate ($\beta = -0.84$, $p = .001$) and elevated ($\beta = -1.43$, $p < .0001$) levels of prenatal depressive symptoms (**Figure 7**). For BITSEA Dysregulation problems, the association between HCC2 and child symptoms occurred for mothers with elevated levels of depressive symptoms ($\beta = -0.76$, $p = .01$, **Figure 7**). A similar pattern seemed to occur with the BITSEA Problem Total score (**Figure 7**), albeit the association for the interaction failed to reach statistical significance ($\beta = -0.11$, $p = .13$).

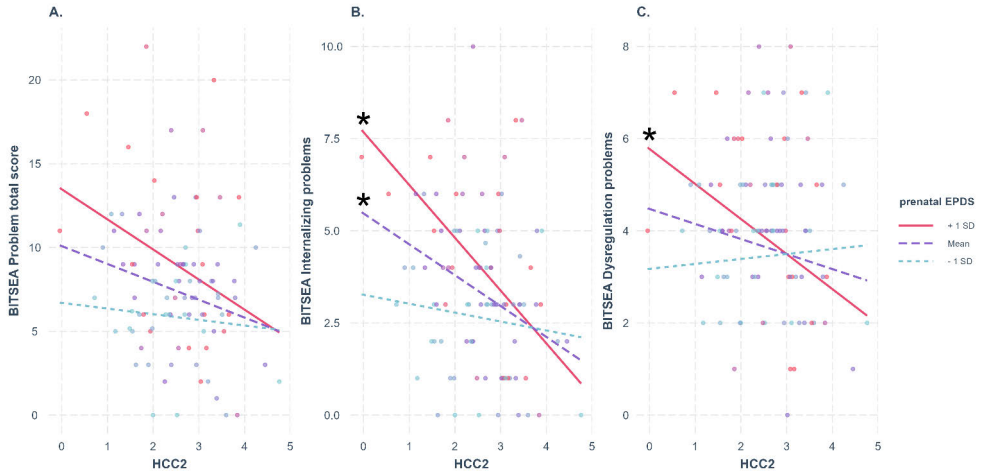


**Figure 7.** Simple slope analyses of interactions between maternal prenatal HCC and depressive symptoms in explaining child socioemotional problems. Significant differences in the association between HCC and child symptoms based on the level of maternal depressive symptoms are marked with asterisks. HCC = hair cortisol concentrations; HCC2 = HCC measured 1–3 days after delivery; BITSEA = Brief Infant-Toddler Socioemotional Assessment; EPDS = Edinburgh Postnatal Depressive Scale.

### 5.4.4 Sex-Specific Associations

In Step 4 of Study III, potential sex-specific associations were explored. The previously observed negative associations between HCC2 and internalizing/emotional symptoms reached significance in boys at 2 years and in girls at 5 years, and while non-significant, the directions and magnitudes of the associations of the other sex were similar in both quantitative and visual examinations (**Figure 8**, **Table 13**, and **Table 15**).). In addition, a novel sex-specific negative association was observed between HCC1 and SDQ Total difficulties score in 5-year-old girls ($\beta = -0.70$, $p = .05$, **Figure 8** and **Table 14**). In descriptive analyses, the subscales that specifically associated with maternal HCC2 in girls were Conduct ($\beta = -0.32$, $p = .03$) and Hyperactivity/inattentive problem subscales ($\beta = -0.38$, $p = .03$; **Figure 8** and **Table 14**).

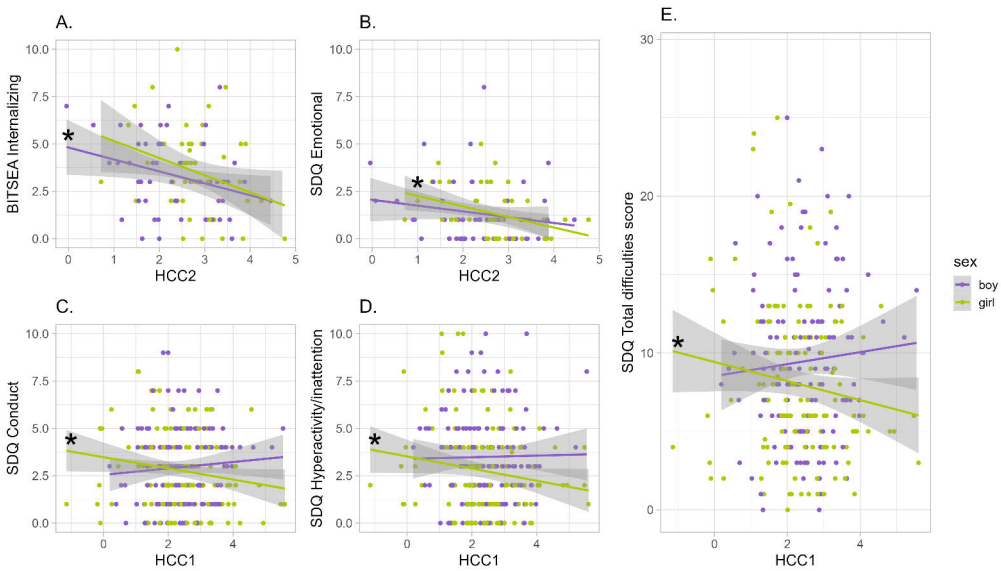


**Figure 8.** An illustration of sex-specific associations between maternal prenatal HCC and child socioemotional problems. Asterisks mark the significant associations between maternal HCC and child symptoms in the marked sex group. HCC = hair cortisol concentrations; HCC1 = HCC measured at gw 24; HCC2 = HCC measured 1–3 days after delivery; BITSEA = Brief Infant-Toddler Socioemotional Assessment; SDQ = Strengths and Difficulties Questionnaire.

## 5.5 Child HCC as a Mediator between Maternal Prenatal HCC and Child Socioemotional Problems

First, the between-method correlations of child HCC from the 70 hair samples that were divided in half and analyzed with both ELISA as well as LC-MS/MS methods were assessed to ensure adequate comparability between maternal prenatal HCC that were analyzed with ELISA and child HCC analyzed with LC-MS/MS. The HCC measured with both methods were correlated ($p < .001$) with an unadjusted correlation coefficient of $r = 0.80$.

