Psychosocial and Biological Profiling of Perinatal Depression

by

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Medicine

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Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree.

The work presented (including data generated and data analysis) was carried out by the author except in the cases outlined below:

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Modelling of psychosocial and lifestyle predictors of peripartum depressive symptoms associated with distinct risk trajectories: a prospective cohort study.

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Abstract

Perinatal depression (PND) has profound consequences for family life, social functioning as well as long-term cognitive development for the offspring. Current screening is ineffective, with half of all cases undetected. Understanding which factors provide the highest risk of PND will benefit screening and the targeting of treatment. PND involves interplay between individual chronic and acute disease burdens, biological and psychosocial environmental behavioural factors. The predictive potential of specific factors and their contribution to severity scores on the Edinburgh Postnatal Depression Scale (EPDS) screening tool is explored here.

This study has employed a multidisciplinary approach to combine psycho-sociodemographic factors with biomarkers from a population of women screened for antenatal (N=1579) and postpartum (N=872) depressive symptoms. Different methods of regression modelling have been explored to determine predictors of perinatal depression, which has also been compared with a machine learning approach. The heterogeneous presentation of symptoms has additionally been explored with statistical analysis.

History of anxiety or depression, young maternal age (18-24) low social status and smoking pre-pregnancy were identified as the strongest contributions to antenatal scores, whereas depressive symptoms in pregnancy and a history of depression or PND exhibited the strongest association with postpartum EPDS. Circulating concentration of IL-10 was significantly associated with antenatal EPDS, in addition to the ratio of IL-6/IL-10. The inclusion of IL-6 and IL-10 data improves prediction of antenatal scores. IL-6 and BDNF concentration were predictive of lower infant birth weights. In general the available covariates are better suited to predict EPDS scores antenatally than postpartum. Risk factor profiles for antenatal and postpartum depression appear to be largely different, supporting the theory that PND is a heterogeneous disease and that underlying pathologies are also heterogeneous.

Abbreviations

PND – perinatal depression

EPDS – Edinburgh Postnatal Depression Scale

APA – average score postpartum to antenatal

DPA – difference score postpartum to antenatal

AND – antenatal depression

PPD – postpartum depression

MDD – major depressive disorder

LBW – low birth weight

SGA – small for gestational age

SES – socioeconomic status

NVB – normal vaginal birth

ELCS – elective caesarean section

EMCS – emergency caesarean section

HPA – hypothalamic-pituitary-adrenal

GR – glucocorticoid receptor

CRH – corticotropin-releasing hormone

CRH-R1/2 – corticotropin-releasing hormone receptor 1/2

ACTH – adrenocorticotropic hormone

SNP – single nucleotide polymorphism

IL-6/10 – interleukin 6/10

BDNF – brain-derived neurotrophic factor

5-HT – 5-hydroxytryptamine (serotonin)

5-mC – 5-methylcytosine

OD – optical density

4-PL – four parameter logistic

CV – coefficient of variation

SD – standard deviation

SEM – standard error of the mean

OR – odds ratio

NPV – negative predictive value

PPV – positive predictive value

HWE – Hardy Weinberg equilibrium

1 Introduction

1.1 Chapter One Abstract

This opening chapter explores the context of the research and the problems which are yet to be adequately investigated in the field of perinatal depression (PND). The relevant literature has been reviewed to identify these gaps in the knowledge and to formulate suitable research questions. Clinical studies and subsequent guidance have emphasised the wide scale problems associated with PND, ranging from psychiatric effects on the mother, father and extended family, to developmental effects both *in utero* and in childhood, with impacts on social, education and health systems. A review of the literature identified two key gaps in the current state of the research: a lack of clarity in understanding the pathogenesis of PND, and a bias towards postpartum studies with fewer studies paying attention to depression during the antenatal period. It is emerging that the effects of PND on the child are profound, with noted effects on infant birthweights, gestational length and cognitive development, which each have implications for later life.

A number of systematic reviews and meta-analyses have investigated psychosocial risk factors for postpartum depression, and the consensus suggests that six key psychosocial risk factors are associated with depression in the postpartum: antenatal depression, antenatal anxiety, major life events, social support levels, depression history and low self-esteem. Fewer studies have investigated psychosocial risk factors for antenatal depression, however a general level of socioeconomic depravation and a lack of social support appear to be important in the development of depression during pregnancy. A crucial problem identified by the literature review is the failure to agree on the definition of perinatal depression; for example the terminology used, the time period this covers, the screening process and the disease classifications are not consistent, leading to difficulties when searching the body of literature. Greater consistency in the field is clearly necessary. Assessment of the current service provision for PND revealed that further inconsistencies are present across maternity services. Since screening for PND is recommended but not mandated, whether or not this is effectively carried out will inevitably vary. The evidence suggests that there is a serious lack of provision for maternal mental health services, and around half of all cases of PND are thought to go undiagnosed.

The aetiology of PND is an area currently lacking clarity, however a few main biological systems carry the strongest evidence for their involvement in the development of maternal depression: the HPA axis, stress-immune interactions (cytokine-glucocorticoid circuit), an underlying genetic risk and DNA methylation. The theories behind each of their involvement is investigated across this chapter. The key gaps in the literature have been scrutinised, leading to the development of this study investigating whether a combination of biological and psychosocial risk factors can predict depressive symptoms in a cohort of women across Coventry and Warwickshire. The aims of this study are presented at the end of this chapter, alongside an overview of the study designed to address them.

1.2 Perinatal Depression

Despite the vast improvement in the physical care of pregnant women in the last century, the same cannot be said for mental health care. Pregnancy and the postpartum period is characterised by increased vulnerability for the onset or relapse of a mental illness, and can present periods of psychological and physical distress. Remarkable physiological and psychological changes occur around parturition which influence maternal health and relationship with the baby. Poor mental health stands as a leading cause of maternal morbidity and mortality, and the foremost cause of maternal death in developed countries, including the UK, is suicide[1].

Depression during pregnancy, around childbirth or within the first year postpartum, is collectively termed perinatal depression (PND). PND has profound consequences for maternal health, family life, social functioning as well as long-term cognitive development for the offspring. Infants of mothers suffering from PND are less likely to be positioned safely while sleeping, receive all recommended immunisations, and be breastfed[2-4]. Anxiety disorders including panic disorder, generalised anxiety disorder (GAD), obsessive compulsive disorder (OCD), post-traumatic stress disorder (PTSD) and tokophobia (extreme fear of childbirth) can present alongside PND as well as on their own[5]. Severe untreated PND can lead to postpartum psychosis, a psychotic illness present in 1-2 in 1000 postpartum women, with symptoms including hallucinations, delusions and suicidal or homicidal thoughts which can become severe rapidly and requires immediate treatment as a psychiatric emergency[6 7]. Since many of its symptoms overlap with those of

depression and anxiety, detection and treatment during pregnancy is vital. PND is now recognised as a major health burden both for the mother, her family and the offspring. The World Health Organisation (WHO) recognises depression as a public health priority[8], with major depression ranked as the world's leading cause of disability[9]. The identification of PND is a critical goal[10], yet half of cases in the UK are undetected[11].

Understanding the origin of the huge under-detection of PND is a timely problem to explore. From a clinical perspective, the identification of depression in the perinatal period is more complex than depression outside of this period. This is in part due to somatic symptoms of pregnancy and the immediate postpartum, such as fatigue and changes to appetite, which can be difficult to distinguish from depressive symptoms and are often attributed to the pregnancy. Depression is surrounded by stigma, and this is especially true for perinatal depression. Societal and cultural beliefs contribute to this stigma and fear surrounding PND, often preventing women from seeking help and hindering its detection. Pregnancy and the birth of a baby carries connotations of happiness, yet for around 1 in 5 mothers[5], this is not the reality.

A lack of awareness exists around PND, not only from a patient and public perspective, but also from health professionals. During pregnancy and postpartum, a mother will have regular contact with trained health professionals, with around twelve antenatal appointments offered[12], providing an excellent opportunity to monitor mental as well as physical health. This is not utilised effectively however, since questions surrounding mental health are encouraged but not mandated, so may often be omitted due to time limitations. A report by the NSPCC on perinatal mental health revealed that 41% of mothers were never asked about depression by their midwife or health visitor[13]. Secondly, this report found that 29% midwives had not received any training in perinatal mental health, and are therefore ill informed to detect symptoms. This is backed by evidence from the Confidential Enquiries into Maternal Death in the United Kingdom, which found a high proportion of midwives received no mental health training[1]. Specialist perinatal mental health midwives have been introduced in some NHS trusts, but the majority of women will not come across these specialists during their care[5].

Depression carries a significant public health impact and is a highly comorbid disease. The rates of obesity, cardiac conditions, heart disease and diabetes are higher in those suffering from depression than in the general population[14]. Mental

ill health is the largest cause of disability in the UK, costing the economy £105 billion per year, which is roughly the entire cost of the NHS, as reported in the Five Year Forward View for Mental Health[15]. This report details the aim to increase NHS funding by £365 million over a five year period to meet the needs of women requiring a specialist perinatal mental health service. Perinatal depression is a specific concern due to a host of health detriments for both mother and child. A first of its kind report into the cost of perinatal mental health problems in the UK found this amounts to £8.1 billion per year, and over 2/3 of these costs relate to effects on the child.[16] The report finds that the average cost of one case of PND to society is £74,000, of which £51,000 relates to impacts on the child. Maternal impacts include health and social care, productivity losses and losses of quality-adjusted life-years, and infant related impacts measured include pre-term birth, infant death, emotional and conduct problems, special educational needs and school qualifications. Several preventative interventions for PND are considered to be cost effective however, as reviewed by Morrell, Sutcliffe *et al.*, further highlighting the importance of detecting women at risk[17].

Postpartum depression (PPD) is closely linked with a higher likelihood of parenting and caregiving difficulties[18 19], yet early treatment can prevent consequences for child development associated with early stress[20]. Infant bonding and parental relationships are compromised, and the detrimental effects on relationships caused by PND have been shown to be multigenerational, extending to both grandmother-parent and grandmother-grandchild dyads[21]. Effective interventions are available to prevent depression postnatally[22], but the inability to identify cases results in lack of treatment and the continuation of symptoms postpartum. A more effective method of identifying high risk women during pregnancy would allow earlier treatment and amelioration of the consequences associated with the continuation of depression postnatally.

Across the general population PND is reported to be experienced by an estimated 10-15% of women[23 24], although the prevalence is likely higher. 30% of PPD persists over a year, with substantial risk of relapse[25 26]. The worldwide prevalence of PND remains unclear, since the vast majority of meta-analyses comprise of women of European ancestry from developed countries, and the rate is estimated to be higher in Low and Middle Income Countries (LMICs).

One recent meta-analysis examined global and national prevalence of PPD, across 291 studies and 56 countries, in addition to risk factors which may vary between

nations[27]. The study found significant heterogeneity exists across nations, ranging from 3% in Singapore to 38% in Chile, and a pooled global prevalence of 17.7%, which is higher than previous estimations. National differences were importantly not explained by methodological differences, but by health and socioeconomic factors. Important factors which increased the national risk of PPD were income inequality, maternal and infant mortality, and women of childbearing age working ≥40hrs per week. This is the largest PPD meta-analysis to date, and lessons must be learnt related to the profound impact of economic disparity and importance of prioritising maternal-child health.

Our current knowledge of perinatal depression is inadequate, and this is a crucial barrier in its detection and treatment, despite the long-lasting effects capable of spanning generations and the cost to society. I have established the need for this novel research contribution, as highlighted by areas which have not yet been adequately investigated. This introductory chapter will continue to introduce the problem of PND and outline any gaps identified within the current literature. This Chapter will cover what is currently know, the limitations and gaps in previous research, unresolved conflicts in the field that still require further investigation, and new developments that will advance the current state of knowledge in the field. The Chapter will conclude with the aims of the current project, a brief description of the research carried out, and an outline of the chapters of this thesis.

1.3 Gaps in the Literature

1.3.1 Pathogenesis of perinatal depression

Depression is a multifaceted disease with no single cause. In order to identify the means to better identify and treat PND, it is imperative to first understand the underlying mechanisms. The pathogenesis of neuropsychiatric disorders such as clinical depression appears to be complex and multifactorial and is a field which warrants further attention. Our knowledge of depressive physiology remains understudied, in particular the aetiology of mental illness in women. Epidemiological studies show that women are twice as likely to develop depression as men[28], yet the majority of preclinical studies in the development of therapeutics focus on males[29]. In particular, our understanding of depression and its mechanisms of disease during pregnancy and the perinatal period remains extremely limited.

Depression outside of the perinatal period has received considerably more attention and multiple categories of biological alterations have been associated with its development. Findings consistently report alterations to the inflammatory system[30] and HPA axis dysfunction[31]. More recent studies have linked genetic and epigenetic alterations with depressive pathogenesis[32]. The complete aetiology of PND is currently unclear, but several hypotheses have been proposed to explain its pathogenesis, involving multiple biological systems (Figure 1.1).

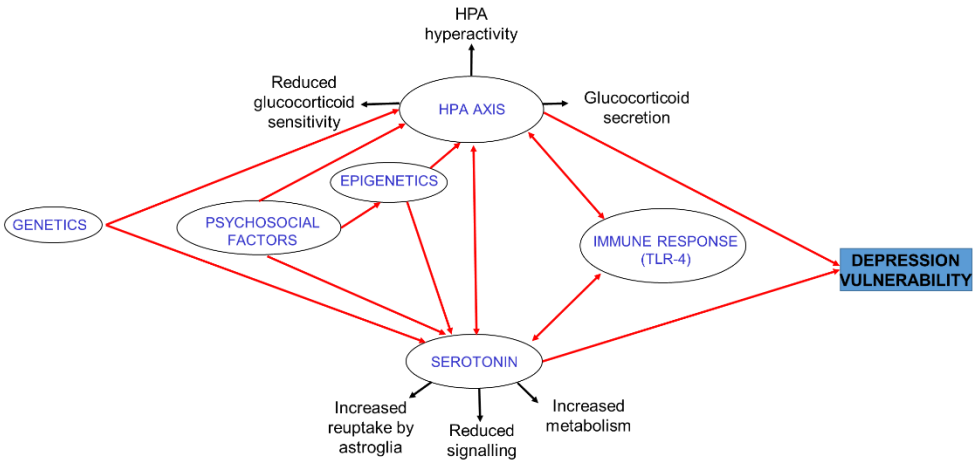

Figure 1.1. The overall pathobiological framework for the development of perinatal depression. The interactions between different systems during PND, namely the immune, neuroendocrine and brain (serotonin), suggest the complexity of this mood disorder. Individual genetic and psychosocial factors interact in this multi-pathway communication network to influence predisposition to depression.

The immune response is becoming increasingly implicated in major depression. The immune system is thought to play a crucial role, and is considered a major triggering factor, mainly characterised by release of cytokines through Toll-like receptor (TLR) signalling. PND has been proposed as an immune-related disorder impinging the brain and neuroendocrine system[33], but an inappropriate inflammatory response can pose harm to both mother and baby during what should be a tightly regulated inflammatory period. There is inconclusive evidence surrounding the role of inflammation in depression during pregnancy and postpartum and this requires further investigation outside of major depression.

The HPA axis is considered central to the pathogenesis of depression[34]. Interconnected individual predispositions including genetics and personal psychosocial factors such as exposure to early adversity are believed to cause adaptive immune, brain and neuroendocrine changes involved in PND. Stress in vulnerable individuals can lead to increased HPA axis activity and high levels of circulatory glucocorticoids due to impaired glucocorticoid receptor function,

although the exact mechanisms of HPA dysfunction in depression are still unclear, as covered in a review by Pariante and Lightman[31]. This review suggests that HPA hyperactivity is not a simple consequence of depression, but is a risk factor predisposing an individual to depression, alongside genetic factors and early life experiences programming molecular changes. They conclude that although altered activity of the HPA axis is one of the most consistent findings in biological psychiatry over the last forty years, it has not been entirely established. It is important to note that this key review covers major depression, and did not assess depression in the perinatal period.

Stress, whether inflammatory, traumatic or emotional, is concurrent with an altered HPA response. Physiological changes to the axis during pregnancy, driven largely by endocrine factors, can lead to an inappropriate response to stress resulting in increased depressive vulnerability. Serotonin dysfunction, which is also thought to drive HPA dysfunction, has long been a key target for depressive physiology research, and is a major target of antidepressant therapies. Brain adaptive responses lead to alterations in serotonin levels which can be a result of reduced signalling, increased metabolism and increased reuptake by astroglia[35]. Both HPA and serotonin dysfunctions are emerging as important areas of investigation in the pathogenesis of PND.

Recently, the role of neuroactive steroids in PND has attracted considerable interest, especially allopregnanolone, a derivative of progesterone synthesised in the adrenal glands, gonads and central nervous system[36]. Experimental studies have linked neuroactive steroids to mood and ability to adapt to stress [37]. Increases in serum allopregnanolone levels during the third trimester have been correlated with alternations in mood, and significantly decreased levels post-partum have been observed in women with symptoms of 'baby-blues' [38]. Allopregnanolone plays a role in the decreased HPA response to stress in rats during pregnancy [39] and this highlights the interaction between neuroactive steroids and the stress system.

Overall, the immune response, HPA axis dysfunction, and alterations to the serotonin system are the three strongest proposed mechanisms for the aetiology of PND. Other predisposing factors include genetics, epigenetics and personal psychosocial factors which appear to interact with the complex and interconnecting systems contributing to PND. Despite their similarities, due to key differences in a number of these systems between the antenatal and postpartum periods, the underlying hypotheses for depression in the two time periods are also thought to

differ. In particular, key differences in the hypothalamic-pituitary-adrenal (HPA) axis function are observed from the pregnant to non-pregnant state, largely due to the growth of the placenta. One of the crucial pathological triggers for PPD is thought to be the rapid decline in hormones such as oestrogen and progesterone following parturition[40]. This rapid decline in hormones is not present during the pregnant state, and therefore the mechanisms for antenatal and postpartum depression are thought to differ. This is further supported by the fact that fathers can too experience postpartum depression, and is highly comorbid with maternal depression, revealing a strong environmental mediating component to PPD such as family environment[41]. The underlying differences will be discussed in further detail later in this chapter.

Further work in perinatal women is required to elucidate the pathobiological mechanisms of PND. The investigation of reported associations between the immune response and PND may provide an opportunity for the discovery of biomarkers to aid its detection. In addition, further distinction between the antenatal and postpartum depressive aetiologies will benefit the debate over whether they represent distinct syndromes and ensure that appropriate biomarkers to detect both antenatal and postpartum depression.

1.3.2 Antenatal depression

The impact of postpartum depression is often more apparent than depression during pregnancy, with more visible consequences including poor mother-infant bonding and an impaired ability to care for the child[18]. It is likely however that the full impact of antenatal depression (AND) has not been realised due to unseen effects *in utero* impacting growth, development, cognitive function and increased psychiatric risk[42 43], which may not be apparent until later in life, and these effects warrant further investigation. Despite established effects of AND on pregnancy outcomes and risk for development of PPD, mental health problems in the antenatal period have received much less attention than PPD with health assessment focusing primarily on maternal and foetal physical health during pregnancy.

Antenatal depression is becoming a leading complication of pregnancy[44]. Fewer studies assess AND, yet it is considered more frequent than PPD, *ipso facto* significantly predicts PPD and carries independent risks[42 45]. Certain psychosocial risk factors have been shown to differ between antenatal and postpartum depression, such as maternal education, which has been found to be a risk factor for postpartum but not antenatal depression[43]. This study by Pearson et al. reports an interaction between PPD and maternal education but no evidence for an interaction between AND and maternal education. Their analyses provided evidence that the effect of PND was limited to mothers with a lower education, whereas effects of AND were present in mothers with both higher and lower education. This study also reported similar patterns of moderation for maternal income, another indicator of socioeconomic status (SES). This suggests that PPD responds more to environmental challenges, with education and income indicative of multiple social and key environmental factors[46], whereas effects of AND may be more strongly linked to biological and genetic mechanisms.

Maternal education indicates multiple sources of psychosocial support and more positive home environment and positive parenting[18], which are likely to protect against depression[47]. The absence of moderation by education on AND is consistent with a biological mechanism *in utero* which is unlikely to be ameliorated by environmental factors. This is further supported by the study's finding that paternal depression is not associated with offspring outcomes. Although the study did not directly test the biological mechanism operating, this provides indirect evidence

that the pathways of AND and PND are different and provides further evidence to support the hypothesis of two distinct syndromes with differing aetiologies.

Antenatal depression appears to pose independent risks for offspring development, such as poor foetal growth and lower birth weights[48], yet the reasons for this are not well defined and the evidence has been inconclusive[49 50]. Developmental effects previously linked to PPD may instead be attributed to AND[51]. Even seemingly inconsequential effects on birth weight can have long-term consequences for cognitive development and responses to stress, as well as general psychological wellbeing in later life and increased risk of mortality[52-54].

Depression in either the antenatal or postpartum period may represent distinct aetiologies, yet perinatal depression can also be considered as a continuous pathological process with similarities and differences. The vast majority of the current literature focuses on one aspect of PND, either the antenatal or postpartum period[55-57]. This approach fails to capture the continuous pathological process than might present as antenatal and/or postpartum depression. Another main limitation of research in the field thus far is the tendency to study one element of susceptibility, for example only the psychosocial/socioeconomic factors, or only genetic factors. In order to really gain a broader understanding of the biology of PND, studies should now become multidisciplinary and examine for example how psychosocial factors interact with genetic factors or biomarker data, since without combining all potential avenues we will not have a full understanding of its aetiology.

1.4 PND and Infant Development

Although the majority of previous research on perinatal mental health has focused on depression in the postpartum period to date, it is known that the antenatal period also represents a time of increased vulnerability for onset or relapse of mental health problems including depression, anxiety, psychosis, post-traumatic stress disorders and eating disorders[58]. All of these problems warrant attention, occurring at any point in the perinatal period, not limited to the postpartum. Biological effects begin to take place *in utero*, and can continue into the period of critical child development after birth, which is explored in the following section.

It is increasingly apparent that the physical and mental health of the offspring starts at conception, and investment in mothers' mental health is key. A recently launched cross party manifesto titled '1001 critical days' galvanises the importance of the first 1001 days of life, spanning from conception until age 2[59]. Following its relaunch in Parliament, a record number of MPs have supported adopting it as Government policy across a number of health-led departments. The NHS Five Year Forward View also establishes the need to invest in maternal mental health[15], with a vast increase in funding for perinatal mental health services over the coming years. These early days of life represent a period of rapid growth, and experiences in this phase underpin future development. There is also strong evidence that this critical period extends to pre-conception[60]. It is becoming increasingly clear that maternal factors during pregnancy, and even pre-conception, can have long-lasting effects on children, further justifying the need to prioritise maternal health.

Untreated depression has been shown to impact the offspring's life, and a number of indicators of infant morbidity are increased[61 62]. Impairment of woman's physical and mental health has acute and longer-term consequences for both mother and child, compromising growth and development of the foetus when mothers suffer with depressive symptoms during pregnancy[63]. For example, AND is linked to low birth weights (LBW), babies small for gestational age (SGA), and pre-term birth[49]. The exact underlying mechanism is not understood, but cortisol is a possible mediator of these relationships, with elevated cortisol levels found in women with AND showing slower foetal growth. The study by Diego *et al.*[49] concluded that prenatal maternal cortisol levels were associated with 30% of the variance in gestational age at birth and 14% of the variance in the rate of foetal growth. A limitation of the study is the estimation of foetal weight and growth from scan data, but it is a useful study in the field of antenatal depression outcomes, since the

majority of studies evaluating offspring outcomes only look at the effects of PPD and therefore what is known about the effects of AND is limited.

Impaired offspring growth and development are also recognised complications of postpartum stress[64]. A review of this subject evaluated the effect of maternal depression on offspring's growth, finding a positive association between exposure to depression and impaired weight and length in the child. This effect on offspring growth is centred on the first year of life[65]. This is likely because children under 1 year are almost entirely dependent on their main caregiver, who is most often the mother, for feeding, sleeping, health promoting and development stimulation, and depressive symptoms may lead to impaired parenting behaviours, less stimulation and less positive mother/child interactions[18 66]. The underlying reasons for this limitation to the first year of life has however not been adequately addressed by these studies and requires further examination. In addition, depression in the postpartum can compromise parenting activities such as breastfeeding, weaning and feeding, in addition to sleeping and health-seeking practices that can affect child growth[47 66]. The quality of maternal care is known to be associated with cognitive and psychological outcomes for the child[67].

LBW/SGA babies experience a number of adverse health effects, including cognitive impairment. Low birth weight babies are more likely to develop attention-deficient/hyperactivity disorder (ADHD) and other behavioural issues. On controlling for a number of potential confounders, ADHD is reported to be three times as likely in LBW babies compared with controls[68]. Socioeconomic status and parental education have been shown to mediate outcome of LBW in some studies, and is thought to represent a biological vulnerability associated with environmental risk and social disadvantage[69 70]. Children of mothers who were depressed while pregnant show developmental delays later in childhood and enhanced susceptibility for diseases in adulthood, identifying programing effects due to deranged development[19]. PND has also been linked to detrimental effects on maternal sensitivity and impaired parenting behaviours in the postpartum period[18].

There are noted biochemical footprints of AND on neonates, including elevated cortisol levels, a product of the hypothalamic-pituitary-adrenal (HPA) axis, fundamental to the hormonal stress response. HPA axis dysregulation is strongly linked to depression[34], and it is thought a continued rise in cortisol levels during pregnancy causes overall dysregulation of the HPA axis[71 72]. The resulting inappropriate response to stress is linked to a susceptibility to physical and mental

illness for the offspring in later life[73 74]. Increased cortisol is additionally associated with compromised foetal growth and neurodevelopmental defects[49], providing further evidence for detrimental effects on infant development. It is increasingly apparent that AND and birth weight are interconnected factors, and this emphasises the need for early detection and subsequent treatment.

Evidence suggests that children exposed to either AND or PPD have raised cortisol levels which continues into adulthood [75 76], although these effects could be reduced by maternal treatment. Placental mechanisms act to protect the foetus from harmful levels of glucocorticoids which can compromise foetal growth and gestational length[77-82], although high levels are thought to overcome the protection provided by the cortisol metabolising enzyme 11β-HSD2 which forms a protective barrier. This is discussed in further detail later in the chapter. Overall, the dysfunction of the maternal HPA axis poses a number of risks for both the mother and the offspring.

Data from the Avon Longitudinal Study of Parents and Children (ALSPAC), a longitudinal study which followed child outcomes of more than 14,000 pregnant women and their children over 18 years, was used to examine the long-term consequences of PPD. Women with moderate to severe PPD at both 2 and 8 months postpartum were also at risk of depression 11 years later. Their children were found to have a higher likelihood of behavioural problems, lower maths scores and increased depression risk aged 18[83]. Other studies have concluded that educational attainment is lower in the children of mothers who suffered from PND[54] and hyperactivity and conduct disorder are more likely[84]. This could be a result of impaired parenting behaviours and the effects of maternal depression in early life[66]. It is also possible that the association is indirect, and is acting through confounders such as socioeconomic status and deprivation, although this is challenging for studies to assess comprehensively.

Antenatal stress and the associated heightened inflammatory response, in particular the increased levels of pro-inflammatory cytokine Interleukin-6 (IL-6), is known to increase the risk of psychiatric illness and behavioural problems in the offspring, and imaging studies in neonates suggest that this acts through effects on the developing foetal brain[85]. In the first human study linking a specific mediator of maternal inflammation and the new-born brain, Graham et al. describes an association between raised systemic maternal IL-6 concentration in pregnancy and larger new-born right amygdala volume and stronger left amygdala connectivity,

which is in turn associated with lower impulse control at 24 months. The amygdala is of specific interest due to associations with phenotypes including social deficits, increased emotional and stress reactivity in animal models, and therefore alterations in amygdala integrity might represent a causal role for maternal inflammation and risk of offspring psychiatric disorders[85]. Maternal inflammation is a strong candidate for a mediating factor in the effect of PND on offspring neurodevelopment. Although cytokines are expressed in the foetal brain during normal development[86], elevated levels for example in response to maternal inflammation trigger alterations in neurodevelopment[87-89]. PND represents an intrauterine condition that influences the offspring's susceptibility to illness later in life, and should be targeted for early intervention and prevention.

The maternal hormonal milieu is clearly critical to foetal development, as depicted in Figure 1.2, representing a developmental window of susceptibility during early gestation. The foetal development hypothesis posits that early-life environmental factors have long-term consequences on ill health. Although many adverse events are unavoidable and unpredictable, recognising the consequences of antenatal stress is a crucial first step to developing interventions for women experiencing adversity and stress during pregnancy. The failure to recognise the intergenerational consequences of maternal stress has far reaching implications if this window of opportunity to intervene and focus on prevention is missed[62].

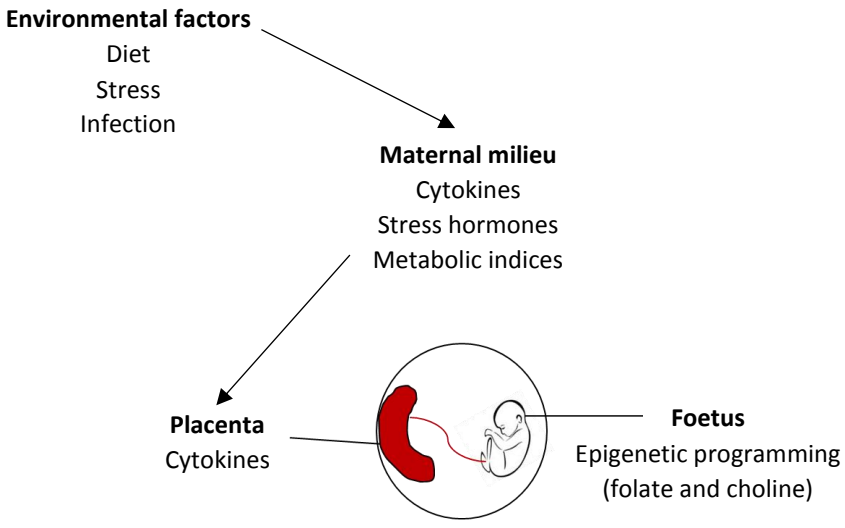

Figure 1.2. The maternal milieu is influenced by environmental factors including antenatal stress. Early in gestation the foetus is vulnerable to external factors which have direct consequences for the placenta and developing foetus. Epigenetic programming is an example of a gene-environment interaction, which is affected by factors such as stress and diet, for which there is a developmental window of susceptibility which can be altered by perinatal depression.

1.5 Psychosocial Risk Factors

There are well established risks which increase the prevalence of PPD, such as a history of depression and socioeconomic depravation, and the difficulties in detecting these especially vulnerable women impedes the current provision of care[90-92]. Previous life events and trauma can increase the risk of depression, and there is an observed association between past abuse and PPD[93]. Additionally there are postpartum risk factors to consider such as traumatic birth experience, poor maternal physical health after birth and a lack of support at home and clearly, antenatal screening cannot identify these. Other important factors include unwanted pregnancy, a specific risk factor for suicide[1], and teenage pregnancy, which doubles the risk of depression[94].

Table 1.1. A summary of the recurring proposed risk factors for perinatal depression split into categories: health, lifestyle, socioeconomic and other.

Health	Lifestyle	Socioeconomic	Other
History of mental disorders	Smoking	Unemployment	Unwanted pregnancy
Family history of PND	Drinking alcohol	Financial insecurity	Early life adversity
Poor maternal health	Young maternal age	Low educational attainment	Past trauma
	Obesity	Poor social support	Cognitive style (e.g. neuroticism)
			Traumatic birth

Previous studies have investigated risk factors for PND, which include psychosocial, personal and biological factors. The most commonly recurring risk factors emerging from the literature are outlined in Table 1.1. Thorough meta-analyses on the subject have suggested the six strongest predictors: antenatal depression, antenatal anxiety, major life events, social support levels, depression history and low self-esteem[23 55 95]. Targeting antenatal depression and anxiety for treatment could eliminate two of these major predictors subsequently preventing the development of postpartum depression. Other factors including unplanned pregnancy, being a first-time mother, poor mother-in-law relationship, and poor

family support have been suggested as important risk factors for PPD[96]. It is acknowledged that social relationships significantly impact perinatal mental health outcomes[97]. Higher levels of support provided by the partner, the family and the social environment is considered crucial for the mother-to-be. The presence of a supportive partner in particular is thought to have a buffering effect against the stress experienced in the transition to parenthood, protecting maternal mental health. Good quality social relationships are also thought to mediate depression through the reduction of the depressive symptoms themselves[98].

Fewer studies have focused on specific risk factors for AND. Lower socioeconomic position has been related to an increased depressive risk in pregnancy[99]. This particular study reported that this association can be moderated by parity, with increased evidence of depressive risk in multiparous women. Social support was shown to mediate the association between socioeconomic position and depressive risk, with a greater mediating effect for nulliparous women. Overall, this study demonstrated a higher risk of AND in women of a lower socioeconomic position, driven by social support, which could in turn be mediated by parity. A recent Australian study identified similar major risk factors for maternal depressive symptoms: a lack of partner support, history of intimate partner violence, and low SES[100]. In addition, antenatal depressive symptoms and assisted delivery were associated with postpartum depressive symptoms.

Maternal sociodemographic factors have been shown to influence AND, such as education and income[56]. It is important to consider how such factors are interrelated, for example, maternal occupation can represent social status, and is strongly tied to education, income and housing. It is therefore important to consider not one single variable but a range of interconnected variables when assessing sociodemographic risk factors involved in PND.

1.6 The Disputed Definition of PND

The definition of perinatal depression is one that invites debate and uncertainty, and even the terminology is one of dispute. The ICD-10[101] (International Classification of Diseases, 10th revision), has no antenatal specifier for depression, but does identify disorders occurring in the puerperium (up to 6 weeks postpartum) with a separate classification. In contrast, the DSM-5[102] (Diagnostic and Statistical Manual of Mental Disorders), does specify major mood disorders "with perinatal onset", which it classifies as within pregnancy or within four weeks postpartum. The National Institute for Health and Care Excellence (NICE) guidelines in the UK however define the postnatal period as 1 year after childbirth[5]. This lack of clarity impedes current screening provision with no clear consensus.

As described above, under some definitions, 'postpartum depression' (PPD) covers pregnancy in addition to the postpartum period, and the timing that is considered 'postpartum' greatly varies from 4 weeks to a year following birth. The term 'perinatal depression' (PND) however more clearly refers to both the antenatal and postpartum period, and this is the preferred term that is used throughout this thesis to describe depression occurring in either the antenatal or postpartum period. The question of whether antenatal and postpartum depression represent distinct syndromes is also unanswered, and remains a topic of uncertainty. The unresolved conflicts in the field surrounding the terminology and timings of onset create difficulties in comparing studies and defining PND, and this requires further work and clarification.

Screening for PND is most commonly carried out by midwives and other health professionals during a woman's routine maternity care, but can be carried out in other setting such as in primary care. Midwives are best placed to discuss mental health with a woman, since ideally a woman will see the same midwife at multiple time points throughout her pregnancy, and a sense of trust and rapport is often built. The presence of PND can only be confirmed following a clinical assessment, however it is commonplace to use a screening tool as a guide. This allows the identification of women who are low risk, and ensures that women at the higher level of risk are prioritised. It also ensures timely identification of depressive symptoms and allows for early intervention during the period of waiting to undergo psychiatric assessment.

The Edinburgh Postnatal Depression Scale (EPDS), which despite the name is validated for use both during pregnancy and postpartum, is a widely used screening tool for PND[103]. It is important to differentiate between a screening tool and a diagnostic one. The EPDS is not a diagnostic tool for depression, but it is used to screen for symptoms, such as sadness and tearfulness, experienced within the last 7 days using a 4 point Likert scale (see Appendix A). The EPDS itself comes with uncertainties, with different cut-off points used as a positive screen for depressive symptoms. In addition, some studies choose to sub-categorise into a screen for minor/major depressive symptoms whereas others select a single cut-off point. The American Academy of Pediatrics recommends classifying an EPDS score of $\geq$10 as a positive screen[104].

The EPDS is considered as a unidimensional tool which provides a raw score of depressive risk. Despite this, it is believed that the EPDS in fact reveals three distinct multiple dimensions of pathology: anxiety, depression and anhedonia[105]. Specific items on the scale have been shown to distinguish between depression and anxiety[106]. Utilisation of this multi-factorial structure of the EPDS would disentangle our understanding of the psychological functioning of postpartum women and improve targeting of treatment.

It is becoming increasingly evident that PND exhibits different disease patterns and heterogeneity of symptoms, resulting in sub-groups of women with distinct timings of onset and remission and different disease progression profiles[107]. It is highly likely that antenatal and postpartum depression represent different clinical sub-types. Clear distinction between sub-types will improve screening, prognosis and treatment of PND. This is especially important in order to identify subtype-specific biomarkers and risk factors and differentiate between women with depression in pregnancy who recover following childbirth, those who appear to be triggered by parturition, and women with depression that continues throughout.

Timing of onset has been a disputed topic in the field, with screening not routinely carried out at consistent points. Despite the recommendation by the American Academy of Pediatrics that well-child visits should be carried out in the first 6 months, it has been shown that PPD can occur any time in the first 12 months and may in fact be most prevalent at the 12 month time point, by which time the provision of screening and care is often absent[108]. This suggests that screening at 12 months postpartum may be beneficial.

It has been reported that the temporal prevalence of AND follows a U-shaped curve, with the prevalence and severity greater in the first and third trimesters[109]. Other studies suggest that screening during the second trimester could be optimal [110 111]. The literature suggests a very wide range of differing screening time-points that no real consensus on the optimal timing exists. One study showed that depressive symptoms at 24 hours postpartum was an effective predictor of depression 4 months later[112], while others suggest screening up to 12 months. Taken together this suggests that monitoring of symptoms at regular intervals, of which there are numerous opportunities when women come into contact with the health service throughout the perinatal period, can only be beneficial to improving detection. Further interrogation of the optimal window of detection is required. Clearly a number of disputes in defining PND, which are outlined in Table 1.2 are currently not resolved, presenting challenges and confusion around PND research and clinical practice, and require addressing.

Table 1.2. A summary of the main challenges in defining perinatal depression.

Terminology	Perinatal/Antenatal/Prenatal/Postnatal/Postpartum Depression. Difficult to search body of literature.
Timing of onset	Antenatal depression not established as homogeneous/heterogeneous and is inconsistently included/not included in studies. 'Postpartum onset' defined differently.
Definition/Disease classification	Differences amongst health professionals and disputes between ICD and DSM classifications.
Screening	Not routinely carried out. Different screening tools used. Different cut-offs used. Timing of screening. EPDS assessing symptoms of both depression and anxiety.
Aetiology	Unclear whether differences represent heterogeneous aetiologies.

1.7 Current Screening and Service Provision

The core features of a depressive episode are a sustained low mood or loss of interest in pleasurable activities for at least two week as defined by the ICD-10 (International Classification of Diseases, 10th revision)[101]. Standardised screening tools can help to identify the key symptoms of depression. Screening for PND can dramatically improve outcomes through early intervention[113]. The 10 question self-report EPDS is the most commonly utilised screening tool for PND, recommended by the National Institute of Clinical Excellence[5]. It excludes physical effects of pregnancy such as fatigue and changes in appetite and is therefore valid for use during the perinatal period. Other tools such as the patient health questionnaire PHQ9 include questions about appetite, fatigue and sleep and might reflect physical effects of pregnancy or the early postpartum rather than depression and are therefore unsuitable in the perinatal period[114]. Nevertheless, medical problems contributing to psychiatric symptoms including thyroid abnormalities, iron deficiency and nausea and vomiting should still be identified and treated since they will likely contribute to the depressive symptoms[44]. Despite concerns surrounding the acceptability of the EPDS for use within different cultures, settings and varying cut-off values, it is generally accepted as the most appropriate tool[115].

The available data is based solely on what is known about prevalence of PND. The true prevalence is likely much higher, since we know that many women fear seeking help or admitting to their depression, and many specifically fear having their child taken away from them. The prevalence rates of PND are estimated to be two to three times higher in Low and Middle Income Countries (LMICs) when compared with high-income countries, with estimated rates from 25% to 40% in some settings[116 117]. This should be taken with caution since PND in LMICs is understudied and differences in study design may contribute to these observed differences, for example the use of self-report measures is higher in LMICs.

The Whooley questionnaire is an alternative to the EPDS screening tool recommended by NICE commonly adopted by midwives to investigate signs of PND. It is a simpler, two question screen asking: “During the past month, have you often been bothered by feeling down, depressed, or hopeless?” and “During the past month, have you often been bothered by little interest or pleasure in doing things?”[118]. Considering the diagnostic accuracy of identification of antenatal depression based on a diagnostic interview, likelihood ratios are 8.2 for the Whooley, and 9.8 for the EPDS, meaning the EPDS performs better in correctly

identifying major depression[119]. The difference in accuracy is relatively minor and the 2-item item Whooley questions are may be preferable in place of the 10-item EPDS in a busy secondary care maternity setting, used as a pre-screen followed by the EPDS if a woman answers yes to the Whooley questions. Regardless of the screening tool used, in order to confirm depression a clinical diagnostic interview is required, and screening is the initial step in identifying possible depression.

Despite the prevalence of PND, screening is not currently mandated or routinely carried out. The inability to reliably detect particularly vulnerable women with certain risk factors impedes the current provision of care. Implementing the assessment of additional crucial risk factors could improve screening. Disparities exist in how PND screening and care is carried out, in particular during pregnancy[120]. Despite the national guidelines, screening is not universally carried out in clinical practice, and as a result PND remains highly undetected. There is strong evidence to suggest high levels of under-provision for maternal mental health, in particular for PND. The literature suggests that only 40% of cases are recognised and diagnosed, only 60% of those receive any treatment, and of those who are treated adequately, only 30% make a full recovery. This amounts to just 3% of all cases of PND ending in full recovery[121]. Clearly, current service provision is inadequate.

The stigmatisation of depression is greater in certain cultures, and some languages do not even have the words to articulate feelings of depression. The charity Acacia[122], who work with women suffering from PND, report that many black, Asian and minority ethnic (BAME) women often do not understand PND or more generally mental health as a concept. Migrant women are a specific population at heightened increased risk of PND, and it has been noted that some migrant women do not have the language to express mental health as a concept, and it is sometimes expressed instead as physical symptoms. This is known as somatisation, the presentation of somatic symptoms with unknown underlying cause. Studies have shown that PND is often misdiagnosed because of this, due to both language barriers and a level of unconscious bias.

A London based perinatal mental health service (South London & Maudsley NHS Trust)[123] prioritises migrant women as a high risk group for PND, and their Parental Mental Health Team have been aiming to promote awareness of maternal mental health and reduce social isolation and improve community engagement in migrant communities. They are specifically using an approach known as the 'Think Family Perspective'[124] to provide early intervention to women high risk of PND. Another

effective intervention used in practice targets young mothers. The Family Nurse Partnership, a home visiting intervention, is offered to young first time mothers with support from specialist trained family nurses[125]. Effective interventions do exist, yet the difficulty is in detecting the high risk women in order to target the interventions to the people most in need.

The perinatal period can be viewed as a window of opportunity. A woman's mental health and wellbeing prior to pregnancy is indicative of her mental health and wellbeing during pregnancy. It is generally accepted that certain groups of women are missing out on the necessary support and it is important to identify these women in order to improve rates of detection. BAME women are the least likely to be asked about their mental health, and this is an example of the Inverse Care Law[126], which states that those most likely to be in need of support and treatment are the least likely to be offered it, and was first set up to improve identification of people at risk of cardiovascular disease. This proposes that "the availability of good medical care tends to vary inversely with the need for the population served."

The inverse care law appears to be especially true for PND. A national survey on PND found that non-white, socially deprived, less educated women were least likely to be asked about their mental health[127]. These women likely have multiple disadvantages, which increase their likelihood of depression, yet they are not being prioritised. It is also thought that the most disadvantaged women are less likely to respond or be able to respond to questions regarding mental health, and women in disadvantaged areas are less likely to be spoken to in a way they understand. Due to these factors hindering detection, it is vital that women are consistently asked about perinatal mental health at every available opportunity.

The treatment of perinatal depression is complex in comparison to depression outside of pregnancy. When considering treatment options, pharmacological intervention can carry risks during pregnancy, however the risk of both the untreated depression and the risk of the drug affecting both the woman and foetus are considered. Alongside the recent increase in screening for PND, the development of new psychotherapy and non-drug treatments has also increased. Personalised treatment of depression has become increasingly commonplace in the general population, however there are no guidelines for this type of personalised approach in PND. A recent review examining non-drug interventions identifies numerous recommended treatments, dependent upon sub-population of PND and personal history[128].

Women with mild PND can generally be treated with psychological interventions including guided self-help, whereas mild to moderate depression is treated with higher intensity psychological therapy such as cognitive behavioural therapy (CBT) and interpersonal psychotherapy (IPT)[129]. Subpopulations of PND are thought to include gonadal hormonal sensitivity, sleep disorders, attachment insecurity, personality disorders, social stressors and trauma history. Subsequent treatments can be tailored accordingly. Although non-drug treatments are preferred in mild to moderate depression, in women with current or past severe depression antidepressants are often necessary. In these cases selective serotonin reuptake inhibitors (SSRIs) are first line drugs in pregnancy for unipolar depression[5].

In addition to questionnaires, future screening could be improved by including testing for potential biomarkers including markers of the HPA axis and a number of genetic variants [130 131]. Biomarker research has achieved great success in clinical fields such as cardiovascular disease and hepatic disorders, yet in psychiatry a biomarker is yet to enter clinical practice, hindered by the lack of a biological gold standard[132]. It is hoped that biomarkers could aid disease diagnosis and prognosis, in addition to improving understanding of pathophysiological mechanisms. Predictive techniques which include biomarkers to identify high risk women could also guide the individual risk benefit analysis used when considering treatment and pharmacological intervention[5]. There is strong evidence that PND is heterogeneous[107], and therefore clear distinction between sub-types will improve screening, prognosis and treatment. This is especially crucial to biomarker studies and most notably to genetic studies.

Despite the effective interventions available[22], detection is often hindered by somatic symptoms of pregnancy such as fatigue, complicating clinical diagnosis[133]. Effective treatment of PPD is associated with normal child development[134], however with severe under detection of women at-risk this cannot be effectively implemented. A more effective method of identifying high risk women during pregnancy would allow earlier treatment and amelioration of the consequences associated with the continuation of depression into the postpartum.

1.8 The HPA Axis and PND

The consideration of psychosocial factors in the development of PND leaves a large proportion of the variance unexplained. Activation of dysregulation of biological mechanisms such as stress plays an important and powerful role in human physiology and behaviour. The body is constantly challenged by intrinsic and extrinsic adverse stressors[135]. The resulting response prepares the body for 'fight or flight' responses, yet prolonged exposure to stressful stimuli is also physiologically damaging and can indirectly affect behaviour in a detrimental way. The concept of allostatic load explains the cumulative and potentially damaging effects of stressors on the brain and body, with a nonlinear 'network of allostasis'[136 137] (Figure 1.3). Homeostasis involves maintaining a complex dynamic equilibrium, but if the stress system becomes dysfunctional, the physiological responses to stress will be inappropriate and eventually lead to psychiatric or somatic disease[135 138]. Psychosocial factors have long been the focus of PND research, but an increasing awareness of the mind-body relationship with human disease is shifting the focus to the contribution of biological factors.

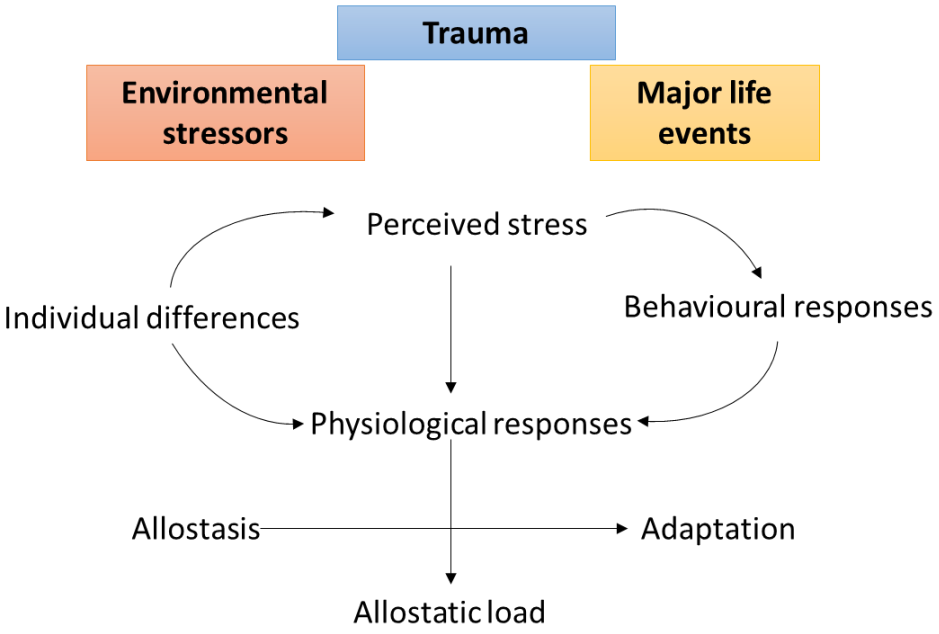

Figure 1.3. The stress response and development of allostatic load, adapted from McEwen (1998)[137]. The perception of stress is influenced by personal experiences, genetics and behaviour. The physiological and behavioural responses triggered by the brain's perception of stress lead to allostasis and adaptation, and allostatic load accumulates over time with overexposure to various forms of stress including immune and endocrine stress.

Stress, whether inflammatory, traumatic or psychological is associated with concurrent activation of the hypothalamic-pituitary-adrenal (HPA) axis[139]. The HPA axis is a fundamental component of the stress response, determining the levels of

glucocorticoids as a physiological response to stress, which are known to play a regulatory role on the basal activity of the HPA axis and termination of the stress response[140]. During pregnancy, the stress system is altered by a number of physiological changes of the HPA axis in pregnancy and postpartum which may contribute to depression (Figure 1.4). The temporal patterns of the HPA axis related to endocrine alterations throughout pregnancy and the postpartum may help to explain the temporal patterns also seen in the presentation of perinatal depressive symptoms. As the axis shifts from the non-pregnant to the pregnant state, placental steroids and the levels of circulating cortisol rise and continue to rise throughout pregnancy, reaching their peak in the third trimester when PND is also thought to have its peak incidence[109]. Dysregulation at this stage could exacerbate symptoms of antenatal depression. HPA axis dysregulation is strongly linked to depression [34], and it is thought a continued rise in cortisol levels during pregnancy causes overall dysregulation of the HPA axis [71 72]. As the axis attempts to revert back to the non-pregnant state, dysregulation coupled with failed re-calibration following delivery may represent a critical maladaptation leaving the mother vulnerable to reproductive steroid-related diseases including PPD.