In assessing the basic assumptions for the mediator analyses with direct correlations between maternal and child HCC and child socioemotional problems, a significant association between maternal prenatal HCC and child socioemotional problems was only observed between the HCC2 and BITSEA Problem total score, a result that was observed similarly in unadjusted correlation analyses ($r = -0.21$, $p = .04$, see **Table 13**) in the final models described previously. In addition, we observed maternal HCC1 to positively correlate with child $HCC_{30m}$ ($r = 0.37$, $p < .001$), but no other association was observed between the HCC of the mother-child dyads. Neither

$HCC_{30m}$ nor $HCC_{5y}$ correlated with the corresponding socioemotional total problem score.

As there were no total effects between HCC1 and child socioemotional outcomes, there were no relationships for mediators to explain, and thus, proceeding with mediator analyses was not achievable. For HCC2, there was a total effect related to the BITSEA Problem Total score, however maternal HCC2 did not associate with child $HCC_{30m}$ and there were no association between $HCC_{30m}$ and the BITSEA Problem Total score. Thus, there was no basis to proceed with these mediator analyses, either. Instead, their relations are visualized in **Figure 9**.

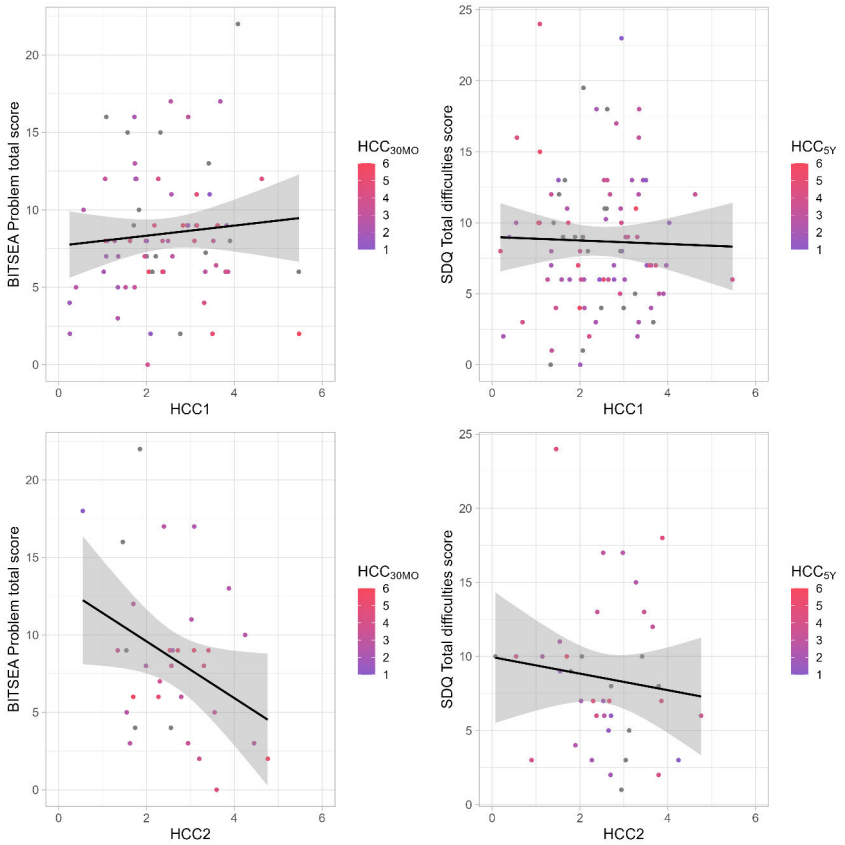


**Figure 9.** Scatter plots of associations between maternal HCC and child socioemotional problems when accounting for the corresponding child HCC. HCC = hair cortisol concentrations; HCC2 = HCC measured 1–3 days after delivery; $HCC_{30mo}$ = child HCC measured at 30 months; $HCC_{5y}$ = child HCC measured at 5 years; BITSEA = Brief Infant-Toddler Socioemotional Assessment; SDQ = Strengths and Difficulties Questionnaire.

# 6 Discussion

## 6.1 Associations between Maternal Prenatal HCC and Maternal Prenatal PD

Recently, studies showing the associations between maternal prenatal PD and HCC have substantially increased leaving a disconnection between the measures of maternal prenatal HCC and experiences of PD. While methodological factors play a significant role in this discordance, even with optimized study designs there would likely be a lack of strong positive associations. Existing evidence does imply that the PD and HCC measures show distinct and only partially interrelated phenomena. Long-term cortisol levels are influenced by a number of factors with only some of them directly linked with experiences of PD, thus the two measures cannot be considered equivalent in a sense that an alteration of either would directly affect the other. However, whether or not there is a link between them is, as such, important. Both distress symptoms and cortisol levels show intrinsic features of maternal prenatal well-being with distinct mechanisms by which they can affect fetal development.

During the past few years, more studies have acknowledged the need to use more finely tuned statistical methods to compare these two fundamentally different measures. Two main tracts exist in how this challenge has been addressed. Some studies focus on modeling chronic, as opposed to temporary, occurrence of PD symptoms and others have shifted the focus to longitudinal within-person alterations in both the cortisol exposure and the concurrent experiences of PD. Depending on the modeling, the studies reporting either of these means and also more traditional momentary between-person associations have presented markedly different results. This implies that disregarding these crucial methodological issues can prevent us from observing the phenomena the data actually show or may only provide a glimpse. This was also observed in Study II, where we observed a positive association between maternal end-of-pregnancy HCC and trajectories of depressive symptoms during the prenatal period, while no associations were found using self-reports of short-term prevalence of maternal PD symptoms regardless of the type of PD. No associations between HCC and depressive or anxiety symptoms were observed when accounting for the symptom prevalence in two consecutive

measuring points, either. We did, however, observe a negative association between mid-pregnancy HCC and cumulative worries about relationships assessed by the Daily Hassles questionnaire, which we could not include in the trajectory analyses because of the metrics of the measure.

In the results drawn from the FinnBrain Birth Cohort, the end-of-pregnancy HCC was associated with the trajectories of depressive symptoms, whereas the mid-pregnancy HCC associated with the cumulative mean of experienced stressfulness related to relationships and dental anxiety (Viitaniemi et al., 2021). Correspondingly, evidence implies that the proportion of significant, either positive or negative, associations was greater during the second and third trimesters than it was when hair samples were acquired after delivery. As the reactivity of the HPA axis is dampened towards the end of pregnancy, it could be that a variety of short-term experiences of inconvenience would be coupled with alterations of the HPA axis functioning during early and mid-pregnancy, while only chronic and cumulative exposure to PD would be related to the end-of-pregnancy HCC. However, the occurrence of both positive and negative associations that both may be relevant for potential offspring outcomes indicates the complexity of the relation.

The trajectory approach in Study II has not, thus far, been used in other studies of the hair cortisol research field. The only other study that, to date, aimed to model the long-term experiences of PD, had two measurement points and calculated the change between them (Orta et al., 2018). In addition, another study assessed the impact of the duration of experienced distress, i.e., a time period of 1 to 3 months or recently, observing that the concordance of PD and HCC measures did not explain the presence or absence of associations (Robertson et al., 2023). Notably, different types of PD were assessed with different questionnaires with varying timelines, thus the lack of associations can result from various reasons. Modeling the trajectories of PD symptoms does seem to provide relevant information on the long-term activity of the HPA axis, thus it would be desirable for the approach to gain more use.

More studies report that maternal prenatal HCC associates with a measure of PD than there are those only reporting null associations. This is especially relevant as the literature review presented here also had studies whose main study questions did not include the direct associations between HCC and PD if still clearly reporting the data on this specific study question. This could reduce the risk for publication bias, as the publication of data with null findings might be more appealing when accompanied with other findings. Nevertheless, when comparing the number of studies reporting positive, null, or negative associations regarding any specific subtype of maternal prenatal PD and HCC, it is systematically the null findings that are most common. This is emphasized in the previous meta-analysis observing only 31 of the total of 242 extracted individual associations to yield significant results (Khoury et al., 2023).

Interestingly, positive associations have been most often reported related to general anxiety symptoms, i.e., four studies with positive associations, none with negative, and seven with null findings. Previously in non-pregnant samples, anxiety symptoms were related to lower HCC, while chronic exposure to stress was associated with elevated HCC (Stalder et al., 2017). This discrepancy could be explained by PTSD-related studies being included as anxiety in that meta-analysis, as PTSD is a severe disorder that is characterized by traumatic and extremely stressful experiences that are prone to chronically alter the functioning of HPA axis. In contrast, prenatal experiences of anxiety symptoms more likely result in acute alterations in stress reactivity or maladaptive patterns in a previously physiologically functioning HPA axis. Depressive symptoms and perceived stress have been nearly as often reported to have positive as negative associations with maternal prenatal HCC, and for both of these measures, null findings have been, by far, most common as there was a null finding for depressive symptoms in 14 studies in comparison to the two with positive and the three with negative findings. There were seven studies with null findings related to perceived stress in contrast to two with positive and two with negative associations. Pregnancy-related anxiety symptoms have been reported not to be associated with HCC in three studies, and a negative association was found in one.

These observations on the effects of the type of PD on the occurrence of associations between maternal prenatal HCC and PD differ from those observed in the recent meta-analysis (Khoury et al., 2023). This study's results did not imply the type of distress to moderate the association, albeit stronger effects were seen with regards to depressive symptoms and self-reported stress. Further analyses of the meta-analyses revealed, however, that this association was related to measures of chronic stress rather than momentary experiences of perceived stress. Anxiety symptoms did not seem to be associated with HCC when assessed with a meta-analytical approach. Statistical analyses were only performed in the meta-analysis and not in the review presented here, thus different studies may have received unequal weight. In addition, the selection of included studies differed slightly between these two reviews. For instance, no presentation abstracts were included here or could additional data from the authors be requested. On the other hand, the literature review conducted for the meta-analysis was last updated in July 2022 compared to January 2024 for the updated review presented in the thesis, thus leading to several papers only being included here. Nevertheless, it may be that the significance of methodological factors outweighs the potential effect of the type of PD. Of note, the supplementary material in the meta-analysis implied there to be inconsistencies in the data used in the analyses concerning the included studies and numbers of subjects in them, however based on personal contact with the first author, those inconsistencies were only related to the supplementary material and did not

affect any of the analyses (e-mail correspondence with Dr Jennifer Khoury in February 2024).

While positive associations between maternal prenatal HCC and PD were remarkably more common, there were also several studies observing negative associations between the two. For depressive symptoms and pregnancy-related anxiety, inverse associations were more common than positive ones. In the context of stress, lower long-term cortisol levels are thought to result from hyporeactivity following an overstimulated period of high allostatic load (McEwen, 1998). When assessing generally homogenous low-risk populations with rather few subjects with extreme levels of stress that would likely disturb the hormonal allostasis for most individuals, the importance of subjective susceptibility to react to and recover from distress is emphasized. However, especially during pregnancy when the increase in cortisol levels is necessary for both the fetal organ maturation and normal progression of the pregnancy and parturition, the role of a hyporeactive HPA axis may be highlighted also in terms of offspring outcomes.

The main study question most often encountered in the studies assessing the associations between maternal prenatal PD and HCC is to assess the long-term alteration in HPA axis reactivity in response to distress. However, the programming of the HPA axis may affect the psychological responses and subjective experiences of distress, anxiety, or depressive symptoms. It can also impact the recovery from stressful experiences or situations and, thus, the chronicity of stress. Even though the negative association between resilience and HCC exists in a meta-analysis (Xiang et al., 2023) and in refugee children (Smeeth et al., 2023), to date, there are no HCC studies assessing the role of resiliency during pregnancy.