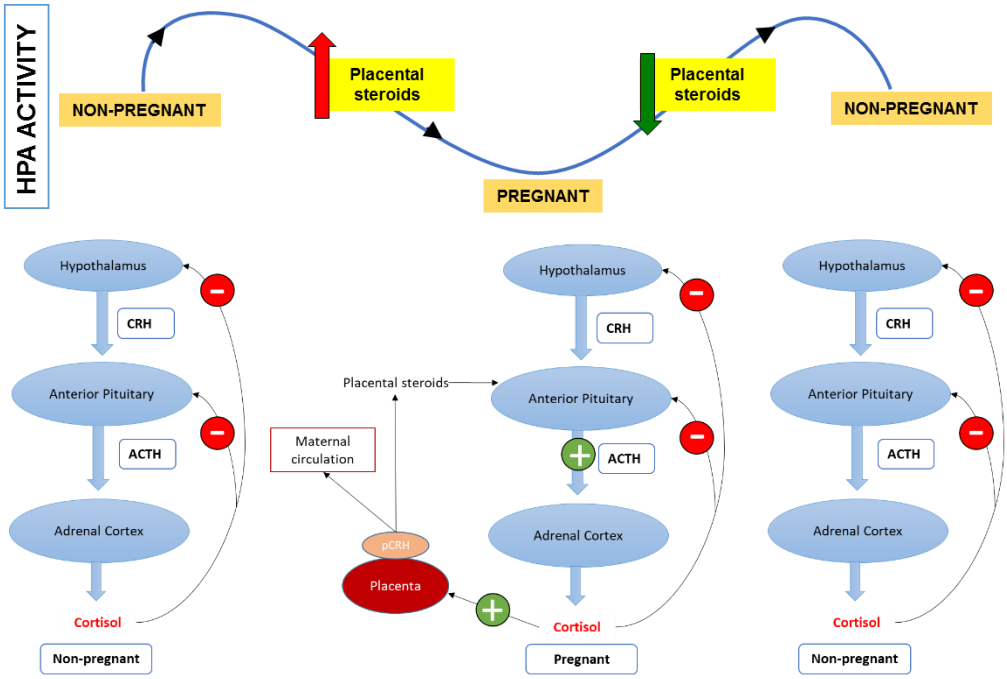

Figure 1.4. Both the non-pregnant and pregnant state of the hypothalamic–pituitary–adrenal axis. The growth of a new organ, the placenta, alters the HPA axis causing a number of physiological changes.

The HPA axis is responsible for regulation of cortisol levels within the circulation. It is activated in response to stress and is central to the biology of PND. The HPA axis is regulated by negative feedback and is acute acting in order to rapidly respond to

acute stress for a limited period to ensure its potent physiological effects are beneficial rather than damaging[141]. The neuropeptide hormone corticotrophin-releasing hormone is the primary regulator of the hormonal response to stress activating the HPA axis. During periods of acute stress, secretion of adrenocorticotrophic hormone (ACTH) and cortisol increases alongside cytokines and other inflammatory mediators to act on hypothalamic, pituitary or adrenal components, potentiating HPA activity[138]. Circulating ACTH and inflammatory markers regulate the release of glucocorticoids, the final effectors of the HPA axis. Corticotropin releasing hormone (CRH), a key mediator of the HPA axis, is released only from the hypothalamus in the non-pregnant state, but is also released from the placenta during pregnancy. In addition, the concentration of CRH binding protein decreases causing further elevations in free CRH. CRH coordinates adaption during stress, and prolonged or increased exposure such as that seen in pregnancy, can be detrimental[141].

It is thought that some women have an increased sensitivity to the changes related to the HPA axis and reproductive steroids occurring in the perinatal period which may leave them vulnerable to depression. Endocrine factors have been a strong candidate for a role in PND aetiology, but direct evidence is lacking. A study by Bloch *et al.*[142] provided the first direct evidence in support of gonadal steroids in development of PPD for a sub-group of women with a history of PPD. In this study the authors simulated hormonal conditions related to pregnancy and parturition in women with and without a history of PPD. They reported that women with a history of PND are differentially sensitive to mood-altering effects of gonadal steroids. This provides evidence for the involvement of reproductive hormones in the development of PND. Despite the interesting findings and first of its kind approach, this was a small study of 16 women, and endocrine levels were not measured during the postpartum period and therefore may not be a complete representation of postpartum depression.

Given the neuromodulatory effects of gonadal steroids and the absence of abnormal hormone levels in women with PPD[143], this may indicate that PPD represents a homeostasis deficiency with the failure to compensate for changes of gonadal steroid levels. This group with an enhanced hormonal sensitivity may represent a subpopulation of women vulnerable to PPD, with altered responses to changes in reproductive steroids which interact with the HPA axis.

HPA axis dysfunction during the antenatal period may be predictive of an extended or more pronounced postpartum HPA refractory period in the puerperium, increasing the risk of PPD. Following delivery of the placenta, there is a rapid drop in placental CRH (pCRH), and this rapid hormonal decline is thought to contribute to depression postpartum[144]. This could explain why AND is a strong predictor of PPD, with a dysfunction during pregnancy leading to failure to readjust the HPA axis back to the non-pregnant state appropriately following delivery.

Unlike the hypothalamic counterpart, cortisol stimulates pCRH production from syncytial cells of the placenta in a dose-response manner, generating a positive feedback loop causing maternal cortisol and pCRH to increase as gestation continues[145]. Circulating cortisol levels continue to rise as gestation continues, increasing three-fold by the third trimester, reaching levels normally expected by the hypothalamic-pituitary portal system under stress [71 146]. The resulting rise in cortisol with the progression of gestation results in levels comparable to that of Cushing's disease and severe depression[71 146]. The foetus is protected from harmful levels of glucocorticoids by the placenta, notably by the action of enzyme 11β-HSD2, which is able to inactivate excess cortisol largely by oxidation[147]. Maternal anxiety is associated with decreased levels of this enzyme, leaving the foetus potentially vulnerable to glucocorticoid exposure and associated risks [82].

Gene-protein x environment interactions may play a role in HPA axis function during pregnancy. It is well established that low maternal socioeconomic status (SES) is associated with disease risk in the offspring[148 149], and it is possible that this may be initiated *in utero*. A low socioeconomic status (SES) has been linked with altered placental mRNA levels of genes involved in glucocorticoid metabolism[150]. The reverse action of HSD11B2, which is thought to provide a placental barrier to the passage of maternal glucocorticoids to the foetus, is the regeneration of glucocorticoids from their inert forms, and this is catalysed by 11-beta-hydroxysteroid dehydrogenase type 1 (HSD11B1) [151]. Low maternal education (indicative of low SES) has been shown to be associated with upregulation of placental glucocorticoid receptor and HSD11B1 gene expression, in a study by Raikkonen et al [152].

One possible explanation for this which was not explored in the study by Raikkonen *et al.* is differences in maternal nutrition, and therefore also epigenetic modifications, which is likely related to SES. Evidence does suggests that DNA methylation is associated with SES and is intergenerational[153]. The resultant

increased HSD11B1 expression in low SES mothers results in glucocorticoid regeneration and therefore higher levels of glucocorticoid availability in the placenta. It is therefore hypothesised that this will in turn increase foetal exposure to glucocorticoids, providing a possible explanation for a number of associations between SES and offspring outcomes.

Antenatal depression represents an intrauterine condition with physiological effects on neonates, including elevated cortisol levels, which is regulated by the HPA axis. Evidence suggests that children exposed to either antenatal or postpartum depression have raised cortisol levels and this continues into adulthood[75 76], although these effects could be reduced by maternal treatment. Dysregulation of the HPA axis during pregnancy may influence exposure of the foetus to stress hormones, and is thought to have the ability to program the foetal HPA axis. This can result in an inappropriate response to stress, which is linked to a susceptibility to physical and mental illness for the offspring in later life[73 74]. In addition, exposure to high levels of glucocorticoids is linked to compromised foetal growth and a shorter gestational length[77-82]. Overall, the dysfunction of the maternal HPA axis poses a number of risks for both the mother and the offspring.

PND appears to exhibit disease heterogeneity, and different symptom profiles are often seen for antenatal and postpartum depression. Their underlying aetiologies appear to be distinct from one another, and may therefore represent different pathologies. As a result it is important to consider that the potential risk factors, causes and biomarkers may also differ. AND is associated with HPA hyper-activation due to increasing levels of cortisol, caused by placental cortisol-releasing-hormone (pCRH) and placental steroids, resulting in the positive feedback of circulating cortisol observed during pregnancy. In contrast to this, PPD is linked to lower cortisol levels due to mild adrenal suppression and a hypo-responsive HPA axis.

It can be hypothesised that antenatal and postpartum depression represent different clinical sub-types. Two recognised forms of depression in the general population are recognised: melancholic and atypical[34]. Melancholic depression is typically defined by a loss of pleasure, depressed mood worse in the morning, insomnia and weight loss. Melancholic depression is associated with HPA hyper-activation. In contrast, atypical depression symptoms are retention of mood reactivity, weight gain, hypersomnia and depressed mood worsening throughout the day. Glucocorticoid suppression has been observed in atypical patients[154]. Differences in HPA profile could be indicative of sub-type, as it appears that the

profile of AND is most similar to melancholic depression, and PPD closer to the atypical type. This would help to explain differences in both symptom profiles and HPA axis.

HPA dysregulation is driven by a number of different factors, thought to include cortisol, cytokines, and serotonin dysfunction. The orchestrated interplay of several neurotransmitter systems in the brain regulates the behavioural, endocrine, autonomic and immune responses to stress. Complex interplay occurs between these elements, and disruption of the delicate balance due to over-activation can alter the HPA axis and its function leading to pathophysiological consequences associated with prolonged production of CRH in non-stressful situations.

Both the immune-inflammatory response and the HPA axis undergo significant change throughout pregnancy and the postpartum, and are thought to be linked by a complex psychoneuroimmune (PNI) response[155]. Cytokines are potent activators of the central stress response, forming a feedback loop through which the immune system and the CNS communicate, and there is evidence that a key inflammatory cytokine Interleukin-6 (IL-6) can alone stimulate the HPA axis[156]. The other inflammatory cytokines, tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are also thought to play a role in stimulation of the HPA axis. Cytokines are thought to act in part on the HPA axis through stimulation of the central catecholaminergic and CRH neuronal systems via ascending spinal pathways, while other inflammatory mediators including serotonin may increase activation via endocrine effects[156].

During pregnancy, a balance must be maintained between protection against pathogens and tolerance against the semi-allogenic foetus, requiring an adaptation to immune system function which is not fully understood. It is now believed that although the majority of pregnancy is concurrent with a shift towards an anti-inflammatory state which acts to protect the foetus from rejection[157], the immune response is split into three stages in pregnancy. The early stages of pregnancy, in the first and early second trimesters, is thought to represent a pro-inflammatory state to assist with implantation and placentation[158]. At this stage an inflammatory environment is required to adequately repair the uterine epithelium and remove cellular debris following to the blastocyst breaking through the epithelial lining of the uterus to implant, and the trophoblast replacement of the endothelium and vascular smooth muscle of the maternal blood vessels to secure the placental-foetal blood supply[159]. The second immunological phase of pregnancy is a period of

rapid foetal growth and development, and the predominant immunological feature is an anti-inflammatory state, while late into the third trimester the mother again enters a pro-inflammatory state, which is believed to prepare for parturition, promoting uterine contraction, delivery of the baby and rejection of the placenta, and this continues postpartum[160].

The inflammatory response and HPA axis, which are both in flux throughout pregnancy and postpartum, interact via the cytokine-glucocorticoid feedback circuit, in order to alert the CNS to a stressor, simultaneously limiting a prolonged pro-inflammatory response and regulating further cortisol secretion[161]. Dysregulation of this cytokine-glucocorticoid feedback circuit has been implicated in the development of PPD, and evidence suggests that cytokine/cortisol dysregulation may in fact be protective against depression postpartum. A study by Corwin et al. found that dysregulation of this circuit after giving birth may protect women against depressed mood[155]. They reported that in healthy women, a sudden increase in pro-inflammatory cytokines did not result in increased cortisol secretion and instead this response is blunted. In women who developed PPD, cytokine stimulation of cortisol is instead robust and negatively affects mood. It therefore seems that these women experience an early postpartum return of hypothalamic responsiveness, whereas in healthy women, the 'dysregulated' paradox of high cytokines and low cortisol is in fact protective against depressive symptoms. The cytokine-glucocorticoid feedback circuit was also found to be inactive in women who did not develop PPD, further suggesting that this 'dysregulation' may in fact be beneficial at this particular time of life. This demonstrates the significant changes to both the innate immune response and HPA axis across the perinatal period, and may help to explain why some women are more prone to developing PND than others.

1.9 Circulating Biomarkers

As described in section 1.7, pregnancy and the postpartum are becoming increasingly recognised as periods of life characterised by tightly regulated inflammatory states[162]. In the general population, a wealth of evidence links major depression and a heightened inflammatory response, yet this is not confirmed in PND. A review of the current literature on the link between postpartum inflammation and PPD assessed in full 19 studies on the subject, and concluded that findings are inconclusive, and both hyper- and hypo- activation of the HPA axis have been associated with perinatal depression[163]. It is thought that disruption of the cytokine-glucocorticoid circuit diminishes the ability to limit inflammation, ultimately resulting in dysregulated cytokine production and cortisol secretion[164] [165].

A key pro-inflammatory cytokine is interleukin-6 (IL-6), a 23.7kDa cytokine with two disulphide bonds that is secreted mainly by T-cells and macrophages. IL-6 induces an acute phase response and plays an essential role in differentiating B cells into immunoglobulin-secreting cells[166]. Interleukin-10, also known as cytokine synthesis inhibitory factor (CSIF), is an important anti-inflammatory cytokine. It is a 20.5kDa glycosylated homodimeric cytokine with two disulphide bonds. It is produced by a range of cell types such as T-cells, macrophages, and mast cells. IL-10 inhibits the synthesis of numerous cytokines that suppress Th1 pro-inflammatory responses and promote phagocytic uptake[167].

It is important to consider not only pro-inflammatory cytokines, as many studies have done, but also the balance between pro- and anti-inflammatory cytokines such as IL-6 and IL-10[155]. Pro-inflammatory cytokines coordinate the non-specific immune response, whereas anti-inflammatory cytokines both provide negative feedback to suppress inflammation and support the type 2 specific immune response[168]. The interplay between these types of inflammatory response, coordinated by opposing cytokines, may prove beneficial in the exploration of cytokines and PND. HPA variability may play a role in the effects of the evoked inflammatory response, and may explain why some women are more sensitive to the inflammatory stimuli of pregnancy and childbirth than others, possibly contributing to the development of depression.

A pilot study[131] conducted by our laboratory prior to the commencement of this current project found that a limitation was the lack of a suitable biomarker to detect

the presence of the underlying disease and hormonal over-activity of the HPA stress axis. This study will investigate whether a reliable circulating biomarker will strengthen the link between genetic susceptibility and disease phenotype, and this is a key aim and novel concept in PND studies. A model for the role of the innate immune system interacting with the HPA axis in the development of PPD has been proposed (Figure 1.5) [169]. Both systems undergo dramatic change during pregnancy and postpartum. Investigating associations between the immune response and PND may provide an opportunity for the discovery of biomarkers. A detailed systematic review by Serati *et al.*[170] summarises the state of the art of biological markers investigated for their involvement in the pathogenesis of depression. The authors cover findings from 1969-2015 on the subject, with 127 papers included in the review following application of inclusion and exclusion criteria. A summary of the findings is given in Table 1.2. The most robust associations have been reported with the HPA axis, hormonal changes, IL-6, vitamin D, fatty acid and BDNF.

Table 1.3. A summary of biomarker studies investigating perinatal depression pathophysiology as detailed in the review by Serati *et al.*[170].

Genetic studies	Biochemical studies	Immunological studies	Endocrinological studies
BDNF	Iron	C-reactive protein	Allopregnanolone
5-HTTLPR	Biopterin	IL-6	Cortisol
FADS1/FADS2	Homocysteine	TNF-α	NR3C1 mRNA
ESR1	Zinc	IL-8/IL-10	pCRH
HTR2A	B12/Folate	Inflammatory cytokines	Estrogen
OXTR	Vitamin D	IgG/IgM/IgA	Testosterone
MAOA/COMT	DHA status	MIF	CRH/ACTH
TPH2	HDLs	Cortisol	ChromograninA/protein
GR	EPA/DHA fish oil		Alpha amylase
CRH-R1	PUFAs		Beta-endorphin
TTC9B/HP1BP3	BDNF		

The release of cytokines associated with depression is thought to cause a response similar to that of a stressor[171]. Interleukin 6 (IL-6) is a pro-inflammatory cytokine, and may represent a suitable biomarker, explaining a possible link between pregnancy driven inflammation and depression. Pro-inflammatory cytokines influence the HPA axis by stimulation of hypothalamic CRH production, in turn causing ACTH and cortisol levels to rise. Increased plasma concentrations of IL-6 have been identified in patients with major depression, and is a potential

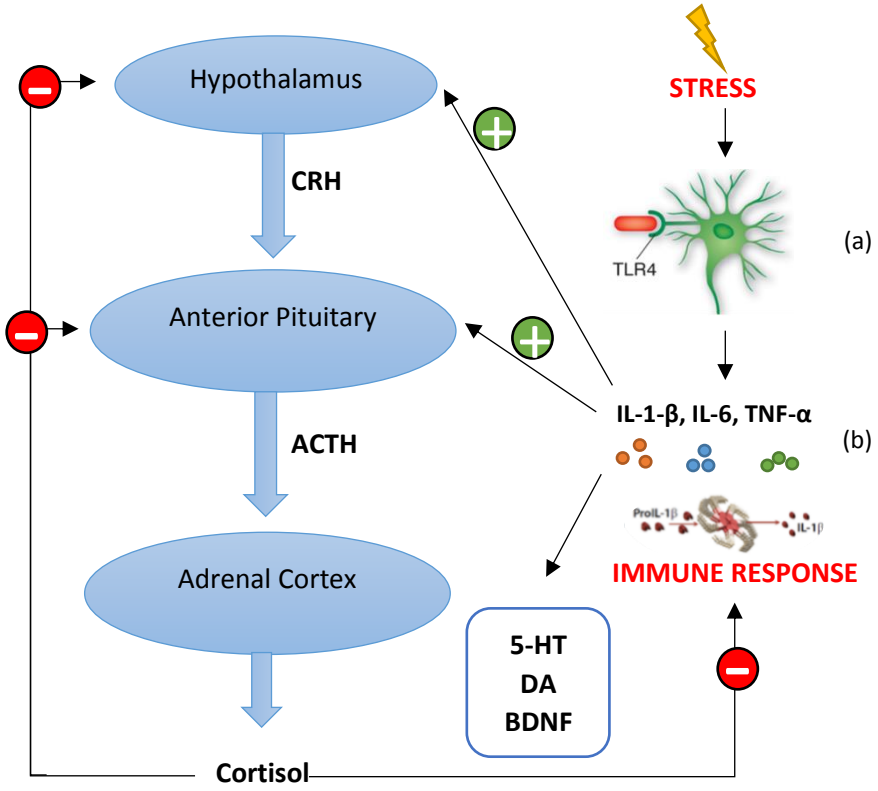

Figure 1.5. Stress-immune interactions. (a) Activation of NF-κβ through Toll-like receptors (TLR) of microglial cells including TLR-4 occurs as a response to a stressor leading to an inflammatory response including (b) the release of pro-inflammatory cytokines mediated by the inflammasome and activation of caspase-1. These cytokines cross the blood-brain barrier to participate in pathways indicated in depression including altered serotonin (5-HT) and dopamine (DA) metabolism, alterations in brain derived neurotrophic factor (BDNF), activation of CRH in the paraventricular nucleus (PVN) and subsequent production of ACTH and cortisol.

contributor to the pathogenesis of depression acting through stress-immune interactions to increase ACTH and cortisol production as seen in Figure 1.5[172]. Pro-inflammatory cytokines are also able to alter the metabolism of neurotransmitters including serotonin and dopamine, and disrupt synaptic plasticity through alterations in growth factors such as BDNF, all of which are thought to be involved in the pathogenesis of depression[173]. A study that investigated immune activation in postpartum blues also found signs of an activated immune response, including increased IL-6 serum levels and markers of other pro-inflammatory cytokines such as IL-1β[174]. A role for macrophage migration inhibitory factor (MIF), also a pro-inflammatory cytokine, has also been implicated in major depression[175].

Alterations in neurotransmitter function of serotonin, norepinephrine and dopamine are primary targets for current antidepressant treatments, but this emerging understanding of the role of inflammation in the pathogenesis of depression could provide targets for novel treatment strategies. Patients with a history of non-responsiveness to conventional antidepressant treatments have been found to have increased plasma concentration of IL-6 when compared with

responsive patients[176], and those with increased inflammation prior to treatment appear less likely to respond to antidepressants[177]. The inhibition of pro-inflammatory cytokines or targeting of antagonists might represent an alternative therapeutic strategy in those who do not respond to traditional treatments, and these individuals may be particularly suited to treating the unique pathophysiology of PND.

Despite generally inconsistent findings in studies investigating the psychoneuroimmune response of depression, elevated expression of peripheral IL-6 is most consistently observed in major depression according to a meta-analysis of 24 studies[178]. The relationship between circulating levels of IL-6 and modulation of depressive phenotype remains under investigation. There is evidence to suggest that IL-6 is able to cross the blood-brain-barrier (BBB) to come into contact with the CNS and alter brain function, either directly on neurons or indirectly via microglia and other immune cells of the CNS[179]. This is further supported by a study finding the intracranial infusion of IL-6 increases depressive type behaviour in animal studies[180]. These studies may lead to therapeutic strategies which target IL-6, but also suggests that the implication of IL-6 in depression could be utilised as a potential biomarker.

Brain-derived neurotrophic factor (BDNF), an important growth factor belonging to the neurotrophic family, may represent another biomarker of interest. It is the most widely expressed neurotrophin in the CNS with the highest levels of expression in the hippocampus, amygdala, neocortex, and cerebellum, and is also expressed by neurons and glial cells[181]. Outside of the nervous system BDNF is expressed in the placenta, heart, lung and skeletal muscle as well as cell types including fibroblasts and platelets, although the majority originates from the CNS. It is produced as proBDNF in response to neuronal activity or inflammatory stimulation and is cleaved before associating into mature homodimeric proteins. It exists both in its free form and bound to its transmembrane receptor, TrkB.

BDNF can cross the blood-brain barrier and a high correlation between serum and hippocampal BDNF has been reported[182 183]. Both pro-inflammatory cytokines and progesterone have been shown to regulate BDNF expression. BDNF is largely recognised for its neurotrophic functions in the developing nervous system, including differentiation, synaptic connectivity and neuronal repair and survival[184]. More recently, a role for BDNF in the pathophysiology of stress and depression has been investigated[185]. It is thought that the function of BDNF in neuron survival is

relevant to the heightened vulnerability of hippocampal neurons following stress, and an early model indicated that BDNF expression in the rat brain is regulated by stress[186].

Following on from earlier animal models, clinical, pharmacological and post-mortem studies have provided evidence for a role for BDNF in depression. Reduced gene expression of BDNF has been described in post-mortem brains of suicide victims[187]. Another study of post-mortem hippocampal tissue found increased BDNF immunoreactivity in subjects treated with antidepressants compared with those who were not, further supporting the notion that BDNF may be regulated by antidepressants and is involved in the pathophysiology of major depression[188]. The exploration of post-mortem brain tissues highlights the importance of BDNF in human psychopathology, yet a method to measure it in a clinically relevant way is required in order to assess and treat depression. BDNF is present in human blood and is stored in platelets, from which it can be released into plasma[189]. Reduced serum levels of BDNF have been observed in drug-free depressed patients, and levels have been shown to be inversely correlated with clinical status, returning to normal following successful treatment with antidepressants[190].

The association between BDNF and depression may be sex dependent, with BDNF levels associated most strongly with depression in women, which may be explained by a reported association between BDNF and circulating sex hormones[191]. In addition to major depression, evidence suggests that BDNF is associated with PPD[192]. A recent study demonstrated a strong association between reduced serum BDNF levels at delivery and depression 3 months postpartum, suggesting BDNF could be predictive in screening[193]. A single nucleotide polymorphism (SNP) in the pro-domain of BDNF results in a substitution of a methionine for valine at codon 66 (Val66Met), which leads to decreased protein secretion, has received much interest in depressive aetiology, but results have been inconsistent[194-196].

1.10 Genetic Risk

It is well established that depressive illnesses are associated with high heritability, and a family history of depression is a key risk factor for PND, suggesting a role for genetic factors in susceptibility. Studies have indicated that perinatal depression may in fact have a higher heritability than depression outside of the perinatal period, with one twin study reported that genetic factors were responsible for 25% of variability for PND diagnoses[197] [185]. Studying genetic factors has the potential to reveal the biological underpinnings of the sub-types of PND and investigate possible genetic differences between them. This could allow for an individual susceptibility to be measured using genetic information in screening.

As described in detail earlier in this chapter, abnormal HPA axis responses to stress have been described in PND, possibly due to enhanced sensitivity to gonadal steroids during pregnancy, and failure to return to normal, post-delivery. The HPA axis is central to the biology of PND, stimulating the release of glucocorticoids. Negative feedback inhibition is required, which is dependent on the presence of functioning receptors. Glucocorticoids act on receptors within target tissues, which includes the hypothalamus and pituitary, causing inhibition of the axis through

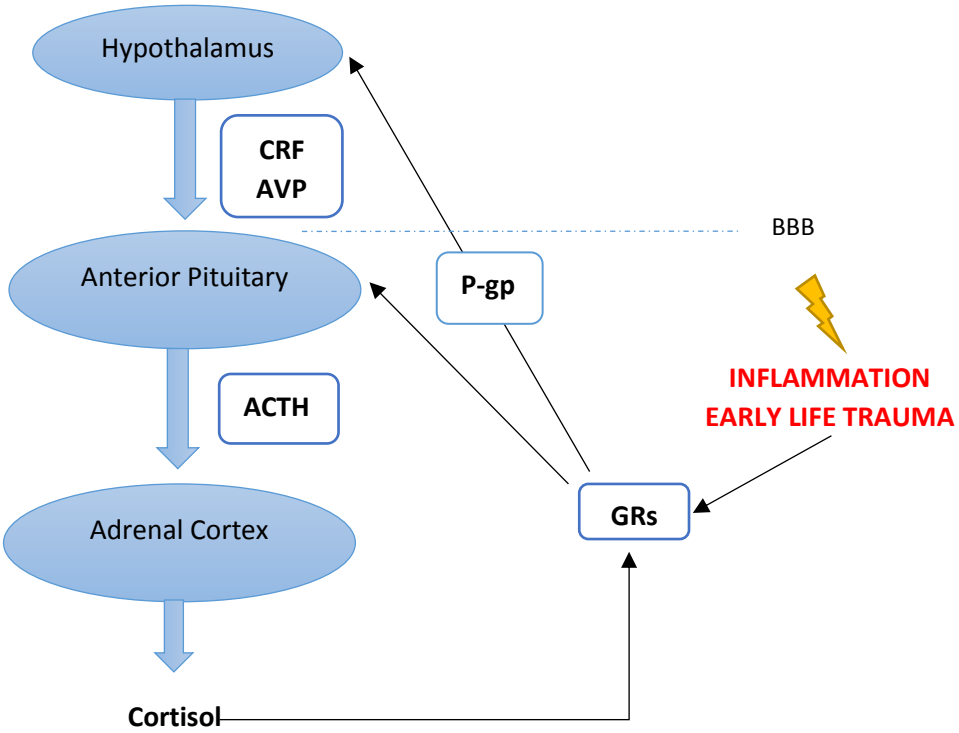

Figure 1.6. Schematic of the HPA axis interaction with glucocorticoid receptors (GRs). Circulating glucocorticoids bind to GR outside the brain (pituitary) and can also cross the blood brain barrier (BBB) to bind inside the brain (hypothalamus). Activated GR induces a feedback inhibition signal leading to reduction of HPA activity. Mechanisms regulating this inhibition include GR expression and function and environmental effects such as early life trauma through epigenetic mechanisms or inflammation, in addition to regulation of P-glycoprotein (P-gp) altering availability of GR in the brain.

feedback inhibition on adrenocorticotrophic hormone-releasing factor (CRF) and vasopressin (AVP) from the hypothalamus, and on ACTH from the pituitary[31]. The mechanism is outlined in Figure 1.6. The hyper-activation of the HPA axis associated with depression is thought to be related to reduced feedback inhibition by endogenous glucocorticoids.

At least two types of glucocorticoid receptor (GR) have been identified within the brain; the mineralocorticoid receptor and glucocorticoid type 2 receptors, which have different affinities for corticosteroids[198]. Type 2 receptors have a lower affinity for cortisol and are believed to have a more important role in stress response regulation when glucocorticoid levels are elevated[199]. The role of GR in stress response regulation during depression is supported by data obtained during antidepressant therapy. Long term antidepressant therapy has been shown to upregulate GR in the brain, and is thought to reverse the inhibition of negative feedback on the HPA axis by glucocorticoids[31]. These exact mechanism of antidepressant effects on HPA regulation is unclear, but it is thought to relate to the multidrug resistance protein transporter, P-glycoprotein, which limits the availability of cortisol in the brain[200].

HPA axis dysfunction has been reported in people with a family history of depression[198 201]. Many studies have investigated polymorphisms of the GR and corticotrophin releasing hormone receptors with depression in a non-pregnant population. The CRH receptors are G protein coupled receptors with seven transmembrane domains. CRH receptors exist in two forms, CRHR1 and CRHR2, which are 70% homologous in sequence[202]. Different isoforms of both receptors have been identified and type 1 receptor is mainly located in cerebellar and sensory centres whereas type 2 is often associated with peripheral tissues[203]. Several studies have investigated polymorphisms of the CRHR1 and CRHR2 gene for association with depression and anxiety disorders[204-212]. Other studies have focussed on the association between CRHR1 receptors and cortisol levels in children[213].

Further evidence from our laboratory suggests an association between SNPs of genes controlling HPA activity (CRHR1 and NR3C1) and increased risk of PND[131]. This prospective cohort pilot study (Coventry and Warwickshire Genetic Association of Postnatal Depression – CW-GAPND) demonstrated that this HPA sensitivity may be genetically determined. The glucocorticoid receptor (NR3C1) and corticotrophin releasing hormone type 1 receptor (CRH-R1) genes are strongly implicated in

depression[208 214]. The pilot study targeted these genes and identified two SNPs, Bcl1 (rs41423247) and rs242939 SNPs of the NR3C1 and CRH-R1 genes, which were associated with raised scores on the Edinburgh Postnatal Depression Scale (EPDS) [131]. This provided the first evidence that specific SNPs of genes involved in stress responses may contribute to an increased risk for PND and may represent promising genetic biomarkers. As this was a relatively small study of 200 patients, further validation is needed.

A systematic review on the subject of genetic factors of PND found a number of potential contributors, although results have been inconsistent[194]. This may be due to a lack of controlling for potential confounding variables, which are crucial to such studies. The review did however indicate that the timing of onset of symptoms may play a significant role in genetic effects. For example, the MAOA and COMT genes were found to be associated at 6 and 8 weeks postpartum but not at 12 weeks[215]. The transient nature of this finding indicates that these polymorphisms may be associated with onset but not persistence of depressive symptoms. This is consistent with a number of other studies which have found genes to be associated with the early postpartum period only, and may explain the mixed results reporting inconsistent or no genetic associations with depression[194].

Overall these association studies suggest that women may be most susceptible to genetic factors during a specific vulnerability window which includes late pregnancy and early postpartum, and it is thought that this may be associated with alterations in oestrogen levels. These findings additionally hint at a gene x environment (G x E) interaction in the development of PND. DNA methylation studies may help disentangle these effects and link environmentally or physiologically challenging events, including hormonal changes, to gene expression levels.

Genetic association studies, of which there has been a surge in recent years, describe potential associations between a number of different genes, which are described in a systematic review by Figueiredo *et al.*[194]. Although those of the glucocorticoid and CRH receptors stand out, recent studies have also linked genes including 5-HTT, FADS1/FADS2, MTHFR, MAOA, COMT and OXTR with PND[194]. BDNF, discussed in the previous chapter as a potential biomarker, has also been genetically linked with PND[196]. The systematic review also noted that almost every study that evaluated associations between SNPs and environmental stressors found a positive association.

Large scale genetic studies are becoming increasingly popular to study depression and anxiety in the general population, and transferring this to the field of perinatal depression would provide an excellent opportunity for research. A current large genetic study of PPD in the US is utilising technology with the PPD ACT app, the first mobile health app used to study psychiatric genetics[216]. Women are able to download a phone application, using it to complete eligibility and informed consent, as well as EPDS screening, and they are sent a saliva collection kit if they wish to participate in DNA analysis. Within its first year, the app has recruited 7344 women with a history of PPD and 2946 DNA samples have been banked. Studies such as this are leading the way in PND genetics research. A limitation of a number of these studies however, is the sole focus on genetics, rather than the interrogation of data with a combination of biomarkers from the different implicated mechanisms of disease. These large scale studies have additionally not addressed the heterogeneity of PND, which now requires investigation in relation to genetic factors.

In summary, it is thought that genetic factors play a role in the susceptibility of perinatal depression. Our laboratory has previously reported an association between SNPs of the NR3C1 (GR) and CRH-R1 genes and increased risk of PND. As described in this Chapter, glucocorticoid and CRH receptors are fundamental to the regulation of the HPA axis and dysfunctions in these genes are implicated in the pathogenesis of depression. It is increasingly recognised that genetic variants with discrete functional effects in genes regulating the HPA axis may alter susceptibility to stress-related psychiatric disorders. A previous study from our laboratory has crucially demonstrated an association between specific SNPs of these genes in postpartum depression. This now requires investigation in a larger cohort, and the combination of multiple other risk factors, to align with the multifaceted nature of PND pathology.

1.11 DNA Methylation

The diathesis-stress model of depression helps to explain why some people are more vulnerable to stress whereas others can withstand stress. Diathesis refers to specific vulnerabilities that affect how individuals respond to stress. These vulnerabilities can include a predisposition caused by genetics, neuro-regulatory systems, personality and cognitive style. The double-hit hypothesis explains that a further second 'hit' or stressor is required in predisposed individuals to trigger the development of the disease, in this case PND. Epigenetics may represent an example of this second hit in PND, depicted in Figure 1.7, whereby early environmental stress triggers a series of epigenetic mechanisms to programme the HPA axis and 5-HT system for survival in a harsh environment as a survival mechanism[217]. These individuals are subsequently maladapted for a 'normal' environment according to the mismatch hypothesis[218], leading to inappropriate stress responses which are known to contribute to depressive pathophysiology.

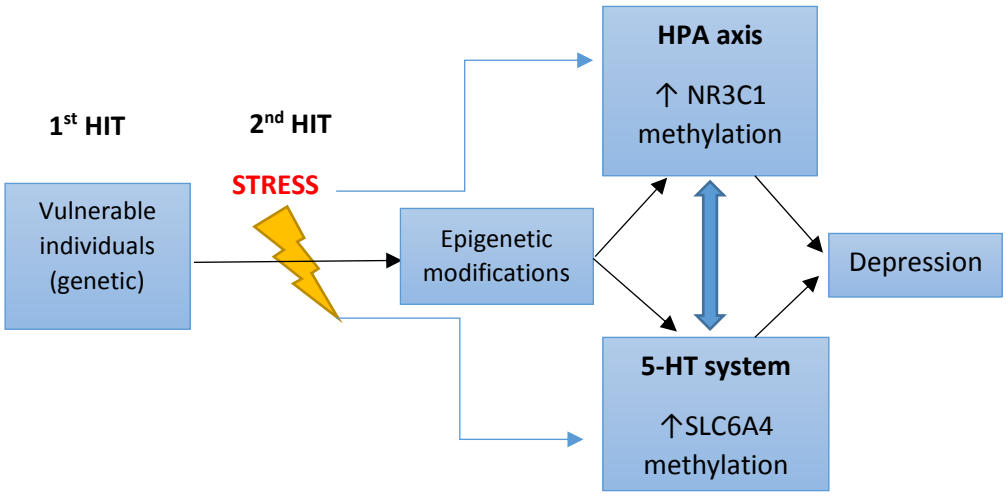

Figure 1.7. The stress-diathesis model of depression vulnerability and 'double hit' hypotheses requiring a secondary stressor to initiate the pathophysiology of depression.

The formation of the epigenome is a critical process largely established during gestation, which directs cell fate and normal development. DNA methylation patterns are formed and shaped during the perinatal period, when the epigenome is sensitive to environmental changes including nutrition, stress, and endocrine disruption[219]. Throughout life a number of fixed and transient changes to the epigenome occur in response to exogenous and endogenous stimuli, leading to its reprogramming. This epigenetic programming begins *in utero,* but continues as an adaptive response throughout life to environmental exposures. Animal studies have demonstrated that the restriction of B12 and folate during pregnancy affects

foetal DNA methylation patterns[220], while humans exposed to famine *in utero* appear to have altered DNA methylation in adulthood[221].

Both the HPA axis and serotonergic (5-HT) system are repeatedly associated with depression[222], yet their dysfunction alone appears inadequate to cause depression and an environmental cue is often implicated. The perinatal environment clearly plays an important role in PND, and epigenetics may provide the missing connection between the HPA axis, 5-HT system and depression. Environmental stress triggers epigenetic alterations, and it is thought that DNA methylation is a key component in depression vulnerability. The epigenetic embedding of inflammatory pathways is a model which links early life adversity and increased risk of maternal depression[223]. This model proposes that early life adversity embeds a pro-inflammatory DNA methylation signature which underlies a predisposition to PND. At a gene specific level, the methylation status of both BDNF and SLC6A4, genes associated with both depression and PND, has differentiated between healthy controls and patients with major depression[224 225], and further studies in a cohort of perinatal women represents a future direction which is yet to be explored. DNA methylation now warrants attention as a candidate for a biomarker of PND risk.

DNA methylation, an essential form of epigenetic variation, is characterised by the addition of a methyl group to the fifth carbon in the cytosine ring, resulting in 5-methylcytosine (5-mC). It is especially prevalent in CpG-rich regions of the genome, CpG islands, which are predominantly found in gene promoter regions[226]. DNA methylation plays an essential role in gene regulation and expression, and is catalysed by methyltransferases[227]. In the study of disease aetiology, the importance of epigenetics is becoming increasingly clear, in particular the role that DNA methylation plays.

The mechanism of DNA methylation is catalysed by DNA methyltransferases (DNMTs), of which a number of different forms exist, including DNMT1, DNMT3a and DNMT3b. Each plays a different important role in the maintenance of DNA methylation patterns. The silencing of gene expression by DNMTs can occur through both direct and indirect mechanisms. The first involves the binding of a transcription factor to a DNA recognition sequence, thus preventing the binding required for gene activation[228]. The second mechanism acts by targeting proteins such as MeCP2, which are known to supress transcription[229]. If DNA methylation occurs at repressor sites then the effect is the reverse, and an increase in

transcription occurs. DNMTs act as epigenetic regulators, with resultant effects on the genome.

DNA methyltransferases use *S*-adenosyl-L-methionine (SAM) as the donor of methyl groups[227], which utilise the folate-dependent *de novo* synthesis of one-carbon units[230]. DNA methylation influences gene expression and depends on the availability of methyl groups from SAM. Humans ingest methyl groups predominantly in the forms of dietary choline, but can also originate from methionine and methyltetrahydrofolate (methyl-THF) in addition to vitamins B-6 and B-12, due to their closely linked metabolic pathways[231]. The pathways intersect at the formation of methionine from homocysteine, where choline is a substrate in the betaine homocysteine methyltransferase pathway. A diet deficient in methyl donors is associated with hypo-methylated DNA both globally and at specific genes. It is therefore important to consider dietary intake of methyl-sources in methylation-based studies.

Pregnancy is characterised by the readjustment of metabolic pathways and micronutrient deficiency, often leading to anaemia. Nutrient metabolism is important in the methylation cycle. Folate, a nutrient involved in methyl group metabolism, can therefore influence epigenetic status. DNA methylation status has been directly correlated with folate levels[232]. The benefits of folic acid in pregnancy are widely recognised, and periconceptual supplementation is recommended by the Department of Health due to its role in the prevention of congenital malformations including neural tube defects (NTDs)[233]. In addition, there is evidence that higher folate intake during pregnancy is associated with improved cognitive ability in the offspring[234]. A deficiency of B12 and folate during pregnancy can lead to anaemia, which has its own set of complications that can affect the unborn offspring, potentially predisposing them to infection and disease later on in childhood and adult life[235].

The role of maternal nutrient intake in offspring neurodevelopment is poorly understood, and the molecular mechanism of folate's role in this is yet to be determined. Epigenetic mechanisms constitute a likely link, and one possible mechanism is DNA methylation. There is evidence for an association between increased folate intake in pregnancy and DNA methylation changes in the offspring[236]. It has also been shown that folic acid supplementation during pregnancy can have significant effects on DNA methylation of genes related to brain function[237], including BDNF, which is closely linked with depression[190].

A recent Japanese study examined the role of diet in psychological distress during pregnancy, finding that fish intake, specifically intake of omega-3 polyunsaturated fatty acids (n-3 PUFAs), significantly reduced the risk[238]. Evidence suggests that n-3 PUFAs can modulate inflammation, and an emerging mechanism for exerting these anti-inflammatory effects is through epigenetic modifications including DNA methylation[239]. Omega-3 fatty acid deficiency is implicated in PND, driven both by its depletion during pregnancy and lactation, and a lack of dietary intake, especially in Western cultures. Micronutrient deficiency, namely omega-3 deficiency, is importantly an easily modifiable factor through supplementation. The demand of omega-3 during pregnancy is very high due to foetal growth and development, and this is particularly high during the third trimester when foetal brain development is occuring[240]. It is thought that omega-3 dietary intake is insufficient even to cover the body's normal demands, and this may explain why levels can decrease by up to 50% during pregnancy and can fail to recover for up to 26 weeks postpartum[241]. Populations consuming more seafood and omega-3 such as Japan and Iceland also have a lower prevalence of PPD[242]. Other environmental factors are associated with omega-3 fatty acids, for example lower levels have been observes in smokers[243].

PND has been linked with altered DNA methylation patterns. Oestrogen driven DNA methylation changes have been found in women at risk of developing PND. Two biomarker loci at the HPIBP3 and TTC9B genes, which both have key roles in oestrogen signalling, could act as indicators of PND, with observed differences in CpG methylation levels within promoter regions[244]. Use of these epigenetic biomarkers predicted PPD with 80% accuracy. The underlying risk in this group might be related to an increased sensitivity for epigenetic change in response to normal levels of circulating hormones during pregnancy. This study supports the idea of a link between pregnancy related steroid hormones, methylation and depression.

Women appear to have an increased susceptibility to epigenetic modifications during specific periods, coinciding with progressively changing oestrogen levels during pregnancy, suggesting oestrogen may drive changes in gene expression. This also supports a number of findings in genetic studies, which suggest a sensitive period to genetic factors during late pregnancy and the early postpartum. This epigenetic reprogramming may represent a molecular mechanism of predisposition to PND risk; exploitation of this mechanism might identify additional predictive biomarkers.

DNA methylation studies can elucidate some of the associations identified in the literature. For example, the study of placental DNA methylation at the leptin receptor locus has elucidated a potential link between antenatal mental illness and poor foetal growth[245]. In addition, DNA methylation profiles within the SLC6A4 serotonin transporter gene have been shown to moderate the association between the 5-HTTLPR polymorphism and cortisol stress reactivity[246]. Recently, methylation at the oxytocin receptor (OXTR) has also exhibited significant associations with PND, and supports the hypothesis of PND specific sensitivity to epigenetic reprogramming at oestrogen targets [247]. A negative relationship between OXTR methylation and antenatal serum oestradiol was identified, and OXTR and oestradiol additionally appear to interact with one another to influence the ratio of progesterone and allopregnanolone. These findings are consistent with other studies which point to an increased sensitivity to oestrogens at an epigenetic level in women at risk of PND[244]. The epigenetic level of variation can provide a more in-depth biological rationale for of a number of the biomarkers proposed in the current literature.

It has been reported that DNA methylation can 'program' child behaviour as a result of prenatal and early exposure to maternal depression[225]. This important study examined the methylation status of the SLC6A4 promoter and found significantly lower methylation levels in pregnant women experiencing depressive mood in the second trimester. Decreased methylation in the infants at two CpG sites in this promoter was also identified. The enzyme Methylenetetrahydro-folate reductase (MTHFR), which regulates the availability of methyl groups from methyl-THF and subsequent levels of methylation, was interrogated and a variant associated with depressed mood during pregnancy identified. This study demonstrated the effect of prenatal exposure to depression on gene-specific DNA methylation patterns, supporting a hypothesis for an epigenetic mechanism for programing offspring development and behaviour. MTHFR is also known to interact with folate status to influence DNA methylation[232], highlighting a further epigenetic alteration associated with PND which is modified by nutritional status. Furthermore, evidence suggests that folate supplementation may represent a protective factor against depression[248].

Human prenatal exposure to depression has also been associated with methylation at the glucocorticoid receptor NR3C1 promoter in new-born infants and alterations to their HPA stress-responses [249]. Maternal care in the rat has been shown to affect the offspring's HPA stress response and this involves changes in methylation to the

glucocorticoid receptor and expression levels of the NR3C1 expression[250]. The effect of maternal care on DNA methylation has also been shown to be broad, and not confined to single candidate gene promoters, demonstrated by microarray analysis in the mouse[251]. DNA methylation appears to be a mediating factor between maternal depression and offspring HPA axis effects. Animal studies have demonstrated that the epigenetic regulation of imprinting in the offspring is influenced by nutrients involved in methyl group metabolism in pregnancy[252 253]. Overall these studies suggest that nutrient metabolism, DNA methylation and PND are interconnected, with potentially long-lasting effects spanning generations.

It is important to note that these epigenetic effects can stem from environmental causes such as environmental stress. An epigenetic component is apparent in psychiatric illness, and in particular in relation to suicidal attempts. This has been demonstrated both through polymorphisms and global DNA methylation levels. Polymorphisms at epigenetic regulatory genes DNMT1 and DNMT3b have been associated with suicidal attempts in psychiatric patients, in addition to increased global DNA methylation in the same patients[254]. Genes associated with depression have also been studied for methylation changes. Increasing methylation at the SLC6A4 promoter has been reported in people with a history of depression, which was higher in females than males[255]. A study investigating maternal depression identified reduced maternal and infant methylation of the SLC6A4 promoter at specific CpG sites[225].

In addition to gene-specific methylation changes, global DNA methylation changes can also represent a biological response to environmental exposures[256]. The response to early life adversity can be observed in both central and peripheral systems and therefore reflects a useful biomarker[217]. A crucial consideration when studying DNA methylation in depression is a suitable and clinically relevant biomarker as a proxy for the brain. Although some DNA methylation patterns are tissue specific, others appear to have similar patterns in peripheral cells as the brain, as is the case with BDNF[224]. Other human studies in mental illness have found similar methylation patterns in peripheral cells as the brain for a number of genes[257 258]. Furthermore, although the pattern cannot be expected to be identical in the brain, it can still provide useful information to indicate an epigenetic response which may represent biological sensitivity to stress.

The epigenome is dynamic and responds to changes of micro- and macro-environments over time[259]. It is closely interconnected with lifestyle, and it is

thought to be associated with maternal exposures. The epigenome provides an interface between environmental influences and the static inherited genome, and can help explain gene-environment interactions. Factors such as age, disease and lifestyle influence our epigenome. The response to nutritional variation is a change in phenotype, and from an evolutionary perspective, producing multiple phenotypes from a single genotype is an important adaptive response.

1.12 Summary of Key Gaps in the Literature

- The inability to detect PND seriously impedes the provision of care, with at least 50% of women suffering from undiagnosed and untreated PND. Therefore, screening and prediction of depressive risk requires a novel approach.
- Risk factors which can predict antenatal and postpartum depressive symptoms are key to prioritising the most vulnerable women.
- In addition to psychosocial risk factors, the literature suggests that screening turns to biomarkers to identify high-risk women.
- A number of avenues are under preliminary investigation to explore biomarkers of PND, which require further validation.
- The literature repeatedly reports the theory of dysregulation of the HPA axis in PND and therefore this is an appropriate focus for emerging biomarker studies.
- Genetic and epigenetic research has identified a number of candidates for contribution to PND, namely that of the glucocorticoid and CRH receptors.
- Studies which focus on a few select targets will be beneficial to assess suitability for use in a hospital laboratory setting.
- An important role for the immune response in PND is emerging, and inflammatory markers could provide a useful window into the inflammatory response underlying depressive symptoms.
- The interaction between psychosocial and biological risk factors in the development of PND is rarely explored simultaneously
- The full temporal progression of depressive symptoms is often not investigated to include both the antenatal and postpartum periods – the majority of studies only focus on one.
- Our understanding of the heterogeneity of PND is limited, in particular the temporal patterns of symptom onset and remission, and this should be addressed when assessing risk factors.

This summarises the key gaps in the knowledge as identified by a review of the literature, which has been explored across this chapter. Research to validate biomarkers and psycho-socioeconomic risk factors has the potential to predict the likelihood of a woman developing PND. This in turn could significantly improve early diagnosis and subsequent treatment, preventing lasting damage which can affect future generations of affected families.

1.13 Brief Description of Study and Project Aims

The central aim of this multidisciplinary project is to profile women at risk of perinatal depression by investigating a number of different susceptibility mechanisms including genetic/epigenetic, immune and psychosocial predictors. I aimed to investigate HPA axis sensitivity by testing the genetic status of patients for SNPs of the glucocorticoid receptor and CRH-R1. An additional sub-study investigates epigenetic susceptibility through the analysis of differences in maternal blood DNA methylation during pregnancy as a preliminary exploration of the usefulness of global DNA methylation in PND. The analysis of circulating cytokines has also been carried out for participants as a potential biomarker for the underlying depressive symptoms. The three main arms of research in the current study based on the literature are psycho-socioeconomic factors, genetic factors, and biomarkers.

This prospective cohort study was designed to investigate distinct patterns of perinatal depressive symptoms associated with recurrence or persistence from pregnancy to the postpartum period and profile psycho-social risk factors in each group in women recruited from NHS secondary care antenatal clinics. Biological samples from these women have been analysed to investigate the underlying pathology of PND. Taken together, this project combines the psychosocial risk factors with biological data and aims improve our understanding of the underlying causes of PND and investigate whether predictors could be used to improve the screening process.

Overall this aims to profile women at risk of perinatal depression by investigating different susceptibility mechanisms. This is addressed by the following core research questions:

1. Can psycho-socioeconomic factors predict antenatal and postpartum depressive symptoms?
2. Is perinatal depression heterogeneous and can the timing of onset/remission be predicted from risk factors?
3. Do cytokines and neurosteroids IL-6, IL-10 and BDNF act as markers for perinatal depression?
4. Does HPA axis sensitivity provide a marker for perinatal depression by genotyping target SNPs?

5. Is there is a difference observed in global DNA methylation patterns between women with perinatal depression and women without?
6. Do the investigated biomarkers improve prediction of perinatal depressive symptoms when compared with psycho-socioeconomic factors?

This thesis answers these research questions across seven Chapters. Following on from this opening chapter which has introduced the context of the research and the problem which has not yet been adequately investigated, I will describe the research and methodology used in the present study to collect research data in Chapter 2. This will be followed by a chapter of results for each of the three arms of the study – psychosocial (Chapter 3), genetic (Chapter 4) and circulating biomarkers (Chapter 5). A final analysis chapter combines the data from each of the three arms and comprises the main analysis of the project in Chapter 6. The thesis concludes with a chapter to discuss of the findings of the project, where this sits within the current literature, and future directions in Chapter 7.