Overall, studies adding our understanding on the factors modifying the individual reactivity to distress during pregnancy are important. While several questions remain regarding associations between PD and HCC in all populations, the pregnancy-related alterations in the functioning of the HPA axis as well as the psychological challenges related to this sensitive transitional phase make the research even more complex. Assuming these findings in non-pregnant individuals to be directly replicable in pregnant individuals may be incongruent. For instance, several studies show hyporeactivity of the HPA axis in relation to experiences of child maltreatment (Hinkelmann et al., 2013; Melhem et al., 2017; Steudte et al., 2013; White et al., 2017), while in pregnant individuals, subjects with a history of two or more adverse childhood events were the only group where positive associations between PD and HCC were observed (Bowers et al., 2018). Without the assessment of maternal prenatal PD, adverse childhood experiences in the expectant mothers' own childhood have been associated with both elevated and lower maternal prenatal HCC (Penner et al., 2023; Swales et al., 2018).

Another source of potential individual differences that has recently been studied is the sex of the fetus. A growing number of studies has assessed whether the child's sex assigned at birth can modify their vulnerability to be affected by maternal prenatal PD or the exposure to altered levels of maternal prenatal cortisol. However, fewer studies focus on the effects of the sex of the fetus on maternal responses even though the communication between the mother and the fetus is known to be reciprocal. Based on studies utilizing hair, salivary, and blood cortisol levels, there are elevated levels and differential patterns in maternal diurnal cortisol when carrying female fetuses (Bleker et al., 2017; Giesbrecht et al., 2015; Romero-Gonzalez et al., 2021). In addition, the reactivity to distress may differ according to the fetal sex, as maternal PD symptoms were associated with HCC only in pregnancies having female fetuses (Freedman et al., 2021).

As the complex relations between maternal prenatal PD and HCC are delicate and affected by numerous factors, it would be unlikely to observe strong associations between the two even with a perfect study protocol. Ethnic factors (Gunnar et al., 2022; Wosu et al., 2015) and maternal physical well-being related factors including prenatal risk conditions (Karakash et al., 2016; van Esch et al., 2020), infections (Gudjonsdottir et al., 2021; Vega Ocasio et al., 2021; Zhang et al., 2022), other medical illnesses (Iob and Steptoe, 2019; Montero-López et al., 2017; Pereira et al., 2019; Smy et al., 2016), and metabolic factors (Jackson et al., 2017; Stalder et al., 2013; Vehmeijer et al., 2021) can all influence HPA axis functioning. In addition, lifestyle habits, such as diet (Vepsäläinen et al., 2021), physical activity (Budnik-Przybylska et al., 2020; Leão et al., 2023), and sleep (El Mlili et al., 2021); and environmental factors like shift work (Manenschijn et al., 2011; Zhu et al., 2022), a demanding work environment (Falco et al., 2023; Steinisch et al., 2014), and factors related to a residential area (Evans et al., 2019; Levhar et al., 2022) play a role in the regulation of humoral stress reactivity. Many of these factors are seldom studied in pregnant individuals. For instance, to date, there are no studies assessing the role of maternal prenatal sleep in maternal prenatal HPA axis functioning and reactivity to PD.

While it is beneficial to learn about the relationship between maternal prenatal PD and concurrent HCC per se, the underlying rationale is to gain an understanding on the potential effects for the offspring development. For this, we need to look further than only the cortisol levels or even the wider glucocorticoid homeostasis, as the maternal signals of distress are transferred to the fetus multifacetedly. An increasing number of studies assess, for instance, the mechanisms related to the maternal and fetal autonomous nervous system, the epigenetic and mitochondrial changes related to placental activity, and the role of maternal microbiota in programming the offspring's gut-brain-axis. The more we understand about the complex, interrelated, and reciprocal effects of all these factors and learn to identify

the subjects with increased vulnerabilities, the better we become in targeting efficient interventions.

## 6.2 Associations between Maternal Prenatal HCC and Child Socioemotional Problems

While numerous studies have been published on the prenatal associations between maternal PD and HCC, longitudinal studies on the relation between maternal prenatal HCC and child PD are scarce. Few indications of direct associations exist between the two (Bosquet Enlow et al., 2017). However, alterations in maternal long-term cortisol levels prenatally could play a role in shaping offspring susceptibility to be adversely affected by maternal PD or by suboptimal parenting (Bosquet Enlow et al., 2017; Galbally et al., 2023b, 2022). Our own results highlight that it is not only elevated, but also lower cortisol levels that may associate with problems in child's socioemotional development.

The only earlier finding of an association between maternal prenatal HCC and a child's socioemotional development was that maternal prenatal HCC was negatively associated with the temperament trait Falling Reactivity at the infant age of six months in unadjusted analyses (Bosquet Enlow et al., 2017). As higher scores in the Falling Reactivity represent a faster rate of recovery from peak distress, excitement, or general arousal, a negative association indicates that children exposed to increased maternal cortisol levels prenatally were more likely to remain in a highly aroused state for a longer time. The study aimed to determine whether maternal lifetime trauma history predicted a child's temperament and whether maternal prenatal HCC acted as a moderator of that association. Thus, no further analyses of that direct correlation were presented, and the finding was not described or discussed in the publication apart from being presented in its correlation table. Hence, this observation can be considered tentative in nature and needs to be interpreted with caution. Nevertheless, as there is a clear hypothetical link between maternal prenatal HPA axis activity and the infant's ease to recover from stress, this association would intuitively seem worthy of further exploration. This finding is contrary to the direction of associations observed in Study III, as Bosquet Enlow et al. (2017) observed the exposure to elevated levels of cortisol to relate to more difficulties in recovery as opposed to less socioemotional problems observed in our results.

Study III was partially explorative in nature as the literature to base our hypothesis on was tenuous. We expected both negative and positive association to be plausible, nevertheless, only observe negative relations between maternal prenatal HCC and child socioemotional problems was surprising. However, the main findings of inverse associations between maternal end-of-pregnancy HCC and child total socioemotional problems at 2 years and both internalizing and emotional symptoms

at 2 and 5 years, respectively, were not without precedence. During the first year of life, elevated end-of-pregnancy HCC have been associated with advantageous offspring outcomes in two previous studies that reported increased HCC to predict accelerated neurodevelopment (Caparros-Gonzalez et al., 2019b; Mariño-Narvaez et al., 2023). Prenatally, placental CRH contributes to the necessary increase in circulating maternal cortisol levels with its positive feedback loop. Thus, a less pronounced increase towards the end of pregnancy could indicate maladaptive regulation of the maternal HPA axis, which could, in turn, be associated with suboptimal offspring developmental trajectories. This could also play a role in recurrent observations of negative associations between maternal prenatal PD and HCC (Bowers et al., 2018; Jahangard et al., 2019; Khoury et al., 2020; Orta et al., 2018; Robertson et al., 2023; Viitaniemi et al., 2021).

The association between elevated maternal cortisol levels and advantageous child outcomes could also derive from non-distress-related increases in cortisol concentrations. Alterations in HPA axis activity are only partly related to the experiences of PD, and there are multiple other reasons for increased cortisol levels. The results of the interaction analyses in Study III highlight a child's emotional problems to be most likely observed in children of mothers with high levels of depressive symptoms but low levels of HCC. In addition, the experiences of stress and distress are distinguished for a reason; not all stress is harmful or related to increased symptoms of psychological distress. Thus, reactivity in HPA axis functioning without significantly elevated PD symptoms during the transitional prenatal period could, in fact, be related to better mental health of the expecting mother. This further highlights that the levels of circulating cortisol and the experienced symptoms of PD are two distinct phenomena that are essential to be acknowledged when assessing the potential effects of prenatal distress.

On the other hand, the pathways of adaptation in fetal programming may not always be straightforward and intuitive. Risk factors generally associate with poorer developmental outcomes. However, as the goal of fetal programming is to enhance the offspring's possibilities to thrive, signals of distress or a harsher environment may be interpreted as a sign to accelerate development and attenuate reactiveness to stress to endure anticipated difficulties. Thus, elevated but non-toxic levels of maternal long-term cortisol could both enhance neurocognitive development (Mariño-Narvaez et al., 2023) as well as help cope with mild levels of distress in early childhood without an increase in socioemotional problems (Mustonen et al., 2024).

The rationale for studying the prevalence of socioemotional problems in small children is that 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 to predict later symptoms

of depression or anxiety is modest in comparison to, for example, hyperkinetic symptoms in preschool-aged children, which more often persevere as similar difficulties in follow-up (Nielsen et al., 2019). This is most likely related to the peak in the incidence of affective disorders in adolescence, whereas traits of neuropsychiatric disorders are typically observed throughout development. Furthermore, the genotypes behind similar behavioral symptoms in toddlerhood can vary greatly thus leading to heterogenic trajectories (Gidziela et al., 2022).

Typically developing two-to-five-year-old children can have a wide variety of different behavioral difficulties that increase and are alleviated at individual paces, and they can be significantly affected by environmental factors. The well-being and behavioral patterns of small children are intrinsically tied to parenting styles and parental well-being. As it was not achievable within the context of this thesis to thoroughly account for all parent-related and other postnatal environmental factors, we opted for assessing both maternal pre- and postnatal depressive symptoms as one key parent-related factor. Further studies are needed to account for other environmental factors such as the wider contexts outside the family of which the child is exposed. For example, the amount of shyness towards strangers at two years of age, which would affect that number of emotional problems reported, varies not only based on individual sensitivity but also the frequency of encountering new situations and people, and whether the child has started out-of-home day-care. This might be especially relevant to account for in studies conducted recently, as the pandemic has remarkably altered the exposure to human contact for most people, although the extents and lengths of this social isolation has varied.

Notably, in the results of Study III, we mostly observed associations that were modest in magnitude. As it was known that both the effects of the prenatal environment on the offspring and the manifestation of problems in socioemotional development in children are complex and multifactorial, this was expected. In addition, the children included in the study were mostly healthy, and high levels of socioemotional symptoms were reported in only a small proportion. The clinical relevance of the observed associations is also somewhat difficult to interpret as logarithm-converted HCC values were used. The beta coefficients for the observed associations ranged from -0.43 for the association between maternal HCC2 and SDQ Emotional symptoms to -1.06 for the association between maternal HCC2 and BITSEA Problem total score, and even -1.43 for the association between HCC2 and BITSEA Internalizing problems in dyads where mothers had elevated levels of prenatal depressive symptoms. As the mean level of problems was generally low (see **Table 6**), a decrease between 0.4 to 1.4 units in symptoms per an increase of one ln-converted HCC unit could be interpreted to represent a clinically relevant difference.

The greatest impact found in Study III was observed in the interaction analyses. Mothers with increased prenatal depressive symptoms and lower HCC, which could be hypothesized to relate to dampened HPA axis reactivity, were most likely to have children who the mothers reported to exhibit heightened amounts of emotional symptoms at 2 years of age. It could be that here, the offspring's HPA axis is also more prone to diminished reactivity. In toddlers, reactive behaviors would more likely be rated as externalizing symptoms and thus, for some individuals, this attenuated reactivity could result in internalizing symptoms being prominent in defining a child's behavioral phenotype. However, this interpretation is merely speculative and further studies with measures of children's HPA axis reactivity are needed.

In the additional data presented in this thesis that was not included in the peer-reviewed papers, we assessed whether alterations in a child's HCC would mediate the associations between maternal HCC and child socioemotional problems. However, as no associations between maternal mid-pregnancy HCC and child socioemotional symptoms were observed in the study population as a whole, and in turn, a child's HCC was only associated with maternal mid-pregnancy and not with the end-of-pregnancy HCC, no mediation analyses could be reliably executed. In addition, the numbers of mother-child dyads included in different subpopulations were rather low, ranging from 33 to 92. Thus, the lack of an observed association should not be interpreted as a confirmation that there could not be a mediating pathway from maternal prenatal to a child's HPA axis functioning further affecting the behavioral outcomes.

As stated, in our studies, we only observed the child's symptoms to be associated with maternal end-of-pregnancy HCC and the child's HCC with maternal mid-pregnancy HCC. When reflected to fetal development, the fetal HPA axis evolves in early pregnancy and is activated already during the prenatal period (Wood and Keller-Wood, 2016). Thus, it is plausible that also its regulation needs to begin early in pregnancy. In contrast, the subtle programming of a child's behavioral phenotypes requires several previous developmental phases of the offspring's nervous system. Therefore, factors affecting brain development early in pregnancy might be related to metrics such as head circumference (Dancause et al., 2011), whereas adjustments related to socioemotional development would relate to the end-of-pregnancy and postnatal environmental cues. Naturally, the significant role of genetics in programming and also the behavioral phenotype of the offspring should not be overlooked.