2 Materials and Methods

2.1 Chapter Two Abstract

This chapter presents the methods used in the study which are designed to answer the research questions and aims as outlined previously in Chapter One. A prospective cohort study was designed, which expands and improves on a preliminary pilot study. The study population is described, with parallel studies running in two different locations, resulting in two separate study cohorts. Restrictions and limiting conditions on the project have been considered, and it will be important to consider these in relation to any findings of the study. The techniques used in the project are described here in detail, including the method to screen for depressive symptoms, utilising the Edinburgh Postnatal Depression Scale, and the laboratory experimental techniques and materials used in the biomarker studies. All variables recorded as part of the study are listed in Tables 2.5 and 2.6. Finally, statistical methods used in the analysis of study data are described, for both psycho-socioeconomic and biomarker data.

2.2 Overview of the Experiment/Design

This study was designed with the central aim to collect data relating to perinatal depression screening and associated risk factors, both biological and psycho-sociodemographic. The study design is a prospective cohort study, in the setting of secondary antenatal care. Women were invited to participate during pregnancy and biological samples were collected in addition to questionnaire data from PND screening and routine NHS data collection. Statistical methods were applied to all data, with the aim to disentangle complex risk factors and quantify their contribution to PND risk.

This project formed part of the Coventry and Warwickshire Genetic Association of Postnatal Depression (CW-GAPND) study, NIHR CRN Portfolio Study 'Biomarkers of Perinatal depression' (Study IRAS ID: 21234). To validate findings of the pilot study in an independent replication cohort, this phase of the study aimed to recruit a further 2000 women from University Hospitals Coventry and Warwickshire (UHCW) and South Warwickshire NHS Trusts, based upon a 70% completion rate. In addition to genetic risk factors, psycho-sociodemographic risk factors have been investigated, in addition to the suitability of circulating biomarkers in screening.

This phase of the study has included a second follow up screening postpartum allowing for postpartum depressive risk to be included, and for disease progression and trajectories in individual women to be followed.

This study includes assays for biomarkers, with the aim to find a relevant biomarker of PND, especially in women with increased genetic susceptibility of HPA dysregulation. In addition, determination of DNA methylation epigenetic markers in maternal blood offers added value and this area of investigation was included in the project. The study will utilise methods which will avoid the need for DNA sequencing, in order to determine suitability as a cost-effective and clinically relevant method for adoption by screening programmes. Ease of use in a hospital laboratory setting will also be assessed.

The research design included six main elements in the methodology:

1. Patient recruitment, screening and preparation of patient samples
2. Analysis of EPDS scores and psycho-sociodemographic data
3. Investigation of genetic susceptibility targeting specific SNPs and their influence on depressive symptoms (EPDS score)
4. Investigation of circulating biomarkers
5. Investigation of epigenetic susceptibility (DNA methylation)
6. Data analysis and development of prediction and statistical modelling

There were three main arms to the project (Table 2.1) – psychosocial risk, genetic risk and the use of a circulating biomarker. A timeline of data collection and analysis is demonstrated in Figure 2.1.

Table 2.1. The three arms of the study and areas of investigation for each aspect.

Mode of susceptibility	Methods of investigation
Psychosocial	Antenatal EPDS
	Postnatal EPDS
	Sociodemographic/psychosocial data
Circulating biomarkers	IL-6, IL-10, BDNF
Genetic	Genetic variation
	SNPs associated with depression risk Bcl1, rs242924, rs242939 Global DNA methylation

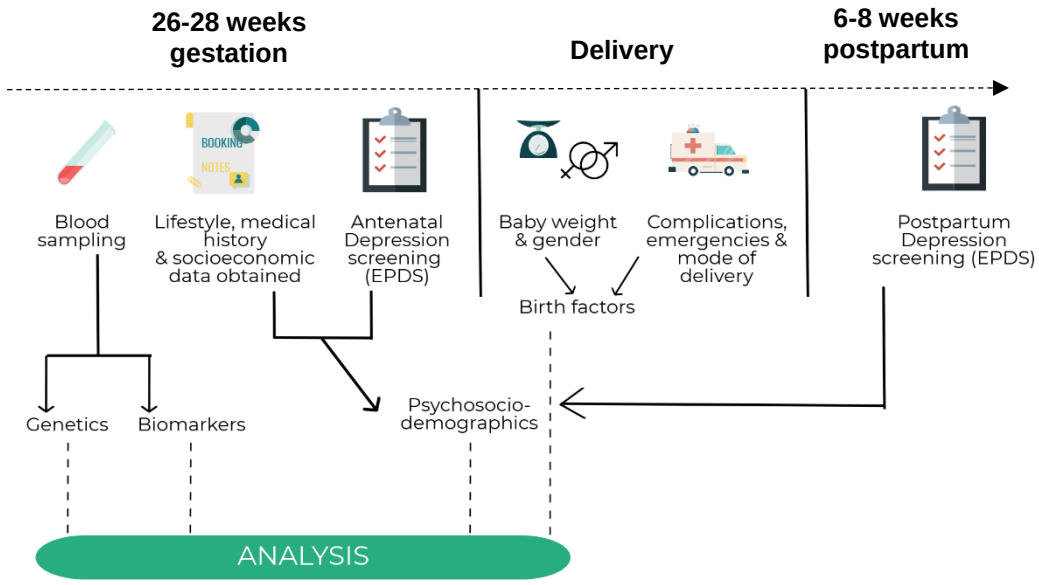

Figure 2.1. Timeline of data collection for the project.

2.3 Population

This thesis uses data collected as part of the Coventry and Warwickshire Genetic Association of Postnatal Depression (CW-GAPND) study. The prospective recruitment of participants to the study was led by research midwives at two hospitals, University Hospital Coventry (University Hospitals Coventry and Warwickshire NHS Trust, UHCW) and Warwick Hospital (South Warwickshire NHS Foundation Trust, SWFT). The study was in the first instance initiated at Warwick Hospital, followed by UHCW. Recruitment began in 2014 and remains ongoing, although data collection for the purpose of this thesis ceased in January 2019.

UHCW is a large University teaching hospital in Coventry, in contrast to Warwick Hospital which is a smaller hospital based in the smaller nearby town of Warwick. Midwives invited women to participate in the study during routine antenatal clinic visits. Ethical approval for this study was obtained from the National Research Ethics Services, UK Health Research Authority (REC reference 09/H1203/69). All participants had given informed consent.

The two cohorts represent two populations of women living in the Coventry and Warwickshire area in the UK. According to the most recent Census data from 2011, the population of Coventry is 66.6% White British, whereas Warwickshire has a

notably higher percentage of White British residents, at 88.5%[260]. The national average is 79.8%, indicative of Coventry's diversity, with a higher proportion of black and minor ethnic population (BME) compared to the national average. Warwickshire is less diverse, with a higher proportion of White British residents than the national average.

Despite their proximity in distance, the two areas are in many ways in stark contrast. Warwickshire is amongst the 20% least deprived areas in the country according to the Index of Multiple Deprivation (IMD) rank of average score[261]. On the other hand, Coventry is the 38th most deprived area; 18.5% of the population live in neighbourhoods which are in the 10% most deprived nationally. Data for this study has been collected from both areas, and differences between our study populations will be investigated.

2.4 Location

All biological samples were sent to the Coventry and Warwickshire Pathology Service (CWPS) at UHCW where they are stored. In this laboratory I can access specialist techniques required for the project that are not available at the University research environment, such as clinical grade molecular diagnostics. It allows access to patient samples and information, in addition to advanced equipment. The laboratories are run to Medical Laboratory accreditation standards as determined by the United Kingdom Accreditation Service (UKAS), and therefore all research delivered here will be to a high standard.

The majority of the equipment needed for this project was available in the pathology department including DNA extracting platforms and LightCycler® PCR for SNP genotyping. All laboratory work was carried out in the UHCW Pathology Department, except for cytokine profiling which was performed using the Meso Scale Diagnostics (MSD) platform at the University's Clinical Sciences Research Laboratories, a Warwick Medical School department based at UHCW.

2.5 Restrictions and Limiting Conditions

Participation in this study is largely random, since all women attending the antenatal clinic were invited to participate. It is however possible that an element of bias is involved in the decision to partake in a research study. For some, stigma unfortunately remains around depression due to cultural or family/personal beliefs, and this may influence the decision whether or not to participate.

The completion of both protocol arms is dependent upon participants responding with their postpartum screening at 6-8 weeks, and failure to do so results in incomplete data. Participants who failed to return the follow up were contacted by telephone and/or post but a number of women still did not complete this and therefore were excluded from postpartum analysis, but were still included in antenatal data analysis. Some women may have moved out of the area or changed contact details and were no longer contactable. It is also possible that those who fail to submit the follow up EPDS are more likely to be suffering from depression.

The protocol includes biological sample collection at one time point during the study, and therefore biomarkers cannot be directly compared from antenatal to postpartum time points. The study has been made as easy as possible for women to complete the protocol, with the follow up by telephone or post. This benefits protocol completion and a further visit to the hospital with a new born baby would certainly result in higher failure to complete cases. Patients were involved in PPI during the preliminary design of the study especially regarding the methods of remote communication post-delivery. This was prior to my involvement, however PPI is a very important part of study design and I would endeavour to include further PPI in future work.

PND screening for the purpose of the study was conducted with the EPDS screening tool, which although widely used and validated is not a diagnostic tool. Therefore we are limited by referring to 'depressive risk' rather than confirmation of PND status. Screening is however not usually mandated and therefore this study benefits all participants by ensuring they are receiving additional care to the standard level in the NHS. All women who screen positive in the study are referred through NHS protocols and will receive treatment that they may not have otherwise had due to lack of detection.

Laboratory work was based at the hospital Pathology laboratories, which are busy clinical environments where clinical work must take priority over research. Space, resources and availability of equipment is dependent upon the clinical workload of the laboratory. During periods with increased operational demand such as the winter pressures, disease outbreaks, and urgent sample testing, access to the laboratory was restricted. The logistics of sample storage in this laboratory also presented difficulties, with blood, saliva, extracted DNA and plasma samples for each participant from two hospitals resulting in thousands of biological samples which were stored and often not readily accessible for quick analysis. The large

scale nature of this study presented challenging logistics for one person to manage in terms of sample processing and analysis.

2.6 Sampling Technique

PND screening

Perinatal depression was assessed during two distinct time points during pregnancy, using the most validated and widely used self-report screening tool for depression during the perinatal period, the Edinburgh Postnatal Depression Scale (EPDS)[103] (Appendix A). The EPDS is designed specifically for postnatal use but is also validated for antenatal use as a pre-screening tool for depression and in particular in research[103]. The EPDS does not include questions about somatic complaints, fatigue and changes in appetite, as these complaints would not help to distinguish depressed from non-depressed women during pregnancy and postpartum[103].

The 10 item EPDS is the most commonly used tool to screen for minor and major depression, and is recommended by NICE[5]. The scale has a maximum score of 30, and a score of 10 or above indicates the presence of depressive symptoms. Although there is no consensus agreement on the most appropriate cut-off, for screening purposes a cut-off score of 10 is widely cited to indicate possible major or minor depressive disorder[103 262], whereas the cutoff of 13 is typically used for identifying major depressive disorder (MDD) (predictive accuracy characteristics reviewed in the Systematic Evidence Review for the US Preventive Services Task Force[263]). In this study women who scored ≥10 were considered as screening positive for PND, as recommended by the American Academy of Pediatrics[104]. This cut-off has a reported sensitivity of 0.81 and 0.86 for detection of both major and minor depression[264].

The time-points at which the EPDS was carried out was based on previous studies to include the peak incidence periods. The antenatal EPDS assessment was carried out between 24–29 weeks gestation (T1) at a hospital visit. Since there is no consensus agreement on the most appropriate assessment time, T1 was chosen to study depressive symptoms early in the third trimester, during the transition period between 2nd and 3rd trimester where women experience most of the uncomfortable physical symptoms of pregnancy, such as tiredness, difficulty eating or sleeping. This period is also associated with the most dramatic changes in hormonal milieu, especially placental hormones that control stress responses and may trigger rapid

mood changes, and has been suggested as an optimal screening time due to the U-shaped pattern of temporal onset resulting in higher prevalence in the third trimester[109]. This stage in pregnancy is where most women have routine antenatal assessments, therefore translation of this research protocol in clinical practice will not impact on resources allocation in the current healthcare model by introducing an additional hospital visit.

All participants were contacted following delivery of their baby and asked to complete a second EPDS at 6–10 weeks postpartum (T2) via post or telephone. Most cases of PPD arise in the 1 to 6 months following childbirth which is the recommended screening period according to the American Academy of Pediatrics[104]. Generally, it is most common for PPD begin sometime within the first 3 months postpartum and therefore T2 was designed to capture the majority of the women who will go to develop PPD[265 266]. The hard copies of EPDS questionnaires were collected, both at the antenatal and postpartum time points, resulting in matched paired observational data.

Blood sampling

Upon giving informed consent for their participation in the study, participants agreed for their biological samples to be used for the purpose of research. Participants provided 2 x 5mL venous blood samples in EDTA collected during an antenatal appointment by a research midwife. One vial of whole blood was stored at 4°C until DNA extraction. The additional sample was centrifuged immediately after receipt in the laboratory and the plasma fraction stored at -80°C until required for biomarker analysis.

2.7 Procedures

DNA extractions

Genomic DNA was extracted from whole blood using the EZ1 extraction instrument (Qiagen, Hilden, Germany) with the Qiagen EZ1 DNA Blood 200µl Kit & DNA Blood Card. Whole blood samples were placed on a blood roller mixer for a minimum of 5 minutes, following which 200 µl blood was pipetted into 2mL polypropylene tubes under sterile air flow cabinets. Cartridges from the Qiagen extraction kit were inserted into the extraction instrument and samples were placed in their corresponding positions, with a labelled 1.5mL elution tube in place to collect eluted genomic DNA. EZ1 machines were UV light treated after each extraction to prevent contamination. DNA samples were eluted in 100 µl elution volumes. The quality and quantity of the extracted DNA was then assessed using Nanodrop spectrophotometer analysis using the A_{260}/A_{280} ratio. The limit of acceptance for samples to be considered high-purity was 1.7-1.8. DNA samples were stored at -80°C prior to SNP genotyping.

SNP genotyping

DNA samples were prepared for allele-specific PCR analysis to genotype participants for the selected SNPs. SNPs genotyped were the Bcl1 SNP of the glucocorticoid receptor NR3C1 (dbSNP NCBI identifier rs41423247), and 2 SNPs of CRH receptor CRH-R1 (rs242924 and rs242939). Genotyping was carried out with the capillary based LightCycler® instrument (Roche Diagnostics Ltd, Burgess Hill, UK), which uses real-time detection to minimise sample handling and contamination risk. The instrument uses heated air to allow rapid ramping. The amplified DNA is quantified using a DNA binding dye. Finally, melting curve analysis is employed to accurately and rapidly detect polymorphisms.

Primers and probes for detection of the chosen SNPs were synthesised for LightCycler® analysis by TIB MolBiol (Berlin, Germany). A set of hybridization probes consists of a pair of oligonucleotide probes which can bind on a DNA template, one labelled with fluorescein at its 3'-terminus [3FL] and the other labelled at its 5'-end with LightCycler® Red dyes [5LC]. HybProbes hybridize on the PCR product and emit a fluorescence signal based on Fluorescence Resonance Energy Transfer (FRET)[267].

For primer design a gap of one to four bases between the probes is recommended. A Forward primer, Reverse primer, Fluorescein probe (Sensor) and Internal LightCycler® Red 640 labelled primer (Anchor) were designed and synthesised for

each SNP to be genotyped (Table 2.2). The Sensor probe is designed to include the target mutation position in the centre of the probe, where it exerts the greatest influence on melting temperature, and the probe spanning the mutation must have a different Tm than the partner anchor probe. Primers and probes for LightCycler® analysis were designed by Tib MolBiol.

Table 2.2. Primer and probe sequences for SNP genotyping with the Roche LightCycler®.

SNP	rs41423247	rs242924	rs242939
Forward	CAA AgA gCC CTA TTC TTC AAA C	AgA CTT AAA Tag AAg gTC CAC AAg C	TCC ACT TCC AgA gTg ATC CTC gT
Reverse	AAA AAA AgA gAA AAA TCA AAC gAA	ggg CTg CCT Agg gCA TgT	ggC Tgg CTg CAA AAg gTg
Sensor [3FL]	CTC TTA AAg AgA TTC ATC AgC AgA CA—FL	ggA CCC TCT CCA TTT TTg C--FL	CTC CTT CAC TTg gAA CCC ACT C--FL
Anchor [5LC]	LC640-AAC TTg TCT ACT TTA Tgg CAA gAA CCC Tg—PH	LC640-Cag Cag Cag CCA TgC CCA ggA- PH	LC640-TgT gTg gCC TCC gTg TTC Agg—PH

All primers and probes were delivered as lyophilised oligonucleotides and were reconstituted in PCR grade H_20 to produce stock solutions. For PCR reactions, a final product of 0.5µM of each primer/probe was used. Optimisation of PCR conditions was carried out as part of the original pilot study and therefore optimal reagent concentrations and cycle conditions had been determined.

The LightCycler® 2.0 Carousel System was used (Roche), and Melting Curve Analysis was employed for genotyping. The genomic DNA samples were added to LightCycler® FastStart DNA Master HybProbe mastermix (Roche), primers and probes. Reaction mixtures were prepared in a sterile PCR grade cabinet and pipetted into pre-cooled glass capillaries (LightCycler® capillaries 20 µl & LightCycler® cooling block, Roche). Genomic DNA samples were added to produce a 10µl total volume for PCR using 2.5 µl genomic DNA as a template and 7.5µl amplification mixture was prepared (Table 2.3).

Table 2.3. Amplification mixture preparation for LightCycler® PCR reactions.

Reagent	Volume per sample (µl)
H_20	3.7
$MgCl_2$	0.8
Primers/probes	0.5 each (Forward, Reverse, Sensor, Anchor)
FastStart DNA HybProbe	1

The PCR mixture was prepared in glass capillaries which were capped and centrifuged at 2000g for 15 seconds. Prepared capillaries were placed into the Carousel and inserted into the LightCycler®.

Chosen parameters for PCR and melting curve analysis consisted of an initial denaturation step of 95°C for 10 min followed by an amplification stage of 40 cycles of 10 s at 95°C, 10 s at 53°C and 20 s at 72°C. After amplification, the melting curve analysis was performed in one cycle of 95°C for 20 s followed by 40°C for 20 s and then ramping to 85°C. A single cooling cycle of 40°C for 30 s was then employed. A temperature transition rate of 20°C/s was used at each step.

Each PCR run included a wild type and heterozygote control confirmed by sequencing, in addition to a negative control (PCR grade H_20 replaced DNA sample), allowing 29 samples to be analysed per run. All laboratory methods were carried out blind to EPDS status. SNP genotype was determined as wild type, heterozygous or homozygous using Melting Curve analysis (Figures 2.2-2.4).

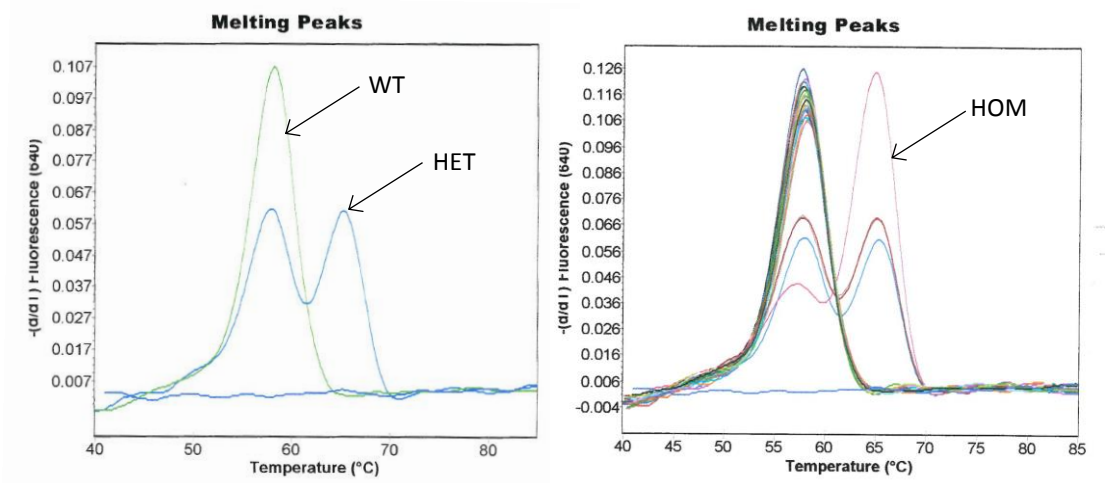

Figure 2.3. (a) Melting curve analysis for rs242939 demonstrating 3 controls used in each PCR run. Negative control (flat line), wild type (WT) (single peak at 57°C) and heterozygote (HET) (one peak at 57°C and one peak at 64°C). (b) An example output from a run including controls and samples, with homozygotes (HOM) producing a single peak at 64°C.

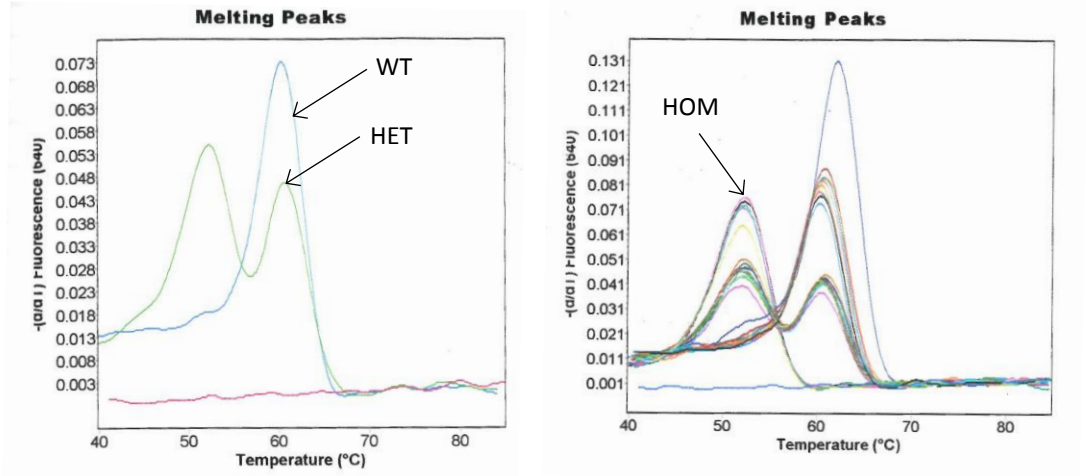

Figure 2.2. (a) Melting curve analysis for rs24294 demonstrating 3 controls used in each PCR run. Negative control (flat line), wild type (WT) (single peak at 60°C) and heterozygote (HET) (one peak at 60°C and one peak at 52°C). (b) An example output from a run including controls and samples, with homozygotes (HOM) producing a single peak at 52°C.

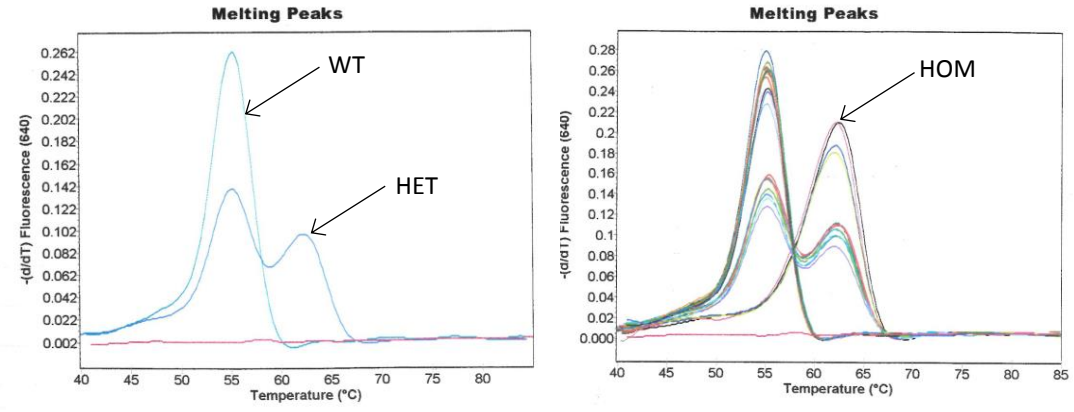

Figure 2.4. (a) Melting curve analysis for Bcl1 demonstrating 3 controls used in each PCR run. Negative control (flat line), wild type (single peak at 56°C) and heterozygote (one peak at 61°C and one peak at 60°C). (b) An example output from a run including controls and samples, with homozygotes (HOM) producing a single peak at 61°C.

Melting peaks of controls were used to correctly determine the genotype of each sample by comparing peaks. A summary of minor allele and genotype is shown in Table 2.4.

Table 2.4. Summary of minor allele mutation and melting temperatures for all 3 SNPs genotyped for wild type (WT) and homozygous (HOM) forms.

SNP	Alleles	WT T_m (°C)	HOM T_m (°C)
CRHR1			
rs242924	C to A	60	52
rs242939	A to G	57	64
GR			
bcl1 (rs41423247)	C to G	56	61

Biomarker analysis

All biomarker analyses were performed on plasma samples during the same 24 hour period for each patient sample to prevent freeze-thaw cycles and minimise variability. Plasma samples were thawed to room temperature and centrifuged at 2000g for 3 minutes to remove particulates. Levels of Brain Derived Neurotrophic Factor (BDNF), pro-inflammatory cytokine Interleukin-6 (IL-6) and anti-inflammatory cytokine Interleukin 10 (IL-10) were quantified. Prior to running each assay, all reagents were brought to room temperature unless otherwise stated.

BDNF

As described in Chapter 1, both free and bound forms of BDNF exist, and an assay was chosen to detect levels of free BDNF. Free rather than bound BDNF was measured in order to ensure the active circulating levels of BDNF were detected, since free BDNF is less affected by protein binding and is considered more biologically active. The measurement of free BDNF is more highly cited in the literature and therefore more comparable data was available. BDNF levels were quantified with an enzyme-linked immunosorbent assay (ELISA) using Human Free BDNF Immunoassay kits manufactured by R&D Systems (Minneapolis, USA). The assay uses a quantitative sandwich enzyme immunoassay technique as depicted in Figure 2.5.

A 96-well ELISA plate was pre-coated with BDNF antibody. Assay diluent was added to each well in addition to 50 µl prepared standard/sample – the plate was covered

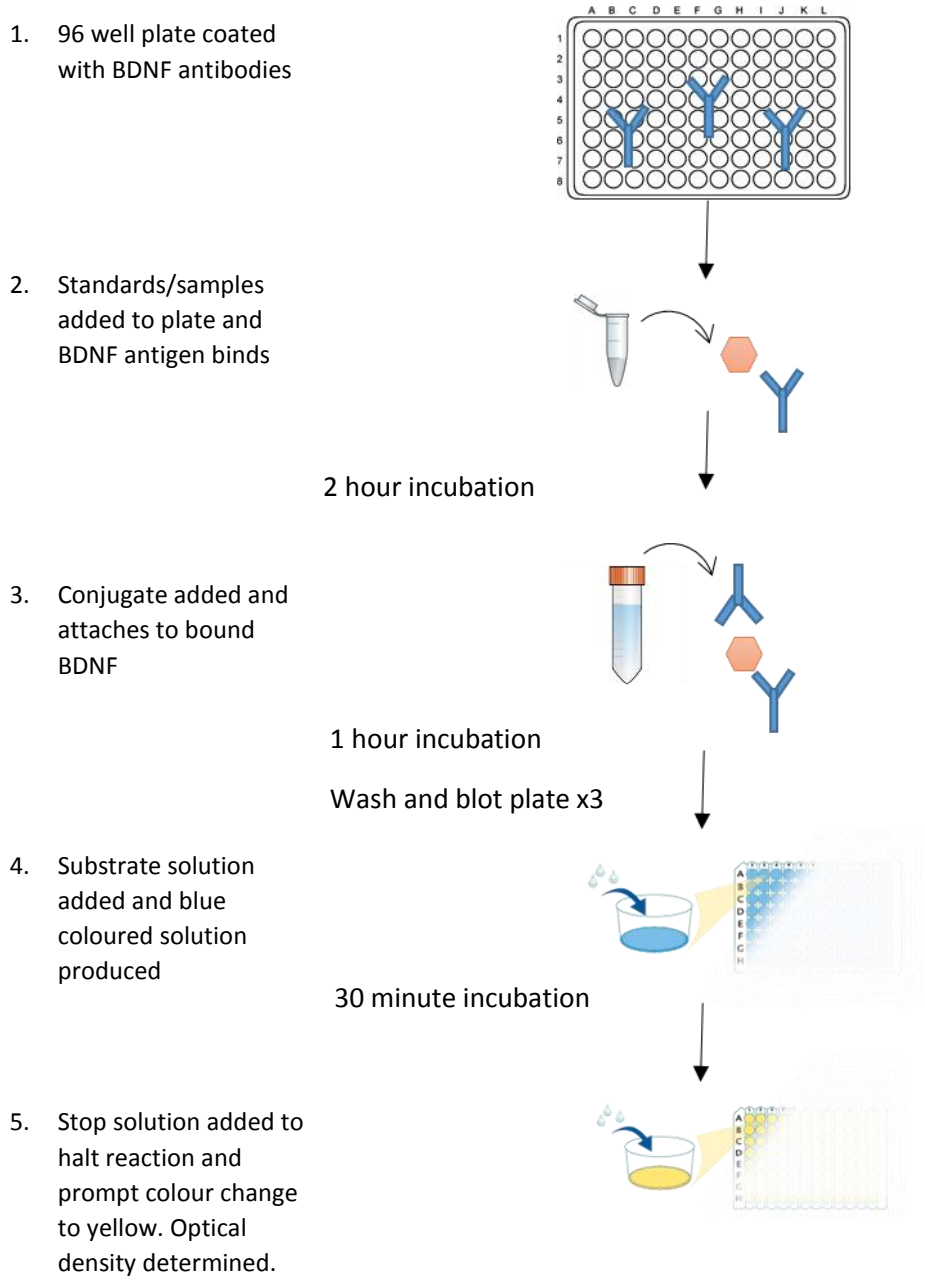

Figure 2.5. The main steps involved in the procedure carried out during the BDNF assay.

with an adhesive plate seal to prevent evaporation and incubated at room temperature for 2 hours. Human Free BDNF conjugate was then added to each well and incubated for a further 1 hour.

Each well was aspirated and washed with wash buffer 3 times – wash buffer concentrate, provided in the kit, was diluted with ddH_2O to produce wash buffer. The plate was blotted following each wash on absorbent paper. After the final wash the plate was thoroughly blotted to ensure complete removal of liquid. Substrate solution was mixed immediately prior to addition due to light sensitivity, and added to the plate following washing for a 30 minute incubation protected from light. After 30 minutes incubation, stop solution was added and the optical density (OD)

of each well was determined using a microplate reader (Tecan, Switzerland) at 450nm, with wavelength correction at 540nm.

Samples were run in duplicate and the mean OD was calculated. The concentration of BDNF for each sample was determined using a 4PL logistic curve based on the concentration of the standards. The coefficient of variation (CV), standard deviation (SD) and standard error of the mean (SEM) were calculated for each sample. CV values <20% were considered acceptable.

IL-6/IL-10

Cytokine quantification of IL-6 and IL-10 were detected using a V-plex multiplex custom antibody detection assay manufactured by Meso Scale Diagnostics (MSD, Maryland, USA), the Proinflammatory Panel 1 Human Kit. This assay is a sandwich immunoassay, pre-coated with capture antibodies on independent and well-defined spots. Specialised custom made plates were pre-coated with both IL-6 and IL-10 antibodies in specific spots in the wells, allowing both assays to be run in

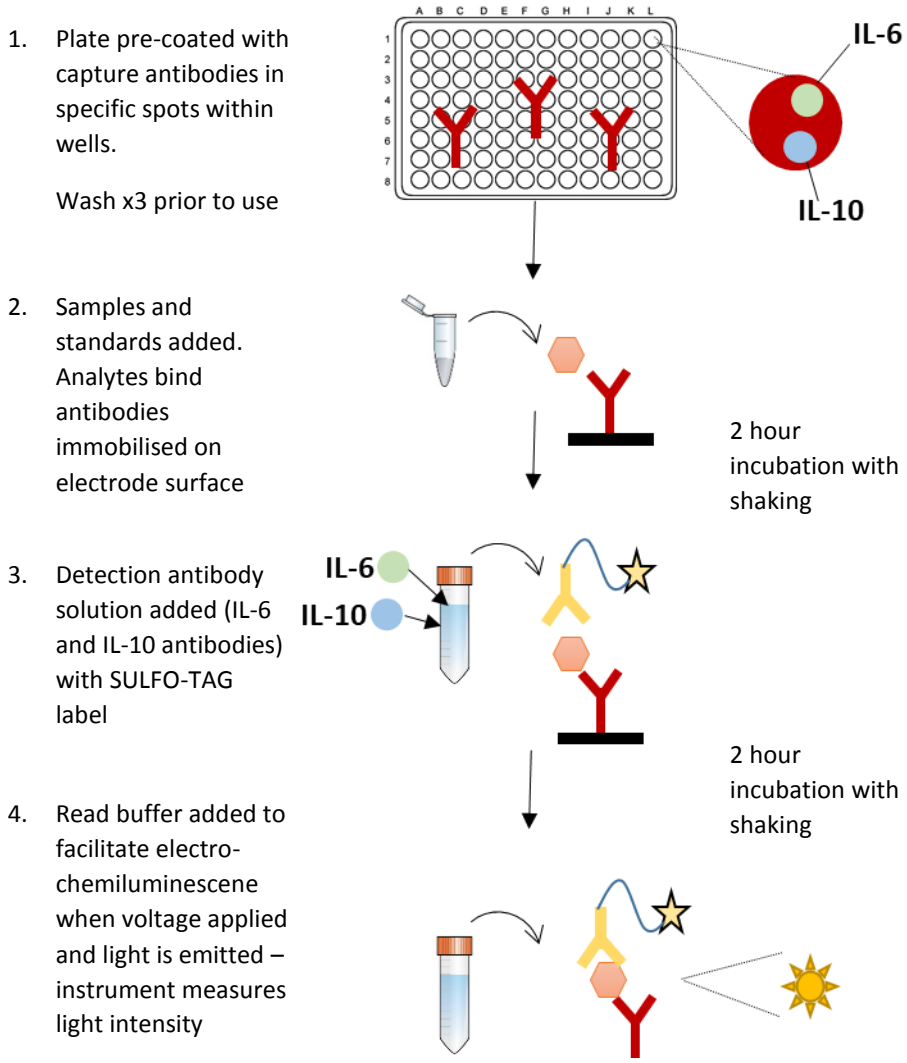

Figure 2.6. Main steps in the method for quantifying IL-6 and IL-10 with the MSD V-plex assay.

duplex on one plate. Both assays were performed simultaneously to ensure consistency and to avoid freeze-thaw of the sample.

Samples and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) were added over a course of incubation periods. Analytes in the sample bind to capture antibodies immobilised on the electrode surface of the plate. A buffer was then added to create the appropriate chemical environment for electrochemiluminescence and the plate was loaded into an MSD instrument where a voltage is applied to the plate causing captured labels to emit light. The instrument then measures the intensity of emitted light which is proportional to the amount of analyte in the sample to provide a quantitative measure.

The plate was washed 3 times with wash buffer (ddH_2O, 5x PBS + 0.05% Tween-20) prior to use. Plasma samples were diluted 2-fold in diluent containing protein, blockers and preservatives, and diluent alone was used as the zero calibrator. An 8-point standard curve was generated by dilution of the calibrator as appropriate. After initial experiments, a number of samples had concentrations at the lower end of the curve, so it was required to add an additional standard to improve accuracy at the lower end of the curve. A total of 9 standards were assayed to generate a standard curve allowing 39 samples to be assayed in each plate.

Samples and standards were added directly to the plate and plates were sealed and incubated shaking (500rpm) on a plate shaker at room temperature for 2 hours. At the end of the incubation period, detection antibody solution containing IL-6 and IL-10 antibodies was added. After washing the plate x3, detection antibody solution was added to each well and incubation was repeated as before for 2 hours. The plate was then washed as before, and read buffer was added to all wells. The plates were read using the MSD Sector Imager analyser. The Discovery Workbench software is used to analyse the signal produced by each well, which corresponds to a concentration as determined by the standards assayed on the plate. A standard curve can then be produced, from which the concentration of all included samples is calculated based on the corresponding signal.

All samples/standards were assayed in duplicate and mean concentrations were calculated. Custom layouts were created for each plate in MSD Discovery Workbench analysis software. Known concentrations of standards were inputted and Standard Curves were calculated, allowing sample concentrations and CV values to be determined.

Global DNA methylation

Global DNA methylation levels were assayed using EpiGentek MethylFlash (New York, USA), a method utilising absorbance-based quantification of global DNA methylation by specifically measuring levels of 5-methylcytosine (5-mC) in an ELISA-based format. The high sensitivity assay was used, with a detection limit of 0.2ng methylated DNA, and no cross-reactivity to unmethylated cytosine or hydroxymethylcytosine[268]. DNA is bound to strip wells with a high DNA affinity. The methylated fraction of DNA is detected with capture and detection antibodies and is quantified by measuring absorbance. Absolute quantification of 5-mC is calculated using a standard curve.

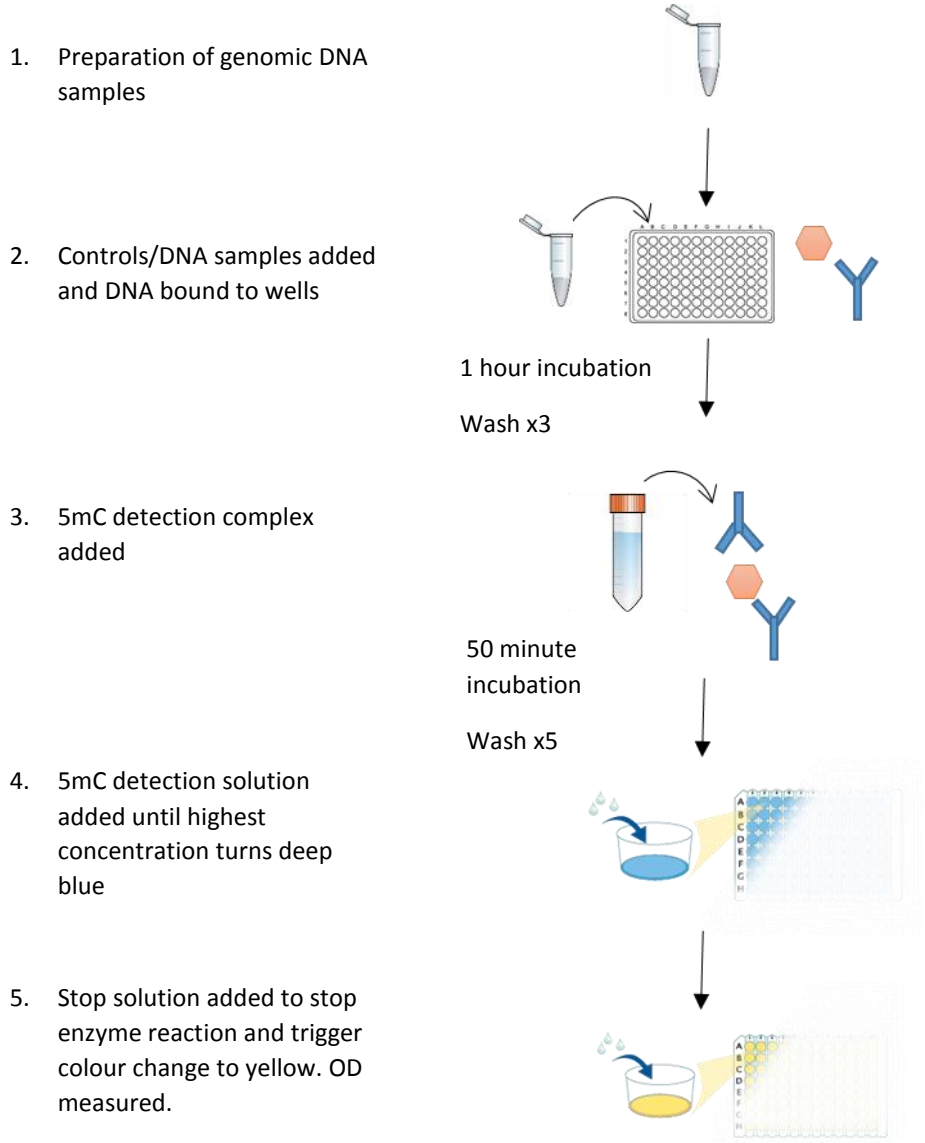

Figure 2.7. Main steps of the method used to quantify 5-mC% with the MethylFlash assay.

100ng genomic DNA was used for each sample as recommended, with a 260/280 ratio >1.6 as the lower limit of acceptance for purity. Controls and standards were prepared in duplicate. Binding solution was added to each well, followed by sample

DNA/controls. Plates were sealed and incubated at 37°C for 1 hour. 5-mC Detection Complex Solution was mixed at the end of the incubation period, containing detection antibodies.

Plates were washed x3 with wash buffer adjusted to pH 7.2-7.5, prior to addition of 5-mC Detection Complex Solution and incubation for 50 minutes at room temperature. Plates were washed x5 and detection solution added for 3-4 minutes, gently shaking, until the colour in the highest concentrated standard turned deep blue in the presence of methylated DNA. Stop solution was then added to all wells to stop the enzyme reaction and a blue to yellow colour change occurs. As soon as the colour change completed, absorbance was measured using a microplate reader (Tecan) at 450nm. The amount of methylated DNA is proportional to the OD reading, which was calculated using the standard curve measurements. Unlike other methods, MethylFlash is able to directly quantify actual levels of global DNA methylation.

The samples were assayed in duplicate, and the mean OD values for each sample was calculated, along with standard deviation and coefficient of variation (CV). The % DNA methylation was calculated using the equation:

*(Average OD of sample – Negative control OD / SLOPE * 100 [ng DNA]) * 100*

Correlation analysis and linear regression modelling were performed using IBM SPSS Statistics Version 24.

A second method was tested to assess DNA methylation which differs from other ELISA-based kits. The LINE-1 Global DNA Methylation Assay from Active Motif (La Hulpe, Belgium) was used in an initial experiment with a small number of samples, which determines the methylation levels of LINE-1 (long intersperses nuclear elements-1) retrotransposons, which are well established as a surrogate for DNA methylation[269]. Fragmented DNA was hybridized to biotinylated LINE-1 probes, which were subsequently immobilized to a streptavidin-coated plate. Washing and blocking steps followed. Methylated cytosines were quantified with an anti-5mC antibody, HRP-conjugated secondary antibody and chemiluminescent detection reagents. Samples could then be compared against a standard curve generated from standards with a known concentration of LINE-1. The assay is able to detect DNA methylation as low as 0.5%. Following the initial experiments this method was not selected for the analysis, partly due to the large quantity of DNA the assay requires (1µg) and the added complexity of the method when compared with the

MethylFlash method, which would not be suitable for quick use in a clinical laboratory. MethylFlash is a more highly cited and comparable method, whereas the LINE-1 assay is a new technique, and therefore MethylFlash was used to quantify DNA methylation in this study.

Psycho-socio-economic data collection

Data surrounding the psycho-socio-economic status of participants were extracted from the NHS Booking forms. Covariates which may be involved in depressive risk were selected for data extraction. A full list of covariates selected is available in Tables 2.5 and 2.6. Data was further extracted from the Health, Social and Family Health forms, which are routinely completed during the initial antenatal booking visit by a midwife. This is used routinely across the NHS using standardised questionnaires, and is designed to obtain information about pre-existing medical conditions and socio-demographic characteristics, in addition to questions about pregnancy and lifestyle. Lifestyle Update forms were also completed at routine antenatal visits as part of normal procedures, from which data was also collected for this study. These forms include a number of known risk factors for PND and these measures were included in the present analysis. These form were completed in hard copy and the responses for each of the variables were extracted and inputted into SPSS (Version 24).

In total responses were available from 1579 participants from the main recruitment site at Warwick Hospital. Postpartum follow-up rate was 55% at the time of data analysis for this thesis, and so complete responses were available for 872 Warwick participants. Responses from University Hospital Coventry participants were available for 448 participants. Postpartum follow-up rate was 67% leaving a final number of 302 available complete responses from Coventry.

2.8 Materials

Name	Supplier	Catalogue number	Use
EZ1 Advanced XL	Qiagen	9001874	Nucleic acid purification
EZ1 DNA Blood 200µl	Qiagen	951034	Nucleic acid purification
EZ1 DNA Blood Card	Qiagen	9015585	Nucleic acid purification
Nanodrop 1000 Spectrophotometer	Thermo Scientific	Product no longer on catalogue	Nucleic acid quantification
LightCycler® 2.0 Carousel System	Roche Diagnostics Ltd	03531414001	Real-Time PCR
LightCycler® Capillaries (20µl)	Roche Diagnostics Ltd	04929292001	Real-Time PCR
LightCycler® FastStart DNA Master HybProbe	Roche Diagnostics Ltd	12239272001	Real-Time PCR
Primers and Probes	TIB MolBiol	Designed to order	Real-Time PCR
MethylFlash Methylated DNA 5-mC Quantification Kit (Colorimetric)	EpiGentek	P-1034-48	Quantification of global DNA methylation
Human Free BDNF Immunoassay Quantikine ELISA	R&D Systems, Biotechne	DBD00	Quantification of human free BDNF
GENios microplate reader	Tecan	16129904	Analysis of ELISA based assays
V-Plex Custom Human Biomarker Kit	Meso Scale Diagnostics	K151A9H-1	Quantification of human IL-6 and IL-10
Sector Imager	Meso Scale Diagnostics	R31QQ-3	Quantification of human IL-6 and IL-10
Vortex Genie 2	Scientific Industries	SI-0236	Sample preparation
Centrifuge MiniSpin	Eppendorf	5452000060	Sample preparation
Vibrax VXR Basic Orbital Shaker	IKA	0002819002	Sample preparation
Heraeus Pico 17 Centrifuge	Thermo Scientific	75002401	Sample preparation

2.9 Variables

All variables selected for inclusion in the study are shown in Table 2.5. Variables were carefully selected based on the literature to include specific risk factors for PND[23 55 95]. For example, 'anxiety' is a known risk factor for antenatal depressive symptoms. This link has been well established and is likely attributable to the pressures of motherhood including the potential financial strain[270 271]. Covariate 'social status' is indicative of socioeconomic status (SES), which is a well-established risk factor for PPD[55 95]. Low social status also often indicates adversity in life.

26 variables were selected for extraction from the forms, 4 of them numerical integer values and 22 factorial covariates with factor levels indicated in Table 2.5. Additional collected variables for the smaller Coventry cohort are displayed in Table 2.6.

Table 2.5 Data extracted for each covariate selected for main study (Warwick Hospital).

Covariate	Question
Education	**To what level have you been in education?**
	Did not take any exams
	>16 years
	>18 years
	Diploma level
	Degree level
	Professional
	Post Graduate
	Doctorate
Social status	**What is your current employment status/level?**
	Higher managerial/professional
	Diploma level professional
	Self employed
	Lower level supervisory/technical
	Semi routine/routine
	Unemployed/student
Ethnicity	**What is your ethnicity?**

Covariate	Question
	White British
	Indian
	Asian
	Other white
	Black Caribbean
	Black African
	Mixed
	Other
Alcohol (pre-pregnancy)	**Did you drink alcohol during the 12 months before conception?**
	Yes
	No
Drinking (in pregnancy)	**Do you currently drink alcohol?**
	Yes
	No
Smoking (pre-pregnancy)	**Were you a smoker during the 12 months before conception?**
	Yes
	No
Cigarettes (in pregnancy)	**Do you currently smoke?**
	Yes
	No
Medication	**Do you currently take any prescription medication?**
	None
	Thyroxine
	Anti-coagulant
	Asthma drugs
	Other
Omega-3	**Do you currently take an omega-3 supplement?**
	Yes
	No
Anxiety	**Have you ever had anxiety?**
	Yes

Covariate	Question
	No
	Do you currently have anxiety?
	Yes
	No
Depression	**Have you ever had depression?**
	Yes
	No
	Do you currently have depression?
	Yes
	No
Past History PND	**Have you ever had perinatal depression?**
	Yes
	No
Family History PND	**Has anyone in your family ever had perinatal depression?**
	None
	1st degree relative
	Other relative
Support	**What is your current support status?**
	Unsupported
	Supported
Parity	**Number of previous children?**
	(N)
Age	**Maternal age at 12 weeks?**
	<18
	18-24
	25-29
	30-34
	35-39
	40-45
	>45
Body Mass Index (BMI)	**Maternal BMI at 12 weeks?**
	<18.5
	18.5-24

Covariate	Question
	25-29
	30-34
	35-39
	40-44
	>45
Gender of baby	Male
	Female
Weight of baby	(grams)
Twins	Yes
	No
Gender of twin 2	Male
	Female
Weight of twin 2	(grams)
Gestational length	(days at delivery)
Mode of delivery	Normal vaginal birth
	Instrument assisted
	Emergency C section
	Elective C section
Induction of labour	**Was labour induced?**
	Yes
	No
Premature baby	**Was the baby delivered prematurely?**
	Yes
	No

Table 2.6. Additional data collected for participants from UHCW.

Covariate	Question
Housing	**What is your housing status?**
	Owns
	Rents
	Parents
	Social Housing
	Friends
Anxiety treatment	**Have you ever received treatment for anxiety?**
	No
	Medication
	Counselling
	Inpatient treatment
Depression treatment	**Have you ever received treatment for depression?**
	No
	Medication
	Counselling
	Inpatient treatment
Obstetric history	None
	Miscarriage
	Stillbirth
	Neonatal death
	Ectopic
	Multiple history
Birth complications	None
	Induced
	Postpartum haemorrhage
	Baby admitted to special care
	Multiple complications

2.10 Statistical Treatment

2.10.1 Psycho-socioeconomic data

Following recruitment, responses from all participants for selected variables were inputted into an electronic data file using SPSS (version 24). The antenatal EPDS score was also available at the stage and this was added to the dataset. At a later stage once postpartum EPDS data was collected the dataset was updated with the postpartum EPDS score in order to maximise data use. Missing data was excluded from the analysis but participants were not excluded on the basis of missing data in order to maximise the available data. The cohorts were approached separately for the statistical analysis – those who completed antenatal EPDS only, and those who completed both antenatal and the follow-up postpartum EPDS.

The EPDS was used in a pre- and post-measures design, and approached as both a linear score, and dichotomised with a cut-off value in order to explore its use as a screening tool. The raw EPDS score was interrogated in order not to constrain the EPDS data to dichotomous groups – this enabled analysis of the relationship between the data collected from the variables and EPDS score. Rather than splitting women into high vs low risk groups, for this part of the analysis we instead interrogated the data to investigate which factors contribute higher scores on the EPDS.

Antenatal and postpartum EPDS scores were recorded, together with the profiling of psychosocial, lifestyle and pregnancy related information. This data is routinely gathered during antenatal hospital visits, and in this prospective study data was utilised to investigate whether distinct patterns of onset and persistence of PND symptoms from pregnancy to the postpartum period are associated with different risk profiles. This stage of the analysis was based on modelling by discretization, by applying well-established cut-offs to determine 'low' or 'high' risk of PND. In addition, data analysis using the full EPDS scale explored the usefulness of covariates as predictors of either antenatal or postpartum EPDS scores in order to identify covariates that can support a "predictive" model capable of forecasting raised EPDS scores. A second aim of the statistical analysis was to detect whether systematic differences between antenatal and postpartum EPDS scores exist, potentially hinting at different aetiologies. Finally, the analysis explored whether data collected based around risk factors for PND can also be used to predict two interconnected offspring outcomes, birth weight and gestational length.

Table 2.7. The main steps in the analysis of data and a summary of the purpose of each method.

Statistical treatment	Purpose
Data input	Hard copies of questionnaires and EPDS forms transferred into SPSS format and variables coded numerically for ease of analysis
Exploratory analysis	Identify prevalence of depressive risk in the study population and to understand the cohort characteristics
Dichotomisation with EPDS	Participants dichotomised into 'high' or 'low' risk dependent upon EPDS cut off score to allow for clinically relevant analysis
Division into risk groups	Risk groups defined by dependent upon timing of onset/remission based on EPDS to allow exploration of trajectories
Chi squared/correlations	Identifies potential correlates/risk factors associated with antenatal and postpartum EPDS scores
Penalised regression	Uses raw EPDS scores to increase statistical power and identify both distinguishing and common covariates across antenatal and postpartum risk. Factors contributing to average score and difference between scores can be explored
Multinomial logistic regression	Allows for 'group analysis' based on risk groups to further understand associations between risk factors and the three groups with distinct trajectories. Relative risk values are generated.
Exploratory linear regression modelling	Exploration of the contribution of specific risk factors to prediction of EPDS scores. Identifies and ranks the most important variables in prediction and quantifies contribution.

Linear regression modelling

Data collection was completed for this project in January 2019. The number of recruited participants from Warwick Hospital, the main recruitment site, totalled 1579 – all of these women had completed the antenatal EPDS. To explore factors associated with antenatal EPDS, variables were prepared for linear regression modelling.

Variables were chosen based on Chi squared (χ^2) analysis for categorical variables, bivariate correlation for continuous variables, and point-biserial correlation for dichotomous variables. Those with significant correlations at this stage were selected for model inclusion. The final variables entered were past history of depression, past history of anxiety, smoking pre-pregnancy, social status and age.

In order to allow for inclusion in linear regression, variables were recoded into dummy variables for social status and age, coded 0/1 for each level of the factor. Remaining variables were dichotomous and therefore eligible for linear regression. All selected variables were entered into the model to assess their prediction of antenatal EPDS score. Baseline categories were selected based upon either 'no' for yes/no answers such as history of depression, or on the modal group such as age category. Hierarchical entry was used, inputting variables in order of theoretical importance based on previous work. Multicollinearity was assessed in addition to residual plots and non-zero variance. Linear regression analysis was repeated for all participants who completed postpartum follow up, with the additional variable of antenatal EPDS score included in the analyses. The smaller study population recruited at Coventry Hospital was analysed in the same manner. There were some differences in data collected by the two different hospitals which can be seen in Tables 2.5 and 2.6.

Detailed statistical analysis sub-group

A sub-group of participants were selected for the most comprehensive analysis – the first 480 women to have completed the postpartum follow up. This cohort has undergone the most in depth statistical analysis based on psycho-socioeconomic data alone, and in addition both biomarker and genetic data has been collected and investigated for these participants.

The main aim of the statistical analysis was to investigate PND heterogeneity according to symptom progression profiles based on timing of onset and remission. This involves the evaluation of the predictive value of risk factors and identification of systematic differences between antenatal and postpartum EPDS scores. This sub-

study analysed paired observational data from participants that completed the protocol resulting in 960 complete observations (n=480).

Classification of risk trajectories

For the first stage of the analysis a cut-off score was used to classify patients as high vs low risk for depression during pregnancy or postpartum. This also allowed us to characterise distribution of scores and subgroup patients according to temporal patterns and possible recurrence of depressive symptomatology. A new variable 'Group' was created and participants were re-categorised into a Group dependent upon EPDS score trajectory (Figure 2.5).

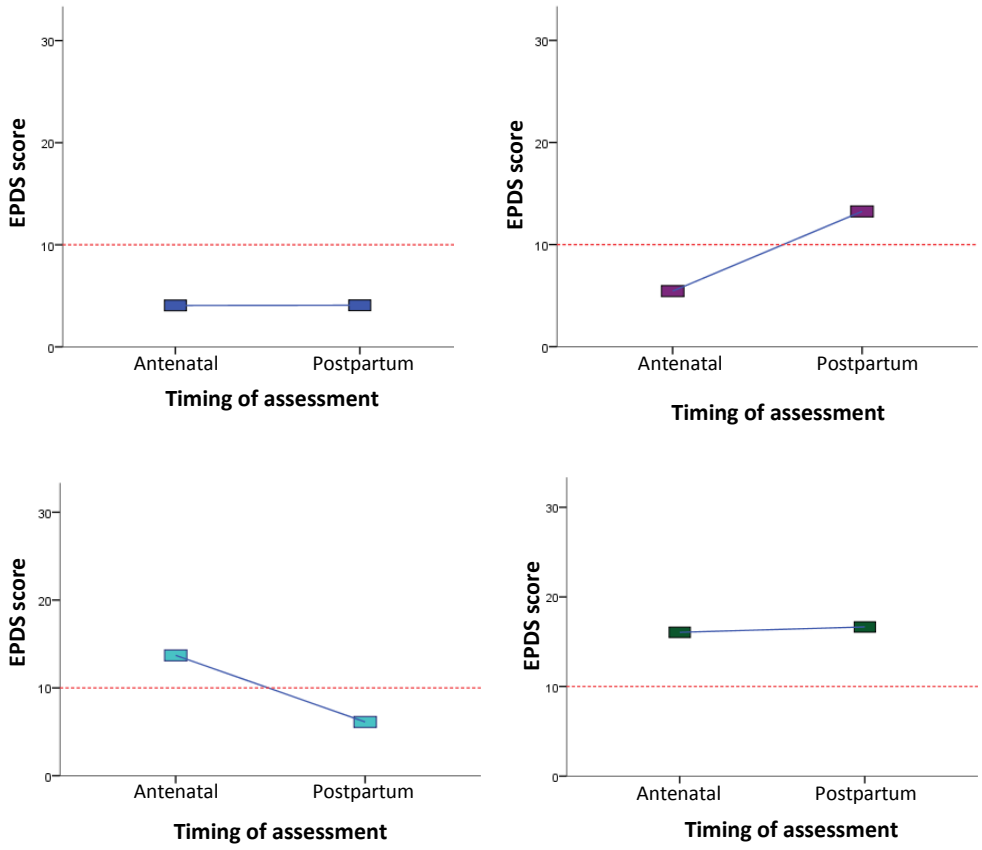

Figure 2.8. There are four possible disease trajectories for PND dependent upon 'Group'. Mean antenatal and postpartum EPDS scores are shown for each group. Red dotted line indicates cut-off for positive screen.

EPDS scores were recoded into variable 'Group' dependent upon EPDS score – as explained in section 2.6, a score of <10 was considered a negative screening results, and a score of ≥10 was considered a positive screening result, for both minor and major depressive symptoms ('high risk' group) (Table 2.8). In addition groups were

interrogated with a higher cut-off score ≥13, which has been recommended to screen for major depression.

Table 2.8. Groups as defined by depression trajectory based upon EPDS screening (cut off ≥ 10).

Group	Antenatal screen	Postpartum screen
0	0 – Negative	0 - Negative
1	0 – Negative	1 - Positive
2	1 – Positive	0 - Negative
3	1 – Positive	1 - Positive

The aim of this statistical analysis had three parts: (i) the investigation of distinct patterns of onset and persistence of PND symptoms from antenatal to the postnatal period; (ii) an exploration of the potential for covariate data gathered routinely from women during hospital visits to predict EPDS scores: (iii) to test the degree to which severity of antenatal and postpartum depression as well as the change in severity across the two periods was associated with distinct covariates risk profiles.

In order to determine the contribution of individual factors to depressive symptoms, a comprehensive statistical analysis was designed with the aim of building a full picture of their prediction of EPDS score. The analysis included (a) dichotomised EPDS scores to define a binary outcome of depressive symptoms in order to obtain estimates of effects and their significance that are more robust to potential misspecifications of the regression models; (b) development of regression models for the raw EPDS scores in order to increase power and to detect subtler effects of covariates; (c) evaluation of the predictive performance of the regression models in comparison to a state-of-the-art machine learning prediction method. The statistical analyses were carried out with the guidance of biostatistician Dr Lorenz Wernisch, a co-author on the publication.

Multinomial logistic regression – group analysis

A number of potential risk factors for PND, which were identified in the published literature and available in this study, were selected for in the present analysis such as age, ethnicity and social status among many others (Table 2.5). All selected variables were first analysed for statistical significance of associations between

potential risk factors and AND/PND through chi-squared ($\chi 2$) tests. Odds ratios (OR) and the associated 95% confidence interval (CI) were obtained for variables that demonstrated statistical significance during chi-squared analysis. Pre- and post-delivery variables for EPDS scores were created to conduct independent analysis between the risk factors.

A p-value of <0.05 was considered indicative of a statistically significant difference. All risk factors with a $p<0.05$ in univariable analysis, either pre-delivery or post-delivery, were identified and included in a multinomial logistic regression model to study the relationship between group classification and risk factors with STATA commands *mlogit* and *mlogit,rrr* to obtain relative risk and *p*-values. To simplify analysis four individuals with missing data were excluded resulting in N=476. Covariates with low frequencies in some levels were merged.

Overall low risk group (Group 0) was used as the baseline category. Relative risk values were calculated to provide a better understanding of the magnitude of association between risk factors and each group. Statistical analyses were conducted with IBM SPSS Statistics Version 24 and STATA/SE 14.0.

Penalised regression and prediction

The first phase of the analysis used multinomial logistic regression to identify the risk imposed by individual factors on each risk group. The next phase was to look in further detail at these contributions and specifically how EPDS score is affected from the antenatal to postpartum time points. Regression modelling should take into account the large number of covariates, many of which are multi-level, and therefore a penalised regression model was selected. To simplify the regression analysis, 6 individuals of the 480 with missing values in some covariates were removed. Since this amounts to about 1% of the data, the effect on the statistical results were considered negligible.

To determine the optimal model of prediction, a linear model with elastic net regularization was used[272]. Parameter alpha was set to 0.5 with equal weight given to Lasso and ridge regularisation in order to improve prediction accuracy of the model. This leads to a spare regression with unimportant variables removed, and highly correlated variables remain in the model when two or more variables explain the outcome equally well. The prediction accuracy may be improved by shrinking coefficients to zero with this approach. R package *glmnet* was used for this model. A standard linear model was also used to obtain corresponding *p*-values.

Differences and commonalities between antenatal and postpartum EPDS scores were also explored. This analysis aimed to investigate specific covariates that would explain a drop or rise in antenatal to postpartum EPDS scores. The 'average' EDPS score [APA score = (T1 + T2)/2] can be seen as an indication of overall perinatal depression not specific to either an antenatal or postpartum time point; identified covariates contributing to the average score would indicate common underlying factors. The 'difference' in EPDS scores [DPA score = (T1 − T2)] corresponds to the improvement or worsening in EPDS score from the antenatal to the postpartum time point; covariates contributing to the difference of T1 to T2 score, would indicate differentiating factors.

Finally the possibility of a stratification of patients based on the results of the questionnaires was explored. To explore whether the regression model would be suitable for prediction, and not only to find important covariates of EPDS scores, predictions derived from the model were compared with predictions from a state-of-the-art machine learning prediction algorithm, extreme gradient boosting, utilising the xgboost R package. In addition, the analysis aimed to establish how far an EPDS score in the third trimester could directly predict an EPDS score at 6-10 weeks postpartum. The predictive power under a range of cut-off values was assessed with measurements of the area under the ROC curve.

Negative Predictive Values (NPV) and Positive Predictive Values (PPV) were calculated to explore the predictive capacity. PPV was calculated using the number of 'true positives' i.e. those with the risk factor who screened positive on the EPDS, and 'false positives' i.e. those with the risk factor but did not screen positive on the EPDS. PPV = (true positives)/ (true positives + false positives). NPV was calculated with the 'true negatives', i.e. those without the risk factor who screened negative on the EPDS, and the 'false negatives', i.e. those who do not have the risk factor but did screen positive on the EPDS. NPV = (true negatives)/ (true negatives + false negatives). Sensitivity and specificity were then calculated. The interpretation of these values will demonstrate the usefulness of this risk factor in the clinic.

2.10.2 Statistical analysis of laboratory data

The laboratory analysis of biomarkers from patient blood samples was carried out for a selection of participants with complete antenatal and postpartum EPDS scores. This group was interrogated for relationships between biomarkers, EPDS

score and psycho-socioeconomic variables. Concentrations of circulating biomarkers were calculated from the results of the IL-6, IL-10 and BDNF assays.

BDNF data

As described earlier in this chapter, BDNF concentration was calculated with the use of a Quantikine ELISA kit. Mean absorbance, calculated as optical density (OD), was determined. The coefficient of variation (CV), standard deviation (SD) and standard error of the mean (SEM) were calculated for each sample. Mean concentration and coefficient of variability (CV) was generated for each sample. The CV for each sample is defined as the ratio of standard deviation (σ) to the mean (μ): $CV=\sigma/\mu$, and is calculated as a percentage (x 100). The average of the individual CVs is reported as the intra-assay CV. CV <20% was considered acceptable. Standard curves were generated from the concentration of standards with known concentrations by using a 4-parameter logistic (4-PL) curve-fit as shown in Figure 2.6. Mean OD of each sample was compared against the curve to determine the sample concentration.

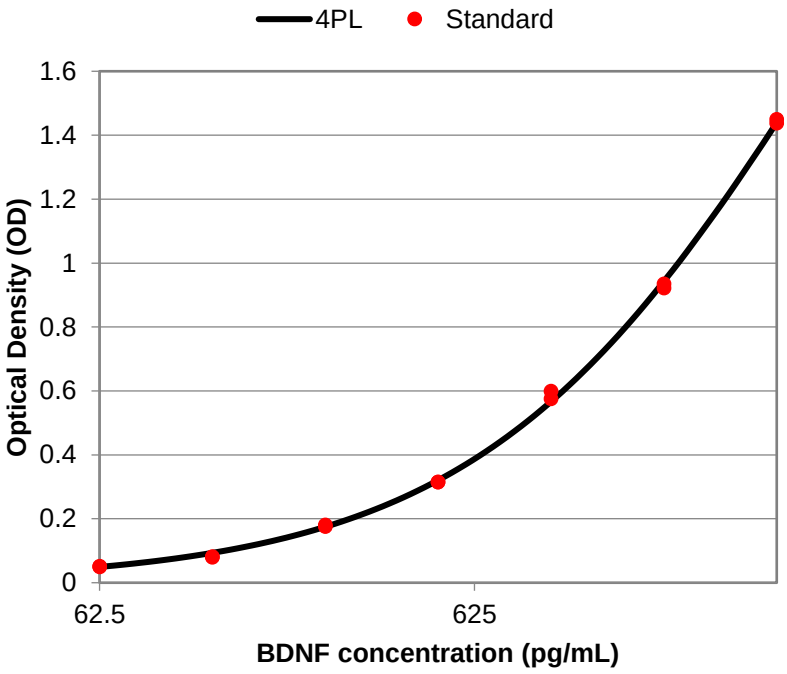

Figure 2.9 A 4-PL logistic curve was generated for each plate following the completion of BDNF assay.

The lowest level of detection (LLOD) is the lowest measurable value that is statistically significant from zero and is calculated by adding two standard deviations to the mean OD value of several zero standards – the corresponding analyte concentration is determined from the standard curve. This was not a

concern for this assay, since BDNF levels tended to be at the higher end of the curve.

IL-6/IL-10 data

IL-6 and IL-10 concentrations were calculated similarly following MSD assays using MSD V-Plex Custom Cytokine assays as previously described. Known concentrations of standards were used to determine unknown sample concentrations based on the standard curve. An example of the standard curve for an IL-6 assay and the associated calculations are shown in Figure 2.7 and Table 2.9. The median LLOD for the MSD assays were 0.06pg/mL for IL-6 and 0.03pg/mL for IL-10.

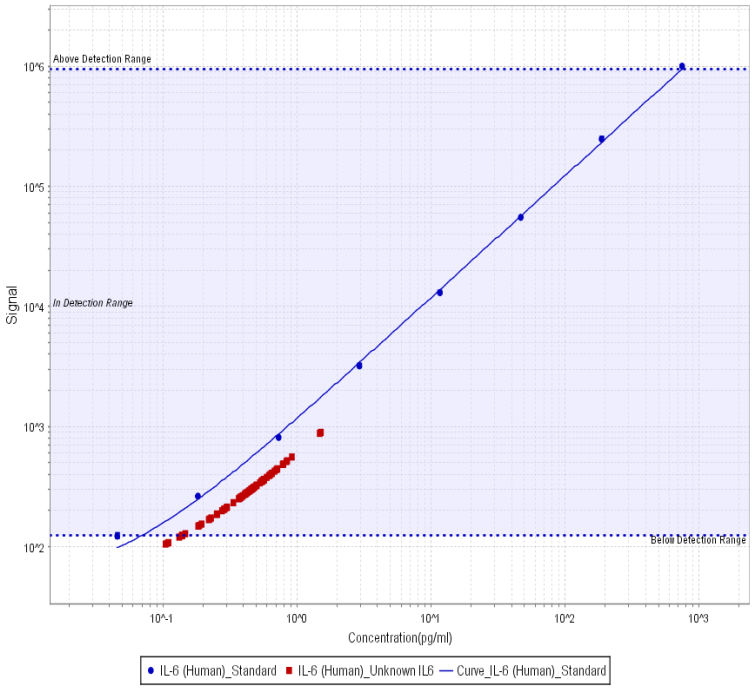

Figure 2.10. The standard curve generated by the standards on an IL-6 MSD assay with calculated sample concentrations plotted next to the standard curve in red.

Table 2.9. Concentrations (pg/mL) of the 9 points of the standard curve based on the corresponding signal generated in each well. Each standard was inputted in duplicate and mean and CV values were calculated.

Sample	Conc.	Signal	Mean Signal	Calc. Conc.	Mean Conc.	% CV (Calc. conc.)
S001	750	1013433	1001322	801.61	792.14	1.69
S001	750	989211	1001322	782.68	792.14	1.69
S002	188	253319	247226	204.56	199.71	3.43
S002	188	241132	247226	194.87	199.71	3.43
S003	46.9	55521	55183	46.00	45.73	0.85
S003	46.9	54844	55183	45.45	45.73	0.85
S004	11.7	12867	13013	10.91	11.04	1.56
S004	11.7	13158	13013	11.16	11.04	1.56
S005	2.93	3269	3202	2.81	2.75	2.95
S005	2.93	3135	3202	2.70	2.75	2.95
S006	0.732	806	811	0.68	0.68	0.82
S006	0.732	815	811	0.69	0.68	0.82
S007	0.183	263	263	0.20	0.20	0.00
S007	0.183	263	263	0.20	0.20	0.00
S008	0.04575	118	123	0.06	0.07	8.52
S008	0.04575	127	123	0.07	0.07	8.52
S009	0	48	46	0.00	0.00	NaN
S009	0	43	46	0.00	0.00	NaN

Concentrations were log transformed to achieve normality in order to prepare for statistical analysis, to aid the interpretation of patterns and to meet the assumptions of statistical testing. Concentrations were calculated for IL-6, IL-10 and BDNF, and a ratio of IL-6/IL-10 was also determined. Reference ranges were calculated for each biomarker to highlight any outliers (±1.96 SD). Positive Predictive and Negative Predictive Values (PPV/NPV) were calculated for the biomarkers using the reference rage as a cut-off as previously described to determine the accuracy of this test. 'Healthy' patients with EPDS <10 were compared with those screening positive for antenatal depression. Associations between biomarkers and EPDS score, as well as psycho-socioeconomic variables were then tested for statistical significance. Patient concentrations of each biomarker were entered as continuous variables into linear regression models to assess whether their addition improved the established model's prediction of EPDS score.

Calculation of biomarker z-scores

In order to generate an overall inflammatory score to input into the analysis, z-scores were calculated using combined data from each of the biomarkers. The method used was based on other studies analysing the effects of inflammatory biomarkers[273]. The study mean (μ) for each biomarker was first calculated based on individual biomarker concentrations (x), in addition to the standard deviation (σ). The z-score for each biomarker was calculated using: $(x - \mu)/\sigma$. These were then combined for IL-6, IL-10, IL-6/IL-10, and BDNF. Direction of expected association was taken into account when generating z-scores.

Genotyping data

Genotyping data was interpreted as either 'presence' or 'absence' of the minor allele, due to the relatively small sample size (N=480). The number of copies of the allele was additionally explored in the analysis in terms of contribution to depressive risk. Frequencies of each of the three genotyped SNPs were determined and compared to the published allele frequencies. Chi squared (χ^2) tests were then used to examine whether differences between observed and expected genotype frequencies are significant using the equation:

$$\chi^2 = \sum \frac{(observed - expected)^2}{expected}$$

Odds ratios and 95% confidence intervals were calculated for each SNP. Correlations were examined to test for any significant associations between the selected variables and SNP status. This was carried out for those participants with and without a family history of PND to test for any differences when a known family association is present. Relationships between genotype and depressive risk were explored, in addition to any genetic associations with Average (APA) and Difference (APA) EPDS scores.

In Chapter 6 of this thesis, the combined analysis of psycho-socioeconomic data and laboratory data is presented. The main study cohort that the laboratory work explores comprises of women who completed the full study protocol. Biomarker data from the laboratory aspect of this study is available for these women, and findings from this cohort will be described in Chapter 4. In addition, genetic data is available for selected participants with complete data; results from this analysis is presented in Chapter 5. For this final group, full EPDS scores, biomarker data and genetic data are available. These cohorts have been analysed separately, as

displayed in Figure 2.11, and modelling has additionally been applied to identify key psycho-socioeconomic risk factors, which can be seen in the relevant Chapters. Combined analysis of biomarker, genetic and psycho-socioeconomic data are presented in Chapter 6.

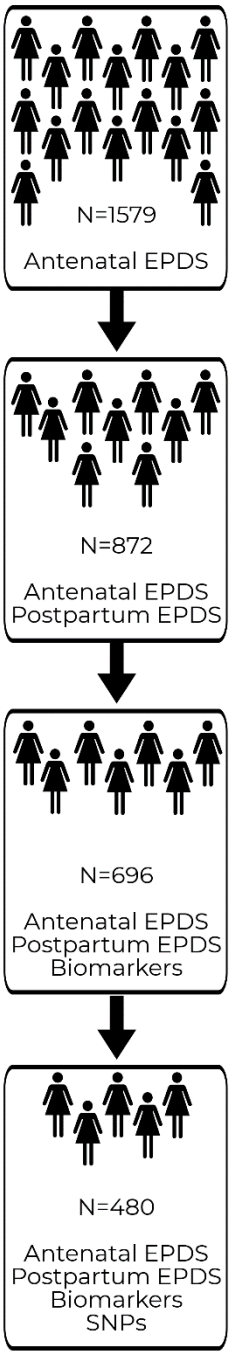

Figure 2.11. The study cohorts involved in the project with tests performed for each group and frequencies in each cohort.

3 Results: Psycho-socioeconomics

3.1 Chapter Three Abstract

This study aims to explore the relationship between certain factors linked with lifestyle, health and social factors, and PND. This chapter address aims 1 & 2: 'Can psycho-socioeconomic risk factors predict antenatal and postpartum symptoms of PND?' and 'Is PND heterogeneous and can timing of onset/remission be predicted from risk factors?' This analysis will investigate independent or alternatively inter-related risks that exert different effects on depression susceptibility during different stages of pregnancy as women progress from the antenatal period to the early postpartum period. This additionally explores the heterogeneity question - whether antenatal and postpartum depression are themselves distinct syndromes, or whether an umbrella category of perinatal depression will suffice.

The study comprises of women who completed the antenatal EPDS at time point 1 (N=1579) and those who completed both the antenatal and postpartum EPDS at time points 1 and 2 (N=872). Analyses are presented in this chapter into two main parts – associations with antenatal EPDS score and associations with postpartum EPDS score. A sub-group of N=480 was first the target of a statistical pilot study based on the predictive potential of psycho-socioeconomic factors. This reveals that a history of anxiety and social status are the two biggest predictors of antenatal depressive symptoms, while a history of depression or PND and a family history of PND contributed most to postpartum depressive symptoms. Figure 3.5 depicts the key factors characteristics to influence both antenatal and postpartum scores as a result of regression modelling. It was found that the covariates recorded in this study are better suited to predicting antenatal EPDS scores than postpartum scores.

The heterogeneity of PND was next explored, with risk groups based on the trajectories of depressive symptoms, supporting the theory of heterogeneity in PND. Important findings from this sub-group are then explored and validated in the larger cohort, which is presented later in the combined analysis in Chapter 6. Laboratory analysis of biomarkers and genetics has also been carried out for the sub-group and is presented in Chapters 5 and 6. The sub-group analysis explores the heterogeneity of PND, with an in depth look at EPDS changes, whereas the main analysis explores the specific contribution of risk factors to EPDS score.

The study comprises of a further group of women recruited from a secondary site, Coventry Hospital (UHCW), and this data has been analysed in the same fashion and is presented in this chapter. Statistical analyses have been carried out for those who completed antenatal EPDS (N=448) and both antenatal and postpartum EPDS (N=302). When comparing the Warwick and Coventry cohorts, both distinct and shared risk factors have been identified, such as a young age, lack of support and a history of anxiety contributing to antenatal depressive symptoms across both cohorts. Differences and similarities between the two study populations are presented in this chapter.

The chapter opens with an examination of the general characteristics of the study cohorts to assess the ability to generalise these findings to a wider population and to explore any potential bias in the results. For the initial statistical analysis, exploratory analyses were carried out, observing differences between controls and cases, defined by a score on the EPDS ≥ 10. A more detailed picture of these associations was then explored further with linear regression modelling, to assess the ability of selected factors to predict EPDS score. This tested whether using the EPDS in pregnancy can predict postpartum outcomes, and to assess whether this is optimised with use of a cut-off score or a raw score. This chapter presents these results of the statistical analyses conducted to test the relationships between psycho-socioeconomic variables and PND.

3.2 Sub-Group Analysis

3.2.1 EPDS score distributions & cohort characteristics

The sub-group consisted of 480 matched pairs with completed antenatal and postpartum EPDS scores recruited from Warwick Hospital. In this group a prevalence of 11.5% was identified for antenatal (n=55) and 14.8% (n=71) for postpartum depressive symptoms. This falls within the estimated 10-15% shown by other studies. Initial analysis showed that the majority of the women in the data set were White British (84.6%), educated to a minimum of degree level (55.9%), were in the 30-34 age group (37.7%), had a 1st trimester BMI of 18.5 – 24 (47.9%), had a social status of higher managerial/professional (35.2%), were not on any medication (84.6%) or Omega 3 supplements (76.3%), and had consumed alcohol before pregnancy (68.3%) but did not drink during pregnancy (97.3%). The majority

did not have a history of anxiety (86.7%), or depression (89.6%), did not smoke at any time prior to pregnancy (88.8%), did not smoke during pregnancy (96.5%) and had no family history of PPD (84.0%). The great majority were supported (98.3%), 44.8% had one child prior to the pregnancy, and 52.1% delivered a baby boy.

Distribution and frequencies of EPDS scores are displayed in Figure 3.1 (studies comparing the antenatal (T1) and postpartum (T2) EPDS scores are also shown in the Penalised Regression analysis and prediction section). Groups were created with the use of a cut-off score to classify patients according to temporal patterns of depressive symptoms. Using the cut-off of 10 to categorize EPDS scores as 'high' or 'low' risk, the risk trajectory defined by EPDS scores during pregnancy and postpartum period determined classification of participants into four groups for the initial analysis (Table 3.1). Group 0 represents a low risk trajectory, Group 3 an overall high-risk trajectory, and Groups 1 & 2 transient risk trajectories with either an increase (group 1) or decrease (group 2) in postpartum EPDS scores.

Table 3.1. Distribution of the cohort within created Group variables.

Group	Total N	%
0 - Overall Low Depressive Risk	379	79.0
1 - Low antenatal risk, High risk postpartum	46	9.6
2 - High antenatal risk, Low risk postpartum	30	6.3
3 - Overall High Depressive Risk	25	5.2

Those with raised EPDS scores postpartum only (group 1) represented the largest group of 'high risk' patients with 45% of the total (n=101). Group 1 had a median antenatal EPDS score of 6 and median postpartum score of 12. The proportion of women with raised EPDS either antenatally (group 2) or at both assessment time points (group 3) was 30% and 25%, respectively. Group 2 has a median antenatal score of 13 and postpartum score of 6, whereas Group 3 exhibited the highest median values with an antenatal EPDS median score of 15 and postpartum EPDS of 16. Patients from group 3 (high risk both antenatally and postpartum) appear to exhibit the most severe symptoms according to EPDS scores; median values of both antenatal and postpartum scores were within the moderate depression range as proposed by McCabe-Beane[274]. In contrast median values of raised EPDS in groups 1, and 2 were within the mild depression range.

Previous studies also investigated predictive accuracy of the antenatal EPDS for postpartum EPDS at different cut-offs. For a comparison with these studies the use of different cut-offs was explored in this study. Using a cut-off of 10, the distribution of 'at-risk' patients across the three groups was 45% in group 1, 37% in group 2 and 28% in group 3. Increasing the cut-off to 13 (to include patients with MDD symptoms only) did not significantly alter the relative distribution of each group, with a similar distribution of 43%, 30% and 27% for groups 1, 2 and 3 respectively (Figure 3.1b). However, decreasing the cut-off to 8 shifted the relative distribution of 'at-risk' patients and resulted in approximately equal numbers across the three groups (34% in group 1, 32% in group 2 and 34% in group 3): this redistribution was characterised mainly by an increase in the size of both groups 2 and 3 and a concomitant reduction by 10% in the size of group 1. Figure 3.1a demonstrates a higher number of women scoring 8/9 in the antenatal EPDS compared to postpartum EPDS, resulting in the inclusion of more women at risk of AND in groups 2 and 3 when the cut-off was lowered to 8.

It has been proposed that the EPDS score correlates with symptoms severity[103 274]: scores below 10 are suggestive of minimal depression, 10-12 of minor depression and ≥13 of major depression. In our cohort of the 55 women screening positive for antenatal depressive symptoms, 45% had scores in the 10-12 range (at risk of minor depression) and 54% had scores ≥13 (at risk of major depression). Of the 71 women

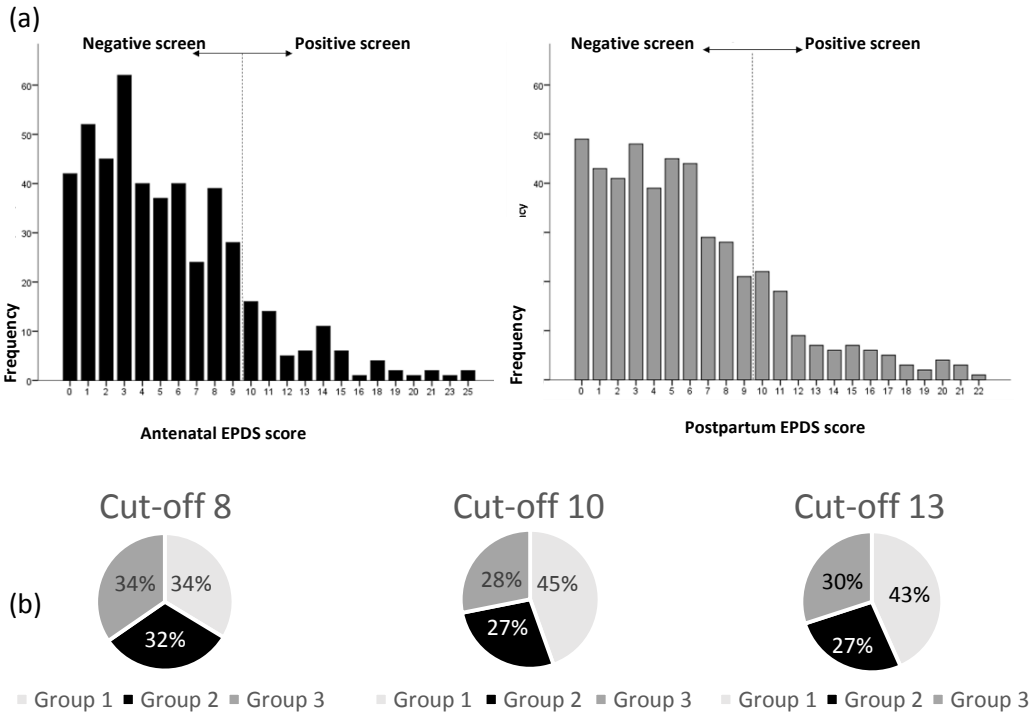

Figure 3.1. (a) Distribution of antenatal and postpartum EPDS scores within the cohort. Dashed line indicates cut-off score of 10 used to indicate depressive risk. (b) Comparison of distributions using cut-off scores of 8, 10 and 12.

screening positive for postpartum depressive symptoms, 48% had EPDS scores in the 10-12 range, whilst 52% scored ≥13.

3.2.2 Shifts between severity risk categories

Table 3.2 further classifies women who screened 'positive' in the EPDS into two risk categories: risk of minor depression (10-12), and major depression (≥13). In addition to moving from a negative to a positive screen for depressive symptoms and vice versa, women also shifted in severity category. These severity shifts were also bidirectional with observed increases as well as decreases in EPDS scores from antenatal to postpartum period.

79% (n=379) of women remained in the low risk category throughout the period of the study (24 weeks of pregnancy up to 10 weeks postpartum), whereas 21% of women had raised risk of PND during pregnancy or postpartum or both. Group 1 included 46 women who were low risk during pregnancy, but moved to high risk postpartum: 64% exhibited EPDS scores in the minor depressive risk category (10-12) and 37% had scores in the MDD risk category (≥13). Likewise, group 2 includes women (n=30) who shifted to low-risk postpartum (<10) from high risk during pregnancy: 60% had antenatal EPDS scores in the minor category, whereas 40% had scores in the MDD category. Group 3 included a small number of women (n=4) who had their antenatal EPDS scores increased from the minor risk category to MDD risk category postpartum, whilst only two women with high (≥13) antenatal EPDS scores shifted to the minor category postpartum. Moreover, in 64% women in group 3, no differences in the severity category were observed.

Table 3.2. Cross-tabulation of antenatal (AND) and postpartum depressive symptoms (PPD) risk according to EPDS risk categories 0-9 (minimal depression), 10-12 (minor depression) and ≥13 (major depression).

		Postpartum score			
		≤ 9	*10-12*	*≥13*	Total
Antenatal score	*≤ 9*	379	29	17	425
	10-12	18	3	4	25
	≥13	12	2	16	30
Total		409	34	37	480

3.2.3 Multinomial logistic regression – group analysis

Initial bivariate chi-squared analysis identified 11 potential correlates associated with antenatal/postpartum depressive symptoms, which were further analysed (Tables 3.3 and 3.4). All risk factors with a $p<0.05$, either pre- or post-delivery, were included in a multinomial regression model for the group analysis. The *p*-value for this model was .0002 and the overall model is therefore statistically significant. The low risk group was used as a baseline category. Relative risk values (RR) were calculated to give a better understanding of the magnitude of association between covariates as risk factors and the three groups with distinct risk trajectories. (Table 3.5).

Table 3.3. Odds ratios and 95% CI for all variables which demonstrated significance in Chi-squared analysis for antenatal depressive symptoms.

Factor	OR	95% CI
Age	1.064421	0.7882357, 1.437377
Social status	1.465595	1.191677, 1.802476
Support	0.4204886	0.0773037, 2.287221
Education	0.7729944	0.6347195, 0.9413929
Medication	1.034493	0.8022022, 1.334048
History of depression	2.295905	1.073715, 4.909291
History of anxiety	2.089739	1.025862, 4.256915
Gestational length	0.9734467	0.955314, 0.9919235
Ethnicity	1.207497	1.00187, 1.455328

Table 3.4. Odds ratios and 95% CI for all variables which demonstrated significance in Chi-squared analysis for postpartum depressive symptoms.

Factor	OR	95% CI
History of PND	3.287746	1.287778, 8.393746
Family history of PND	1.530038	0.8929046, 2.621797
History of depression	3.208556	1.661414, 6.196427

Table 3.5. Relative Risk (RR) values for significant variables within each at risk group.

Group	Risk factors
1 – High postpartum risk	Past history of PPD (p=0.017, RR=3.78) Past history of depression (p=0.034, RR= 2.76)
2 – High antenatal risk	Past history of anxiety (p=0.013, RR= 3.55) Ethnicity - Other (p=0.006, RR= 5.19) Age - <24 (p=0.010, RR= 6.39)
3 – Overall high risk	Past history of depression (p=0.045, RR=3.51)

For group 1 (high postpartum score only), significant risk factors were found to be past history of PPD (N=27) and past history of depression (N=50). In group 2 (high antenatal score only), risk factors were a past history of anxiety (N=64), age <24 (N=44) and ethnicity other (N=43), although it is important to note that 84.6% of the cohort were White British. An age of 40+ also increased the risk of being in Group 2 (RR=4.17) but this association was not quite significant and was relevant to <10% of the cohort. In group 3 (high antenatal and postpartum score), past history of depression was the only significant risk factor.

3.2.4 Penalised regression analysis and prediction

In the previous section covariates were identified which constitute major risk factors for either group 1 (history of PPD, history of depression), group 2 (history of anxiety, ethnicity, age), or group 3 (history of depression). It is likely that dichotomising EPDS scores results in loss of statistical power; it also depends on specific cut-off values. The regression analysis was then repeated on the full range of EPDS scores without dichotomisation. The relationship between the antenatal

(T1) and postpartum (T2) EPDS scores, with a correlation of 0.5002, is shown in Figure 3.2. The deviance from perfect correlation and alignment can be attributed to any systematic change (drop or increase) in depressive symptoms from before to after delivery, but also to unexplained variability (noise) in the acquisition of EPDS scores. This analysis aimed to elucidate the underlying characteristics contributing to this observed deviation between antenatal and postpartum EPDS scores by looking at the association between severity, timing and covariates. We examined distinguishing as well as common covariates across antenatal and postpartum scores

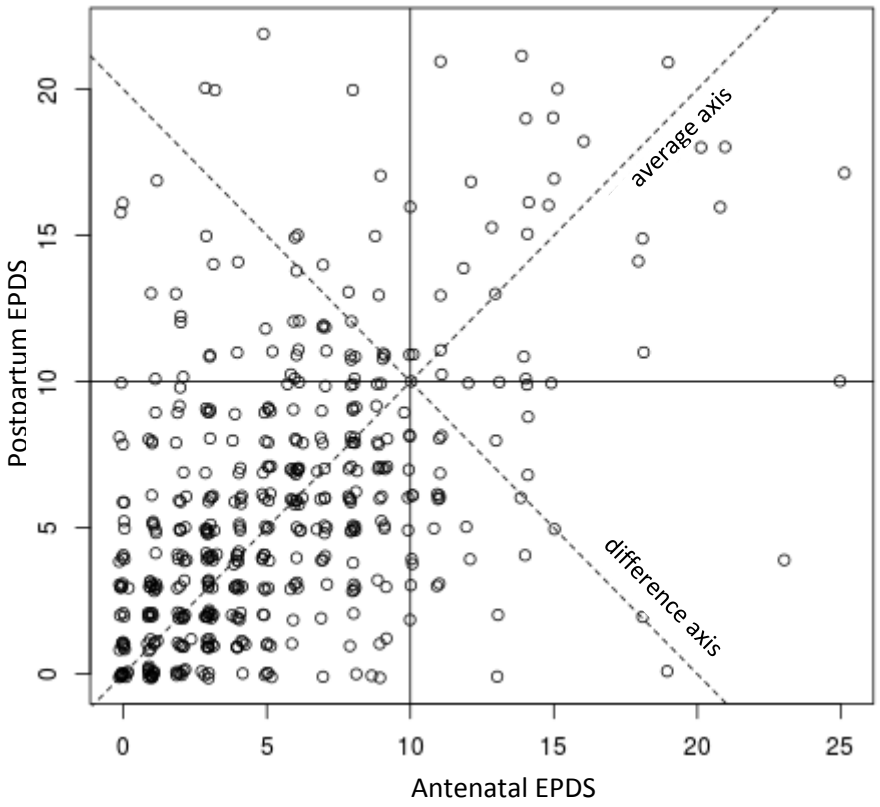

Figure 3.2. Ante- vs postnatal EPDS scores for 474 individuals (correlation 0.5002). Points are jittered randomly for better visibility. Sections of the graph represent groups created in the previous analysis. Mean EPDS scores are shown for each group.

In the following section linear as well as nonlinear regression analyses are presented for the antenatal and postpartum EPDS scores, for the difference between them and finally for their average. The purpose of these four analyses is to explore whether covariates contributing to a high antenatal score are different from covariates contributing to a high postpartum score and which covariates contribute to both.

Antenatal EPDS score

Variables contributing to a linear model for antenatal EPDS scores were selected using penalised regression. Coefficients for the selected variables were calculated by standard linear regression. Coefficients with a $p < 0.05$ are shown in Table 3.6.

Table 3.6. Significant covariates of a linear model for antenatal EPDS scores. Positive or negative coefficients indicate by how much the EDPS score increases or decreases with the covariate.

Covariate	Coefficient	p-value
Social status - Routine/semi-routine	1.768	<0.001
Social status - Unemployed/student	3.887	0.004
Education > 18 years	-1.373	0.033
BMI ≥30 - Yes	-0.581	0.037
Alcohol pre-pregnancy - Yes	0.813	0.049
Family history of PPD, 1st degree relative - Yes	1.391	0.012
Past history of anxiety - Yes	2.314	<0.001

Covariates that appeared particularly important in the prediction of antenatal EPDS scores were 'past history of anxiety' contributing around 2 points toward the EPDS score, the most significant contribution in terms of a *p*-value, with further contributions identified from social status of 'unemployed/student', contributing almost 4 points towards the antenatal EPDS score, and 'semi-routine/routine' work contributing about 1.8 points. Other significant contributions come from a 'family history of PPD' as well as 'alcohol consumption'. Marginal protective effects were identified from education beyond 18 years and a higher BMI. A model was also fitted adding all pairwise interactions. However, the cross validated mean squared prediction error was unchanged indicating that adding interactions does not improve predictive power and therefore such models or any models with higher orders of interaction were not considered.

Postpartum EPDS scores

As previously described a linear model for postpartum EPDS scores was selected using penalised regression. Coefficients with a $p<0.05$ are shown in Table 3.7. Fewer covariates were identified as significant compared to the antenatal EPDS score model. 'Past history of depression', 'past history of PPD' and 'family history of PPD' contributed about 2.8, 2.2, and 1.8 points to the postpartum EPDS, respectively. Information about pre-existing illness and associated medication as well as level of education improved prediction accuracy although their effect was not statistically significant.

Table 3.7. Significant covariates of a linear model for postpartum EPDS scores.

Covariate	Coefficient	p-value
Past history of PPD - Yes	2.204	0.014
Past history of depression - Yes	2.788	<0.001
Family history of PPD - 1st degree relative - Yes	1.779	0.003

3.2.5 Differences and commonalities between antenatal and postpartum EPDS scores

Systematic differences between antenatal and postpartum EPDS scores were next explored in terms of potentially different sets of covariates, and covariates that would explain the drop or rise in antenatal to postpartum EPDS scores observed in many subjects. Therefore, we investigated within-person patterns of change by analysing the contribution of covariates to the differences and averages of postpartum (T2) and antenatal (T1) EPDS scores. The 'average' EDPS score [APA score=(T1+T2)/2] can be seen as an indication of overall perinatal depression not specific to either an antenatal or postpartum time point; identified covariates contributing to the average score would indicate common underlying factors. The 'difference' in EPDS scores [DPA score= (T1-T2)] corresponds to the improvement or worsening in EPDS score from the antenatal to the postpartum time point; covariates contributing to the difference of T1 to T2 score, would indicate differentiating factors. The relationship between difference and average score is illustrated in Figure 3.3a. The two components are also indicated as average and

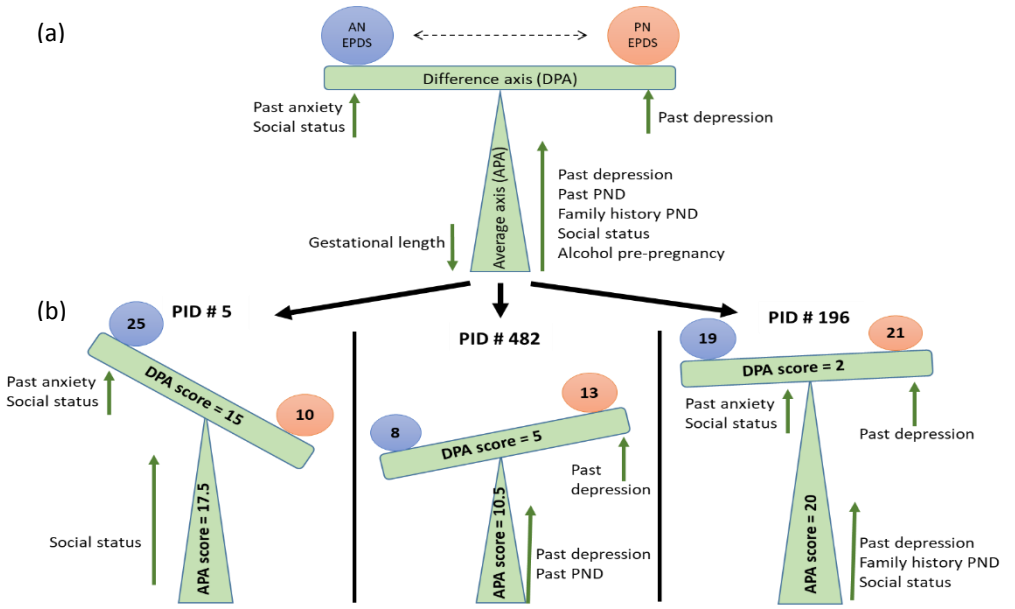

Figure 3.3. (a) A schematic of the relationship between contribution of covariates identified by a linear model to the differences and averages of postpartum and antenatal EPDS scores. The 'average' EDPS score at T1 and T2 time points (APA score) can be seen as an indication of overall perinatal depression not specific to either an antenatal or postpartum time point. The 'difference' in EPDS scores (DPA score) corresponds to shifts in EPDS score from the antenatal to the postpartum time point indicative of improvement or worsening. (b) Representative case studies of cohort patients with covariates that shift DPA and APA scores. Subject #5 who had two covariates with negative coefficients, a 'previous history of anxiety' and a 'social status of student/unemployed', exhibited a significant drop in the DPA score. Subject #482 had a covariate with positive coefficient, a 'previous history of depression' and exhibited an increase in the DPA score. In this patient, the covariates 'past history of PPD' as well as 'past history of depression' might contribute to the APA score. Subject #196 had covariates with both negative and positive coefficients, a previous history of anxiety' and 'previous history of depression' and exhibited a small increase in the DPA score. In this patient, the APA score was also influenced by at least 3 covariates with positive coefficients, such as 'family history of PPD', 'past history of depression' and 'social status'.

difference axes in Figure 3.3b shown with real example case studies. Their correlation with different sets of predictors is discussed in the following section.

Difference between postpartum and antenatal EPDS score

Covariates which contribute to the DPA score were explored in order to find correlates explaining why some individuals exhibited a drop in score from before to after delivery while others showed the opposite effect. Table 3.8 shows significant coefficients of a linear model selected by penalised regression. Negative coefficients indicate a decrease from antenatal to postpartum EPDS score, while positive coefficients indicate an increase. The clinical significance of a 1 point change in EPDS score represents a "small change", 2-3 points a "medium change" and 4 points or higher a "large change"[275].

Based on this regression analysis one would expect covariates that contribute to T1 EPDS score (Table 3.6) but not T2 EPDS score (Table 3.7) to exert negative coefficients in this analysis, since they can explain antenatal but not postpartum depressive symptoms. Conversely covariates that show up in Table 3.7 but not Table 3.6 are expected to have positive coefficients in the difference analysis, since these covariates are associated with postpartum but not antenatal depressive symptoms. This was confirmed in our analysis: two differentiating factors between antenatal and postpartum scores were a 'past history of anxiety' or a social status of 'student/unemployed'. In the current analysis they were both associated with a drop in the EPDS score by over 2 points from before to after delivery. The reverse was also observed, and a 'past history of depression', which was significant for postpartum EPDS score only (Table 3.7) contributed to an increase in the EPDS score by about 2 points.

Table 3.8. Significant covariates of a linear model for the difference of antenatal to postpartum (DPA) EPDS scores.

Covariate	Coefficient	p-value
Social status - unemployed/student	-2.664	0.040
Past history depression - yes	2.036	0.003
Past history of anxiety - yes	-2.565	<0.001

Average of postpartum and antenatal EPDS scores

Significant covariates, which contribute to the average of postpartum and antenatal EPDS scores indicate subject characteristics underlying both scores and are shown in Table 3.9. As expected, a 'past history of PPD' as well as 'past history of depression' and a 'family history of PPD' contribute to a higher average EPDS score. In contrast, a longer gestation is associated with a reduction in score. Slightly surprising, obesity might contribute marginally to a reduction in score, but the overall effect was weak. These findings are summarised in Figure 3.3, depicting examples of representative case studies.

Table 3.9. Significant covariates of a linear model for average antenatal and postpartum (APA) EPDS scores.

Covariate	Coefficient	p-value
Social status - routine/semi-routine	0.890	0.027
BMI ≥30 - yes	-0.198	0.023
Alcohol pre-pregnancy - yes	0.764	0.039
Past history of PPD - yes	1.737	0.021
Past history depression - yes	2.027	0.001
Family history of PPD - 1st degree relative	1.574	0.002
Gestation length / days	-0.029	0.029

Prediction of EPDS scores

Finally, we explored the possibility of a stratification of patients based on the results of psychosocial covariates alone without taking EPDS scores into account. To explore whether the regression model is powerful enough for prediction of EPDS scores as well (and not only for finding important covariates of EPDS scores), we compared predictions derived from the four linear models (for antenatal and postpartum EPDS, and average and difference EPDS) with predictions from a state-of-the-art machine learning prediction algorithm, extreme gradient boosting (xgboost).

Table 3.10 shows the result of a ten-fold cross validation (repeated 10 times) of the correlation of predicted scores with the measured scores. Interestingly, the linear

models perform comparably (or even slightly better) than the state-of-the-art machine learning prediction algorithm, which depends very little on any statistical assumptions. This indicates that a simple linear model is able to capture most of the signal in the data that is suitable for prediction. Prediction accuracy is highest for antenatal EPDS score, lower for the postpartum score, and in between for the APA score. The available covariates are better suited to predict depression before rather than after birth.