If it were, in fact, more likely that maternal long-term cortisol levels during mid-pregnancy were associated with a child's HPA axis programming and the end-of-pregnancy cortisol levels with a child's socioemotional development, then, it might be revealed in future studies by assessing prenatal trajectories of maternal HCC.

Additionally, it is important to note that if the child's reactivity to stress is what is hypothesized to mediate the association between maternal long-term cortisol and child socioemotional problems, then that should also be what is measured. In other words, momentary cortisol measures, such as salivary samples, should be used to assess stress reactivity instead of the cumulative cortisol output of the past months measured by HCC, as its increased levels can derive from multiple sources, such as elevated basal levels, high peaks in cortisol as a response to different exposures, or decelerated recovery from stress.

In Study III, we assessed whether some associations were only observed in either of the sexes. While no overall associations with the mid-pregnancy HCC emerged, we did observe more socioemotional problems to be reported in girls when maternal HCC during mid-pregnancy were lower. The number of Total difficulties in 5-year-old girls was inversely associated with maternal mid-pregnancy HCC, and in the descriptive analyses, it was the Conduct problems and Hyperactivity/inattentive behaviors that drove this association. Studies suggest that girls are more vulnerable to the effects of prenatal stress (Carpenter et al., 2015; Sandman et al., 2013). It has been hypothesized that the female placenta is more responsive to alterations in stress signals early in pregnancy and that the female offspring were more prone to fine-tuned and plastic alterations in fetal programming. In contrast, the male fetuses would be more straightforwardly steered into either evolutionarily "good quality" or "poor quality" offspring, and thus, they would either not be affected at all by the subtle signals of early prenatal stress or the effects would be more robust (Clifton, 2010; Sandman et al., 2013).

Importantly, while we conducted sex-stratified analyses, we did not assess the sex of the child as a moderator of potential associations between maternal HCC and child socioemotional problems. This decision was based on that the developmental psychopathological outcomes or phenotype presentations may vary based on the biological sex of the child. For instance, affective symptoms are more prevalent in females and externalizing symptoms in male offspring. By only assessing a child's sex as a potential moderator we might not have observed some associations that were related to these sex differences in the outcome presentations. However, it was not possible to test for sex differences and we cannot state whether a child's sex would moderate these associations.

To conclude, this thesis's findings are novel, as only one preliminary report of an association between maternal prenatal HCC and a trait in child socioemotional development exists. However, the reliability of our results was supported by corresponding observations in both 2- and 5-year-old children. There are earlier observations on the interplay between maternal well-being and prenatal HCC related to problems in a child's socioemotional development, however as no measures of

prenatal PD have previously been included, our results bring new light on this interaction.

## 6.3 Strengths, Limitations, and Future Directions

This study has several strengths. The FinnBrain Birth Cohort Study is a uniquely interdisciplinary prospective longitudinal study with a relatively large study population. It is one of the first large-scale cohorts to begin collecting repeated data for HCC analyses both generally and especially in the context of pregnancy cohorts. Even when accounting for the accumulated literature during the last years, the FinnBrain Study's maternal prenatal HCC data set alone is still amongst the most comprehensive data collections worldwide. Furthermore, the uniqueness of the data lies in its multidisciplinary and longitudinal prospects as they allow the researchers to use the maternal prenatal HCC data both to gain more understanding on the mechanisms related to prenatal fetal exposures as well as, even more importantly, to examine their associations with the offspring's short- and long-term health outcomes. The studies included in this thesis only present a fraction of the potential the Cohort data have to offer, thus future plans within the Cohort are also relevant.

The studies included in this thesis have used a longitudinal study design related to both the mechanisms transmitting the potential effects of maternal PD to the fetus as well as the associations to offspring outcomes. Our data on maternal and child hair samples as well as the validated and widely utilized questionnaires on maternal and child distress were gathered repeatedly during the prenatal and postnatal periods and thus, we can offer new insights on the role of timing for both the exposure and the outcomes. We have assessed the prenatal associations between maternal HCC and PD not only cross-sectionally at separate timepoints during pregnancy but also by assessing the trajectories of PD symptoms, which is yet to be repeated within the study field. In addition, the utilization of maternal prenatal HCC to predict features in a child's socioemotional or psychosocial development is still somewhat exceptional as only one study group before has focused on these associations and others have only included these measures adjacent to their mostly parenting-related main study questions. The potential mediating role of a child's HCC on the association between maternal prenatal HCC and child socioemotional problems has not been previously studied.

Considering the wider prenatal PD literature from a child psychiatry point of view, during the first few years of life, it is perhaps more common to assess infant temperament rather than measuring traits in behavior or emotion regulation, which are more frequently assessed during preschool age (Madigan et al., 2018). Thus, a major strength of Study III was utilizing instruments that account for wider behavioral patterns and emotion regulation skills already at the age of 2 years. The

smaller the child is, the larger the impact of innate temperamental traits. However, already during the first year of life, self-regulation capacity and behavioral reactivity develop and adjust according to the environment and the support the child receives. As those factors are crucial also when assessing the future risk of psychosocial difficulties and psychiatric illnesses, we would miss out on an important facet of information if we would only focus on attributes related to temperament. Simultaneously, it is important to note it is not possible to reliably separate the two theoretical constructs especially with parental report questionnaires, as the ways they associate with phenotypes in a child's behavior and the parents' interpretations of them are overlapping and interrelated.

Socioemotional measuring tools such as the BITSEA and the SDQ in 2- to 5-year-old children show distinct traits of socioemotional difficulties in small children, in whom the phenomena are universal in nature. That means similar behavioral problems can arise from a variety of genotypes and environments, and based on one attribute alone, it is not possible to determine their roots or their consequences. During those years, children are in a stage of rapid development of their self-regulating skills and their capacity to express themselves and reciprocally interact with parents and peers, which can crucially influence the ways a child responds in different situations. For example, a child with rapid language development and stronger effortful control would be more likely to respond in socially accepted ways in difficult situations, whereas a child with less functioning regulation and communicative skills might demonstrate behaviors that would be depicted as either internalizing or externalizing problems, even though their emotional response to the situation itself would be the same. Thus, it is understandable that the psychometric properties of BITSEA, for instance, present a lower value of internal consistency. Assessing socioemotional behaviors of small children is of importance, as they form the basis for the trajectories of mental health in later childhood, adolescence, and adulthood. In the future, complementing this viewpoint with information of the individual's other assets and challenges in development is needed for further understanding of the factors that shape the risk profiles.