Table 3.10. Ten-fold cross validated correlation of prediction of EPDS scores with original scores based on predictors using penalised linear regression (elastic net), linear regression (linear), and extreme gradient boosting (xgboost). In brackets the standard deviation based on 20 random iterations is displayed. AN= antenatal; PP= postpartum

	elastic net	linear	xgboost
AN EPDS	0.25 (0.02)	0.27 (0.02)	0.24 (0.02)
PP EPDS	0.19 (0.02)	0.18 (0.02)	0.16 (0.02)
DPA EPDS	0.13 (0.03)	0.15 (0.03)	0.13 (0.02)
APA EPDS	0.21 (0.02)	0.22 (0.02)	0.22 (0.02)

It seems particularly difficult to predict the DPA score, i.e. the change in EPDS score from before to after delivery, however a comparison of using psychosocial covariates alone versus antenatal scores to predict the postpartum score has been done. This reveals a correlation of 0.19 predicting from psychosocial covariates, compared with a correlation of 0.50 when predicting from antenatal scores. This demonstrates that antenatal EPDS scores are still much better predictors of postpartum EPDS scores than generic psychosocial covariates.

Another way to assess prediction accuracy from a more practical perspective is to inspect PPV and NPV when turning predicted scores into predictions of depressive symptoms at an EPDS cut-off ≥10 (or ≥ 0 for the DPA score). In this analysis the PPV was defined as the percentage of women predicted to have more severe depressive symptoms, who actually exhibit EPDS scores ≥10, while the NPV is the percentage of women predicted to have minimal symptoms who actually have EPDS<10. The result is shown in Table 3.11 for the linear and the xgboost predictor (due to the penalisation the absolute score value of the elastic net regression is not representative). The comparatively high correlation for antenatal EPDS score in Table 3.10 translates into a PPV of around 45% and a NPV of about 85% for the linear model and the xgboost predictor. As expected from the weaker correlation, predictive performance for the other scores is less impressive.

Table 3.11. Ten-fold cross validated positive predictive value (PPV) and negative predictive value (NPV) for prediction of depression based on predictors using linear regression (linear), and extreme gradient boosting (xgboost). In brackets the standard deviation based on 20 random iterations is displayed. AN= antenatal; PP= postpartum

PPV / NPV	linear %	xgboost %
AN EPDS	43(8) / 86(0.1)	47(5) / 86(0.1)
PP EPDS	32(7) / 81(0.1)	37(6) / 81 (0.1)
DPA	61(1) / 50(2)	61(1) / 48(2)
APA	38(7) / 88(0.1)	36(5) / 88(0.1)

Previous studies also investigated predictive accuracy characteristics of the antenatal EPDS at different cut-offs[276]. To carry out a comparison study the analysis aimed to establish the predictive performance of the antenatal EPDS score at various cut-off values (Table 3.12). To assess the predictive power under a range of all possible cut-offs the area under the ROC curve was measured obtaining a value of 0.76 shown in Figure 3.4, which confirms previous studies[276]. Further details on predictive performance of the antenatal and postpartum EPDS score are shown in Table 3.12. These results are essentially identical to previous studies albeit requiring a slight shift in the EPDS cut-off to obtain similar levels of NPV and PPV.

Table 3.12. Predictive performance of antenatal EPDS score at various cut-offs for a postpartum EPDS score ≥ 10 (standard error in brackets). The area under the ROC curve for all cut-offs (see Figure 3.4) is 0.756.

	Cut-off ≥ 15	Cut-off ≥ 10	Cut-off ≥ 5
Sensitivity % (95% CI)	16.3 (3.9)	39.1 (5.1)	79.3 (4.2)
Specificity % (95% CI)	99.0 (0.5)	90.8 (1.5)	56.5 (2.5)
PPV % (95% CI)	78.9 (9.4)	50.7 (5.9)	30.5 (3.0)
NPV % (95% CI)	83.1 (1.8)	86.1 (1.7)	91.9 (1.8)

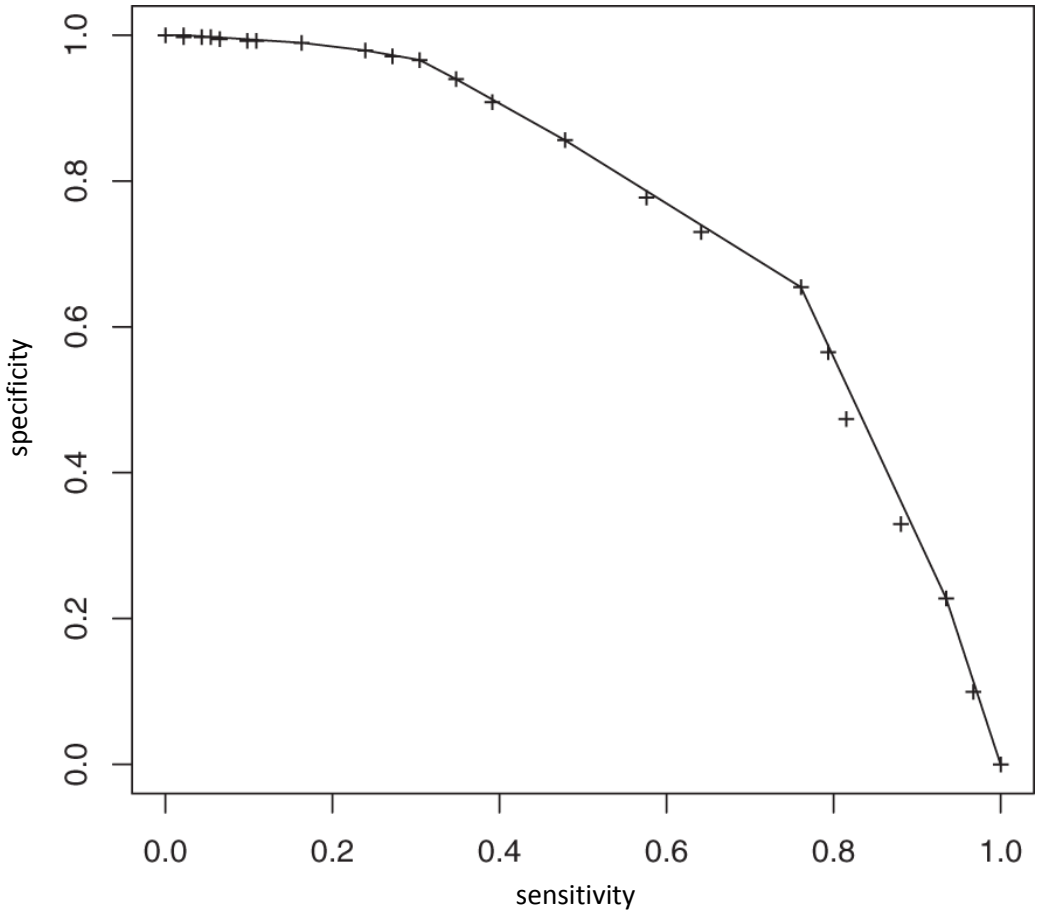

Figure 3.4. ROC curve (convex hull) for prediction of postpartum EPDS score ≥ 10 from antenatal EPDS scores (AROC 0.756) at various cut-offs (crosses).

3.3 Warwick Hospital Cohort

Recruitment of participants at Warwick was completed in the five year recruitment window from 2014-2019. A total of 1579 participants were recruited and screened for PND. Mean gestation at recruitment was 28 weeks. Although this study is still open, at the time of writing, postpartum follow up rate was 55%, with 872 complete postpartum screening results.

3.3.1 Antenatal EPDS – exploratory analysis

EPDS scores ranged from 0-26. In this cohort 16.7% of women screened positive for AND, using a score ≥10 on the EPDS. The mean antenatal EPDS score was 5.68 (SD=4.395). Cohort characteristics for all participants who completed antenatal screening (N=1579) are presented in Table 3.13. Women from this cohort were generally White British, 30-34 year old first-time mothers with a high social status and educated to degree level. They tended to be of good health as non-smokers, not taking medication, with a healthy BMI.

Table 3.13. Cohort characteristics of the study population. Continuous variables are expressed as the mean (standard deviation) and categorical variables are expressed in N (%) at each factor level. National averages are shown where available[277].

Covariate	N (SD) / Mean (%)	National average
Gestation - recruitment (days)	195.9 (5.9)	
Total gestation – delivery (days)	276.3 (11.4)	280
Baby gender		
Female	708 (48.6)	
Male	750 (51.4)	
Birth weight (g)	3473.69 (540.3)	3000-3400g
Parity		
0	689 (43.6)	43%
1	626 (39.6)	
2	171 (10.8)	
3	55 (3.5)	
4	23 (1.5)	
5	8 (0.5)	
6	5 (0.3)	

Covariate	N (SD) / Mean (%)	National average
7	1 (0.1)	
8	1 (0.1)	
Age		
<18 years	5 (0.3)	
18-24 years	152 (9.6)	4% (<20 years)
25-29 years	385 (24.4)	29%
30-34 years	547 (34.6)	31%
35-39 years	380 (24.1)	17%
40-45 years	106 (6.7)	4%
>45 years	4 (0.3)	0%
Ethnicity		
White British	1263 (80.1)	65%
Indian	102 (6.5)	
Asian	47 (3.0)	9%
Other White	108 (6.8)	
Black Caribbean	4 (0.3)	4% (Black)
Black African	9 (0.6)	
Mixed	27 (1.7)	2%
Other	17 (1.1)	4%
Education		
No exams	21 (1.3)	
>16 years	196 (12.4)	
>18 years	165 (10.4)	
Diploma level	360 (22.8)	
Degree level	507 (32.1)	
Professional	92 (5.8)	
Post Graduate	206 (13.0)	
Doctorate	31 (2.0)	
BMI		
<18.5	46 (2.9)	3%
18.5-24	626 (36.9)	46%
25-29	388 (24.6)	28%
30-34	329 (20.8)	23% (30+)

Covariate	N (SD) / Mean (%)	National average
35-39	118 (7.5)	
40-44	48 (3.0)	
>45	17 (1.1)	
Twin		
No	1562 (97.8)	
Yes	17 (0.01)	
Social Status		
Higher Managerial/Professional	612 (38.8)	
Diploma level Professional	236 (14.9)	
Self Employed	96 (6.1)	
Low level supervisor/Technical	199 (6.1)	
Semi routine/Routine	381 (24.1)	
Unemployed/Student	53 (3.4)	
Supported		
No	47 (3.0)	
Yes	1528 (97.0)	
Alcohol in pre-pregnancy		
No	540(34.2)	
Yes	1039 (65.8)	
Alcohol in pregnancy		
No	1546 (97.9)	
Yes	33 (2.1)	
Smoking in pre-pregnancy		
No	1319 (83.5)	83%
Yes	260 (16.5)	12%
Smoking in pregnancy		
No	1511 (95.7)	
Yes	68 (4.3)	
Medication		
No	1338 (84.7)	
Thyroxine	82 (5.2)	
Anti-coagulant	41 (2.6)	
Asthma drugs	31 (2.0)	

Covariate	N (SD) / Mean (%)	National average
Analgesia	6 (4)	
Other	81 (5.1)	
Taking any medication		
No	1338 (84.7)	
Yes	241 (15.3)	
Omega-3 supplement		
No	1174 (74.4)	
Yes	405 (25.6)	
Past history of depression		
No	1319 (83.5)	
Yes	260 (16.5)	
Past history of PND		
No	1454 (92.1)	
Yes	125 (7.9)	
Family history of PND		
No	1365 (86.4)	
1st degree relative	197 (12.5)	
Other family	17 (1.1)	
Past history of anxiety		
No	1280 (81.1)	
Yes	299 (18.9)	
Mode of delivery		
Normal vaginal birth	790 (50.0)	57%
Instrument assisted	184 (11.7)	11%
Elective C section	279 (17.7)	14%
Emergency C section	196 (12.4)	16%
Induction of labour		
No	969 (67.0)	
Yes	68 (33.0)	
Premature baby		
No	1380 (95.3)	
Yes	68 (4.7)	

Women were most commonly (34.6%) aged 30-34. A high majority of the sample were White British (80%). The majority of the sample (43.6%) were primiparous (first time mothers). Most women were of a 'higher managerial/professional' level social status (38.8%). A very high proportion (97%) of women identified as 'supported'. Women were most commonly educated to degree level (32.1%).

A majority (65.8%) drank alcohol in the 12 months prior to conception, although almost all (97.9%) stated that they did not drink alcohol during pregnancy, with only 2% of women reporting drinking alcohol in pregnancy. 83.5% of women were non-smokers in the 12 months prior to conception – this rose to 95.7% non-smokers during pregnancy, with just 4% stating they smoked during pregnancy. 84.7% of women were not taking medication during pregnancy. Of those who did take medication, the majority took Thyroxine (N=82) or 'Other' (N=81). 25.6% of women were taking an Omega-3 supplement during pregnancy. BMI was most commonly in the category 18.5-24 (39.8%).

7.9% of women had a past history of PND. 16.5% of the sample had a past history of depression. 18.9% had a history of anxiety. 13.6% had a family history of PND. Mean total gestational length was 39.4 weeks. Mean birth weight was 7.66 lbs (3473.69 grams). 51.4% of babies were male. 50% of deliveries were natural vaginal births. Labour was medically induced in 33% of women. 4.7% of babies were born premature.

The first stage of exploratory analysis involved a comparison of controls (N=1315) and cases (N=264), defined by EPDS ≥ 10, which found significant differences for a number of the selected covariates between the groups. A comparison of the two groups is displayed in Table 3.2. Chi-squared and correlation analyses were conducted as appropriate.

3.3.2 Chi squared & correlation analysis

A comparison of cases and controls was first carried out to identify variables displaying statistical significance between the two groups (Table 3.14). Results of bivariate analyses are presented in Tables 3.15 and 3.16.

Table 3.14. Characteristics of study participants (EPDS cut off 9/10) for antenatal depression. Significance of Chi squared/correlation statistics are presented.

Covariate	**Controls (n=1315) Mean (SD) / N (%)**	**Cases (n=264) Mean (SD) / N (%)**	***p* value**
Gestation - recruitment (days)	195.89 (6.142)	195.93 (4.547)	0.886
Total gestation – delivery (days)	276.68 (11.099)	274.51 (12.682)	**0.007**
Baby gender			0.318
Female	599 (49.1)	109 (45.6)	
Male	620 (50.9)	130 (54.4)	
Birth weight (g)	3486.25 (536.302)	3410.07 (556.991)	0.071
Parity			0.930
0	577 (43.9)	112 (42.4)	
1	521 (39.6)	105 (39.8)	
2	142 (10.8)	29 (11.0)	
3	43 (3.3)	12 (4.5)	
4	18 (1.4)	5 (1.9)	
5	7 (0.5)	1 (0.4)	
6	5 (0.4)	0	
7	1 (0.1)	0	
8	1 (0.1)	0	
Age			**0.001**
<18 years	4 (0.3)	1 (0.4)	
18-24 years	116 (8.8)	36 (13.6)	
25-29 years	307 (23.3)	78 (29.5)	
30-34 years	468 (35.6)	79 (29.9)	
35-39 years	336 (25.6)	44 (16.7)	
40-45 years	82 (6.2)	24 (9.1)	

Covariate	Controls (n=1315) Mean (SD) / N (%)	Cases (n=264) Mean (SD) / N (%)	*p* value
>45 years	2 (0.2)	2 (0.8)	
Ethnicity			0.962
White British	1051 (80.0)	212 (80.3)	
Indian	85 (6.5)	17 (6.4)	
Asian	39 (3.0)	8 (3.0)	
Other White	91 (6.9)	17 (6.4)	
Black Caribbean	4 (0.3)	0	
Black African	7 (0.5)	2 (0.8)	
Mixed	21 (1.6)	6 (2.3)	
Other	15 (1.1)	2 (0.8)	
Education			**0.000**
No exams	18 (1.4)	3 (1.1)	
>16 years	153 (11.6)	43 (16.3)	
>18 years	138 (10.5)	27 (10.2)	
Diploma level	272 (20.7)	88 (33.3)	
Degree level	446 (33.9)	61 (23.1)	
Professional	78 (5.9)	14 (5.3)	
Post Graduate	181 (13.8)	25 (9.5)	
Doctorate	28 (2.1)	3 (1.1)	
BMI			0.393
<18.5	39 (3.0)	7 (2.7)	
18.5-24	535 (40.8)	91 (34.5)	
25-29	313 (23.8)	75 (28.4)	
30-34	268 (20.4)	61 (23.1)	
35-39	100 (7.6)	18 (6.8)	
40-44	42 (3.2)	6 (2.3)	
>45	13 (1.0)	4 (1.5)	
Twin			0.401
No	1302 (99.0)	260 (98.4)	
Yes	13 (1.0)	4 (1.6)	
Social Status			**0.000**

Covariate	Controls (n=1315) Mean (SD) / N (%)	Cases (n=264) Mean (SD) / N (%)	*p* value
Higher Managerial/Professional	537 (40.9)	75 (28.4)	
Diploma level Professional	205 (15.6)	31 (11.7)	
Self Employed	77 (5.9)	19 (7.2)	
Low level supervisor/Technical	163 (12.4)	36 (13.6)	
Semi routine/Routine	290 (22.1)	91 (34.5)	
Unemployed/Student	41 (3.1)	12 (4.5)	
Supported			**0.000**
No	33 (2.5)	14 (5.3)	
Yes	1280 (97.5)	248 (93.9)	
Alcohol in pre-pregnancy			0.919
No	449 (34.1)	91 (34.5)	
Yes	866 (65.9)	173 (65.5)	
Alcohol in pregnancy			0.807
No	1287 (97.9)	259 (98.1)	
Yes	28 (2.1)	5 (1.9)	
Smoking in pre-pregnancy			**0.000**
No	1130 (85.9)	189 (71.6)	
Yes	185 (14.1)	75 (28.4)	
Smoking in pregnancy			**0.004**
No	1267 (96.3)	244 (92.4)	
Yes	48 (3.7)	20 (7.6)	
Medication			0.154
No	1118 (85.0)	220 (83.3)	
Thyroxine	71 (5.4)	11 (4.2)	
Anti-coagulant	31 (2.4)	10 (3.8)	
Asthma drugs	21 (1.6)	10 (3.8)	
Analgesia	5 (0.4)	1 (0.4)	
Other	69 (5.2)	12 (4.5)	
Taking any medication			0.487
No	1118 (85.0)	220 (83.3)	
Yes	197 (15.0)	44 (16.7)	

Covariate	Controls (n=1315) Mean (SD) / N (%)	Cases (n=264) Mean (SD) / N (%)	*p* value
Omega-3 supplement			0.508
No	982 (74.7)	192 (72.7)	
Yes	333 (25.3)	72 (27.3)	
Past history of depression			**0.000**
No	1155 (87.8)	164 (62.1)	
Yes	160 (12.2)	100 (37.9)	
Past history of PND			**0.000**
No	1232 (93.7)	222 (84.1)	
Yes	83 (6.3)	42 (15.9)	
Family history of PND			0.055
No	1149 (87.4)	216 (81.8)	
1st degree relative	153 (11.6)	44 (16.7)	
Other family	13 (1.0)	4 (1.5)	
Past history of anxiety			**0.000**
No	1117 (84.9)	163 (61.7)	
Yes	198 (15.1)	101 (38.3)	
Mode of delivery			**0.003**
Normal vaginal birth	673 (55.5)	117 (49.6)	
Instrument assisted	159 (13.1)	25 (10.6)	
C section in labour	168 (13.8)	28 (11.9)	
Elective C section	213 (17.6)	66 (28.0)	
Induction of labour			0.745
No	813 (67.2)	156 (66.1)	
Yes	397 (32.8)	80 (33.9)	
Premature baby			0.182
No	1160 (95.6)	220 (93.6)	
Yes	53 (4.4)	15 (6.4)	

Results of bivariate analyses are presented in Tables 3.3 and 3.4. Ten covariates were identified as having a significant association with a positive antenatal EPDS screen: education, social status, supported, smoking pre-pregnancy, smoking in

pregnancy, past history of depression, past history of PND, past history of anxiety, mode of delivery and gestational length.

Table 3.15. Correlation coefficient r and significance reported for continuous variables with p<0.05 for antenatal depression, using EPDS ≥ 10.

Covariate	r	*p*-value
Total gestation (days)	-0.082	0.015

Table 3.16. Significant values in chi-squared analysis - antenatal depression. For variables with >2 categories, odds ratios (OR) are presented to compare to a selected baseline group ([a]). OR values were not calculated for variables with any expected counts <5 ([b]).

Covariate	*p* value	X^2	*Odds ratio*
Age	**0.001**	23.900	
<18 years[b]			
18-24 years			1.63
25-29 years			1.38
30-34 years[a]			
35-39 years			0.58
40-45 years			1.50
>45 years[b]			
Education	**0.000**	31.715	
No exams[b]			
>16 years			1.48
>18 years			0.97
Diploma level			1.92
Degree level[a]			
Professional			0.89
Post Graduate			0.66
Doctorate			0.53
Social Status	**0.000**	27.211	
Higher Managerial/Professional[a]			
Diploma level Professional			0.57
Self Employed			0.72
Low level supervisor/Technical			1.25
Semi routine/Routine			1.12
Unemployed/Student			1.86

Covariate	*p* value	X^2	*Odds ratio*
Supported	**0.014**	6.043	2.19
No			
Yes			
Smoking in pre-pregnancy	**0.000**	32.872	2.42
No			
Yes			
Smoking in pregnancy	**0.004**	8.221	2.16
No			
Yes			
Past history of depression	**0.000**	105.669	4.40
No			
Yes			
Past history of PND	**0.000**	27.780	2.80
No			
Yes			
Past history of anxiety	**0.000**	77.095	3.50
No			
Yes			
Mode of delivery	**0.003**	13.935	
Normal vaginal birth[a]			
Instrument assisted			0.76
C section in labour			0.81
Elective C section			1.72

3.3.3 Regression modelling

Results of the linear regression modelling are presented in Table 3.17. In the final model (R^2 =.14***), variables which improved the model's prediction of EPDS score were history of depression and anxiety, smoking pre-pregnancy, age, education, mode of delivery, social status, past history of PND and BMI. 14% of the variance is explained by the included predictors. The most important predictors are a past history of depression and anxiety, contributing over 2 points to the score each.

Unemployment adds 1.5 points to the score, and age of either 18-24 or 40-45 adds around 1.4 points. Smoking pre-pregnancy adds 1.1 point to the score.

The link between educational level and EPDS score suggests that the lowest level of education (no exams) is associated with protection against depressive symptoms, but this was not found to be statistically significant although it improved the model, and must be interpreted with caution due to the small sample size of this category (N=21, 1.3%). Women with a planned Elective C-section had a significantly increased risk, although interpretation of this is limited since it is unknown whether the C-section was planned prior to EPDS screening. Higher social status is associated with lower antenatal EPDS (higher managerial and supervisor), whereas all levels below this have a risk of increased EPDS score. BMI is difficult to interpret, with a higher BMI appearing to be related to lower EPDS scores.

The optimal model shown includes all variables that improve prediction, based on exploratory regression modelling. The initial stepwise modelling found the optimal model explains 15% of the variability in scores. For categorical variables, levels which improve the model's prediction are shown.

Table 3.17. Results of a linear regression model with antenatal EPDS score as the outcome (N=1579) with a number of selected predictor variables based on exploratory modelling.

	B	*SE B*	*β*
Constant	4.22	.23	
Age			
(baseline - 30-34)			
18-24	1.37	.38	.09***
25-29	0.84	.25	.08**
40-45	1.41	.42	.08**
Education			
(baseline – degree level)			
No exams	-1.19	.91	-.03
Mode of delivery			
(baseline – normal vaginal birth)			
Elective C section	0.810	.27	.07**
Social status			
(baseline – Higher managerial/professional)			
Semi routine/routine work	0.74	.25	-.07**

	B	*SE B*	*β*
Unemployed/student	1.49	.59	-.06*
Past history PND	0.88	.40	.05*
Smoking pre-pregnancy	1.12	.29	.09***
Past history depression	2.13	.30	.18***
Past history anxiety	2.11	.28	.19***
BMI			
(baseline (18.5-24)			
30-34	-0.42	.26	-.04
35-39	-0.78	.40	-.05*
40-44	-1.50	.59	-.06*

R^2 = .14, F=18.253. *p<.05, **p < .01, ***p < .001.

3.3.4 Postpartum EPDS – exploratory analysis

872 participants of the study completed the postpartum EPDS. This screening identified a prevalence of 19.8% (N=173) for PPD, based on EPDS ≥ 10. Scores on the EPDS ranged from 0-26 with a mean score of 5.96 (SD= 4.640). Cohort characteristics of all participants who completed the postpartum EPDS are displayed in Table 3.18.

Table 3.18. Cohort characteristics for all participants who completed the study protocol with postpartum follow up. Continuous variables are expressed as the mean (standard deviation) and categorical variables are expressed in N (%) at each factor level.

Variable	**N (SD) / Mean (%)**
Gestation - recruitment (days)	196.05 (5.966)
Total gestation – delivery (days)	276.62 (11.129)
Baby gender	
Female	422 (49.0)
Male	439 (51.0)
Birth weight (g)	3474.32 (537.021)

Variable	N (SD) / Mean (%)
Parity	
0	384 (44.0)
1	370 (42.4)
2	80 (9.2)
3	26 (3.0)
4	9 (1.0)
5	2 (0.2)
6	1 (0.1)
7	0
8	0
Age	
<18 years	1 (0.1)
18-24 years	59 (6.8)
25-29 years	333 (38.2)
30-34 years	235 (26.9)
35-39 years	69 (7.9)
40-45 years	69 (7.9)
>45 years	2 (0.2)
Ethnicity	
White British	708 (81.2)
Indian	44 (5.0)
Asian	29 (3.3)
Other White	61 (7.0)
Black Caribbean	2 (0.2)
Black African	2 (0.2)
Mixed	15 (1.7)
Other	11 (1.3)
Education	
No exams	2 (0.2)
>16 years	86 (9.9)
>18 years	96 (11.0)
Diploma level	162 (18.6)
Degree level	328 (37.6)

Variable		N (SD) / Mean (%)
	Professional	56 (6.4)
	Post Graduate	119 (13.6)
	Doctorate	23 (2.6)
BMI		
	<18.5	28 (3.2)
	18.5-24	393 (45.3)
	25-29	207 (23.9)
	30-34	159 (18.3)
	35-39	57 (6.6)
	40-44	18 (2.1)
	>45	5 (0.6)
Twin		
	No	859 (98.5)
	Yes	13 (1.5)
Social Status		
	Higher Managerial/Professional	380 (43.6)
	Diploma level Professional	139 (15.9)
	Self Employed	50 (5.7)
	Low level supervisor/Technical	90 (10.3)
	Semi routine/Routine	192 (22.0)
	Unemployed/Student	21 (2.4)
Supported		
	No	16 (1.8)
	Yes	855 (98.1)
Alcohol in pre-pregnancy		
	No	276 (31.7)
	Yes	596 (68.3)
Alcohol in pregnancy		
	No	849 (97.4)
	Yes	23 (2.6)
Smoking in pre-pregnancy		
	No	771 (88.4)
	Yes	101 (11.6)

Variable	N (SD) / Mean (%)
Smoking in pregnancy	
No	852 (97.7)
Yes	20 (2.3)
Medication	
No	732 (83.9)
Thyroxine	41 (4.7)
Anti-coagulant	29 (3.3)
Asthma drugs	19 (2.2)
Analgesia	4 (0.5)
Other	47 (5.4)
Taking any medication	
No	732 (83.9)
Yes	140 (16.1)
Omega-3 supplement	
No	637 (73.1)
Yes	235 (26.9)
Past history of depression	
No	761 (87.3)
Yes	111 (12.7)
Past history of PND	
No	815 (93.5)
Yes	57 (6.5)
Family history of PND	
No	752 (86.2)
1st degree relative	108 (12.4)
Other family	12 (1.4)
Past history of anxiety	
No	739 (84.7)
Yes	133 (15.3)
Mode of delivery	
Normal vaginal birth	458 (53.6)
Instrument assisted	112 (13.1)
C section in labour	119 (13.9)

Variable	N (SD) / Mean (%)
Elective C section	166 (19.4)
Induction of labour	
No	592 (95.7)
Yes	37 (4.3)
Premature baby	
No	819 (95.7)
Yes	37 (4.3)

Women were most commonly primiparous, aged 25-29, White British, educated to Degree level and in a Higher Managerial/Professional occupation. Women were generally healthy with a BMI of 18.5-24 and not taking any Medication. 68.3% of women did drink alcohol in pre-conception, falling to 2.6% during pregnancy. 88.4% were non-smokers, rising to 97.7% in pregnancy. 12.7% had a past history of depression, 6.5% of PND, and 15.3% of anxiety. A family history of PND was present in 13.8% of women. Babies were most commonly born by normal vaginal delivery (53.6%), with 19.5% electing for a C section. Labour was not induced in the vast majority of women, and 95.7% gave birth to babies at term.

3.3.5 Chi squared & correlation analysis

A comparison of cases and controls was first carried out to identify variables displaying statistical significance between the two groups (Table 3.19). Results of bivariate analyses are presented in Tables 3.20 and 3.21. Significant variables associated with postpartum EPDS based on correlation or Chi-squared analyses were: antenatal EPDS score, positive antenatal EPDS screen, total gestation (days), parity, education level, past history of depression, past history of PND, and past history of anxiety.

Table 3.19. Characteristics of study participants (EPDS cut off 9/10) for postpartum depression. Significance of chi-squared analysis is reported / point-biserial correlations for continuous variables.

	Controls (n=699) Mean (SD) / N (%)	Cases (n=173) Mean (SD) / N (%)	*p* value
Antenatal EPDS	4.57 (3.353)	8.57 (5.094)	**0.000**
Positive antenatal screen	640 (91.6)	107 (61.8)	**0.000**
	59 (8.4)	66 (38.2)	
Gestation - recruitment (days)	196.05 (6.338)	196.08 (4.146)	0.924
Total gestation – delivery (days)	277.04 (10.757)	274.91 (12.410)	**0.023**
Baby gender			0.182
Female	346 (50.1)	76 (43.9)	
Male	344 (49.2)	95 (54.9)	
Birth weight (g)	3487.04 (531.547)	3422.46 (557.388)	0.116
Parity			**0.001**
0	302 (43.2)	82 (47.4)	
1	302 (43.2)	68 (39.3)	
2	71 (10.2)	9 (5.2)	
3	20 (2.9)	6 (3.5)	
4	3 (0.4)	6 (3.5)	
5	1 (0.1)	1 (0.6)	
6	0	1 (0.6)	
7	0	0	

		Controls (n=699) Mean (SD) / N (%)	Cases (n=173) Mean (SD) / N (%)	*p* value
	8	0	0	
Age				0.275
	<18 years	0	1 (0.6)	
	18-24 years	47 (6.7)	12 (6.9)	
	25-29 years	140 (20.0)	33 (19.1)	
	30-34 years	267 (38.2)	66 (38.2)	
	35-39 years	193 (27.6)	42 (24.3)	
	40-45 years	51 (7.3)	18 (10.4)	
	>45 years	1 (0.1)	1 (0.6)	
Ethnicity				0.591
	White British	569 (81.4)	139 (80.3)	
	Indian	33 (4.7)	11 (6.4)	
	Asian	21 (3.0)	8 (4.6)	
	Other White	49 (7.0)	12 (6.9)	
	Black Caribbean	2 (0.3)	0	
	Black African	2 (0.3)	0	
	Mixed	12 (1.7)	3 (1.7)	
	Other	11 (1.6)	0	
Education				**0.033**
	No exams	1 (0.1)	1 (0.6)	
	>16 years	63 (9.0)	23 (13.3)	
	>18 years	79 (11.3)	17 (9.8)	
	Diploma level	120 (17.2)	42 (24.3)	
	Degree level	265 (37.9)	63 (36.4)	
	Professional	50 (7.2)	6 (3.5)	
	Post Graduate	104 (14.9)	15 (8.7)	
	Doctorate	17 (2.4)	6 (3.5)	
BMI				0.275
	<18.5	23 (3.3)	5 (2.9)	
	18.5-24	314 (45.2)	79 (45.9)	
	25-29	163 (23.5)	44 (25.6)	

	Controls (n=699) Mean (SD) / N (%)	Cases (n=173) Mean (SD) / N (%)	*p* value
30-34	135 (19.4)	24 (14.0)	
35-39	40 (5.8)	17 (9.9)	
40-44	15 (2.2)	3 (1.7)	
>45	5 (0.7)	0	
Twin			0.268
No	687 (98.3)	172 (99.4)	
Yes	12 (1.7)	1 (0.6)	
Social Status			0.516
Higher Managerial/Professional	313 (44.8)	67 (38.7)	
Diploma level Professional	110 (15.7)	29 (16.8)	
Self Employed	41 (5.9)	9 (5.2)	
Low level supervisor/Technical	66 (9.4)	24 (13.9)	
Semi routine/Routine	153 (21.9)	39 (22.5)	
Unemployed/Student	16 (2.3)	5 (2.9)	
Supported			0.072
No	10 (1.4)	6 (3.5)	
Yes	689 (98.6)	166 (96.0)	
Alcohol in pre-pregnancy			0.293
No	227 (32.5)	49 (28.3)	
Yes	472 (67.5)	124 (71.7)	
Alcohol in pregnancy			0.446
No	682 (97.6)	167 (96.5)	
Yes	17 (2.4)	6 (3.5)	
Smoking in pre-pregnancy			0.992
No	618 (88.4)	153 (88.4)	
Yes	81 (11.6)	20 (11.6)	
Smoking in pregnancy			0.558
No	684 (97.9)	168 (97.1)	
Yes	15 (2.1)	5 (2.9)	
Medication			0.595

	Controls (n=699) Mean (SD) / N (%)	Cases (n=173) Mean (SD) / N (%)	*p* value
No	593 (84.8)	139 (80.3)	
Thyroxine	29 (4.1)	12 (6.9)	
Anti-coagulant	24 (3.4)	5 (2.9)	
Asthma drugs	14 (2.0)	5 (2.9)	
Analgesia	3 (0.4)	1 (0.6)	
Other	36 (5.2)	11 (6.4)	
Taking any medication			0.150
No	593 (84.8)	139 (80.3)	
Yes	106 (15.2)	34 (19.7)	
Omega-3 supplement			0.402
No	515 (73.7)	122 (70.5)	
Yes	184 (26.3)	51 (29.5)	
Past history of depression			**0.000**
No	638 (91.3)	123 (71.1)	
Yes	61 (8.7)	50 (28.9)	
Past history of PND			**0.000**
No	669 (95.7)	146 (84.4)	
Yes	30 (4.3)	27 (15.6)	
Family history of PND			0.129
No	611 (87.4)	141 (81.5)	
1st degree relative	79 (11.3)	29 (16.8)	
Other family	9 (1.3)	3 (1.7)	
Past history of anxiety			**0.003**
No	605 (86.6)	134 (77.5)	
Yes	94 (13.4)	39 (22.5)	
Mode of delivery			0.623
Normal vaginal birth	373 (54.4)	85 (50.3)	
Instrument assisted	88 (12.8)	24 (14.2)	
C section in labour	97 (14.1)	22 (13.0)	
Elective C section	128 (18.7)	38 (22.5)	
Induction of labour			0.998

	Controls (n=699) Mean (SD) / N (%)	Cases (n=173) Mean (SD) / N (%)	*p* value
No	475 (69.2)	117 (69.2)	
Yes	211 (30.8)	52 (30.8)	
Premature baby			0.784
No	657 (95.8)	162 (95.3)	
Yes	29 (4.2)	8 (4.7)	

Table 3.20. Correlation coefficient r and significance reported for continuous variables with p<0.05 for postpartum depression, using EPDS ≥ 10.

Covariate	**r**	***p*-value**
Antenatal EPDS score	0.339	0.000
Total gestation (days)	-0.077	0.033

Table 3.21. Variables significant in chi-squared analysis - postpartum depression. For variables with >2 categories, odds ratios (OR) are presented to compare to a selected baseline group ([a]). OR values were not calculated for variables with any expected counts <5 ([b]).

Covariate	***p* value**	**X^2**	***Odds ratio***
Positive antenatal screen (≥10)	0.000	99.679	6.69
Parity	0.001	22.527	
0[a]			
1			0.85
2			0.49
3			1.22
4[b]			
5[b]			
6[b]			
7[b]			
8[b]			
Education	0.033	15.265	
No exams[b]			
>16 years			1.55

Covariate	*p* value	X^2	*Odds ratio*
>18 years			0.86
Diploma level			1.55
Degree level[a]			
Professional			0.47
Post Graduate			0.54
Doctorate[b]			
Past history of depression	0.000	50.811	4.25
No			
Yes			
Past history of PND	0.000	29.062	4.12
No			
Yes			
Past history of anxiety	0.003	8.876	1.87
No			
Yes			

3.3.6 Regression modelling

Results of the optimal linear regression model selected for prediction of postpartum EPDS scores are shown in Table 3.22. In the final model (R^2 = .24***), eleven predictors made significant contributions to postpartum EPDS score: screening positive in antenatal screening (EPDS≥10), past history of depression, past history of PND, parity (4 or 5 previous children), age <18, smoking, drinking, gestational length and BMI (35-39). Despite the contribution of educational level and medication to prediction in the model, these variables did not reach statistical significance. 24% of the variance in EPDS scores can be explained by the model.

Table 3.22. Results of a linear regression model with postpartum EPDS score as the outcome (N=872) with a number of selected predictor variables.

	B	*SE B*	*β*
Constant	11.76	3.52	
Positive antenatal screen	3.88	0.42	.29***

	B	*SE B*	*β*
Past history depression	2.88	0.45	.21***
Past history PND	1.43	0.59	.08*
Parity (baseline – 0)			
4	4.51	1.39	.10**
5	5.99	2.91	.06*
Education (baseline – Degree)			
Diploma level	0.31	0.36	.03
Age (baseline – 30-34)			
<18	10.97	4.11	.08**
Smoking pre-pregnancy	-0.68	0.44	-.05
Drinking pre-pregnancy	0.74	0.30	.07*
Medication	0.71	0.38	.06
Total gestational length	-0.03	0.01	-.07*
BMI (baseline 18.5-24)			
35-39	1.13	0.57	.06*

R^2 = .24. *p < .05, **p < .01, ***p < .001.

To explore whether the addition of raw EPDS scores would improve the model, the model was re-run with antenatal EPDS score as a further predictor variable in place of the cut-off screen (Table 3.23).

Table 3.23. Results of a linear regression model with postpartum EPDS score as the outcome (N=872) predicted by a number of selected predictor variables, including antenatal EPDS score.

	B	*SE B*	*β*
Constant	9.14	3.33	
Antenatal EPDS score	0.47	0.03	.43***
Past history depression	2.70	0.42	.19***

	B	*SE B*	*β*
Past history PND	1.16	0.55	.06*
Parity (baseline – 0)			
4	4.18	1.31	.09**
5	6.84	2.75	.07*
Education (baseline – Degree)			
Diploma level	0.16	0.34	.01
Age (baseline – 30-34)			
<18	9.61	3.87	.07*
Smoking pre-pregnancy	-0.91	0.42	-.06*
Drinking pre-pregnancy	0.66	0.29	.07*
Medication	0.49	0.36	.04
Total gestational length	-0.02	0.01	-.06*
BMI (baseline 18.5-24)			
35-39	1.22	0.54	.07*

R^2 = .32. *p < .05, **p < .01, ***p < .001.

Overall the model now predicts 32% of the variability in postpartum EPDS scores (R^2 = .32), which is an improvement on 24% when using the cut-off in place of raw score. The model was also run excluding antenatal EPDS completely, to test the importance of adding this to the model. The model predicts just 15% of the variability without antenatal EPDS, which demonstrates its predictive power in the model. Overall, prediction is improved with the inclusion of antenatal EPDS screening in the model, and is further improved still by inputting EPDS as a raw score rather than a dichotomised cut-off value.

In this cohort a number of important variables for both antenatal and postpartum EPDS have been identified by linear regression modelling. Since antenatal EPDS exerts a strong influence over postpartum EPDS scores, variables predicting antenatal EPDS can be considered as indirectly influencing postpartum EPDS score, as demonstrated in Figure 3.5.

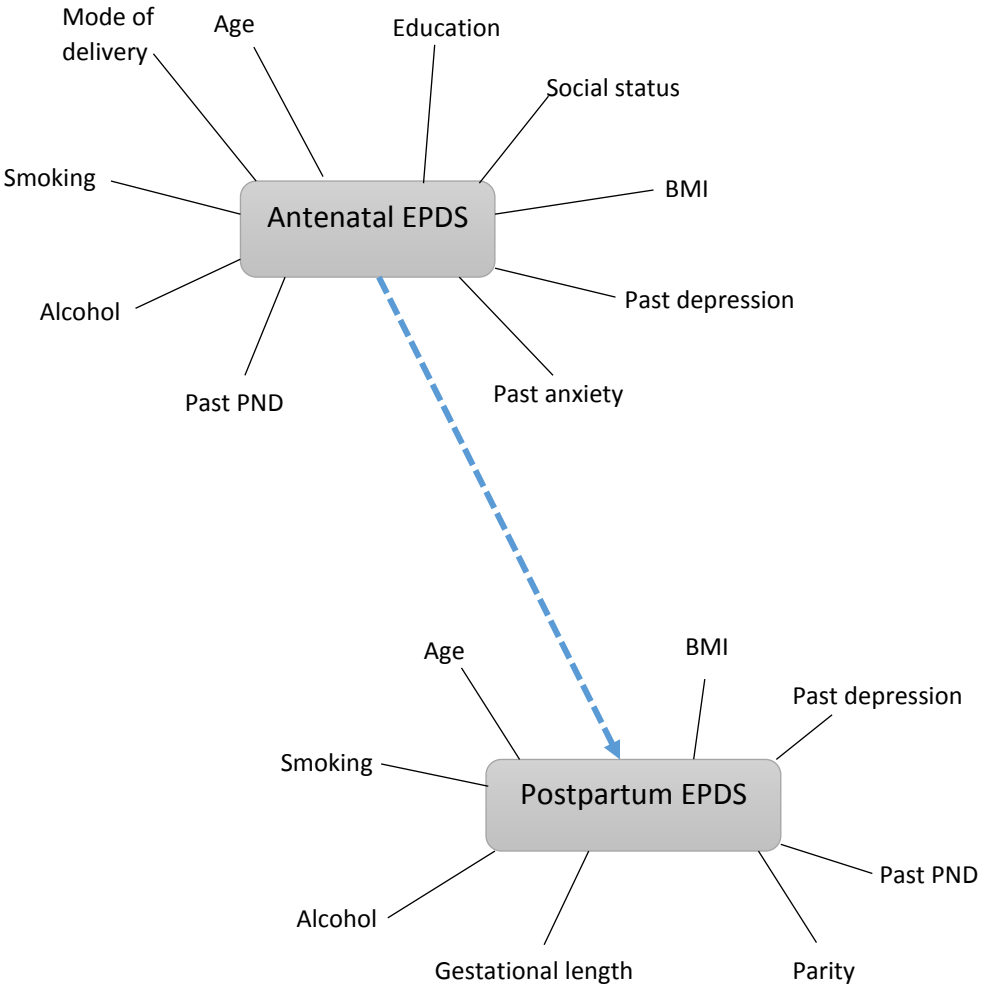

Figure 3.5. Variables predicting antenatal EPDS in regression modelling indirectly influence postpartum EPDS score.

3.4 Coventry Hospital Cohort

A total of 448 women were recruited at the antenatal stage at University Hospital Coventry and Warwickshire (UHCW), known as the Coventry Hospital cohort. Postpartum follow up rate for this cohort was 67% resulting in 302 participants with complete postpartum screening results. Mean gestation at recruitment was 29 weeks.

3.4.1 Antenatal EPDS – exploratory analysis

Screening identified a prevalence of 18.1% for AND in the study population, using a score ≥10. EPDS scores ranged from 0-21 with a mean score of 5.85. Cohort characteristics are presented in Table 3.24.

Table 3.24. Cohort characteristics of the study population. Continuous variables are expressed as the mean (standard deviation) and categorical variables are expressed in N (%) at each factor level.

Covariate	N (SD) / Mean (%)
Gestation - recruitment (days)	202.59 (22.36)
Total gestation – delivery (days)	271.55 (19.40)
Baby gender	
Female	174 (50.4)
Male	171 (49.6)
Birth weight (g)	3338.84 (606.17)
Parity	
0	148 (48.7)
1	92 (30.3)
2	44 (14.5)
3	12 (3.9)
4	4 (1.3)
5	2 (0.7)
6	1 (0.3)
7	0
8	1 (0.3)
Age	
<18 years	3 (0.7)
18-24 years	53 (11.8)
25-29 years	128 (28.6)

Covariate	N (SD) / Mean (%)
30-34 years	147 (32.8)
35-39 years	88 (19.6)
40-45 years	28 (6.3)
>45 years	1 (0.2)
Ethnicity	
White British	316 (70.5)
Indian	39 (8.7)
Asian	22 (4.9)
Other White	29 (6.5)
Black Caribbean	2 (0.4)
Black African	18 (4.0)
Mixed	10 (2.2)
Other	12 (2.7)
BMI	
<18.5	9 (2.0)
18.5-24	170 (38.0)
25-29	126 (28.2)
30-34	88 (19.7)
35-39	38 (8.5)
40-44	11 (2.5)
>45	5 (1.1)
Twin	
No	339 (97.1)
Yes	10 (2.9)
Housing	
Owns	232 (53.0)
Rents	147 (33.6)
Parents	40 (9.1)
Social housing	16 (3.7)
Friends	3 (0.7)
Employment status	
Full time	242 (55.0)
Part time	111 (25.2)

Covariate	N (SD) / Mean (%)
Unemployed	77 (17.5)
Student	10 (2.3)
Supported	
No	8 (2.7)
Yes	286 (63.8)
Alcohol in pre-pregnancy	
No	202 (68.5)
Yes	93 (31.5)
Alcohol in pregnancy	
No	293 (99.3)
Yes	2 (0.7)
Smoking in pre-pregnancy	
No	260 (88.4)
Yes	34 (11.6)
Smoking in pregnancy	
No	278 (94.6)
Yes	16 (5.4)
Family history of PND	
No	268 (88.4)
1st degree relative	35 (11.6)
Other family	0
Mode of delivery	
Normal vaginal birth	191 (55.4)
Instrument assisted	29 (8.4)
Elective C section	50 (14.5)
Emergency C section	75 (21.7)
Obstetric history	
None	212 (69.7)
Miscarriage	74 (24.3)
Stillbirth	7 (2.3)
Neonatal death	2 (0.7)
Ectopic	3 (1.0)
Multiple history	6 (2.0)

70.5% of women were White British. The majority were aged 30-34, with a BMI of 18.5-24. Most were in full time employment (55%). 53% of women owned their own housing. 48.7% were first time mothers. 68.5% of women did not drink alcohol pre-pregnancy. 99.3% did not report drinking during pregnancy. The majority of women were non-smokers (88.4%), rising to 94.6% during pregnancy. 11.6% of women had a family history of PND. Almost all women (97.3%) were supported in their pregnancy.

The majority of women had no obstetric history (69.7%), although 24.3% had suffered with previous miscarriage. 50.4% of babies born were female. The majority of deliveries were normal vaginal deliveries (55.4%). Mean total gestation was 38.8 weeks and average birth weight was 7.36lbs (3338.84 grams).

Chi squared & correlation analysis

A comparison of cases and controls was first carried out to identify variables displaying statistical significance between the two groups (Table 3.25).

Table 3.25. Characteristics of study participants from the Coventry cohort for cases and controls (EPDS ≥ 10) for antenatal depression. Significance of Chi squared/correlation statistics are presented.

Covariate	Controls (n=367) Mean (SD) / N (%)	Cases (n=81) Mean (SD) / N (%)	*p* value
Gestation - recruitment (days)	202.62 (23.32)	202.47 (17.47)	.905
Total gestation – delivery (days)	271.65 (19.51)	271.10 (19.05)	.766
Baby gender			.940
Female	143 (50.5)	31 (50.0)	
Male	140 (49.5)	31 (50.0)	
Birth weight (g)	3347.60 (592.13)	3298.84 (670.27)	.495
Parity			.987
0	123 (49.2)	25 (30.9)	
1	75 (30.0)	17 (31.5)	
2	35 (14.0)	9 (16.7)	
3	10 (4.0)	2 (3.7)	
4	3 (1.2)	1 (1.9)	
5	2 (0.8)	0	
6	1 (0.4)	0	
7	0	0	
8	1 (0.4)	0	
Age			
<18 years	3 (0.8)	0	**.000**
18-24 years	28 (7.6)	25 (30.9)	
25-29 years	103 (28.1)	25 (30.9)	
30-34 years	132 (36.0)	15 (18.5)	
35-39 years	78 (21.3)	10 (12.3)	
40-45 years	22 (6.0)	6 (7.4)	
>45 years	1 (0.3)	0	
Ethnicity			**.044**

Covariate		Controls (n=367) Mean (SD) / N (%)	Cases (n=81) Mean (SD) / N (%)	*p* value
	White British	260 (70.8)	56 (69.1)	
	Indian	32 (8.7)	7 (8.6)	
	Asian	17 (4.6)	5 (6.2)	
	Other White	26 (7.1)	3 (3.7)	
	Black Caribbean	2 (0.5)	0	
	Black African	16 (4.4)	2 (2.5)	
	Mixed	4 (1.1)	6 (7.4)	
	Other	10 (2.7)	2 (2.5)	
BMI				.898
	<18.5	7 (1.9)	2 (2.5)	
	18.5-24	143 (39.0)	27 (33.3)	
	25-29	103 (28.1)	23 (28.4)	
	30-34	69 (18.8)	19 (23.5)	
	35-39	30 (8.2)	8 (9.9)	
	40-44	10 (2.7)	1 (1.2)	
	>45	4 (1.1)	1 (1.2)	
Twin				.515
	No	278 (96.9)	61 (98.4)	
	Yes	9 (3.1)	1 (1.6)	
Housing				**.000**
	Owns	207 (57.8)	25 (31.3)	
	Rents	112 (31.3)	35 (43.8)	
	Parents	28 (7.8)	12 (15.0)	
	Social housing	9 (2.5)	7 (8.8)	
	Friends	2 (0.6)	1 (1.3)	
Employment status				.583
	Full time	203 (56.5)	39 (48.1)	
	Part time	88 (24.5)	23 (28.4)	
	Unemployed	60 (16.7)	17 (21.0)	
	Student	8 (2.2)	2 (2.5)	

Covariate	Controls (n=367) Mean (SD) / N (%)	Cases (n=81) Mean (SD) / N (%)	*p* value
Supported			.136
No	5 (2.1)	3 (3.7)	
Yes	237 (97.9)	49 (60.5)	
Alcohol in pre-pregnancy			.924
No	166 (68.6)	36 (67.9)	
Yes	76 (31.4)	17 (32.1)	
Alcohol in pregnancy			.236
No	241 (99.6)	52 (98.1)	
Yes	1 (0.4)	1 (1.9)	
Smoking in pre-pregnancy			.066
No	217 (90.0)	43 (81.1)	
Yes	24 (10.0)	10 (18.9)	
Smoking in pregnancy			.157
No	230 (95.4)	48 (90.6)	
Yes	11 (4.6)	5 (6.2)	
Family history of PND			**.005**
No	227 (90.8)	41 (77.4)	
1st degree relative	23 (9.2)	12 (22.6)	
Other family	0	0	
Mode of delivery			.764
Normal vaginal birth	153 (54.1)	38 (61.3)	
Instrument assisted	25 (62.9)	4 (6.5)	
Elective C section	42 (14.8)	8 (80.6)	
Emergency C section	63 (22.3)	12 (19.4)	
Obstetric history			.886
None	175 (70.0)	37 (68.5)	
Miscarriage	60 (24.0)	14 (25.9)	
Stillbirth	5 (2.0)	2 (3.7)	
Neonatal death	2 (0.8)	0	
Ectopic	3 (1.2)	0	
Multiple history	5 (2.0)	1 (1.9)	

Results of the bivariate analyses are presented in Table 3.26. Four covariates had significant associations with a positive antenatal screen: age, ethnicity, housing and a family history of PND.

Table 3.26. Significant values in chi-squared analysis - antenatal depression, Coventry cohort. For variables with >2 categories, odds ratios (OR) are presented to compare to a selected baseline group ([a]). OR values were not calculated for variables with any expected counts <5 ([b]).

Covariate	*p* value	X^2	Odds ratio
Age	**0.000**	40.394	
<18 years[b]			
18-24 years			5.40
25-29 years			1.14
30-34 years[a]			
35-39 years			0.52
40-45 years			1.25
>45 years[b]			
Ethnicity	**0.000**	31.715	
White British[a]			
Indian			0.99
Asian			1.35
Other White			0.50
Black Caribbean[b]			
Black African			0.55
Mixed			7.26
Other			0.90
Housing	**0.000**	27.211	
Owns[a]			
Rents			1.73
Parents			2.11
Social housing			3.76
Friends[b]			
Family history of PND	**0.014**	6.043	
No			
1st degree relative			2.89

Regression modelling

Exploratory linear regression modelling identified the optimal model to predict antenatal EPDS score (Table 3.27).

Table 3.27. Results of a linear regression model with antenatal EPDS score as the outcome (N=285) with a number of selected predictor variables based on initial exploratory modelling.

	B	*SE B*	*β*
Constant	9.93	1.46	
Age (baseline - 30-34)			
18-24	2.57	0.76	.19*
Ethnicity (baseline - White British)			
Mixed	4.83	1.58	.17*
Supported	-4.83	1.48	-.18*
Family history of PND	1.37	0.76	.10

R^2 = .12. *p < .01.

In the final model (R^2 = .12*), three predictors made significant contributions to antenatal EPDS score in the Coventry cohort: Age 18-24, Mixed Ethnicity and Support. Young age and mixed ethnicity both increased EPDS score, whereas women who were supported had decreased EPDS scores, i.e. protected from PND. Despite the contribution of family history of PND to the model's prediction, this variable was not statistically significant. 12% of the variance in antenatal EPDS scores can be explained by the final model.

Due to a large proportion of missing data for this cohort, the model was re-run excluding variables with missing data to include N=448 (Table 3.28). This now excluded obstetric history, mode of delivery, gender, parity, supported, alcohol, drinking, smoking, cigarettes, family history and twin pregnancy. The variables now included were age, ethnicity, housing status, employment status and BMI.

Table 3.28. Results of a linear regression model with antenatal EPDS score as the outcome (N=448) with a number of selected predictor variables.

	B	*SE B*	*β*
Constant	5.07	0.26	
Age (baseline - 30-34)			
18-24	2.12	0.62	.16**
Ethnicity (baseline - White British)			
Asian	1.89	0.94	.09*
Mixed	5.32	1.37	.18***
Housing (baseline – Owns)			
Rents	0.98	0.43	.10*

R^2 = .08. *p < .05, **p < 0.01, ***p < .001

In this final model (R^2 =.08), four predictors contributed significantly to antenatal EPDS for this Coventry cohort: age 18-24, Asian/Mixed ethnicity and living in rented housing. Despite the inclusion of more participants in the model (N=448), due to the loss of variables only 8% of the variation in antenatal EPDS scores can be explained by the model.

3.4.2 Postpartum EPDS – exploratory analysis

302 participants completed the postpartum follow-up EPDS with an identified prevalence of 10.6% for PND based on EPDS ≥ 10. Mean EPDS score was 4.57, with a range of 0-21, and standard deviation 4.047. Cohort characteristics of all participants who completed the postpartum EPDS are presented in Table 3.29.

Table 3.29. Cohort characteristics of the study population for participants who completed postpartum EPDS from the Coventry cohort. Continuous variables are expressed as the mean (standard deviation) and categorical variables are expressed in N (%) at each factor level.

Covariate	N (SD) / Mean (%)
Gestation - recruitment (days)	202.52 (23.06)
Total gestation – delivery (days)	271.34 (20.09)
Baby gender	
Female	134 (48.6)
Male	142 (51.4)
Birth weight (g)	3361.02 (619.60)
Parity	
0	116 (47.5)
1	83 (34.0)
2	32 (13.1)
3	10 (4.1)
4	1 (0.4)
5	1 (0.4)
6	0
7	0
8	1 (0.4)
Age	
<18 years	3 (1.0)
18-24 years	31 (10.3)
25-29 years	89 (29.5)
30-34 years	98 (32.5)
35-39 years	58 (19.2)
40-45 years	22 (7.3)
>45 years	1 (0.3)
Ethnicity	

Covariate	N (SD) / Mean (%)
White British	225 (74.5)
Indian	20 (6.6)
Asian	14 (4.6)
Other White	16 (5.3)
Black Caribbean	2 (0.7)
Black African	11 (3.6)
Mixed	7 (2.3)
Other	7 (2.3)
BMI	
<18.5	6 (2.0)
18.5-24	108 (35.8)
25-29	94 (31.1)
30-34	53 (17.5)
35-39	30 (9.9)
40-44	8 (2.6)
>45	3 (1.0)
Twin	
No	271 (97.8)
Yes	6 (2.2)
Housing	
Owns	163 (55.3)
Rents	93 (31.5)
Parents	28 (9.5)
Social housing	10 (3.4)
Friends	1 (0.3)
Employment status	
Full time	172 (58.1)
Part time	73 (24.7)
Unemployed	43 (14.5)
Student	8 (2.7)
Supported	
No	8 (3.4)
Yes	228 (96.6)

Covariate	N (SD) / Mean (%)
Alcohol in pre-pregnancy	
No	159 (67.1)
Yes	78 (32.9)
Alcohol in pregnancy	
No	236 (99.6)
Yes	1 (0.4)
Smoking in pre-pregnancy	
No	260 (88.4)
Yes	34 (11.6)
Smoking in pregnancy	
No	215 (90.7)
Yes	22 (9.3)
Family history of PND	
No	221 (90.6)
1st degree relative	23 (9.4)
Other family	0
Mode of delivery	
Normal vaginal birth	154 (55.8)
Instrument assisted	24 (8.7)
Elective C section	46 (16.7)
Emergency C section	52 (18.8)
Obstetric history	
None	168 (68.9)
Miscarriage	62 (25.4)
Stillbirth	5 (2.0)
Neonatal death	1 (0.4)
Ectopic	3 (1.2)
Multiple history	5 (2.0)

Women in this cohort were recruited on average at 28.9 weeks gestation. The mean delivery was at 38.8 weeks. Mean birth weight was 7.4lbs. The majority of women were primiparous, aged 25-29, White British, in full time employment, owning their own housing. Modal BMI was 18.5-24, 67.1% of women did drink alcohol in pre-

conception but only 0.4% reported drinking alcohol during pregnancy. Women were generally non-smokers in both pre-conception (88.4%) and pregnancy (90.7%). 9.4% of women had a family history of PND. Normal vaginal delivery was the most common mode of delivery. 68.9% of women had no prior obstetric history, with 25.4% of women who had a history of miscarriage.

Chi squared & correlation analysis

A comparison of cases and controls was first carried out to identify variables displaying statistical significance between the two groups (Table 3.30).

Table 3.30. Characteristics of study participants from the Coventry cohort cases and controls (EPDS cut off 9/10) for postpartum depression. Significance of Chi squared/correlation statistics are presented.

Covariate	**Controls (n=270) Mean (SD) / N (%)**	**Cases (n=32) Mean (SD) / N (%)**	***p* value**
Antenatal EPDS	5.32 (4.10)	9.25 (5.39)	**.000**
Positive antenatal screen	38 (14.1)	14 (43.8)	**.000**
Gestation - recruitment (days)	202.37 (23.35)	203.81 (20.69)	.403
Total gestation – delivery (days)	271.54 (20.29)	269.69 (18.58)	.609
Baby gender			.827
Female	120 (48.8)	14 (46.7)	
Male	126 (51.2)	16 (53.3)	
Birth weight (g)	3380.01 (585.75)	3205.27 (844.82)	.235
Parity			.814
0	102 (47.0)	14 (51.9)	
1	72 (33.2)	11 (40.7)	
2	31 (14.3)	1 (3.7)	
3	9 (4.1)	1 (3.7)	
4	1 (0.5)	0	
5	1 (0.5)	0	
6	0	0	
7	0	0	
8	1 (0.5)	0	
Age			.873
<18 years	3 (1.1)	0	

Covariate		Controls (n=270) Mean (SD) / N (%)	Cases (n=32) Mean (SD) / N (%)	*p* value
	18-24 years	26 (9.6)	5 (15.6)	
	25-29 years	80 (29.6)	9 (28.1)	
	30-34 years	90 (33.3)	8 (25.0)	
	35-39 years	51 (18.9)	7 (21.9)	
	40-45 years	19 (7.0)	3 (9.4)	
	>45 years	1 (0.4)	0	
Ethnicity				**.015**
	White British	205 (75.9)	20 (62.5)	
	Indian	18 (6.7)	2 (6.3)	
	Asian	10 (3.7)	4 (12.5)	
	Other White	15 (5.6)	1 (3.1)	
	Black Caribbean	2 (0.7)	0	
	Black African	11 (4.1)	0	
	Mixed	4 (1.5)	3 (9.4)	
	Other	5 (1.9)	2 (6.3)	
BMI				.093
	<18.5	4 (1.5)	2 (6.3)	
	18.5-24	100 (37.0)	8 (25.0)	
	25-29	78 (28.9)	16 (50.0)	
	30-34	49 (18.1)	4 (12.5)	
	35-39	28 (10.4)	2 (6.3)	
	40-44	8 (3.0)	0	
	>45	3 (1.1)	0	
Twin				.642
	No	242 (98.0)	29 (96.7)	
	Yes	5 (2.0)	1 (3.3)	
Housing				**.043**
	Owns	149 (56.7)	14 (43.8)	
	Rents	82 (31.2)	11 (34.4)	
	Parents	25 (9.5)	3 (9.4)	
	Social housing	6 (2.3)	4 (12.5)	

Covariate	Controls (n=270) Mean (SD) / N (%)	Cases (n=32) Mean (SD) / N (%)	*p* value
Friends	1 (0.4)	0	
Employment status			**.007**
Full time	157 (58.1)	15 (46.9)	
Part time	67 (25.4)	6 (18.8)	
Unemployed	32 (12.1)	11 (34.4)	
Student	8 (3.0)	0	
Supported			.892
No	7 (3.3)	1 (3.8)	
Yes	203 (75.2)	25 (96.2)	
Alcohol in pre-pregnancy			.258
No	139 (65.9)	20 (76.9)	
Yes	72 (34.1)	6 (23.1)	
Alcohol in pregnancy			.725
No	210 (99.5)	26 (81.3)	
Yes	1 (0.5)	missing data	
Smoking in pre-pregnancy			.311
No	190 (90.0)	25 (96.2)	
Yes	21 (10.0)	1 (3.8)	
Smoking in pregnancy			.888
No	204 (96.7)	25 (96.2)	
Yes	7 (3.3)	1 (3.8)	
Family history of PND			.697
No	198 (90.8)	23 (88.5)	
1st degree relative	20 (9.2)	3 (11.5)	
Other family	0	0	
Mode of delivery			.663
Normal vaginal birth	140 (56.9)	14 (46.7)	
Instrument assisted	20 (8.1)	4 (13.3)	
Elective C section	40 (16.3)	6 (20.0)	
Emergency C section	46 (18.7)	6 (20.0)	
Obstetric history			.785

Covariate	Controls (n=270) Mean (SD) / N (%)	Cases (n=32) Mean (SD) / N (%)	*p* value
None	147 (67.7)	21 (77.8)	
Miscarriage	57 (26.3)	5 (18.5)	
Stillbirth	4 (1.8)	1 (3.7)	
Neonatal death	1 (0.5)	0	
Ectopic	3 (1.4)	0	
Multiple history	5 (2.3)	0	

Results of the bivariate analyses are presented in Table 3.31. Five covariates had significant associations with a positive postpartum screen: antenatal EPDS score, positive antenatal screen (EPDS ≥ 10), ethnicity, housing and employment status.

Table 3.31. Significant values in chi-squared analysis - postpartum depression, Coventry cohort. For variables with >2 categories, odds ratios (OR) are presented to compare to a selected baseline group ([a]). OR values were not calculated for variables with any expected counts <5 ([b]).

Covariate	*p* value	X^2	*Odds ratio*
Positive antenatal screen	**0.000**	17.676	4.75
Ethnicity	**0.000**	27.211	
White British[a]			
Indian			0.93
Asian			3.71
Other White			0.55
Black Caribbean[b]			
Black African[b]			
Mixed[b]			
Other			3.53
Housing	**0.043**	9.862	
Owns[a]			
Rents			1.20
Parents			1.01
Social housing			6.29
Friends			

Covariate	***p* value**	**X^2**	***Odds ratio***
Employment status	**0.007**	11.987	
Full time[a]			
Part time			0.69
Unemployed			3.90
Student[b]			

Regression modelling

Results of linear regression modelling are presented in Table 3.32. Variables were selected by exploratory backward elimination modelling, and entered into a final linear regression model based on the optimal model.

Table 3.32. Results of a linear regression model with postpartum EPDS score as the outcome for the Coventry cohort (N=236) with a number of selected predictor variables based on exploratory modelling with the cohort.

	B	*SE B*	*β*
Constant	1.88	1.30	
Positive antenatal screening	3.83	0.64	0.34***
Age (baseline - 30-34)			
18-24	-1.47	0.84	-.11
Ethnicity (baseline – White British)			
Asian	5.08	1.17	.25***
Employment status (baseline – Full time)			
Student	-3.73	1.31	-.16**
BMI (baseline – 18.5-24)			
40-44	-2.86	1.37	-.12*
Mode of delivery (baseline – NVB			
Emergency C section	1.40	0.61	.13*
Parity	-0.60	0.23	-.15**
Supported	2.38	1.29	.10
Twin	3.19	1.82	.10
Housing (baseline – Owns)			
Social housing	6.07	1.47	.25***

R^2 =0.31. *p < .01, **p < .001

In the final model (R^2 = .31*), seven predictors made significant contributions to antenatal EPDS score in the Coventry cohort: positive antenatal screening, ethnicity, employment status, BMI, mode of delivery, parity and housing status. Despite the contribution of age, support and twin pregnancy, these variables were

not statistically significant in the model. 31% of the variance in postpartum EPDS scores can be explained by the final model.

To test whether raw antenatal EPDS scores are a better predictor than using the cut-off, the model was re-run replacing 'positive antenatal screen' with 'antenatal EPDS (Table 3.33). This improved the model, which now explains 35% of the variance in postpartum EPDS scores, in comparison to 31%. The model was additionally compared to a model excluding antenatal EPDS score entirely, and the exclusion of this variable resulted in R^2 of 20%. Overall, adding an EPDS screen to the model does improve its prediction, which is benefitted further by adding the raw score.

Table 3.33. Results of a linear regression model with postpartum EPDS score as the outcome for the Coventry cohort (N=236) with a number of selected predictor variables.

	B	*SE B*	*β*
Constant	-0.58	1.34	
Antenatal EPDS	0.381	0.05	.41***
Age (baseline - 30-34)			
18-24	-1.42	0.81	-.10
Ethnicity (baseline – White British)			
Asian	4.83	1.14	.24***
Employment status (baseline – Full time)			
Student	-3.94	1.26	-.17**
BMI (baseline – 18.5-24)			
40-44	-2.15	1.33	-.09
Mode of delivery (baseline – NVB)			
Emergency C section	1.18	0.59	.11*
Parity	-0.58	0.22	-.14**
Supported	3.34	1.27	.15**
Twin	2.84	1.76	.09
Housing (baseline – Owns)			
Social housing	6.56	1.42	.27***

R^2 = .35. *p < .05, **p < .01, ***p < 0.001.

Significant predictors in the final step of this model were antenatal EPDS score, Asian ethnicity, student occupation, emergency C section, parity, support and living in social housing.

3.4.3 Differences and similarities between the cohorts

The different study populations recruited from the two hospital sites, Coventry and Warwick, were largely similar. A more complete picture of risk factors is available for the larger Warwick cohort, with more data collected relating to mental health history and family history compared with the Coventry cohort. The Warwick cohort was also larger, and therefore statistical analyses have more power and scope to find significant results. A larger number of risk factors have been identified in the Warwick cohort. Both cohorts had similar average EPDS scores, with an antenatal average of 5.85 for Coventry and 5.68 for Warwick, although the postpartum average EPDS was slightly higher in Warwick at 5.96 compared with 4.57 in Coventry. In the Coventry population, a prevalence of 18.1% was identified in antenatal depressive screening, falling to 10.6% in postpartum depressive screening. In contrast, prevalence in the Warwick cohort was 16.7% for antenatal symptoms, rising to 19.8% for postpartum symptoms. When examining the demographics of the two cohorts, a couple of key differences stand out. The larger Warwick cohort is predominantly comprised of White British participants (80%), whereas this is less dominant in the Coventry cohort (70% White British). This is compared with a national average of 65%. Unemployment rates are notably higher in the Coventry population, with 20% of participants unemployed/student compared with just 3% of Warwick participants.

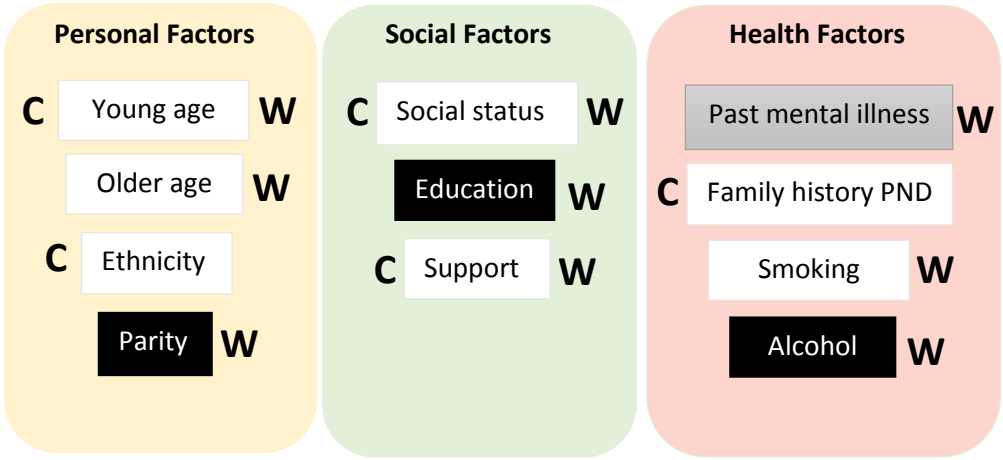

Figure 3.6. A representative profile of key risk factors emerging from the analysis for antenatal EPDS (white) and postpartum EPDS (black), or both (grey), categorised into personal, social and health factors. Those present in the Coventry cohort are labelled 'C', and those present in the Warwick cohort are labelled 'W'.

Three risk factors are in agreement across the cohorts for increased risk of antenatal depressive symptoms: young age, social status and lack of support. An age of 18-24 is a risk factor for antenatal EPDS in both cohorts, and in the Warwick cohort age 40-45 was identified as an additional risk factor. Social status is important in both cohorts for antenatal EPDS. Living in social housing or rented accommodation increased EPDS, and a social status of student/unemployed increased EPDS in the Warwick cohort. Identifying as 'unsupported' is another shared risk factor for antenatal EPDS between the two cohorts. Smoking status, both during and pre-pregnancy was a risk factor in the Warwick cohort only, and an Asian or Mixed Ethnicity was a risk factor in the Coventry cohort only. A family history of PND was a risk factor for the Coventry cohort only, and although personal mental health history was a risk factor for Warwick, this was not recorded for the Coventry cohort and therefore cannot be compared.

Risk factors for postpartum EPDS differ between the two cohorts. For both cohorts, antenatal EPDS was the strongest predictor, but no other risk factors were shared. Warwick risk factors were education, past mental health history, parity and alcohol pre-pregnancy, whereas Coventry risk factors were ethnicity, housing status, unemployment, emergency C-section, lack of support and twin pregnancy. Findings from both cohorts point to social disadvantage as a risk factor for PPD. A depiction of similarities and differences in risk factors between cohorts is shown in Figure 3.6.

4 Results: Circulating Biomarkers

4.1 Chapter Four Abstract

Depression and inflammation are linked by an intricate neuroimmune network, providing a means to assess the underlying inflammation as a proxy for depression. As described in detail in Chapter 1, both hyper- and hypo- activation have previously been associated with PND, and therefore the study of both pro- and anti-inflammatory cytokines is warranted. Increasingly studies dissecting inflammatory responses highlight that both pro- and anti-inflammatory arms are important for the development and resolution of inflammation[155 178 278]. In this study of biomarkers and their predictive potential for PND two key cytokines were selected for analysis based on the literature, pro-inflammatory cytokine Interleukin-6 (IL-6) and anti-inflammatory cytokine Interleukin-10 (IL-10). These two cytokines can be measured independently to assess key markers of the inflammatory response, and can also be analysed as a ratio in order to assess the balance between pro- and anti-inflammatory cytokines in circulation. The third selected biomarker is brain derived neurotrophic factor (BDNF), an important growth factor belonging to the neurotrophic family, for which a role in the pathophysiology of stress and depression is proposed.

As detailed in the Methods chapter, all circulating biomarkers were measured in human plasma samples from 688 participants during pregnancy. Firstly, concentrations in individual samples were calculated based on a standard curve generated for each individual experiment. This was carried out for IL-6, IL-10 and BDNF. The ratio of IL-6/IL-10 was additionally calculated. Measurements for all biomarkers were recorded for every participant included in the biomarker study. Once measurements were complete and sample concentrations were calculated, concentrations were entered as continuous variables into the linear regression models described in Chapter 3.

In Chapter 3, exploratory modelling of psycho-socioeconomic variables was carried out to determine the optimal models. The purpose of this present study is to assess whether additionally measuring selected biomarkers will improve the prediction of EPDS scores. This addresses aim 3 of the study – 'Do cytokines and neurosteroids IL-6, IL-10 and BDNF act as markers for PND?' In order to assess this, the new variables IL-6, IL-10, IL-6/IL-10 and BDNF will be included to expand on the

established model. This statistical modelling will utilise the participants for whom complete biomarker data plus full EPDS data at both time points is available. This modelling will be presented later in Chapter 6. Firstly, this present chapter explores the independent associations between the analysed biomarkers and EPDS score.

The biomarker study revealed interesting associations for each of the selected markers. Raised IL-6 is associated with lower social status, and is highly correlated with maternal BMI. IL-10 levels are lower in participants at risk of major depression (EPDS≥10), and the ratio of IL-6/IL-10 is also associated with depressive risk, in particular the risk of depressive symptoms worsening in the postpartum period. The above biomarkers were assessed as indicators of depressive risk, and their usefulness as a negative predictor for those women at low risk of depression is promising. Levels of BDNF were similarly found to be associated with PND risk in this study, suggesting that a level of protection may be offered by high circulating BDNF, since women with raised BDNF scored low on the EPDS. It appears that this protection may be generated by both psychosocial and biological factors, which will be explored later in Chapter 6. Finally, a sub-study investigating DNA methylation and PND explores aim 5 – is there a difference observed in global DNA methylation patterns between women with and without PND? The findings of this experiment suggest that antenatal depressive symptoms are associated with methylation levels, which may be in turn related to relevant social factors such as social status and housing type.

4.2 Cytokines: IL-6 and IL-10

Complete biomarkers were measured in 688 participants. IL-6 levels ranged from 0.09-4.72pg/mL, with a mean concentration of 0.59pg/mL (SD 0.51). IL-10 ranged from 0.01-5.69pg/mL, with a mean concentration of 0.33pg/mL (SD 0.41). Mean IL-6/IL-10 ratio was calculated as 2.88pg/mL (SD 3.35).

IL-6 and IL-10 data were log transformed prior to statistical analysis to achieve normality (Figures 4.1 and 4.2).

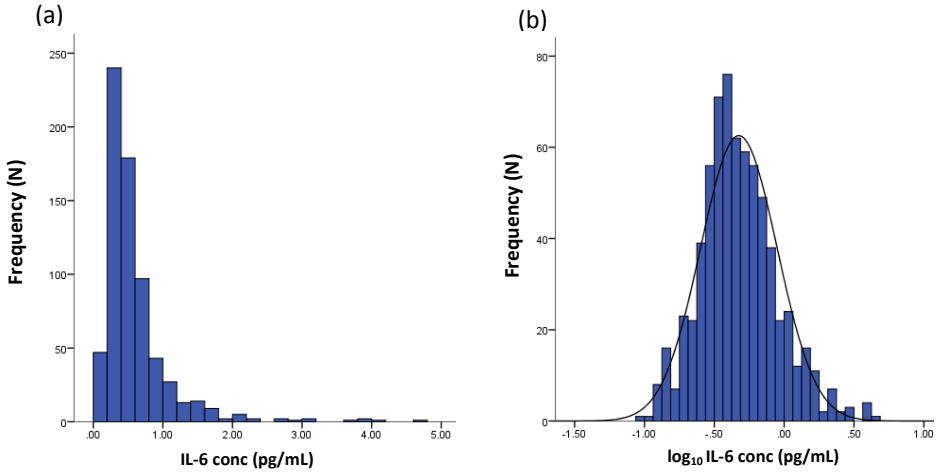

Figure 4.1. (a) IL-6 data prior to log transformation and (b) post log transformation to achieve normality prior to statistical tests.

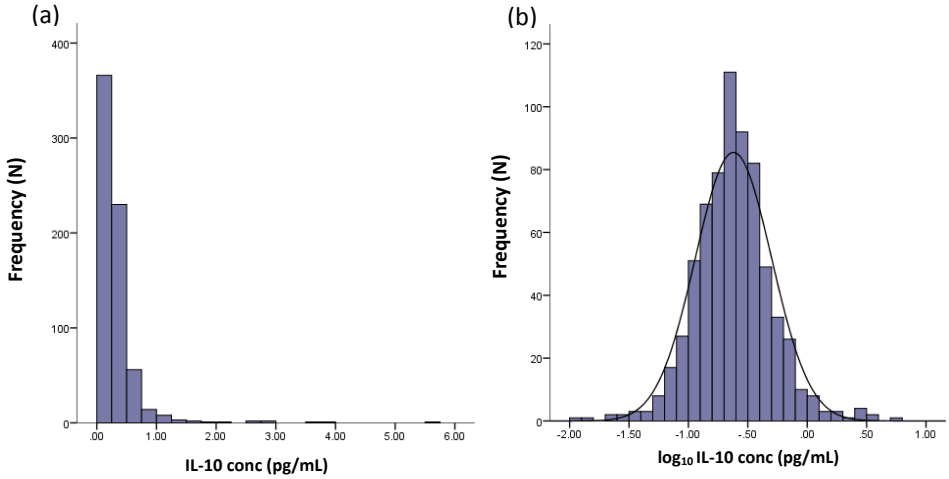

Figure 4.2. (a) IL-10 data prior to log transformation and (b) post log transformation to achieve normality prior to statistical tests.

IL-6 levels were not found to be significantly different in cases vs. controls using EPDS ≥ 10. The analysis additionally experimented with other EPDS cut off values of 8, 13 and 15, none of which were found to be significantly associated with IL-6 concentration. IL-6 was however significantly correlated with social status (r=.08), with the lowest levels observed in women of the highest social status. IL-6 had the

strongest and most significant observed relationship with BMI (Figure 4.3) (r=.301), significant at the .01 level.

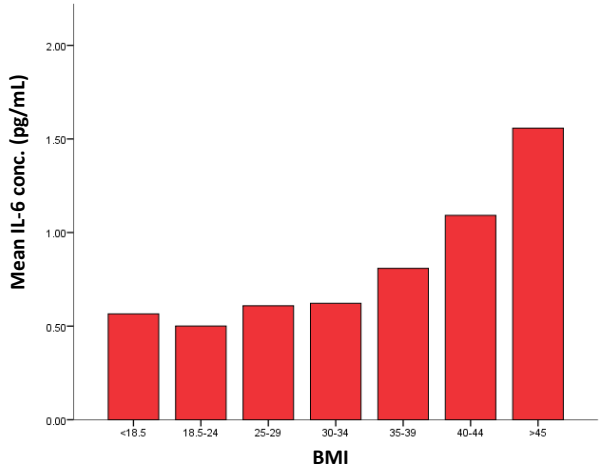

Figure 4.3. Relationship between IL-6 concentration (pg/mL) and BMI category.

IL-10 levels were significantly correlated with EPDS score, and with severity category, as defined by EPDS score (Figure 4.4). Reduced IL-10 levels (r= -.10) are seen in the highest severity category (EPDS ≥ 15, risk of major depression).

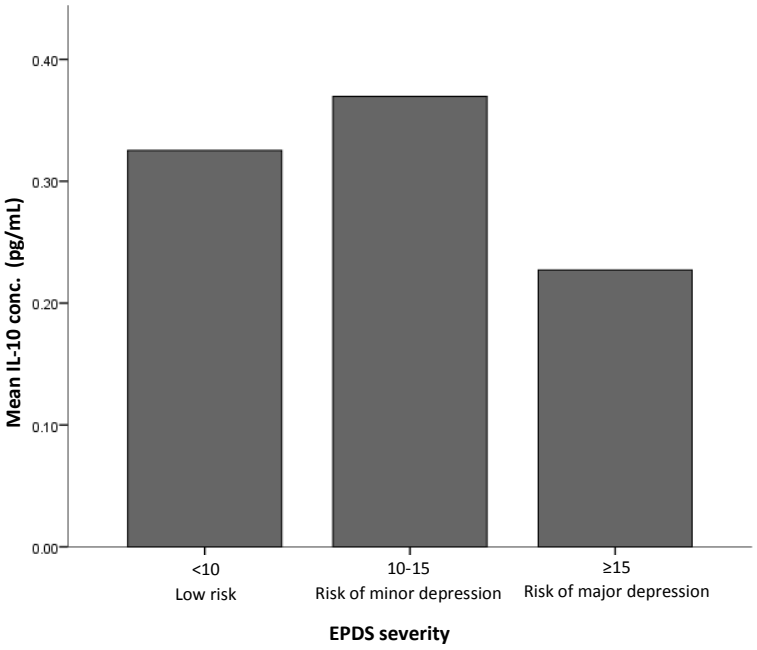

Figure 4.4. The relationship between IL-10 levels and EPDS risk category as defined by EPDS score. A score under 10 represents a low depressive risk, whereas a score between 10 and 15 represents a risk of minor depression, and a score above 15 indicates a risk of major depression. Mean IL-10 concentration was 0.33 (SD=0.36) for Low risk, 0.37 (SD=0.66) for Risk of minor depression, and 0.23 (SD=0.32) for Risk of major depression.

IL-6/IL-10 ratio was associated with depressive risk (r=.10, *p*=.01). Additionally, the ratio of these markers was significantly associated with a postpartum outcome, mode of delivery (r=.09). The ratio is lowest in women delivering normally, and rises in those who went on to have a more complicated delivery. Highest IL-6/IL-10 ratio

is observed in women who went on to have emergency caesarean section (C-section) (Figure 4.5). It has been reported that maternal BMI is associated with emergency C-section, leading to a 30% increased risk, which may explain the link between mode of delivery, BMI and IL-6 observed here[279].

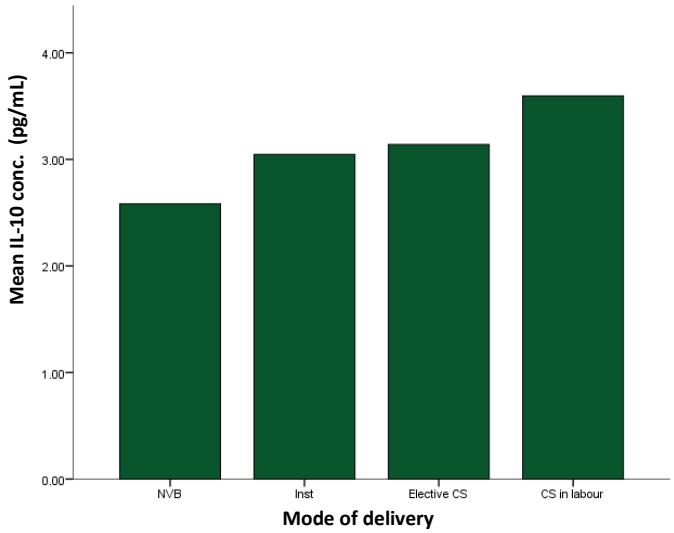

Figure 4.5. Relationship between IL-6/IL-10 ratio (pg/mL) and mode of delivery.

As described in Chapter 3, in addition to exploring the effects of variables on either antenatal or postpartum EPDS scores, it is also possible to explore effects on either the average (APA) or difference in (DPA) EPDS scores. All biomarkers were analysed for significant relationships with APA and DPA scores, and it was found that the IL-6/IL-10 ratio is significantly correlated with an increased DPA score (r=.09, *p*=.017). This can be interpreted as a likelihood of depressive symptoms worsening in the postpartum for women with a higher IL-6/IL-10 ratio during pregnancy. Relationships with DPA and APA scores were investigated for other biomarkers but significant no significant effects were seen.

Following log transformation reference ranges were calculated for all biomarkers using the parametric method of mean ± 1.96 SD using data from healthy controls (Figure 4.6). For IL-6, the mean was calculated as 0.47pg/mL, the lower reference range as 0.14 and the upper reference range as 0.61. Published reference ranges for cytokine levels during pregnancy are not readily available, but the manufacturer of the kits used for analysis (MSD) suggests that normal human EDTA plasma samples test in the range of 0.12-0.99pg/mL. Calculation of reference ranges was repeated for IL-10 with a mean of 0.33pg/mL, lower limit of 0.06 and upper limit of 0.98. Mean IL-6/IL-10 ratio was 2.88, with a lower limit of 0.33 and an upper limit

of 9.68. Any samples measuring outside of this range were referred to as 'abnormal'. 37 samples (5.4%) fell outside of this range for IL-6, and 40 fell outside for IL-10 (5.8%). Of the 'cases', 5% had abnormal IL-6 levels, 13% had abnormal IL-10 and 7% had an IL-6/IL-10 ratio outside of the range.

Due to the association found between these markers and antenatal EPDS score, the reference ranges were calculated to explore the use of a cut-off value for these biomarkers in screening. The positive predictive value (PPV) and negative predictive value (NPV) were calculated (Tables 4.1 and 4.2). IL-10 outside the reference range had a NPV of 86% for predicting antenatal depressive risk with a PPV of 29%, and IL-6/IL-10 ratio outside the reference range had NPV 86%, with a PPV of 18%. NPV describes the true negative rate, and therefore both tests perform well as a negative screen with 86% certainty they will be low risk for antenatal depression if they score within the reference range.

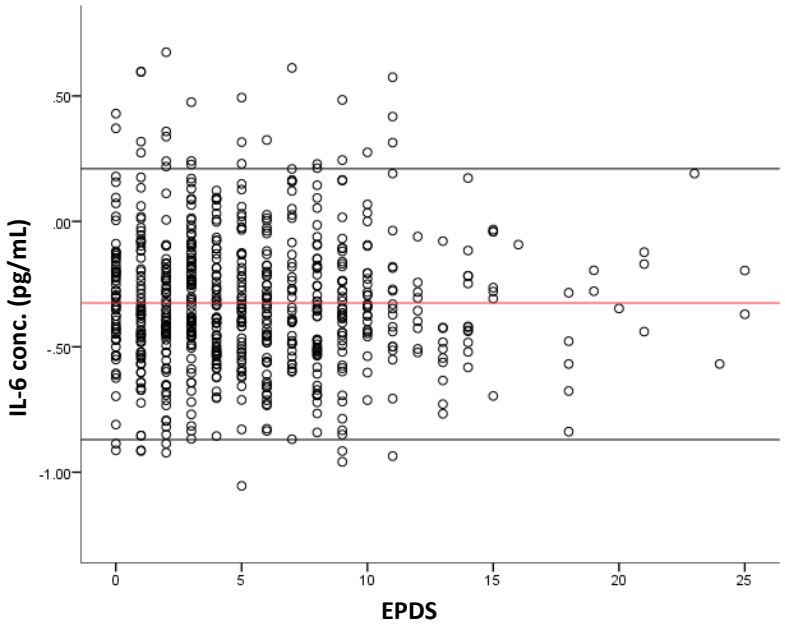

Figure 4.6. Reference ranges calculate by the mean (central reference line) ± 1.96 SD (higher and lower reference lines).

IL-10

Table 4.1. A cross-tabulation of patients with abnormal IL-10 concentration and antenatal depressive risk as determined by the EPDS allows calculation of PPV and NPV.

	Antenatal Depressive Risk	
IL-10 outside ref range	**High Risk**	**Low Risk**
Yes	13	32
No	88	563

PPV= 13/ (13+32) = 29%

NPV= 563/ (88+563) = 86%

IL-6/IL-10 ratio

Table 4.2. A cross-tabulation of patients with abnormal IL-6/IL-10 ratio and antenatal depressive risk as determined by the EPDS allows calculation of PPV and NPV.

	Antenatal Depressive Risk	
IL-6/IL-10 outside ref range	**High Risk**	**Low Risk**
Yes	7	32
No	94	563

PPV= 7/ (7+32) = 18%

NPV= 563/ (94+563) = 79%

Bayes' theorem was applied to test the suitability of the biomarkers, a statistical rule based on probability theory which described the probability of an event based on prior knowledge of conditions that might be related to the event[280]. This equation was applied in this case to test the chance that somebody will have depression given that they have a biomarker reading outside the reference range (the positive test result). The probability of having depression given a positive test result *(Pr(H|E))* was calculated using Bayes' theorem with equation:

$$\Pr(H|E) = \frac{(\Pr(E|H))(\Pr(H))}{(\Pr(E|H)\Pr(H))+((\Pr(E|notH))(\Pr(notH))}$$

Where *P(E|H)* is the chance of a true positive (having PND with a positive test result), and *Pr(H)* is the chance of PND (estimated as 15% in the general population). Using the probability of PND, this allows the suitability of the test in a real world population to be estimated.

For an IL-10 test, based on those scoring outside of the calculated reference range, *Pr(H|E)* = 26.77%, and for the IL-6/IL-10 ratio test, *Pr(H|E)* = 13.14%, meaning around a quarter of those with 'abnormal' IL-10, and around 13% of those with 'abnormal' IL-6/IL-10 ratio will have PND. Bayes' theorem corrects for errors and accounts for false positives resulting in the relatively low chance of depression given a positive test. These results therefore suggests that these biomarker screens would be best used as a negative screen, to rule out those who are low risk for PND based on IL-6/IL-10 concentration scoring within the reference range.

4.3 BDNF

For each sample, raw OD was converted into its corresponding mean concentration and % CV was calculated as before with values <20 considered acceptable. Mean BDNF (N=688) was 2032pg/mL (SD 2246), with a range of 46-18370pg/mL.

BDNF is significantly correlated with EPDS score (*p*=.025, r=.09). On visual inspection this appears to be a positive association, with BDNF levels increasing with depressive risk. On plotting a scatter plot of the BDNF data however, it was observed that although the correlation is a negative one, a downward trend is in fact apparent outside of the 'normal' data (Figure 4.7).

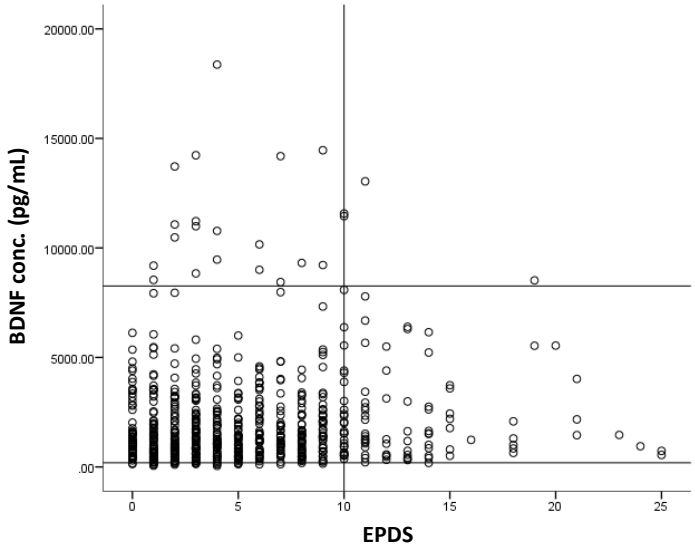

Figure 4.7. A plot of EPDS score against BDNF concentration. The vertical reference line indicates EPDS cut-off 10, and a horizontal line indicates the upper BDNF reference range (mean ±1.96 SD).

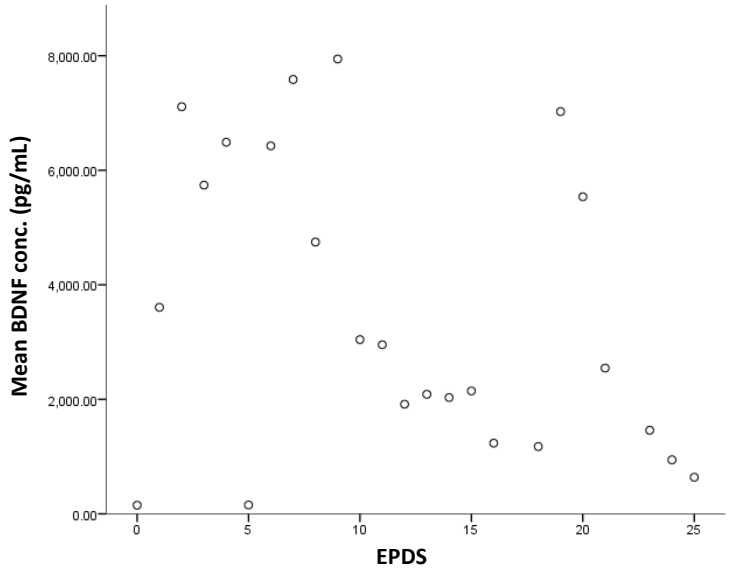

Figure 4.8. Plot of 'abnormal' data points - scoring either in the depressed category or outside BDNF reference range.

Data points inside the lower left quadrant are from participants scoring in the 'healthy' EPDS category and the 'healthy' BDNF category (Figure 4.7). The vast majority of points lie within this quadrant (93.6%), however it is perhaps more interesting to view the 'abnormal' data (N=44) separately to explore the relationship between BDNF and PND (Figure 4.8). It is clear that the majority of samples with very high BDNF levels are from the non-depressed EPDS category.

The reference range for BDNF concentration was determined with the healthy control group (low depressive risk). Values were log-transformed to achieve normality and the parametric method of reference range calculation was then used. This was calculated as 190-8260ng/mL, based on a mean of 1963ng/mL, and 44 samples (6.4%) fell outside of this range. After calculating the reference range for BDNF, statistical associations between 'abnormal' samples and PND were investigated. For this analysis, only those with EPDS ≥ 10 or BDNF outside the reference range were included. The plot of this data can be seen in Figure 4.10. Using only these data points, the correlation, which is now visible from the scatterplot, is negative ($r=-.25$, $p<.01$). It is apparent that high BDNF levels are generally associated with low depressive risk, whereas low BDNF levels are associated with high depressive risk. A couple of data points do not follow this trend but the correlation remains significant. Figure 4.9 presents a comparison those at risk of depression in the antenatal period compared with the postpartum period, and BDNF levels are reduced in the postpartum risk group.

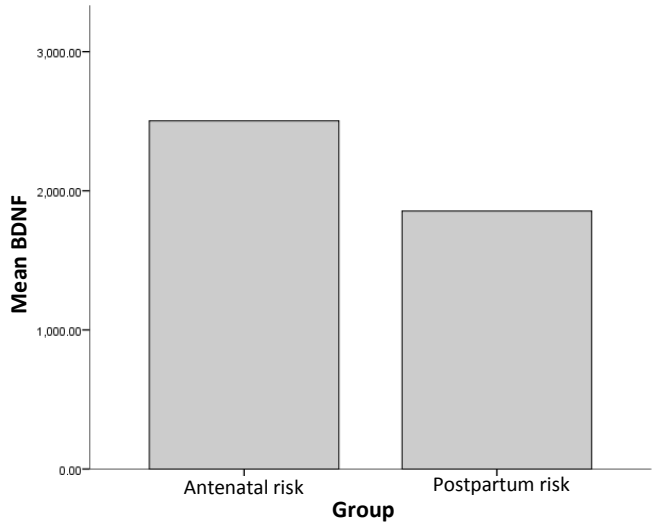

Figure 4.9. Mean BDNF concentration (pg/mL) split by depressive risk group (antenatal or postpartum only).

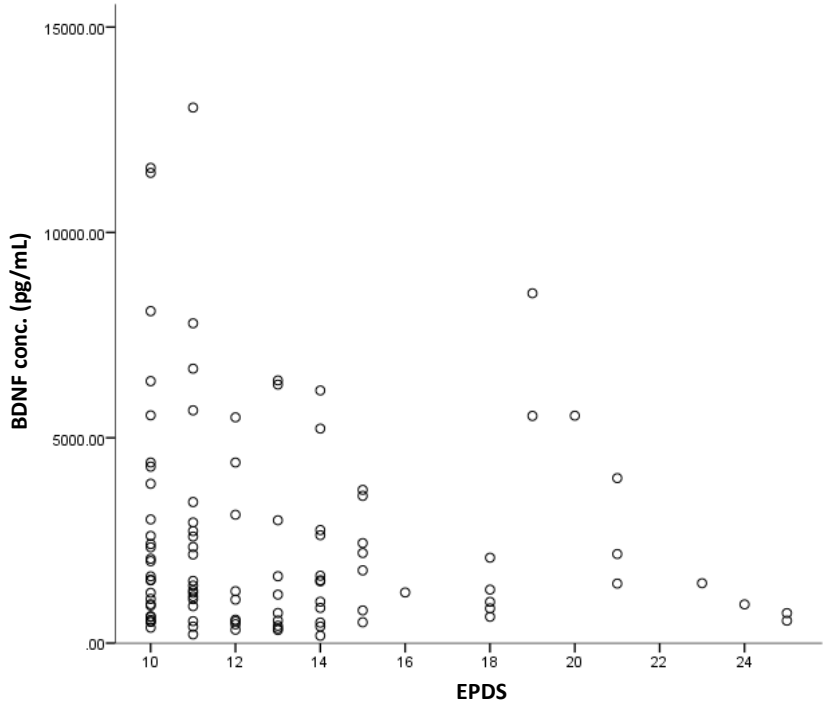

Figure 4.10. Plot of 'abnormal' data points - scoring either in the depressed category or outside BDNF reference range.

Using the calculated reference ranges to explore the use of a cut-off value for these biomarkers in screening, the positive predictive value (PPV) and negative predictive value (NPV) were calculated. For this investigation, the aim was to test whether a high BDNF value exceeding the reference range can predict low depressive risk (EPDS<10). This test has a specificity, or true negative rate, of 96.8%, (CI 95.04-98.06) and a NPV of 85.5% (CI 85.01-86.06%). This means that we can say with high probability that if BDNF exceeds the upper reference range, this participant will be in the low depressive risk group. As a negative screen it performs with 96.8% certainty. The overall accuracy of this test is 83.29% (CI 80.3-85.99).

Of the participants who had high BDNF levels above the reference range, case-wise analysis was carried out and observations recorded. Raised levels of BDNF are proposed to protect against PND. In this sample of women with high BDNF (N=23), the majority of the participants (N=19) screened negative on the EPDS. It is possible that the mechanism of protection is provided both by positive psycho-socioeconomics and biological effects via high BDNF. Another possible mechanism for protection is the role of BDNF alone, acting despite the presence of certain psycho-socioeconomic risk factors. This relationship will be explored later in Chapter 6.

Summary of biomarker associations

Table 4.3. All investigated biomarkers with their corresponding significant correlations, p-value to demonstrate significance level, and r to show the direction and magnitude of correlation.

Biomarker	Significant correlations	*p-value*	r
BDNF	Antenatal EPDS	.025	0.09
	(EPDS – 'abnormal BDNF' only)	.003	-0.25
IL-10	Antenatal EPDS	.033	-0.8
	EPDS risk category	.013	-1.0
IL-6	Social status	.048	0.08
	BMI	<.001	0.3
IL-6/IL-10	Antenatal EPDS	.01	0.1
	EPDS risk category	.07	0.1
	BMI	<.001	0.25
	Mode of delivery	0.02	0.9

Table 4.3 provides a summary of all significant associations identified between biomarkers and psychosocial factors or EPDS score. Statistical analysis and modelling did not reveal any significant association between the calculated combined biomarker z-score and EPDS score. No statistical associations between biomarkers and postpartum EPDS scores were found. No associations between IL-6, IL-10 or their ratio with BDNF were found. Analysis of BDNF and DPA/APA score identified no significant associations.

4.4 DNA Methylation

A pilot study was conducted with a sub-group of participant samples to assess whether global DNA methylation changes are associated with perinatal depressive risk as determined by EPDS score. DNA methylation is characterised by the addition of a methyl group to the fifth cytosine ring, resulting in 5-methylcytosine (5-mC). The percentage of 5-mC in DNA from study participants was measured. Relationships with levels of folate and B-12 were additionally explored for participants with this data available, due to known links between folate and DNA methylation, and closely linked metabolic pathways between B-12 and the ingestion of methyl groups[190 231]. Genomic DNA was extracted from participant blood samples collected at 24-29 weeks gestation as described in the methods and assayed to calculate % 5mC. Absorbance measurements were compared against the generated standard curve to calculate % 5mC in the samples.

Experiment 1 included 38 random samples from the study population. Antenatal EPDS score was significantly correlated with 5-mC% (r=0.39, *p*=.016); increased levels are observed in those with EPDS ≥ 10 (Figure 4.11). Mean 5-mC% in women in the low risk group was 3.81, compared with 5.98 in the high risk group. Methylation levels in this group were also found to be significantly correlated with social status (*p*=.046).

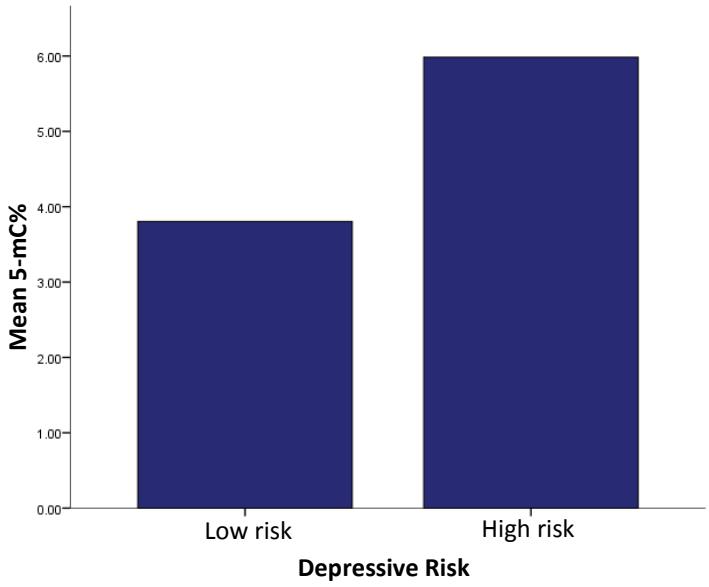

Figure 4.11. Mean levels of global DNA methylation are significantly increased in women with raised EPDS (≥10).