In addition to the variation due to differential developmental stages, small children are dependent on the support of the environment and are thus prone to react to alterations in their daily life and the well-being of their caregivers. However, as we are interested in the potential adaptive and programming processes that derive from fetal exposure to alterations in maternal long-term cortisol levels, the level of reactivity of the child is of great interest. Longitudinal study designs with repeated measures help to separate the children whose sensitivity to react is chronically heightened from those responding to a sporadic, major stressor. In addition, the effect of differential susceptibility in determining whether or not the offspring is affected

by prenatal exposures remains to be studied in this context even though there are means to seek to identify those more vulnerable (Assary et al., 2023).

The relatively large number of subjects with relevant measurements enabled the observations of subtle associations, which was significant, as it is known the studied phenomena are multifaceted and maternal cortisol levels only account for a part of the entity. Most other studies in the field have included less than a hundred or a 100 to 200 subjects with HCC measures, thus the maternal prenatal HCC sample size here is amongst the largest ones. This is true despite the significant number of collected samples that were left without a reliable HCC result, mostly due to inadequate hair sample size. This affected especially the final amount of HCC1 (i.e., mid-pregnancy) samples, as the problem in sample collection was noticed and corrected in time to affect both the collection of HCC2 samples as well as postnatal child and parental hair samples. In addition to the hair sample size issue, there were some samples we were unable to use due to uncertainties in the laboratory process, as the hair sample analysis protocol was under revision during the period the maternal prenatal HCC samples were being analyzed. For instance, a few samples were analyzed with an unnecessarily heavy sample weight, which hindered the comparability of the final concentrations and resulted in these samples being excluded. On the other hand, participating in the methodological revising process offered a remarkable learning possibility for the doctoral candidate and has likely improved her abilities to understand and interpret the HCC data.

The methodological development of HCC analyses has been rapid, which is conceivable as the research field of hair analyses has emerged during the last 20 years with hair sample collection in FinnBrain beginning only six years after the first publication. In the beginning, most studies were conducted using ELISA while nowadays, LC-MS/MS analyses are considered the golden standard and measuring steroid hormones other than cortisol is possible. This is in line with the analytical plan executed in the FinnBrain Study, as the maternal prenatal hair analyses were conducted with ELISA, and the analysis method of all postnatal hair samples, here referring to child HCC at 30 months and 5 years, was decided to be exchanged to LC-MS/MS simultaneously including measures of hair cortisone and DHEA. In our data, according to the hair samples that were assessed in duplicate with these two measures, the comparability of the HCC appears satisfactory. In addition, in the early days, there was controversy about whether it is necessary to utilize a ball mill to ensure an evenly grounded hair matrix for reliable steroid extraction or if finely cutting the hair strands would suffice. Thereafter, it has proved applicable to extract cortisol from uncut, whole hair that has streamlined the sample preparation process. Overall, the data presented in the thesis can be considered as one of the pioneering works of large-scale HCC studies, which can be seen both as a strength of the study but also possesses limitations due to the inevitable learning process.

Other limitations of the study include a comparably homogenous general population sample comprising little ethnic variation and socioeconomical statuses biased towards the higher end that limits the generalizability of the findings. Fortunately, although there was some selective attrition, the levels of child and maternal distress and HCC did not differ between the subjects of the whole Cohort and the final study populations. In addition, as the findings were observed regardless of the relatively low number of mothers with high levels of prenatal PD and the small proportion of children with clinically elevated levels of socioemotional problems, it can be assumed that the findings would more likely be confirmed and strengthened in a higher-risk population rather than them being present only within this Cohort.

In terms of child outcomes, Study III only included maternal-report-questionnaires that depicted the child development, which may generate bias related to parental interpretations of their child's behavior. In FinnBrain, observational data from the study visits and assessments from schoolteachers concerning older children are available and can be utilized in future studies, however for the aims of this thesis we selected these outcome measures to best secure eligible sample sizes while still gathering relevant data on child socioemotional development. To account for potential reporting bias that could result from concurrent maternal depressive symptoms, we conducted sensitivity analyses with maternal postnatal EPDS as a covariate, which did not alter the observation of a negative association between maternal end-of-pregnancy HCC and child emotional symptoms at 2 years.

As another limitation, the study only focused on the mother-child dyad and paternal HCC and paternal reports of the child were not included. There are data available on paternal HCC from gw 24, however they could not be utilized as the aims of the study were to assess the mechanisms potentially transmitting the effects of maternal prenatal PD to the fetus. Paternal reports of child socioemotional problems could have, on the other hand, provided a balance to any potential reporting bias on the maternal side, however, as there were already several collateral models, we were unable to include them within the scope of these studies. In future studies, the role of father's HPA axis functioning in programming offspring development through genetic and epigenetic mechanisms as well as postnatally shaping the reactivity and regulative capacities of the child should be assessed.

In the supplemental analyses included in the thesis, we used the cortisol concentrations measured from a child's hair sample. In this context, other steroid measures were decided to be omitted to avoid overcomplicating the analyses. However, further studies may benefit from a broader perspective on the HPA axis functioning as the ratios of cortisol to cortisone and cortisol to DHEA can show the individuals current state of stress reactivity in more detail.

In this study, the trajectories of maternal cortisol levels throughout pregnancy could not be modeled as the number of subjects with both HCC1 and HCC2 results,

and necessary questionnaire data were relatively low. By selecting to use 5-cm segments we could, however, cover the entire length of pregnancy, even though simultaneously a trimester-wise approach could not be used.