Experiment 2 included samples from the Coventry cohort (N=40). For these participants, Folate and B12 measurements were recorded and entered into the analysis. EPDS score was also significantly correlated with 5-mC% in this cohort (r=0.31, *p*=.025), and this remained significant in a linear regression model (*p*=.016). Despite an observed association between EPDS and Folate, this relationship failed to reach significance in this population (*p*=.059). No relationship with B12 was observed.

A highly significant relationship was observed between methylation levels and housing status (r=0.46, *p*=.006), with twice the 5-mC% in women staying with friends compared with owning their home (Figure 4.12). No women in this group were living in Social Housing and so this association could not be tested.

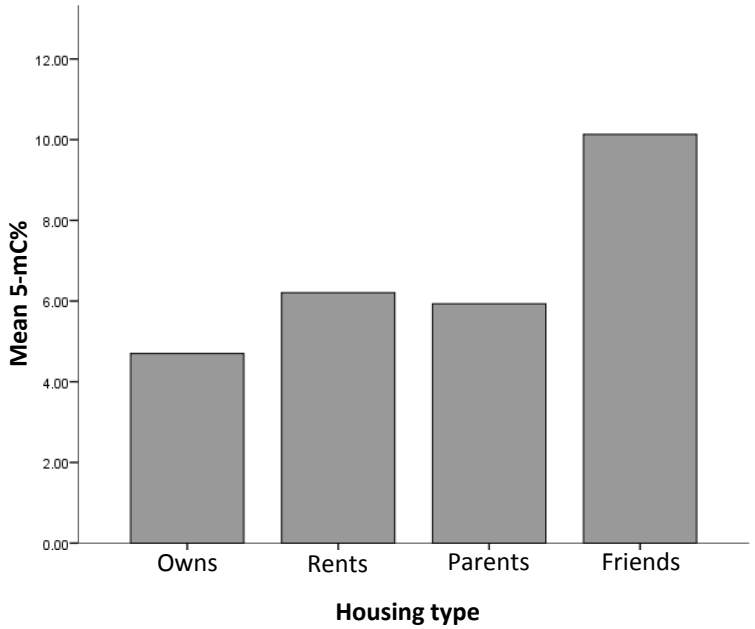

Figure 4.12. Global DNA methylation levels are associated with housing status, with the lowest %5-mC in women who own their home and the highest %5-mC in women who have no permanent home staying with friends.

5 Results: Genotyping

5.1 Chapter Five Abstract

This thesis investigates risk factors for perinatal depression; in Chapter 3 psycho-socioeconomic risk factors have been explored, and in Chapter 4 the involvement of an acute inflammatory response was investigated through the use of circulating biomarkers. Depression is related to a broad range of physiological processes in the body, some acute and short-term, such as the release of pro-inflammatory cytokines, and other more pre-determined factors, such as genetics. The acute response is in turn associated with genetic predisposition, and this may be true for the stress response as determined by the hypothalamic-pituitary-adrenal (HPA) axis.

There is strong evidence for the existence of a predisposition to depressive risk, specifically relating to the HPA axis. Aim 4 of the study asks - 'Does HPA axis sensitivity provide a marker for PND by genotyping target SNPs?', and this will be addressed within this chapter. A link between three single nucleotide polymorphisms (SNPs) related to HPA axis function (Bcl1, rs242924 and rs242939), and risk of PND has been explored. The initial aim of this genetic study was to test whether previous findings were replicated in this larger cohort. A second aim was to test the clinical feasibility of genotyping in the laboratory. The overall aim was to test whether the inclusion of these genotyping results, when combined with the rest of the collected data, improves prediction of depressive risk. This study additionally aimed to test the feasibility of this analysis as a routine test in a hospital laboratory.

As detailed in Chapter 2, DNA samples were extracted and purified from participant's blood samples and women were genotyped for the presence of three key SNPs. In this Chapter, the results of the genotyping study are presented. Participants from the main Warwick cohort, for whom both biomarker and psychosocial variables were available, were included in the genetic sub-study (N=480). Genotyping was carried out for women from this cohort, 21% of which were at-risk of some form of PND, either antenatal or postpartum. This included 46 women from Group 1, 30 from Group 2, and 25 from Group 3. In addition a further 14 'high risk' samples were included in the SNP analysis from the larger cohort in order to increase sample size in the smaller at-risk groups. SNP frequencies of each

of the 3 SNPs were determined and compared to published variant allele frequencies, and χ^2 tests were used to test whether any difference between observed and expected genotypes is significant. Odds ratios and 95% confidence intervals were calculated for SNPs. A case-control analysis first compared EPDS scores between genotypes, assessing any significant associations with presence of the minor allele. Participants were categorised for this according to EPDS ≥ 10 to establish low risk and high risk groups.

In the analysis of the whole study population, no statistically significant relationships were observed between the SNPs and PND risk. Participants were then split into those with and without a family history of PND, to further explore the genetic component of PND aetiology. This analysis reveals some interesting findings, such as a higher likelihood of past depression, a key risk factor for PND, in women with the Bcl1 minor allele. This was explored further, revealing an indirect association with IL-6/IL-10 ratio, which in turn has an effect on depressive risk as described in the previous chapter. The rs242939 SNP was also found to be associated with EPDS scores in women with a family history, more specifically with a likelihood of symptoms easing postpartum. This SNP could therefore be overrepresented in women at risk of antenatal depression rather than postpartum. Overall there is insufficient evidence from this genetic study to support the use of SNPs in the prediction of PND, although the findings related to family history are promising and warrant further investigation in a larger cohort of women with a family history of PND to better explore its genetics.

5.2 SNP 1 - Bcl1 (rs41423247, NR3C1)

The Bcl1 polymorphism is a G>C single nucleotide variation, with variant allele frequency 0.34. The population is in Hardy-Weinberg equilibrium (HWE) ($\chi^2 = 0.34$, $p=0.85$) calculated from the observed and expected frequencies of alleles (Table 5.1).

Table 5.1. Observed and expected genotype frequencies for Bcl1 SNP for calculation of HWE.

Allele	Observed frequency	Expected frequency
C/C	193	199
C/G	209	205
G/G	55	53

Mean antenatal EPDS for wild type (WT) carriers is 5.8, compared with 5.6 for heterozygotes (HET) and 4.4 from homozygotes (HOM). The minor allele for Bcl1 is protective (OR 0.74, CI 0.43-1.27). For postpartum EPDS scores, mean WT score was 6.1, HET score 5.9 and HOM score 4.6. The minor allele appears to be somewhat protective with slightly lower EPDS for carriers, (OR 0.94, CI 0.57-1.53) but scores are reduced less postpartum than in the antenatal period. Chi-squared analysis did not find a significant association between genotype and EPDS score for Bcl1.

The presence of one or two copies of the minor allele is considered 'positive' and analysis with this variable is the least stringent model which maximises chance of statistical significance in relatively small samples sizes. The mean EPDS scores for 'positive' and 'negative' Bcl1 status is shown in Table 5.2. Considering the 'positive' SNP (Figure 5.1d), difference in mean EPDS score both at the antenatal and postpartum time points was negligible and was not found to be significant.

Table 5.2. Mean EPDS score by time point, dependent upon Bcl1 status.

Time-point	EPDS	Bcl1 status
Antenatal	5.63	Negative
Antenatal	5.86	Positive
Postpartum	5.36	Negative
Postpartum	5.67	Positive

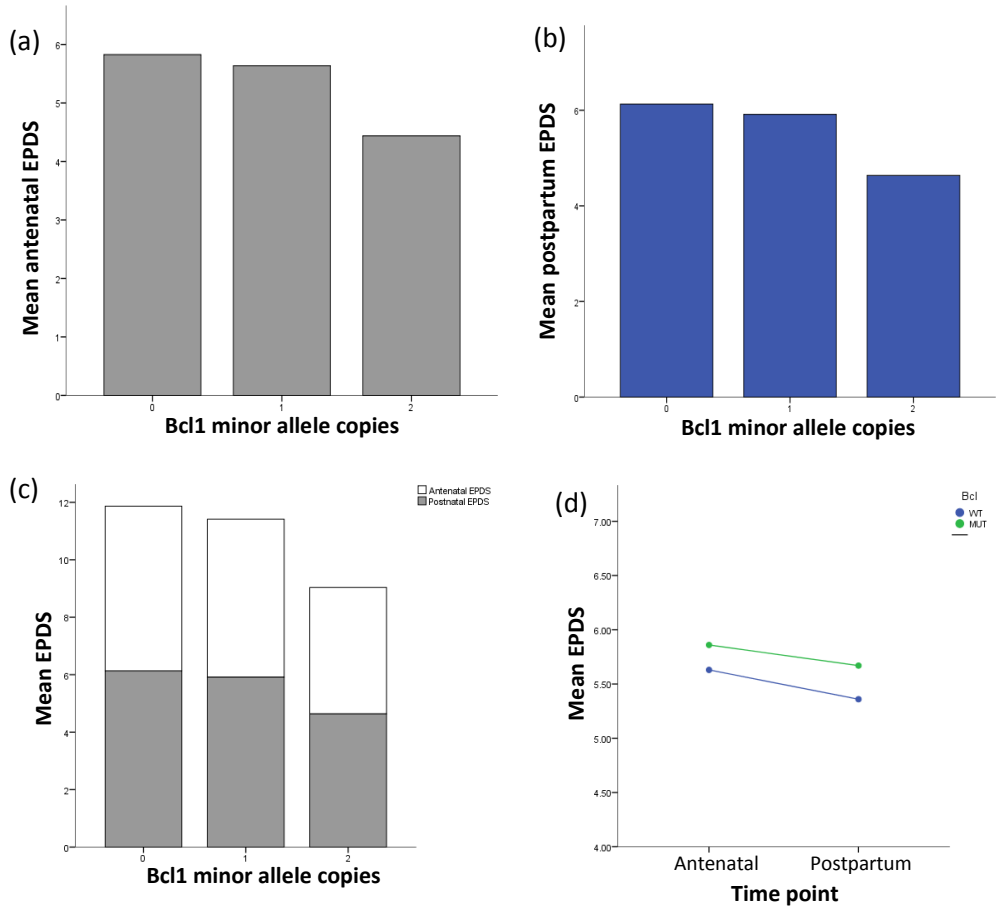

Figure 5.1. (a) Mean antenatal EPDS score split by bcl1 minor allele copy number. (b) Mean postpartum EPDS score split by bcl1 copy number. (c) The overall effect on EPDS score for bcl1 copy number at both time-points. (d) The association between bcl1 status of 'presence' (MUT) or 'absence' (WT) of the minor allele and the overall effect on EPDS score over time. None of the effects were found to be statistically significant.

To further investigate an association between the selected SNPs and family history, the participants were next split by whether they reported a family history of PND. It was found that in women who have a family history of PND (N=77), Bcl1 status was significantly associated with their personal history of depression in correlation analysis (r=.25, *p*=.035). In women without a family history, presence of the minor allele was associated with an 8% incidence of depression, whereas in women with a family history, the minor allele was associated with a 48% incidence of depression. It is possible that genetic status is acting indirectly by predisposing to depression outside of pregnancy, which is in turn a strong risk factor for developing PND. The presence of the Bcl1 minor allele may represent a predisposition to depression in general, most prominent when there is a family history. The sample size of women with a family history in this study is relatively low, although in line with other studies assessing family history as a risk factor for PND[281], and a larger powered study would be required to further explore the effects of these SNPs.

When women with a family history are excluded, Bcl1 is significantly associated with a slightly reduced average EPDS score (r=-.09, *p*=.049), demonstrating a protective effect. When the associations with the measured inflammatory biomarkers were investigated in the group of women with a family history of PND, a link between Bcl1 and IL-6/IL-10 ratio was identified in correlation analysis (r=-.22, *p*=.029). This link also remains significant in the whole study population (r=-.08, *p*=.041). The presence of the Bcl1 minor allele is significantly associated with a mildly reduced IL-6/IL-10 ratio, and may indirectly protect against symptoms of PND. Partial correlation analyses reveal that this association is mediated through IL-6/IL-10 ratio, and this has a greater influence on EPDS scores than the Bcl1 status.

5.3 SNP 2 - rs242924, CRHR1

The rs242924 polymorphism is a G>T single nucleotide variation, with variant allele frequency 0.47. The population is in Hardy-Weinberg equilibrium. ($\chi^2 = 0.27$, $p=0.87$), as calculated from Table 5.3.

Table 5.3. Observed and expected genotype frequencies for rs242924 SNP for calculation of HWE.

Allele	Observed frequency	Expected frequency
G/G	122	127
G/T	228	225
T/T	101	99

Mean antenatal EPDS for WT carriers is 5.73 compared with 5.39 for HET and 5.22 for HOM. The mean EPDS scores for 'positive' or 'negative' SNP status is shown in Table 5.4. The minor allele is associated with a mild reduction in antenatal EPDS (OR 0.88, CI 0.49-1.56). For postpartum scores, WT mean is 5.93 compared with 5.66 for HET and 6.27 for HOM (Figure 5.2). Overall, for all genotyped samples, the minor allele now associated with a slight increases risk of PPD (OR 1.06, 0.62-1.79). There was no significant association identified in Chi squared analysis for either antenatal or postpartum EPDS scores.

Table 5.4. Mean EPDS score by time point, dependent upon rs242924 status.

Time-point	EPDS	rs242924 status
Antenatal	6.05	Negative
Antenatal	5.64	Positive
Postpartum	6.31	Negative
Postpartum	6.11	Positive

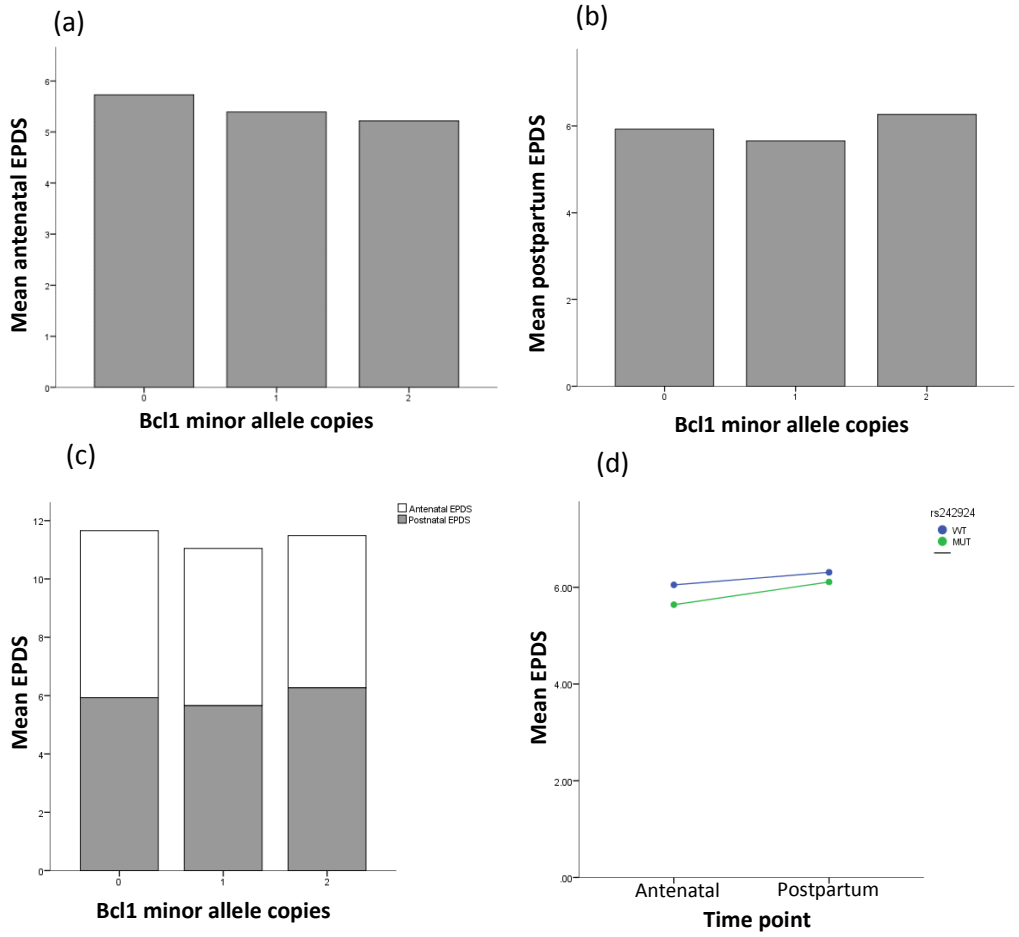

Figure 5.2. (a) Mean antenatal EPDS score split by rs242924 minor allele copy number. (b) Mean postpartum EPDS score split by rs242924 copy number. (c) The overall effect on EPDS score for rs242924 copy number at both time-points. (d) The association between rs242924 status of 'presence' (MUT) or 'absence' (WT) of the minor allele and the overall effect on EPDS score over time.

An association between genetics and family history was explored by splitting participants according to family history. In women with a family history of PND, the rs242924 minor allele appears to be related to a personal history of PND. In the general population of women who report no family history, the prevalence of past PND is low at around 6%, however in those with a family history of PND, the prevalence of personal PND rises to 16%. Women with the rs242924 minor allele are more likely to have a personal history of PND, but this just fails to reach significance at *p*=.055. Although this SNP isn't directly associated with EPDS, its effect may be indirectly acting through family and personal history of PND, a very important risk factor in the development of future PND. The lack of a confirmed relationship with depressive symptoms in this study may be attributed to the small sample size of women with a family history.

5.4 SNP 3 – rs242939, CRHR1

The rs242939 polymorphism is a C>T single nucleotide variation, with variant allele frequency 0.06. Due to the rare frequency of genotype T/T, calculation of Hardy-Weinberg with χ^2 is inappropriate in this sample size with an expected value <5. There is however some observed deviation from expected values as seen in Table 5.5.

Table 5.5. Observed and expected genotype frequencies for rs242939 SNP.

Allele	Observed frequency	Expected frequency
C/C	402	393
C/T	39	54
T/T	8	2

Table 5.6. Mean EPDS score by time point, dependent upon rs242939 status.

Time-point	EPDS	rs242939 status
Antenatal	5.84	Negative
Antenatal	5.10	Positive
Postpartum	6.09	Negative
Postpartum	6.43	Positive

Participants WT for rs242939 have a mean antenatal EPDS score of 5.52, and a mean postpartum score of 5.80. This is compared with HET carriers with mean antenatal score of 4.77 and postpartum score 6.36. Mean EPDS score by 'positive' or 'negative' SNP status is shown in Table 5.6. Overall, for all genotyped samples, presence of the minor allele associated with a slight reduction in EPDS score in both the antenatal (OR 0.97) and postpartum (OR 0.65) time points, with the effect considerably more noticeable at the postpartum stage. Homozygotes for this SNP had antenatal mean score 4.88 and postpartum score of 5.84. The exploration of genotype effect on score is shown in Figure 5.3. Chi-squared analysis did not find a significant association between genotype and EPDS score for rs242939.

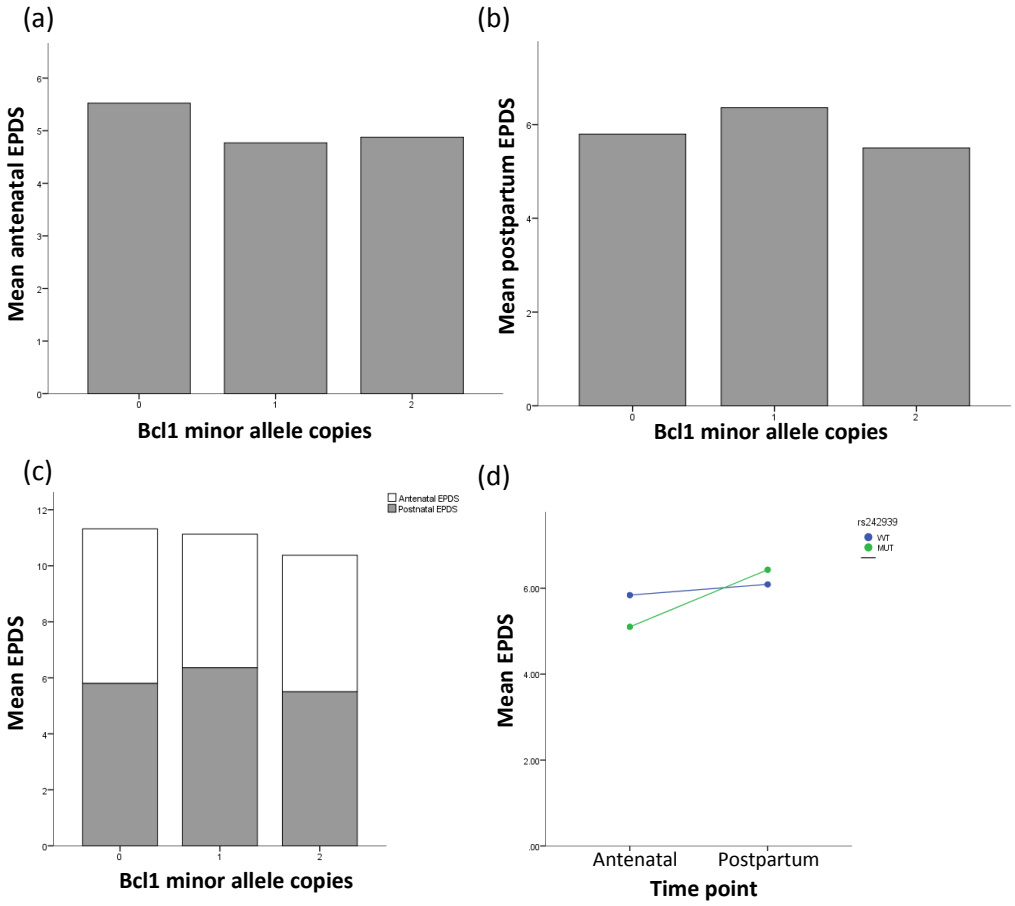

Figure 5.3. (a) Mean antenatal EPDS score split by rs242939 minor allele copy number. (b) Mean postpartum EPDS score split by rs242939 copy number. (c) The overall effect on EPDS score for rs242939 copy number at both time-points. (d) The association between rs242939 status of 'presence' (MUT) or 'absence' (WT) of the minor allele and the overall effect on EPDS score over time.

The relationship between the rs242939 SNP and family history of PND was investigated by splitting the participants into those with and without a family history. The analysis revealed that this SNP is significantly associated with an easing of depressive symptoms postpartum, known as the DPA score. This SNP is associated with a difference of an average -1.6 points (*p*=.049*)*, meaning that symptoms are likely to ease postpartum compared with in the antenatal period. This SNP may be more associated with 'Group 1' type women who are at high risk of antenatal but not postpartum depression.

A one-way fixed effects analysis of variance (ANOVA) was conducted to evaluate the null hypothesis that EPDS means were equal across the 3 selected SNPs. A statistically significant difference was not found for any of the SNPs. Despite the lack of statistical significance, a protective effect by the presence of the minor allele was observed. For each of the three SNPs, mean antenatal EPDS score was lower in the presence of the minor allele. The largest protective effect was found for Bcl1 (OR 0.74, 0.43-1.27), followed by rs2424924 (0.88, 0.49-1.56) and a very mildly protective effect was observed for rs242939 (0.97, 0.41-2.28).

For postpartum EPDS scores, Bcl1 minor allele still exhibited a protective effect, but this was smaller in magnitude (0.94, 0.57-1.53). The rs242924 SNP mildly increased the risk of depression, (OR 1.06, 0.62-1.79), conversely to its protective effect on antenatal depression. The rs242939 minor allele remained protective, but now to a greater extent in the postpartum period (0.65, 0.28-1.53). These effects were not found to be statistically significant. Overall, there is not enough evidence in this study to support the use of these three SNPs in biomarker analysis for perinatal depression.

6 Analysis

In the previous three results chapters, relationships between a number of investigated factors and perinatal depressive risk have been explored. In Chapter 3, key psycho-socioeconomic risk factors were identified for both antenatal and postpartum depressive symptoms, based on exploratory regression modelling. As described in Chapter 4, the analysis of circulating biomarkers identified a relationship between cytokines IL-6/IL-10 ratio and antenatal depressive symptoms. Secondly, an association between Brain Derived Neurotrophic Factor and antenatal depressive symptoms was identified. In Chapter 5, the effect of genetic factors was explored, with observations suggesting that SNPs may offer some level of protection/risk for depressive risk, in particular in women with a family history of PND. Taken separately, the interpretation of each of the individual risk factors explored is limited. Taken together however, this analysis may start to unpick the relationships between the various factors and perinatal depressive risk as well as any interplay between risk factors.

The aim of this Chapter is to combine the results from the previous three chapters into the final analysis. This analysis addresses aim 6 – do the investigated biomarkers improve the prediction of PND symptoms when compared with psycho-socioeconomic risk factors? First the established model identified in Chapter 3 will be extended to this larger cohort of interest to validate findings and explore the value of the selected psycho-socioeconomic factors in predicting risk. Each of the circulating biomarkers analysed will then be added to this model in a stepwise approach to determine the optimal inclusion of variables. This will investigate whether adding this data improves the prediction of the model, and if so, the importance of each of the markers. The risk/protection provided by the biomarkers will be quantified by the subsequent effect on EPDS score. The approach to this analyses is demonstrated in Figure 6.1.

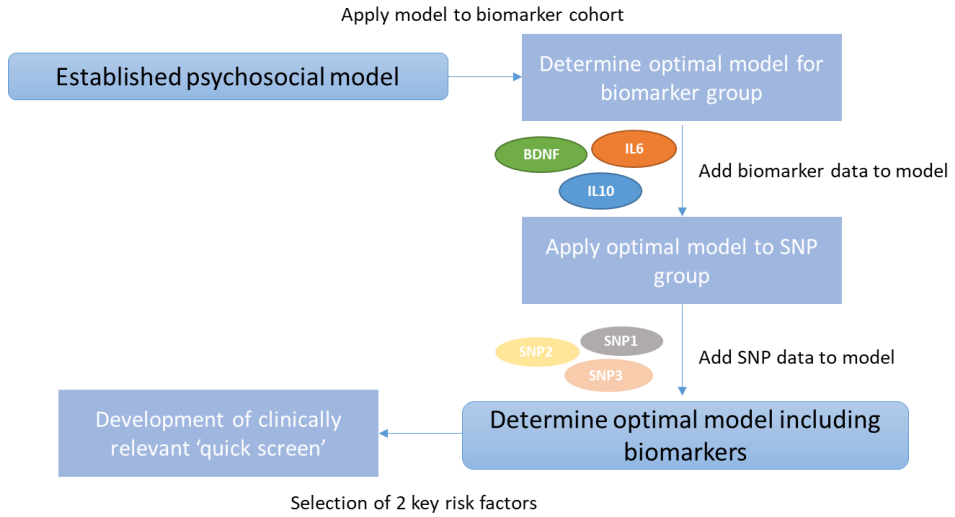

Figure 6.1. Schematic demonstrating the approach to analysis combining the optimal model for psychosocial risk factors with biomarker and SNP data.

6.1 Psycho-socioeconomic Factors

As described in Chapter 3, psycho-socioeconomic risk factors were first modelled dependent upon time course and progression of depressive symptoms to categorise participants into risk groups. This was repeated for the larger group to now include N=696, all participants with biomarker data measured.

Table 6.1. Results of the multinomial regression analysis which models risk factors dependent upon Group. For each significant variable, p values, Relative Risk scores, and confidence intervals (CI) are shown.

Group	Risk Factors	Protective Factors
1 – High Postpartum risk	Past history of PND (p=.001, RR=4.10, CI 1.801-9.28) Past history of depression (p=.009, RR= 2.70, CI 1.28-5.69)	Education (baseline 16) – professional level (p=.027, RR=0.14, CI 0.03-0.80)
2 – High Antenatal Risk	Past history of anxiety (p=.002, RR= 3.20, CI 1.54-6.67)	
3 – Overall high risk	Past history of depression (p=.000, RR=7.86, CI 3.55-17.38) Past history of PND (p=.003, RR=4.13, CI 1.61-10.60)	

Variables which reached significance in the model are shown in Table 6.1. For Group 1, risk factors were a past history of PND and a past history of depression, adding 4 and 3 points to EPDS score respectively. Higher educational level was a mildly protective factor. Other factors which did not reach significance but do contribute to EPDS score was a past history of anxiety (+1 point), age 30-34 (-0.8 points) and higher level of education (-0.7 points for degree level).

For group 2 there was one significant risk factor, a past history of anxiety, which contributes 7 points to the score. Other risk factors which did not reach the level of significance were past history of depression (+1.8 points), past history of PND (+1.7 points), feeling unsupported (+1.4 points), and smoking pre-pregnancy (+1.4 points). Employment was a protective factor when compared with unemployment, with even a low level routine job decreasing EPDS by -0.7 points. Age >24 was also protective compared with women under 25.

For group 3, the highest risk group, two factors were statistically significant: past history of depression (+7 points) and past history of PND (+4 points). Further notable contributing factors which increased score but did not reach significance were a past history anxiety (+1.7 points), age > 39 (+1.1 point), feeling supported (-0.2 points), higher educational level (Diploma level +1 point), and employment (Supervisor level +0.8 points). All levels of employment were protective when compared with unemployment. Overall the findings of the group analysis in this larger cohort are very similar to the results of the smaller sub-study analysed previously.

6.1.1 Prediction of antenatal EPDS score

For the second stage of the analysis, linear regression modelling was applied based on the optimal model established in Chapter 3 to the larger Cohort A. One factor from the optimal model (Education – no exams) was excluded from the model since no participants in this smaller cohort belonged to this level. This model with the psycho-socioeconomic variables alone accounted for 16.4% of the variance in EPDS scores in this cohort of N=696 (Table 6.2).

Table 6.2. Results of a linear regression model to predict antenatal EPDS score with variables included based on the optimal model previously established.

	B	*SE B*	*β*
Constant	4.14	0.24	
Age			
(baseline - 30-34)			
18-24	1.62	0.63	.10*
25-29	-0.13	0.40	-.01
40-45	0.63	0.57	.04
Mode of delivery			
(baseline – normal vaginal birth)			
Elective C section	0.90	0.40	.08*
Social status			
(baseline – Higher managerial/professional)			
Semi routine/routine work	1.09	0.37	.11**
Unemployed/student	4.07	1.06	.14***
Past history PND	1.28	0.63	.07*
Smoking pre-pregnancy	0.58	0.51	.04
Past history depression	1.89	0.52	.14***
Past history anxiety	2.53	0.47	.20***
BMI			
(baseline (18.5-24)			
30-34	-0.69	0.40	-.06

	B	*SE B*	*β*
35-39	-0.66	0.63	-.04
40-44	-1.18	1.04	-.04

R^2 = .16.4 F=18.253. *p<.05, **p < .01, ***p < .001.

6.1.2 Prediction of postpartum EPDS score

The linear regression model was repeated with postpartum EPDS score as the outcome based on the previously established optimal model. This model explains 33.3% of the variance in EPDS scores in this study population (Table 6.3). This can be further increased to 35% when all levels of categorical variables are inputted into the model.

Table 6.3. Results of a linear regression model to predict postpartum EPDS score with variables included based on the optimal model previously established.

	B	*SE B*	*β*
Constant	8.91	3.67	
Antenatal EPDS score	0.47	0.04	.43***
Past history depression	2.63	0.50	.17***
Past history PND	1.30	0.62	.07*
Parity (baseline – 0)			
4	4.10	1.34	.09**
5	6.81	2.80	.07*
Education (baseline – Degree)			
Diploma level	0.43	0.38	.01
Age (baseline – 30-34)			
<18	9.64	3.94	.08*
Smoking pre-pregnancy	-1.15	0.48	-.08*
Drinking pre-pregnancy	0.71	0.32	.07*

	B	*SE B*	*β*
Medication	0.46	0.41	.04
Total gestational length	-0.02	0.01	-.06*
BMI (baseline 18.5-24)			
35-39	1.69	0.61	.09**

R^2 = .33. *p < .05, **p < .01, ***p < .001.

6.2 Addition of Biomarkers to Prediction Modelling

The original model predicting antenatal EPDS scores included psycho-socioeconomic variables only, and accounted for 16.4% of the variation in antenatal EPDS scores. In the next stage of the analysis laboratory data was additionally entered into the model to assess whether any of the biomarkers measured for the participants would improve the model's prediction.

6.2.1 Ratio of IL-6/IL-10

IL-6/IL-10 ratio was added into the antenatal model's predictor variables, with a resultant prediction of 17.4%. IL-6/IL-10 was then entered in a backward stepwise approach with the established predictor variables to assess its importance amongst the psycho-socioeconomic variables. The optimal model selected with the backward approach included IL-6/IL-10 as an important influence on the model. According to this approach, the 9 key factors to assess are: age 18-24, Elective C-section (ELCS), unemployment, low level routine work, past history PND, past history depression, past history anxiety, BMI 30-34 and, interestingly, IL-6/IL-10 ratio (Table 6.4). The ratio of these cytokines ranks above a Past history of PND, Elective C-section, and BMI 30-35 in this model.

If all levels of the categorical variables are inputted into the model, for example all age categories rather than just the 18-24 category of interest, in addition to IL-6/I-L-10 ratio, prediction rises to 19.5%. Removal of IL-6/IL-10 results in prediction of 18.7%.

Table 6.4. Resuts of a stepwise linear regression model to predict antenatal EPDS score with the addition of IL-6/IL-10 ratio into the established psychosocial model.

		B	*SE B*	*β*
Step 1				
	Constant	4.85	0.17	
	Past history anxiety	3.40	0.46	.27***
Step 2				
	Constant	4.23	0.25	
	Past history anxiety	3.35	0.45	.27***
	Social status (baseline Higher)			
	Low level supervisory	1.27	0.54	.09*
	Routine	1.58	0.40	.16***
	Unemployed/student	4.58	1.05	.16***
Step 3				
	Constant	4.13	0.25	
	Past history anxiety	2.76	0.47	.22***
	Social status (baseline Higher)			
	Low level supervisory	1.20	0.54	.08*
	Routine	1.47	0.39	.15***
	Unemployed/student	4.79	1.04	.17***
	Past history depression	2.08	0.52	.15***
Step 4				
	Constant	4.16	0.31	
	Past history anxiety	2.77	0.47	.22***
	Social status (baseline Higher)			
	Low level supervisory	1.23	0.54	.09*
	Routine	1.30	0.41	.13**
	Unemployed/student	4.08	1.09	.14***
	Past history depression	2.07	0.52	.15***
	Age (baseline 30-34)			
	18-24	1.32	0.65	.08*
Step 5				
	Constant	3.71	0.33	

		B	*SE B*	*β*
	Past history anxiety	2.78	0.47	.22***
	Social status (baseline Higher)			
	Low level supervisory	1.22	0.54	.09*
	Routine	1.25	0.41	.12**
	Unemployed/student	3.96	1.08	.14***
	Past history depression	2.06	0.52	.15***
	Age (baseline 30-34)			
	18-24	1.63	0.65	.10*
	Mode of delivery			
	Elective C-section	1.22	0.41	.11**
Step 6				
	Constant	3.70	0.36	
	Past history anxiety	2.76	0.47	.22***
	Social status (baseline Higher)			
	Low level supervisory	1.31	0.54	.09*
	Routine	1.28	0.41	.13**
	Unemployed/student	4.21	1.09	.15***
	Past history depression	2.11	0.52	.15***
	Age (baseline 30-34)			
	18-24	1.70	0.65	.10**
	Mode of delivery			
	Elective C-section	1.28	0.41	.12**
	BMI			
	30-34	-0.54	0.42	-.05
Step 7				
	Constant	3.46	0.37	
	Past history anxiety	2.75	0.47	.22***
	Social status (baseline Higher)			
	Low level supervisory	1.31	0.54	.09*
	Routine	1.27	0.41	.13**
	Unemployed/student	4.23	1.09	.15***

		B	*SE B*	*β*
	Past history depression	2.13	0.52	.16***
	Age (baseline 30-34)			
	18-24	1.64	0.65	.10*
	Mode of delivery			
	Elective C-section	1.21	0.41	.11**
	BMI			
	30-34	-0.64	0.43	-.06
	IL-6/IL-10 ratio	0.11	0.05	.09*
Step 8				
	Constant	3.41	0.37	
	Past history anxiety	2.65	0.47	.21***
	Social status (baseline Higher)			
	Low level supervisory	1.28	0.54	.09*
	Routine	1.21	0.41	.12**
	Unemployed/student	4.07	1.09	.14***
	Past history depression	1.99	0.52	.15***
	Age (baseline 30-34)			
	18-24	1.70	0.65	.10**
	Mode of delivery			
	Elective C-section	1.10	0.41	.08*
	BMI			
	30-34	-0.64	-0.06	-.06
	IL-6/IL-10 ratio	0.12	0.05	.09*
	Past history PND	0.63	0.63	.07*

		B	*SE B*	*β*
Step 9				
	Constant	3.41	0.37	
	Past history anxiety	2.64	0.47	.21***
	Social status (baseline Higher)			
	Low level supervisory	1.27	0.54	.09*
	Routine	1.17	0.41	.12**
	Unemployed/student	3.88	1.11	.14**
	Past history depression	1.96	0.52	.14***
	Age (baseline 30-34)			
	18-24	1.62	0.66	.10*
	Mode of delivery			
	Elective C-section	1.11	0.41	.10**
	BMI			
	30-34	-0.65	0.42	-.06
	IL-6/IL-10 ratio	0.11	0.05	.09*
	Past history PND	1.31	0.63	.08*
	Smoking pre-pregnancy	0.43	0.52	.03

Note R^2 = .07 for Step 1; ΔR^2 = .046 for Step 2, ΔR^2 =.020 for Step 3, ΔR^2 = .017 for Step 4, ΔR^2 = .014 for Step 5, ΔR^2 = .011 for Step 6, ΔR^2 = .007 for Step 7, ΔR^2 = .005 for Step 8, ΔR^2 = .001 for Step 9. R^2 at Step 9 = .195
*p < .05, **p < .01, ***p < .001.

In exploratory modelling, biomarkers were additionally inputted to determine order of importance. IL-6/IL-10 was the most important of the biomarkers, and made a small but significant contribution to EPDS score with p=.001. One other biomarker demonstrated significance in a backward stepwise approach – IL-6 alone.

6.2.2 IL-6

In the exploratory modelling stage, IL-6 made a significant contribution to antenatal EPDS prediction (p=.018). In contrast to the IL-6/IL-10 ratio, the relationship between IL-6 and antenatal EPDS score is negative, meaning a raised IL-6 score is

associated with a decreased EPDS score (*B*= -0.9). A model including both IL-6/IL-10 and raw IL-6 now accounts for 19.9% of the variation in EPDS score. With IL-6 alone this slightly falls to 18.5%. Entered into a model containing all factor levels for chosen psycho-socioeconomic variables, in addition to IL-6 and IL-6/IL-10, prediction is highest at 20%. The two biomarkers are both significant in this final model.

Contributions of IL-10 or BDNF were not found to be significant in the modelling analysis. A minor contribution to prediction was observed, however this was negligible and since these variables were non-significant they were excluded from the modelling.

The optimal model to predict antenatal EPDS score has now been established. This includes the following variables: age, mode of delivery, social status, past history of PND, past history of depression, past history of anxiety, smoking pre-pregnancy, BMI, IL-6 concentration, and IL-6/IL-10 ratio. The significant levels of these variables are presented in Table 6.5. The addition of the biomarkers has raised the prediction of the model from 16.4% to 20%, as demonstrated in Figure 6.2. The overall significance level of the model is <.001 in ANOVA.

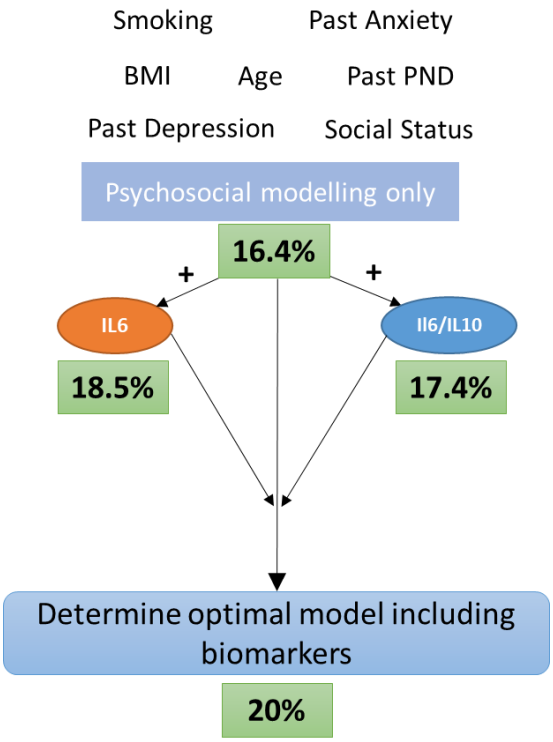

Figure 6.2. Prediction of antenatal EPDS scores using the psychosocial model alone results in 16.4% explanation of the variance. The prediction improves with the addition of IL-6 into the model, and IL-6/IL-10 ratio. When the psychosocial model is combined with these two biomarkers prediction has risen overall from 16.4% to 20%.

Table 6.5. Resuts of a linear regression model to predict antenatal EPDS score with the addition of IL-6/IL-10 ratio and IL-6 concentration into the established psychosocial model.

	B	*SE B*	*β*
Constant	3.65	0.39	
Age			
(baseline - 30-34)			
18-24	1.60	0.65	.10*
25-29	-0.31	0.43	-.03
40-45	0.64	0.58	.04
Mode of delivery			
(baseline – normal vaginal birth)			
Elective C section	1.13	0.41	.10**
Social status			
(baseline – Higher managerial/professional)			
Semi routine/routine work	1.21	0.41	.12**
Unemployed/student	3.88	1.11	.14***
Past history PND	1.26	0.63	.07*
Smoking pre-pregnancy	0.39	0.52	.03
Past history depression	1.96	0.52	.14***
Past history anxiety	2.62	0.47	.21***
BMI			
(baseline (18.5-24)			
30-34	-0.60	0.42	-.05
35-39	-0.82	0.66	-0.5
40-44	-0.80	1.06	-.03
IL-6/IL-10 ratio	0.16	0.05	.13**
IL-6 concentration	-0.71	0.34	-.08*

R^2 = .20. *p<.05, **p < .01, ***p < .001.

The most important risk factor in this model for antenatal depressive risk remains as a history of anxiety (p=<.001). This is closely followed by unemployment (p=<.001) and a past history of depression (p=<.001). IL-6/IL-10 ratio is the next most important risk factor in this model (p=.003), with an increased ratio in women with raised EPDS scores. Another factor which contributes to the model is 'elective C-section (ELCS)' (p=.006). This is an unusual variable in the model, since planned ELCS may represent increased levels of stress in anticipation of a more complex delivery and recovery period, however this finding must be interpreted with caution, since it is unknown whether the EPDS screen preceded the C-section being planned and may therefore be an invalid association to make. Other important factors in the model were young maternal age (p=.015), low social status indicated by occupation of low skill level (p=.003), and IL-6 concentration levels (p=.04).

Biomarker concentrations were entered into regression models to test the prediction of postpartum EPDS scores in the same way, but it was found that this did not improve the postpartum model's prediction. Since all circulating biomarkers were measured in blood samples taken during pregnancy, and postpartum samples were not available in this study, it was expected that any associations between circulating biomarkers and depressive symptoms would be acute and therefore predictive of current antenatal symptoms rather than symptoms occurring months later postpartum.

Regression models were also used to predict the temporal patterns of depressive symptoms, using the average EPDS score (APA) and difference in EPDS score (DPA), but the addition of biomarkers to these models did not improve prediction. It was however found that the APA score, the average throughout, can be predicted in this cohort very well, with a model including a select number of significant psychosocial variables only based on exploratory modelling (EPDS, Parity, Past PND, Past Depression, Past Anxiety and BMI), explaining 75% of the variability in APA score. This can be interpreted as the psychosocial factors explaining the average EPDS score very well. The DPA, the change in EPDS score, was more difficult to predict, with an optimal model explaining 29% of the variability.

6.2.3 SNPs

Modelling was next applied to the cohort for which both SNP genotyping and biomarker analysis was carried out. The established antenatal model, described earlier in this chapter was first applied to this smaller cohort to test whether the same associations are true.

The model was run with this dataset and prediction is the same as with the larger dataset, with 20% of variation in antenatal EPDS scores explained by the current model. The addition of SNP data for all three SNPs, based on presence of the minor allele, raised the prediction of the model slightly to 22%, $p<.001$. Individually however, the SNP data was not statistically significant. The presence of the minor allele for all three SNPs slightly reduced the EPDS score. The effect was minor for all SNPs (Bcl1 -0.4, rs242924 -0.4, rs242939 -0.6). When inputted into backward regression models, the optimal model does not include the SNP data.

Experimental modelling further assessed whether additional genotyping data (WT, HET or HOM) would improve prediction in the model compared with presence or absence of minor allele alone. The model predicts 21% of the variance with this data, and is therefore not an improvement compared with simpler presence/absence data. This did however allow quantification of effect on EPDS score – it is noted that Bcl1 HET genotype exerts a very small effect (-0.1) whereas HOM genotype is 10 times more influential (-1.0), although non-significant here. This effect is not seen in the other two SNPs.

Both IL-6 and IL-6/IL-10 remain in the optimal model for this smaller cohort (Table 6.6) and both variables are significant (N=480).

Table 6.6. Resuts of a linear regression model to predict antenatal EPDS score in subgroup of N=480 with the addition of IL-6/IL-10 ratio and IL-6 concentration into the established psychosocial model.

	B	*SE B*	*β*
Constant	3.77	0.49	
Age			
(baseline - 30-34)			
18-24	1.46	0.76	.10
25-29	-0.43	0.54	-.04
40-45	0.61	0.74	.04
Mode of delivery			
(baseline – normal vaginal birth)			
Elective C section	1.49	0.52	.13**
Social status			
(baseline – Higher managerial/professional)			
Semi routine/routine work	1.48	0.51	.15**
Unemployed/student	4.28	1.30	.16**
Past history PND	1.24	0.85	.06
Smoking pre-pregnancy	-0.10	0.66	-.01
Past history depression	1.52	0.66	.11*
Past history anxiety	2.36	0.59	.18***
BMI			
(baseline (18.5-24)			
30-34	-0.94	0.53	-.08
35-39	-1.78	0.96	-.09
40-44	-0.71	1.29	-.03
IL-6/IL-10 ratio	0.20	0.06	.16**
IL-6 concentration	-0.96	0.40	-.12*

R^2 = .20. *p<.05, **p < .01, ***p < .001.

The findings in this smaller cohort are similar to those of the larger cohorts, but a few differences are observed. Age is no longer a significant factor (18-24 new *p*=.054), but remains influential in the model. Smoking pre-pregnancy has decreased in importance and is no longer significant and its influence negligible. Elective C-section has now increased in importance, as has social status. Past history of PND is not statistically significant in this model, but its influence remains similar and it improves overall prediction. IL-6/IL-10 ratio (*p*=.002) and IL-6 concentration (*p*=.017) have both become increasingly important in this cohort.

The rank of risk factor importance in this cohort is now slightly altered. A past history of anxiety remains as the most important factor to consider. The ratio of IL-6/IL-10 is now the joint second most influential risk factor, alongside social status. Elective C section ranks next, followed by IL-6 concentration levels. Past history of depression has reduced in importance and now ranks after IL-6.

6.2.4 Clinical transferability of biomarker data

Within the clinical setting, it is important to consider the usefulness of biomarker data and how it can be adopted into screening for PND. Rather than inputting all data into models, in order for the results to be clinically relevant, a quick and simple method of assessing risk is needed. As described in Chapter 4, reference ranges can be calculated based on the standard deviation to assess samples testing within range and out of range, or normal and abnormal. The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of a screen can then be calculated to assess its suitability in detecting cases, or in detecting those who are low risk with the true negative rate. This can aid screening and prioritisation of resources to women who are most at-risk.

In Chapter 4 the reference range was calculated for IL-6/IL-10 ratio, and use of this biomarker data alone results in NPV of 79% for antenatal depressive risk, performing fairly well as a negative screen. A two-step screen is an alternative which may provide an optimal screening test, combining both a key psycho-socioeconomic factor and a biomarker test result. This might represent a useful and fast screening tool, which could be an alternative to the EPDS.

A cross tabulation (Table 6.7) demonstrates the frequency of participants with either IL-6/IL-10 levels outside of the reference range, or with a history of anxiety (N=128). All participants with either of these two risk factors were coded 1. This new variable is 'Antenatal Screen 1'.

Table 6.7. Calculation of Positive Predictive Value and Negative Predictive Value based on a cross tabulation of true positive/negative results and false positive/negative results.

	EPDS result	
Antenatal quick screen	Positive	Negative
Positive	37	91
Negative	64	504

The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated for this screening tool. PPV= 29% and NPV=89%. This true negative rate is 10% higher than using IL-6/IL-10 alone. This test was repeated using past history of anxiety alone as a screening tool, and the NPV is identical at 81%, indicating that if a woman does not have a history of anxiety, she is 81% likely not to develop PPD. The addition of the IL-6/IL-10 screening, based on scoring outside of the reference

range, brings this NPV value to 89% as a negative screen for those with IL-6/IL1-0 inside the reference range. This is an example of a fairly simple method of combining a psychosocial variable with a biomarker to improve prediction of depressive risk. This could be used with relative ease in a clinical setting, and can be expanded to include other important risk factors. Here we have combined the most important biomarker from the analysis, IL-6/IL-10 ratio, and the most important psychosocial risk factor in terms of influence on prediction, a history of anxiety. Using this two-step approach screening can be done quickly and with ease, but additional risk factors can be added as required. Although 29% is relatively low as a predictive screen, 89% is a good negative predictive value, although in order to prevent 11% of women being incorrectly classified as low risk, screening with the EPDS would still be optimal.

6.3 BDNF and Psycho-socioeconomics – Case Studies

High levels of BDNF are thought to be protective of PND, based on both this present study and previous literature. Although BDNF did not play a significant role in the regression modelling part of the analysis, some interesting observations can be made from this data. On initial inspection, a statistical association between BDNF and antenatal EPDS suggests a positive relationship. As described in Chapter 4, the data is in fact skewed by the high proportion of participants scoring low on the EPDS. Reference ranges were calculated and the 'abnormal' data was inspected separately, elucidating a negative relationship between BDNF and EPDS in this sample ($r=-0.25$, $p<.01$). Those participants with high BDNF levels as determined by the reference range (N=23) were explored in more detail as case studies with an in depth assessment of their patient notes. The theory that high BDNF can protect against PND despite the presence of key risk factors was explored.

Four participants with high BDNF did not fit with this theory, and screened positive on the antenatal EPDS (#196, #317 and #959). It was noted however that three of these women had a previous mental health history. For example, case study #959, had a history of depression, anxiety and also PND. Despite the high 'protective' BDNF, her personal mental health history is a very strong risk factor, as demonstrated by the present analysis, and appears to play a more important role in depressive risk. One participant, #196, who had a history of both depression and anxiety was also a smoker, and was therefore excluded from the BDNF analysis due to a link between smoking and BDNF levels[282 283].

The other case study that did not fit with this theory was a woman of an ethnic minority (#84), and this participant screened positive on the EPDS despite high BDNF. No other key risk factors were present, but being of an ethnic minority was identified in our analysis of increasing depressive risk. High BDNF levels did not protect against screening positive in this case, although severity may have been reduced since the EPDS score was 10, the minimum score for a positive screen.