While not yet replicated in other maternal prenatal HCC studies, the trajectories of maternal prenatal distress in the FinnBrain Study, with one publication conducted in collaboration with a pregnancy cohort from University of California, have since been associated, for instance, with neonatal amygdala functional connectivity, infants' negative affect and fear reactivity at eight months, and 2- and 5-year-old children's socioemotional problems (Korja et al., 2024; Marr et al., 2023; Nolvi et al., 2019). As the inclusion of subjects was separately selected for each study and the subpopulations have thus differed, one cannot conclude that this would mean that maternal prenatal HCC would also directly associate with these measures. However, for future studies, the findings do present a promising basis. In addition, although thus far only published as a presentation abstract, we have observed concurrently elevated maternal end-of-pregnancy HCC and elevated prenatal depressive symptoms to be associated with decreased fractional anisotropy values in the corpus callosum as measured from 2–5-week-old infants' brain MRI data suggestive for slower maturation and less organization of the structure (Mustonen et al., 2019b). The analyses were originally conducted with a rather small subpopulation of n = 30 mother-infant dyads, however, now with all data readily available, the findings seem to replicate with a slightly larger sample size of n = 40 (manuscript under preparation).

In future studies regarding maternal prenatal PD and HCC, it should be carefully assessed how they contribute to the field. For example, we do not need more studies with only partially compatible PD and HCC measures and altering sampling times during the prenatal period as we already know the critical importance of these methodological factors. Nevertheless, with high-quality longitudinal studies that carefully account for the concurrence of PD symptom occurrence and cortisol accumulation as well as the chronicity and co-occurrence of different types of maternal PD, we can gain more understanding on the factors that account for the hyper - and hyporeactivity of the HPA axis. As there is remarkable between-subject variation in the cortisol levels in both non-pregnant individuals and prenatally, within-subject-assessments may also prove to be important.

The research field on a child's socioemotional outcomes requires more basic research, and there are several points of view that need further examination. Thus far, little is known on the type of behavioral or stress regulation domains that would be most vulnerable to be affected when exposed to altered long-term cortisol levels. A further understanding about the role that lower cortisol levels play is needed, and to concurrently observe both maternal experiences of PD and HCC is needed. Because of the complexity of the mechanisms that transmit maternal signals of PD

to the fetus, studies that simultaneously observe biological measures related to more than one pathway would offer new insights to the interplay between them. For instance, accounting for the mediating role of the placental activity could shed light on the actualized fetal exposure of cortisol. Studies not relying on parental reports of a child's socioemotional development offer more objective interpretation of child behavior. Multidisciplinary studies that include measures of salivary cortisol in depicting HPA axis reactivity to stress or hair cortisol together with data on socioemotional or psychosocial outcomes and, for instance, neuroimaging data would add relevant information on the mechanisms related to the child's risk for future psychopathology. In addition, longitudinal studies extending the follow up further from the preschool years are warranted to establish the clinical significance of the findings.

# 7 Summary/Conclusions

This doctoral dissertation aimed to assess maternal prenatal HCC as a potential indicator of the link between maternal prenatal PD and a child's socioemotional problems. A systematic review on the associations between maternal prenatal HCC and PD was conducted and updated for the purposes of this thesis, and the associations between maternal prenatal HCC and a) maternal prenatal PD and b) a child's socioemotional problems were investigated in the FinnBrain Birth Cohort Study population.

Based on the results presented here, it can be concluded that utilizing maternal prenatal HCC offers essential novel information on both the long-term alterations in HPA axis functioning as a response to distress during pregnancy, and on the problems in offspring socioemotional development when being exposed to altered maternal long-term cortisol levels prenatally. This offers a new understanding on the mechanisms that potentially mediate offspring exposure to maternal prenatal PD. Specifically, these results add to the evidence using maternal prenatal HCC emphasizing the integral role of maternal cortisol levels as one part of the complex mechanistic pathway transferring maternal distress signals to the fetus. In addition, they highlight that higher cortisol levels should not be unquestionably considered detrimental. While HCC should not be conceived as a direct biomarker for either prenatal PD or a child's socioemotional problems, HCC measurements have significantly benefitted the research field by not needing to rely on momentary cortisol measures in attempts to estimate chronic cortisol levels. The methodologies used in the literature vary, however the means to best capitalize on HCC's advantages are actively being clarified.

When aiming to assess links between maternal prenatal PD measures and HCC, accounting for the co-occurrence of measured PD experiences and cortisol exposure plays a key role. This can be accounted for by utilizing a trajectory approach to show prenatal PD symptom prevalence and by studying the within-person changes in maternal prenatal PD and HCC longitudinally. Some of the null findings in the literature may have resulted from methodological issues, such as a mismatch between the PD and HCC timelines. However, PD symptoms do not seem to substantially steer the maternal prenatal HPA axis functioning, but, rather, the

interplay between the two is subtle and complex with several individual factors influencing whether positive, negative, non-linear, or any associations are present.

The prenatal functioning of the maternal HPA axis is delicately regulated by bidirectional communication between the mother and the fetus. If accompanied by other indications of risks, both elevated and decreased long-term cortisol levels may be interpreted as a signal of harsher expected postnatal environment. On the other hand, the adaptation to an expectedly stressful environment may also result in accelerated offspring development or decreased internalizing symptoms, although thus far, whether these seemingly beneficial adaptations only associate with advantageous later health outcomes remains understudied. Furthermore, the connection or disconnection between prenatal PD and long-term cortisol secretion may account for a significant proportion of how the maternal signals to the fetus are interpreted.

Further studies on the role that maternal prenatal PD plays on offspring outcomes need to account for the complexity of the phenomena and measure at least both maternal prenatal HCC and experiences of long-term PD symptoms. In addition, studying different parallel mechanisms, such as placental functioning, epigenetic signaling, and maternal immune markers is recommended. The impact of fetal sex on both influencing maternal responses and steering the vulnerability of the offspring to be affected by maternal distress signals should not be underestimated. In terms of offspring socioemotional outcomes, wide knowledge gaps remain. Studies assessing child development longitudinally and multimodally, i.e., including, for instance, neuroimaging or HPA axis functioning measures together with behavioral assessments or observations, are warranted. Importantly, understanding which children are more likely to be affected by prenatal exposures and targeting them in future studies would help to clarify the complex phenomena.

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