Two case studies with high BDNF (#52 and #1154) had a history of anxiety but not depression, and these women screened negative on the EPDS. This suggests that a personal history of depression exhibits a more powerful effect on depressive risk in pregnancy than a history of anxiety. It may be possible that high BDNF can counteract the risk of anxiety, but not depression. A further case study #110 did have a history of PND, but despite this had high BDNF and also screened negative on the EPDS. High BDNF levels could be one possible explanation here for this low risk despite having a key risk factor.

Three case studies (#437, #489 and #1154) had a family history of PND, an important risk factor, however all screened negative on the EPDS, suggesting the possibility that the detrimental effect of their family history was outweighed by protective high BDNF. Six case studies fell into the low social status risk categories. 100% of these women screened negative on the EPDS despite this. Once again, this protection may be provided by high levels of BDNF. One case study (#437) identified as being unsupported in her pregnancy, one of the key risk factors for PND. Despite this, the EPDS screen was negative. High BDNF may have protected against the risk posed by this lack of support. Twelve women with high BDNF did not have any of the key risk factors and also screened negative on the EPDS. It is likely that these women have a combination of biological and psycho-socioeconomic protective effects, which are linked, leading to low EPDS scores.

In summary, high levels of BDNF are proposed to protect against PND. In this sample of women with high BDNF (N=23), the majority of the participants (N=19) screened negative on the EPDS. The majority of these women appear to be protected by both positive psycho-socioeconomics and biological effects potentially via high BDNF. In some cases however we see a number of the risk factors typically associated with PND. It is hypothesised that these women are protected from depression, despite their personal psycho-socioeconomic risk factors, due to their high levels of circulating BDNF. In particular, it seems that a history of anxiety, a history of PND, a family history of PND and low social status all

have their effects ameliorated by high BDNF levels, which is illustrated in Figure 6.3. In this way the individual threshold has been altered and the response to insults has a lesser effect. In contrast, women with a history of depression all screened positive on the EPDS despite their high BDNF levels. I have demonstrated in this study that depressive history is the strongest risk factor for PND, and therefore it is sensible to suggest that its effect is so strong that high levels of BDNF cannot counteract the subsequent increased risk.

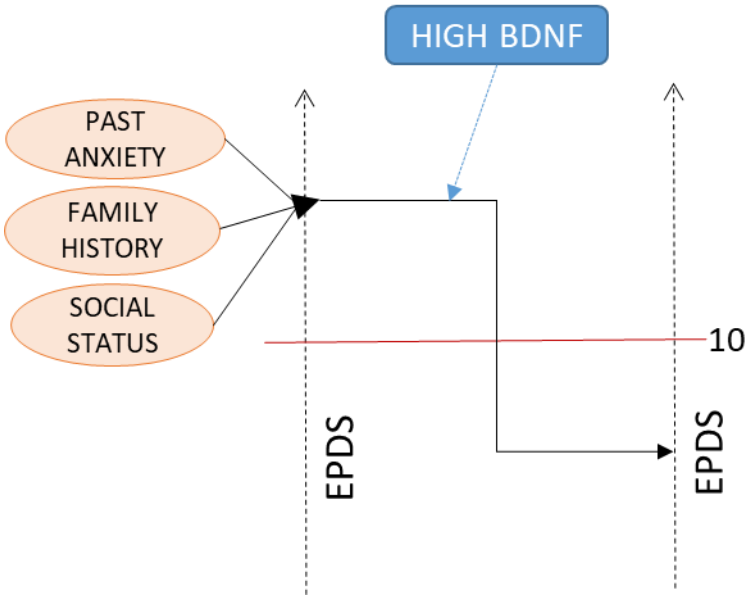

Figure 6.3. Women with risk factors including past anxiety, family history of PND and low social status are predicted to score highly on the EPDS, and have an increased risk of scoring ≥ 10 on the EPDS, the cut-off score for PND risk. It seems that in some women, high levels of circulating BDNF lead to amelioration of these effects and results in a lower than expected EPDS score.

In Chapter 4 an interaction between DNA methylation and social status was also explored, identifying a further interaction between a biomarker and a psycho-socioeconomic risk factor. A statistically significant relationship was found (p=.006) between global DNA methylation levels (5-mC %) and housing status. When compared with women owning their home, women without a permanent home who identified as 'staying with friends' had twice the mean 5-mC %.

6.4 Birth Weight & Gestational Length – Regression Modelling

Birth weight, perinatal depression, and psycho-socioeconomic risk factors are complex and interlinked factors. A further aspect of this study explores whether data on risk factors for PND are also related to baby birth weight and could therefore be used in prediction of birth weight or gestational length. In Chapter 4, a significant correlation was identified between postpartum depressive risk and shorter gestational length, which is in line with previous studies finding that pre-term infants increase maternal stress[284 285]. In linear regression modelling, shorter gestational length was also significantly associated with raised postpartum EPDS scores. Regression modelling was carried out to identify factors related to gestational length and birth weight.

Prediction of birth weight

The first exploratory model tested associations between risk factors for PND and birth weight. The optimal model included all variables included in Table 6.8.

Table 6.8. Results of a linear regression model to predict birth weight outcome based on selected predictors including IL-6 concentration, BDNF concentration and selected psycho-socioeconomic variables.

	B	*SE B*	*β*
Constant	3215.81	57.65	
IL-6 concentration	-91.93	40.02	-.09*
BDNF concentration	-0.02	0.01	-.07*
Alcohol pre-pregnancy	103.61	42.29	.09*
Smoking in pregnancy	-290.58	125.85	-.09*
Age (baseline 30-34)			
40-45	-150.66	72.18	-.08*
Education (baseline Degree)			
>16	151.67	70.75	.09*
Mode of delivery (baseline NVB)			
Instrumental	241.46	63.77	.14***

	B	*SE B*	*β*
Social status (baseline Higher Managerial/Professional)			
Low level routine work	-85.63	48.91	-.07
Unemployed/Student	-298.12	137.88	-.08*
BMI (baseline 18.5-24)			
25-29	175.41	49.40	.14***
30-34	264.38	54.06	.19***
35-39	163.34	85.08	.07
40-44	241.78	134.38	.07
≥45	566.90	235.46	.09*
Parity (baseline 0)			
1	313.87	44.41	.28***
2	342.60	72.57	.18***
3	229.54	114.70	.08*
6	1082.70	518.97	.08*

R^2 = .15. *p<.05, **p < .01, ***p < .001.

Important factors were found to include two of the measured biomarkers: IL-6 and BDNF, which might play different roles. Higher concentrations of both markers were associated with lower birth weights. Other factors associated with lower birth weights are smoking during pregnancy, maternal age 40-45, and lower social status. Alcohol pre-pregnancy, lower educational level (>16), higher BMI and having previous children were all associated with increased birth weights.

The initial exploratory modelling excluded gestational length, which is a known clear predictor of birth weight. The modelling was then repeated to include gestational length, and important factors were now slightly altered in order to adjust for the influence of gestational length. This can help to disentangle whether risk factors are directly associated with birth weight, or indirectly via gestational length. IL-6 and BDNF were no longer included in the optimal model for birth weight, nor were pre-pregnancy alcohol or educational level. This new model including gestational length greatly improves the model's prediction from 15% to 41% (Table 6.9).

Table 6.9. Results of a linear regression model to predict birth weight outcome based on selected predictors including IL-6 concentration, BDNF concentration, gestational length and selected psycho-socioeconomic variables.

	B	*SE B*	*β*
Constant	-4502.41	415.62	
Gestational length	27.94	1.50	.58***
Smoking in pregnancy	-214.25	101.86	-.06*
Age (baseline 30-34)			
≥45	812.22	430.12	.06
Mode of delivery (baseline NVB)			
Instrumental	241.46	55.54	.06
EMCS	116.80	50.29	.07*
ELCS	125.72	44.44	.09**
Social status (baseline Higher Managerial/Professional)			
Low level routine work	-105.01	39.78	-.08**
BMI (baseline 18.5-24)			
25-29	93.71	40.81	.07*
30-34	196.20	44.92	.14***
35-39	207.68	69.11	.09**
≥45	381.18	194.02	.06*
Parity (baseline 0)			
1	266.38	37.66	.24***
2	271.81	60.41	.14***
3	339.46	95.23	.11***
4	353.24	147.64	.07*
5	568.97	306.95	.06
6	1087.40	430.29	.08*

R^2 = .41. *p<.05, **p < .01, ***p < .001.

Gestational length is by far the best predictor or birth weight in this model. Other important factors are parity, BMI, social status, mode of delivery, age ≥ 45 and smoking during pregnancy. The influence of biomarkers IL-6 and BDNF is now absent. This is likely due to an indirect relationship with gestational length, which

in turn influences birth weight. This was explored next in further modelling with gestational length as the outcome.

Prediction of gestational length

Modelling of gestational length identified a number of predictor variables, including BDNF, both with and without the inclusion of birth weight in the model. When birthweight is absent from the model, BDNF becomes increasingly significant, indicating that the influence of BDNF is acting mainly through effects on gestational length but also in part directly on birth weight (Table 6.10).

Table 6.10. Results of a linear regression model to predict gestational length based on selected predictors including BDNF concentration and selected psycho-socioeconomic variables.

	B	*SE B*	*β*
Constant	278.89	0.94	
BDNF	-0.00	0.00	-.10**
Postpartum EPDS	-0.23	0.09	-.10**
Mode of delivery (baseline NVB)			
Instrumental	5.17	1.34	.15***
EMCS	-0.60	1.24	-.02
ELCS	-5.16	1.07	-.18***
Social status (baseline Higher Managerial/Professional)			
Low level routine work	1.44	0.97	.06
BMI (baseline 18.5-24)			
35-39	-3.47	1.68	-.08*
Parity (baseline 0)			
1	1.20	0.87	-.05
3	-5.02	2.32	-.08*
4	-4.30	3.70	-.04

R^2 = .11. *p<.05, **p < .01, ***p < .001.

BDNF concentration still improves the overall prediction of the model when birthweight is included as a predictor (Table 6.11). Postpartum EPDS was also identified as a significant variable in the optimal model which includes birthweight.

This model overall now has R^2 of 0.40, accounting for 40% of the variation in gestational length.

Table 6.11. Results of a linear regression model to predict gestational length based on selected predictors including BDNF concentration, birthweight and selected psycho-socioeconomic variables.

	B	*SE B*	*β*
Constant	240.45	2.28	
Birthweight (grams)	0.01	0.00	.55***
BDNF	0.00	0.00	-.05
Postpartum EPDS	-0.16	0.07	-.07*
Mode of delivery (baseline NVB)			
Instrumental	2.74	1.12	.08*
EMCS	-1.96	1.02	-.06
ELCS	-5.32	0.89	-.19***
Social status (baseline Higher Managerial/Professional)			
Low level routine work	1.83	0.80	.07*
BMI (baseline 18.5-24)			
35-39	-3.98	1.38	-.09**
Parity (baseline 0)			
1	-1.43	0.73	-.06
3	-6.25	1.92	-.10**
4	-5.72	3.04	-.06

R^2 = .40. *p<.05, **p < .01, ***p < .001.

Variables which were found to improve prediction of the model in exploratory backwards regression modelling were included in the final optimal model. Birthweight had the strongest association with gestational length, with a clear direct positive relationship which was expected. Other variables positively associated with gestational length in the model are Instrumental delivery (forceps) and lower social status. Variables with an inverse association with gestational length are Elective C-section, Emergency C-section, Parity, high BMI, postpartum EPDS and BDNF concentration.

7 Discussion

7.1 Introduction

This study had six central aims, outlined in Chapter One, which were designed to further understand and delineate the contribution of both biological and psychosocial risk factors to the prediction of perinatal depressive risk. Each of those aims has been addressed in this thesis.

Aim 1 – Can psycho-socioeconomic risk factors predict antenatal and postpartum depressive symptoms?

Previous studies have focused on prediction of postpartum EPDS scores, and a clear association between antenatal and postpartum scores has been previously identified[276]. The present analysis identifies the same relationship, and goes beyond previous work to analyse the contribution of psycho-sociodemographic covariates to antenatal, postpartum and perinatal EPDS scores and presents novel data. The AUC and correlation analyses in this study demonstrate that this relationship is also present in this dataset. The limited correlation of 0.50 between antenatal and postpartum EPDS scores, combined with the extreme differences between these two scores for some participants also indicated that there are other factors contributing to this difference.

Initially EPDS scores were dichotomised to define a binary outcome of depressive symptoms in order to obtain estimates of effects and their significance that are more robust to potential misspecifications of the regression models. This approach is also more likely to be relevant for routine healthcare risk assessment approaches designed around the use of cut-offs; this was followed by development of regression models for the raw EPDS scores in order to increase exploratory power and to detect more subtle effects of covariates without the constraint of cut-offs. Finally the predictive performance of the regression models was evaluated in comparison to a state-of-the-art machine learning prediction method. This complementary but distinct statistical analysis identified a similar set of covariates. Both approaches to the analysis resulted in qualitatively similar conclusions about the contributions of various psychosocial covariates.

It was next explored how far psychosocial factors alone could predict antenatal EPDS scores in a clinical setting. As a general pattern antenatal EPDS scores are

better explained by the available covariates and are associated with covariates indicating a general level of worry and anxiety, lack of support, overindulgence in alcohol and smoking and lower social status. These covariates also predict that signs of depression might ease after delivery. On the other hand, postpartum depression is less reliably predicted by the available covariates and is mostly associated with a previous history or a family history of depression and PND. These findings hinted at a possible genetic component for PND. Postpartum scores are however aided by the inclusion of antenatal scores in their prediction.

In the main study population a prevalence of 16.7% was identified for antenatal depressive risk and 19.8% for postpartum depressive risk. These values slightly exceed the 10-15% estimate in the general population, although it is widely considered that the rates are underestimates. Other studies report similar rates[23] [24]. A possible explanation for this is that every woman partaking in the study is screened using the EPDS as part of the study protocol, whereas in normal clinical practice screening is not mandated and a number of cases will be missed. Study participants who screened positive on the EPDS in the study were referred on for further treatment. It is likely that a number of these women would not have been screened under their regular antenatal care, and this further emphasises the need to routinely screen for symptoms in all women under maternity care.

Aim 2 – Is perinatal depression heterogeneous and can the timing of onset/remission be predicted?

Previous studies have suggested that PND may not represent a homogenous disorder as previously thought, and instead exhibits different patterns of onset, severity and symptoms and aetiologies[107] [286]. In order to explore the possible variable course of depression, the analysis also classified women into distinct sub-groups dependent upon their risk of depression. Each at-risk group showed distinct trajectories of timings of onset and remission (ante/post-natal), in addition to different risk and/or protective factors which suggests that they may represent sub-types of PND. The findings demonstrate that the course of perinatal depression can vary, and is bi-directional, as shown in Figure 7.1.

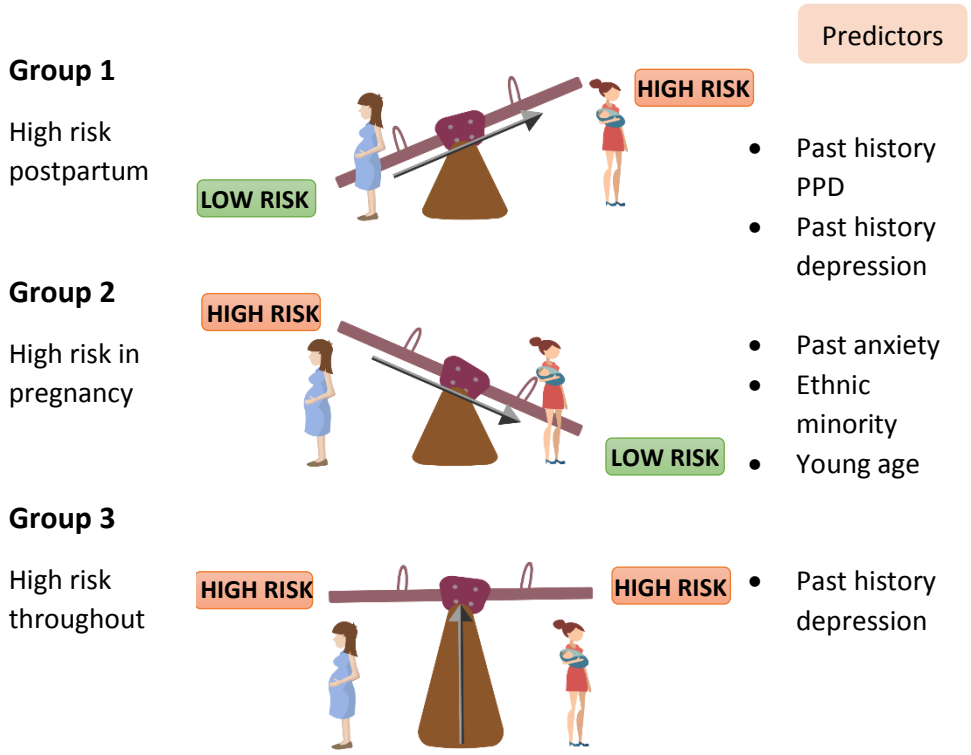

Figure 7.1. The heterogeneity of perinatal depression can have a bidirectional course of progression, with risk either increasing, decreasing, or remaining consistent from the antenatal to postpartum periods. Group specific risk factors are associated with distinct groups.

In an ongoing debate over whether differing trajectories represent distinct conditions, these findings support the heterogeneous view of PND. This heterogeneity may be due to different sensitivity thresholds, different 'insults' or 'stressors', and different responses to these insults, which may include for example a detrimental inflammatory response. Studies to date have focused on postpartum depression (PPD), and one view is that PPD is biologically distinctive from major depressive disorder, with vulnerability due to increased sensitivity to gonadal hormone fluctuations, and that this increased risk is only present in the immediate postpartum. Alternatively PPD can be described more simply as an episode of major depression manifesting within the postpartum period. This debate in the field has important implications for disentangling contributing factors and better understanding the course of PND. This study further supports the theory of heterogeneity for PND, and explores the clinical problem as a whole where most studies have focused on exclusively postpartum depression.

In addition to categorising into a risk group, EPDS scores were used to assess within-person patterns of change of depressive symptoms severity at two distinct time points antenatally (T1) and postpartum (T2). Using a single score cut-off (≥10) we identified three subgroups according to onset and duration or remission of symptoms. An antenatal vs postpartum EPDS score correlation of 0.5002

demonstrates that although there is a clear association, there is also a clear deviation of antenatal EPDS score from postpartum EPDS supporting the hypothesis of a multifactorial aetiology[286].

Aims 3-6 - Do biomarkers (circulating biomarkers, genetic SNPs and DNA methylation) act as markers for perinatal depression?

A crucial aim of the analysis was to evaluate the additional predictive capacity with the inclusion of biomarker data from participants, utilising the ability of a statistical driven approach to further interrogate heterogeneous data and therefore the heterogeneity of PND. Biomarkers were further explored for their individual contributions to EPDS scores and associated psychosocial risk factors. The findings report that biomarkers can provide additional valuable information to predicting perinatal depressive risk. In particular, a role for inflammatory markers is supported. Findings related to each individual biomarker are discussed in section 7.4. Biomarker data has been collected and analysed alongside the psychosocial variables, in a novel approach to combining a wide variety of data to interpret contribution to the risk of PND.

In addition to the identification of risk factors, this study aimed to assess two important offspring consequences of PND, gestational length and birth weight, and the role that the neuro-inflammatory system plays by exploring the relationships between maternal biomarkers and these offspring outcomes. It is now established that two thirds of the costs relating to maternal mental illness in the UK are directly related to the child, including costs of pre-term birth, special educational needs, low educational attainment and emotional problems[16]. These detrimental and costly effects on offspring of affected mothers demonstrates that this research is urgently required, and addresses a specific gap in the knowledge identified in the current literature. The analysis suggests that biological markers of neuroinflammation are involved in the pathogenesis of developmental impairment in children of mothers suffering from PND.

7.2 Analysis – Benefits of Approaches

The perinatal depression disease trajectory contains two main parts – one antenatal and one postpartum. One of the main strengths of the approach to the analysis in this study is the focus on antenatal as well as postpartum depressive symptoms, since most studies do not approach the clinical problem as a whole in this way. The statistical analysis has utilised the data to its full potential, looking at both EPDS cut-off scores, which are clinically useful, and raw scores which can provide a more detailed picture of score prediction. Analysis focused firstly on EPDS score dichotomised using a cut-off value, and a second analysis utilised raw EPDS scores to increase power. Both analyses reached qualitatively similar conclusions made about the contribution of selected psychosocial variables, providing reassurance around these findings.

The analysis employed different types of regression and machine learning methods. Initially EPDS scores were dichotomised to define a binary outcome of depressive symptoms in order to obtain estimates of effects and their significance that are more robust to potential misspecifications of the regression models. This approach is also more likely to be relevant for routine healthcare risk assessment approaches designed around the use of cut-offs; this was followed by development of regression models for the raw EPDS scores in order to increase exploratory power and to detect more subtle effects of covariates without the constraint of cut-offs. Finally the evaluation of the predictive performance of the regression models was carried out in comparison to a state-of-the-art machine learning prediction method. This complementary but distinct statistical analysis identified a similar set of covariates.

The EPDS was used in multiple ways to assess risk, including a group analysis approach which would be appropriate for use in the clinic. The 'quick screen' approach was also explored, in order to simplify risk prediction with the use of Positive Predictive Values and Negative Predictive Values to test how far this approach could be used to classify risk. Overall the analyses performed in this study have been targeted towards clinical transferability, starting with more complex exploratory analysis and finishing with a simplified version of screening with the most relevant risk factors for ease of use in a clinical setting.

7.3 Limitations

A number of study limitations should be considered in the interpretation of these results. A high majority of the main study population were White British and women were generally well educated, of a high social status and identified as 'supported'. The findings are therefore limited in their generalisation and care must be taken when comparing to other populations. Depressive symptomatology was assessed through the use of a self-report screening instrument rather than clinical assessment, although the EPDS has been reported to have high sensitivity in a large number of studies both in the antenatal period and postpartum. A number of recorded variables were binary (yes/no) limiting the level of detail and the conclusions for some covariates. A further weakness of the study is that there was no record of previous life experiences that could influence coping mechanisms and responses to stress. Additionally other medical factors such as gestational diabetes were not recorded which could act as confounding variables.

Although the study design assessed depressive symptoms at two key time points during pregnancy and postpartum, this cannot capture the full temporal spectrum of symptoms onset or duration; for example women who experience transient symptoms during the first trimester or beyond 3 months postpartum since there was no follow-up post- 6–10 weeks postpartum, and therefore onset of depressive symptoms after the study period will not have been included in the analysis. The lack of multiple sampling points, including the measurement of postpartum biomarkers, also did not allow application of modelling tools for identifying multiple un-observed sub-populations, describing longitudinal change within each sub-population, and examining differences in change among sub-populations. Such growth models typically require at least three repeated measures per individual. The completion rate of the postpartum EPDS screening was relatively low at 55% in this ongoing study, which also limited the sample size and statistical power of postpartum analyses.

The findings of the statistical analyses are limited in their interpretation since the inclusion of a large number of variables can result in significant findings by chance alone. Corrections for multiple comparisons such as Bonferroni were not included here since it was an exploratory analysis to find all possible contributing factors, however it is acknowledged that a significant *p*-value does not represent a causative relationship.

7.4 Risk factors – Interpretation and Implications

Can psycho-socioeconomic risk factors predict antenatal and postpartum depressive symptoms? (Aim 1)

Age

The peak time in a woman's life to experience depression is at childbearing age[287]. Young age, and more specifically teenage pregnancy, has previously been identified as a specific risk factor for depression, doubling a woman's risk[94]. Recent reports suggests that the biological adolescent phase of life in current society continues to the age of 24, rather than a previously suggested age of 19[288]. In alignment with this, the findings of the present study indicate that women aged under 25 are particularly vulnerable to PND. It is thought that women of this age are still undergoing biological growth, including brain maturation, in addition to experiencing major social role transitions, meaning suitability for motherhood may be compromised. The implications of this finding point to the additional support required by young mothers, and expanding the risk category up to the age of 25 for prioritisation and close monitoring of depressive symptoms to better detect those at higher risk of PND.

In the largest cohort analysed of this study (N=1579) the modal age category was 30-34. Women belonging to this category were identified as most protected from antenatal depressive risk. Age is an important, highly significant variable in antenatal depressive risk (p=.001). 18-24 year olds were at the highest risk of raised antenatal EPDS in this cohort, 1.63 times more likely to fall into the 'high risk' category. Regression analysis identified an increase of 1.4 points to the antenatal EPDS score for women in this age category. Young maternal age consistently transpired to be a risk factor across the different cohorts, although age appears to have a diminished role in postpartum risk.

The analysis reveals that is not only young maternal age that increases a woman's risk of PND. Women over 40 were similarly found to be at increased antenatal risk, with 1.4 points also added to their EPDS scores for women aged 40-45. Therefore there is an optimum period of reduced vulnerability for depression following a U-shaped pattern, with risk increasing at the lower and higher ends of the age spectrum. The 40-45 age category was also associated with lower birth weights in addition to depressive risk. Higher maternal age is associated with an increased risk of obstetric complications including gestational diabetes, pre-eclampsia, miscarriage, congenital abnormalities such as Down's syndrome, babies small or

large for gestational age, and complications during delivery, and are also more likely to have undergone assisted conception[289 290]. The underlying reasons for older maternal age as a risk factor for PND may be related to other associated health complications acting indirectly to increase in stress during pregnancy. Older maternal age, specifically in the same age category of 40-45, has been reported as risk factors for depression in previous studies[291 292].

Overall, young maternal age is a consistent risk factor for PND in this analysis, particularly in the antenatal period and this is true across different populations and cohorts. One preventative intervention for PND used in the UK involves specialist home visits for teenage mothers[125], and it can be argued that this research points to expanding this programme to include first time mothers under 25 who clearly require additional support in their pregnancies. Likewise, older maternal age is apparent as a risk factor, and these mothers should also be prioritised. It is a common misconception that women in their 40s, with increased life experience, are better equipped to cope with the stress of pregnancy and the postpartum, yet this finding indicates that the opposite is true, and clearly support at either end of the age spectrum is warranted, and is corroborated by previous findings[292 293].

Anxiety

Anxiety is a common disorder affecting at least 10% of Western populations, with the highest prevalence seen amongst women[294 295]. The impact of anxiety is all too often dismissed, and the impact on health misunderstood. It is generally considered less detrimental than depression, yet anxiety presents a great challenge for the biological stress systems, most notably that of the HPA axis. The influence of a history of anxiety on antenatal symptoms in this cohort is consistent with the noted dysregulation of the HPA axis associated with AND[296]. Long-term modification of HPA axis activity as a result of early life stress, which is highly related to anxiety, is thought to contribute to a heightened psychological vulnerability of perinatal women. There is strong evidence that maternal anxiety influences child development[82 218]. Maternal anxiety is thought to influence infant temperament, which in turn affects parental stress, and in this way the emotional states of mother and child interact with one another in a bidirectional mechanism of influence[297]. The way in which the stress system reacts to challenge can affect the developing foetus/infant and it is important to consider this when investigating the implications of perinatal anxiety.

In the study population, 19% of women had a past history of anxiety. Past anxiety indicates an underlying susceptibility to stress, and a likelihood of anxiety-related personality traits, which are known to be associated with the 5-HT system[298]. Certain personality types linked with anxiety such as neuroticism have also been associated with the development of PPD[91]. In the women scoring high risk on the antenatal EPDS, rates of past anxiety were more than double that of healthy controls. Most notably, a history of anxiety stands out in the present analysis as the single strongest predictor of antenatal depressive risk. This finding has implications for both the developing offspring and the mother which are likely to contribute to PPD and the consequences that brings without intervention. The EPDS has been shown to be a good measure of anxiety in addition to depression and therefore may represent underlying anxiety worsening during the perinatal period[299]. An anxiety sub-scale within the EPDS questions has been previously identified, with specific anxiety dimensions addressed by particular questions on the screening tool[300]. Based on the findings of this study and previous studies, it can be recommended that healthcare professionals focus on these anxiety dimensions of the EPDS in order to identify a key risk factor for PND.

Women with a history of anxiety were identified as 3.5 times more likely to be high overall risk for depression throughout pregnancy and postpartum (group 3), and regression modelling quantified an additional 2.1 points added to the EPDS score for women with past anxiety. Anxiety was also a specific risk factor for antenatal depressive symptoms – women with a history of anxiety were 7 times more likely to be high risk for AND (group 2). In the analysis that did not split women into risk groups, anxiety was also one of the strongest risk factors for antenatal EPDS scores, but not postpartum scores. Interestingly, anxiety plays a lesser role postpartum in this analysis and appears only to contribute to prediction of scores in pregnancy. The importance of anxiety in pregnancy adds weight to the idea of a highly biological mechanism acting in the development of AND, relying heavily upon the increased HPA activation and higher cortisol levels likely in anxiety prone individuals.

The lack of association postpartum indicates that during pregnancy is the time in which women with a history of anxiety require intervention for depressive symptoms, but the results show that these effects are likely to subside postpartum. This highlights the increased need to support and monitor this group of women antenatally, rather than postnatally when it is too late to mitigate the consequences for the child. The importance of anxiety is highlighted by its major role in AND,

which in turn is one of the biggest predictors of PPD. Anxiety is therefore also indirectly contributing to PPD, which carries its own risks for mother, child and family unit, including relationship breakdown and poor mother-infant attachment.

If women who come into contact with maternity services are routinely asked about a history of anxiety, this will provide the optimal opportunity to intervene and offer additional support and guidance throughout pregnancy relating to any symptoms of PND which occur. This study, along with the previous literature[55 301 302], demonstrates the increased risk of depression during pregnancy for this group of women who are prone to anxiety. Moreover, a number of women will come into contact with NHS professionals prior to conception to discuss family planning. The results here indicate that this family planning discussion could be the optimal time to recognise women with a history of anxiety. Mental health prior to conception is a good indicator of perinatal mental health, and prevention of PND is more likely with the right intervention pre-conception where possible.

Past history of depression

This analysis identified that women with a past history of depression are consistently at increased risk of both antenatal and postpartum depression. A past history of depression is additionally associated with higher average EPDS score between antenatal and postpartum periods, as well as bigger shifts between scores. This association is in agreement with the literature, and prospective population based studies suggest that the risk of PPD is more than 20 times higher for women with a history of depression compared to women without, as previously described in the literature[23 293].

A history of depression is one of the most influential and significant risk factors identified in this study, with an odds ratio of 4.4 of scoring 10 or above on the antenatal EPDS and 4.3 postpartum. In prediction modelling a history of depressions contributes over 2 points to the EPDS antenatally and around 3 points postnatally. In the group modelling, a past history of depression is a significant risk factor for two groups: high postpartum risk (group 1) and overall high risk (group 3). It appears to play a stronger role in sub-types which involve depression in the postpartum, but not when only antenatal symptoms occur. The opposite was true for a past history of anxiety, with increased vulnerability in sub-types involving depression antenatally, demonstrating a further difference in underlying aetiology representing biologically distinct processes.

It is hypothesised that antenatal and postpartum depression represent different clinical sub-types. Two forms of depression are recognised, melancholic and atypical[34]. Melancholic depression is typically defined by a loss of pleasure, depressed mood worse in the morning, insomnia and weight loss, and is associated with HPA hyper-activation. In contrast, atypical depression symptoms involve retention of mood reactivity, weight gain, hypersomnia and depressed mood worsening throughout the day, and is linked to a hypo-responsive HPA axis and glucocorticoid suppression[154]. Differences in risk profile could be indicative of sub-type, since the profile of antenatal depression is most similar to that of melancholic depression, and postpartum depression closer to the atypical type. This may explain the observed differences.

Sub-groups of women may be genetically predisposed to depression of either the melancholic or atypical type, influencing their risk of depression at specific stages of the perinatal period. Atypical depression seems to be triggered postpartum[45]. Women with a vulnerability to the atypical type may be at-risk of PPD due to the withdrawal of cortisol, and there is some evidence for HPA axis changes similar to atypical depression seen in the postpartum period[303].

A past history of PND has been identified in the analysis as an additional important risk factor to consider, although not as influential as depression outside of this period. Past PND was a specific risk factor for postpartum symptoms. This does not however appear to play a role in antenatal symptoms. This finding suggests that women who have experienced PND previously should be closely monitored, especially in the postpartum period, as they are at increased risk following a future pregnancy.

The highly influential nature of past depression and anxiety in the development of PND highlights the need to identify these two risk factors as early as possible during pregnancy, or pre-conception wherever possible. The positive aspect of these two risk factors is that they can be identified and targeted early on based on patients' notes and history. The results of this analysis show that women with a history of mental illness, including anxiety and depression, are increasingly vulnerable in the perinatal period. In addition, sub-groups can be identified based on these risk factors, which can guide interventions accordingly. Further work to understand differences in their underlying pathology is required.

If the three important variables discussed so far, 'age', 'past history of anxiety' and 'past history of depression', are used alone in regression modelling to predict

antenatal EPDS score, it is noted that prediction is almost as accurate as when using the nine variables selected in the optimal model. The model with all nine variables explains 14% of the variation in EPDS scores (R^2), whereas when these three variables alone are used in prediction, the R^2 only reduces slightly to 12%, meaning that these variables have the majority of the influence on scores. Age, Anxiety and Depression history may therefore represent an easy initial screen for AND. When these three variables are used alone to predict PPD, prediction is less accurate, since antenatal EPDS is the best predictor of postpartum scores.

Social status

This study found that social status is an important risk factor for PND, which is in agreement with previous findings[92]. The analysis of the main cohort finds social status to be a highly significant covariate. Social status of 'unemployed/student' carries an odds ratio of 1.86 in relation to scoring high on the antenatal EPDS (≥ 10). Prediction modelling identifies an additional 1.5 points added to antenatal EPDS score for women in this category. This finding is in agreement with previous studies also reporting that AND is more prevalent in unemployed women[56 270]. Analysis of postpartum scores finds no association with social status.

Lower social status, indicated in this study by level of occupation, often indicates adversity in life. Two main stress hypotheses exist, the traditional 'cumulative stress' hypothesis, where the vulnerability increases as life history of stress increases, and the 'mismatch' hypothesis (Figure 7.2)[304]. The two hypotheses can also be integrated into one model dependent upon an individual's exposure to early programming effects. In the mismatch hypothesis, problems will only occur when

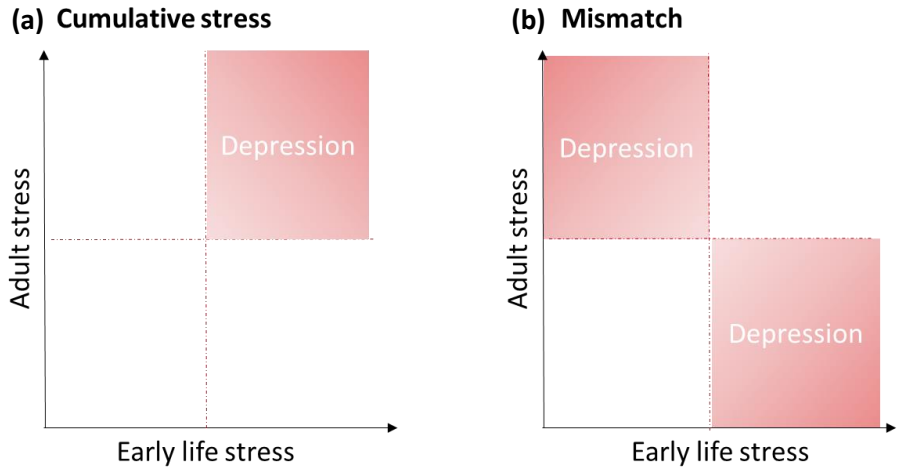

Figure 7.2. Hypotheses of exposures to an adverse environment leading to either (a) cumulative damage and increased risk for disease or (b) a mismatch of early life programming experiences and adult experiences increases likelihood of disease, whereas a match of early life and adult conditions results in resilience due to early life programming. Figure adapted from Nederhof & Schmidt (2012) .

there is mismatch between the early life and adult environment, or the pre/post-natal environment (Figure 7.2 (b). Adversity either prenatally or in early life adapts the individual to stress in later life as a protective mechanism. This could however render the individual maladapted to 'normal' environments, producing a stress response when unnecessary. If a mother with a low social status had also experienced early life adversity, this could help explain their increased vulnerability to depression. Further information regarding current and previous life circumstances would be required to investigate this in this cohort.

A higher social status is protective against an overall risk of depression in this study. Social status is indicative of socioeconomic status (SES), which is a well-established risk factor for PPD[55 95]. These results show that unemployment is a key risk factor for antenatal depressive symptoms, which likely reflects financial worries and instability. Similarly in the smaller Coventry cohort, insecure housing was a significant risk factor for both antenatal and postpartum symptoms. Findings indicate that more options for working mothers would be beneficial, as women with a lower job level or in unemployment had a significantly raised likelihood of overall depressive risk. Additionally, the rising costs of childcare contributes to financial stress on families looking to return to work. Both employers and governments should consider this in their policies on parental leave and childcare support.

Social status not only has an impact on a woman's risk of PND, but also on the consequences of maternal depression for the child. Socioeconomic status (SES) has been found to be an independent risk factor for offspring depression into adulthood, indicating that treating the mother during pregnancy should be prioritised[43]. The extent of the consequences can be moderated by SES. The children of mothers with the same degree of PPD but higher SES are less likely to be affected, whilst the offspring of lower educated mothers have an increased risk of adolescent depression [43]. Why this moderation exists is currently unknown.

Interestingly, a low socioeconomic status (SES) has also been linked with altered placental mRNA levels of genes involved in glucocorticoid metabolism[150]. It is possible that the effects of maternal SES on the offspring are initiated in the womb. It seems that a higher SES can mitigate both maternal and offspring effects of PND. It is important to note that the moderation of outcome by SES is not evident in antenatal depression. This suggests a pathway predominantly involving biological consequences *in utero*, in contrast to a highly environmental mechanism for PPD.

Significant associations were found between social status and a number of the measured biomarkers related to stress adaptability in this study, including IL-6/IL-10 and global DNA methylation. These biomarker associations will be discussed in more detail later in this chapter.

Educational level

Across almost all aspects of health, those with a higher educational level experience better outcomes, adopt healthier behaviours and live longer, and studies have shown that at least part of this relationship is considered causal[305]. Education is additionally indicative of multiple social factors. In general, previous studies find an inverse relationship between level of education and likelihood of PND. The findings related to this covariate in our study are somewhat ambiguous, but is likely influenced by the fact the main study population is generally highly educated, with over half of women educated to a minimum of degree level. The majority of studies in this area examine low education at a level without passing school examinations, yet only 1% of women in the study did not attain any qualifications, and therefore care should be taken when comparing to less educated populations.

In the main study population, education was only significant for prediction of postpartum EPDS scores, which is similar to what is reported in the literature. Although not statistically significant, educational level did still play a role in antenatal EPDS score, and was in fact the inverse of the relationship postpartum, with lower educational attainment (no exams) associated with a slight decrease in EPDS score. Due to a lack of significance and the small number of cases (EPDS ≥ 10) in this category this result is considered negligible. The finding that education to aged 16 only is associated with a 1.5 point increase in EPDS score is more in fitting with what might be expected.

Low maternal education has been highlighted as a risk factor for postpartum but not antenatal depression[43]. Low maternal education (indicative of low SES) has been shown to be associated with upregulation of placental glucocorticoid receptor and HSD11B1 gene expression[152]. Increased HSD11B1 expression results in glucocorticoid regeneration and therefore higher levels of glucocorticoid availability in the placenta. It is therefore hypothesised that this will in turn increase foetal exposure to glucocorticoids, providing a possible explanation for a number of associations between SES and offspring outcomes.

Educational level, socioeconomic status and depressive risk appear to be interconnected. In terms of effects on offspring, the investigation of paternal

depression in fathers can help elucidate the different mechanisms. There is currently no evidence for an association between paternal antenatal depression and offspring depression, or that the effects are moderated by his education. There is however an association between paternal postpartum depression and offspring depression, which similarly to maternal depression and SES, is limited to offspring with low education[43]. This provides further indirect evidence that the pathways of antenatal and postpartum depression are different. SES is closely linked with educational level, which is an additional risk factor for PND indicated by the analysis.

Cigarette and alcohol consumption

Smoking is identified in the main cohort as a risk factor, specifically for antenatal depressive symptoms. Smoking uptake is known to be higher among those with low socioeconomic status and quit attempts are more likely to be unsuccessful[306]. Studies have also demonstrated educational inequalities in smoking, finding that a quarter of those with high school or less education smoke, compared with 11% of those with an undergraduate degree and 5.6% with a graduate degree[307 308]. Both SES and educational level are also implicated in perinatal depressive risk both in this study and in previous literature. Smoking both in the 12 months pre-conception and during pregnancy were significantly associated with EPDS scores ≥ 10, with respective odds ratios of 2.2 and 2.4. In terms of significance level, smoking pre-conception was the most statistically significant. In the antenatal prediction model adjusting for a number of related covariates, smoking pre-conception remained a statistically significant influence at the $p<.001$ level, contributing 1.1 points to the score. Smoking during pregnancy is not included in the optimal regression model based on exploratory work after controlling for factors including social status and education level. In analysis of postpartum EPDS scores, smoking either pre-pregnancy or in pregnancy, had no apparent association.

Drinking alcohol during the 12 months pre-conception is associated with postpartum EPDS scores, but not antenatal scores, which is the reverse of the association with smoking. In the postpartum prediction modelling for the largest cohort, pre-pregnancy alcohol was a statistically significant influence on the modelling of score prediction, adding around 0.7 points to the score. This is a relatively small but significant contribution. Combined with a number of other key risk factors, this could be enough to push the boundary into the depressive risk category.

Both smoking and the consumption of alcohol are widely known to be detrimental to health. It is also widely accepted that smoking and drinking are especially harmful to an unborn child and women who are trying to conceive are advised to avoid these substances prior to conception. Alcohol consumption is often higher in those suffering with depression, and is additionally harmful to mental health, and therefore the direction of this association is difficult to assess since women scoring higher on the EPDS are experiencing symptoms of depression and anxiety[309]. The prevalence of smoking is also higher in people with major depression, and cessation more difficult and likely to lead to further depressive episodes[310 311].

Although research relating to harmful effects for the foetus are inconclusive, it is though that long-term health risks are greater the more a woman drinks. Clear evidence also shows that heavy alcohol consumption during pregnancy can lead to fetal alcohol spectrum disorder (FASD), affecting intellectual ability, birth defects, behaviour, fine motor skills and mental health[312]. For low-to-moderate alcohol consumption, the latest evidence suggests that this is related to increased risk of having a baby small for gestational age (SGA), which present numerous health detriments of its own, when compared with abstinence[313]. Until recently UK health guidelines advised avoiding alcohol while trying to conceive and during the first trimester, but also suggested consumption should be limited to '1 to 2 units, once or twice a week'[314]. This has now been updated and the Chief Medical Officers for the UK recommend that both pregnant women and those planning to become pregnant entirely abstain from alcohol to keep risks to a minimum as a precaution due to lack of robust evidence[315].

The association between alcohol consumption and symptoms of depression and anxiety has been identified by other studies, and may largely reflect the impact of other factors such as low socioeconomic status, lower education and smoking status[316 317]. In the current study, alcohol consumption pre-conception is a risk factor for PND. Although evidence into the reasons behind this is currently lacking, it is reasonable to suggest that women who report drinking alcohol pre-conception are more likely to have had an unplanned pregnancy, which carries its own risks relating to PND, and are less likely to adopt health promoting behaviours around the time of conception and pregnancy. Women who consume alcohol and cigarettes around pregnancy face compromising their own and their child's health, and these behaviours may represent underlying symptoms of depression and anxiety[309]. It is important to note that detailed information on units of alcohol or

number of cigarettes is unavailable in the present study and so this analysis compares abstinence with any self-reported smoking/drinking.

The identification of both smoking and drinking alcohol as risk factors for PND have implications which are readily transferrable to the clinic. It seems that the public consensus is that smoking and drinking in pregnancy are known to be harmful, but the risk of these factors pre-conception are misunderstood. Perhaps most importantly, all women of childbearing age should be aware of this association. It is important to emphasise this should be discussed prior to pregnancy, since the key factor here is the consumption of cigarettes and alcohol pre-conception, and therefore specifically women who are coming off contraception or planning a pregnancy should be targeted. In addition, an association with smoking in pregnancy is apparent in the analysis, highlighting the additional benefits of smoking cessation as early as possible in pregnancy. The current findings suggest that increased provision of support to stop smoking and drinking during pregnancy would be beneficial to maternal mental health.

Mode of delivery

The chosen mode of delivery is discussed with midwives and doctors administering a woman's maternity care to decide the optimal type of delivery for their pregnancy. It is based on a number of factors including health of the mother and baby and any complications in a previous or current pregnancy. Some women will opt for a planned, or elective caesarean section (ELCS), meaning they know in advance that they will deliver their baby by section. In 2012-2013, over a quarter of all babies in England were delivered by caesarean, almost half of which were ELCS, and this has almost doubled in the previous decade[318]. There is further evidence that rates of ELCS are continuing to rise, with the latest reported national average at 30% caesarean births, half of which were ELCS[277]. Caesarean operations result in a prolonged stay in hospital when compared with spontaneous birth, normally a minimum of two days after the ELCS, representing a financial and care burden on the NHS associated with the peri- and post-operative care required for an ELCS.

The increased recovery period for ELCS when compared with spontaneous delivery is likely to increase anxiety in an expectant mother, with increased worries about the procedure and difficulties in caring for a new-born child. It is also more likely that women opting for ELCS have experienced pregnancy associated complications. Mode of delivery has received little attention in the field of PND, although a small

number of studies suggest a link between mode of delivery and perinatal depressive symptoms and wellbeing[319 320]. The directionality of this relationship requires further investigation, and can perhaps be aided by the measurement of biomarkers, which has been applied here and will be discussed later in this chapter.

In the present study, mode of delivery was a significant factor in antenatal depressive symptoms. Women opting for ELCS were 1.72 times more likely to score ≥ 10 on the antenatal EPDS compared with those opting for normal vaginal birth (NVB). Delivery by ELCS is a significant factor influencing the optimal prediction model, increasing score by 0.8 points. No associations between postpartum scores and mode of delivery were identified. Although mode of delivery is a postpartum outcome which would not usually be taken into account in the prediction of antenatal symptoms, an exception was made for ELCS due to the planned nature of the procedure which may increase levels of anxiety and anticipation throughout the pregnancy. Additionally, there are confounding factors to consider such as health complications which may also act to increase risk of PND. It is also worth considering that the directionality of this association is the reverse, with antenatal depression leading to pregnancy complications which may in turn to result in opting for an ELCS. These findings must also be interpreted with caution since it is unknown whether the planning of the ELCS preceded the data at which the EPDS screening took place, since in some cases this will happen later in pregnancy, and therefore conclusions are limited. Further work would be required in order to investigate this relationship.

Overall the influence on depressive symptoms antenatally point to the increased level of worry and anxiety during pregnancy surrounding thoughts of a more complex birth and prolonged recovery period. These findings indicate that women who are planning to deliver by ELCS are at increased risk of AND and require increased monitoring and support throughout pregnancy.

Do cytokines and neurosteroids act as markers for PND? (Aim 3)

Biomarkers

A number of the relationships identified with the psychosocial covariates in this analysis are complicated by multiple associations with other social, psychological and physiological factors. The interrogation of biomarkers in this process aimed to help disentangle these effects and assess the underlying physiological response related to perinatal depressive symptoms. The three biomarkers of interest in this

study, IL-6, IL-10 and BDNF, all appear to have interesting associations with the selected psychosocial covariates.

The analysis reports an inverse association between IL-6 and one of the key risk factors for PND, social status. On closer inspection, the relationship between IL-6 and depressive symptoms was investigated. Although no clear association was identified between IL-6 and EPDS, when the ratio of IL-6/IL-10 is calculated, a significant association with antenatal EPDS is apparent. This finding demonstrates that the balance of pro- and anti-inflammatory cytokines is important in depressive pathophysiology, and is also related to sociodemographic factors such including social status. IL-10, the anti-inflammatory counterpart to IL-6, is significantly associated with EPDS in this study, and has an inverse relationship. High circulating IL-10 levels appear to protect against depressive symptoms while lower levels of IL-10 were found in those at risk of major depression (EPDS ≥ 15).

Raised IL-6/IL-10 in pregnancy was also found to be significantly associated with mode of delivery, with raised levels found in those with more complex deliveries. The highest ratio was found in women who went on to deliver by Emergency Caesarean Section (EMCS), and the lowest in women delivering by Normal Vaginal Birth (NVB). This high ratio of IL-6/IL-10 may represent a biomarker of general levels of inflammation and complications with the pregnancy that can result in a more complex birth requiring emergency intervention.

Maternal BMI was highly associated with both IL-6 and IL-6:IL-10 in in this analysis. A BMI of 35-39, which falls into the obese category, was also a significant predictor of postpartum depressive risk in the optimal prediction model in this study, increasing EPDS score by 1.13 points. It has been reported that in overweight and obese women, there is a reduction in NVB rate with increasing BMI[279]. Obesity is associated with higher rates of EMCS, with an observed 30% increased risk, and therefore this may help to explain the links between mode of delivery, BMI, IL-6 and IL-10 reported in this analysis. This finding could represent an indirect effect of high BMI, acting through increased circulating pro-inflammatory cytokines, exacerbating or contributing to depressive symptoms in pregnancy, summarised in Figure 7.3.

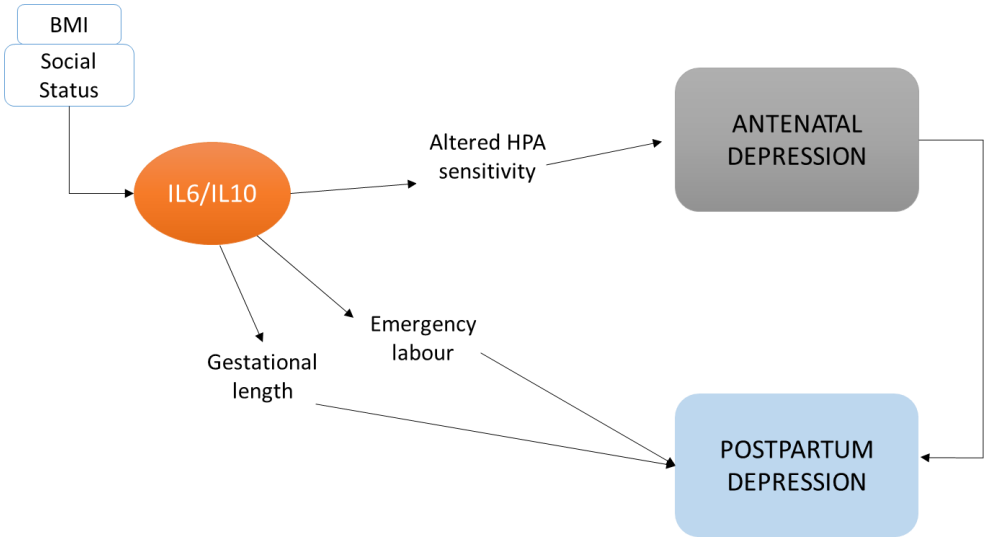

Figure 7.3. Insults such as high BMI or low social status can alter the ratio of IL-6/IL-10. This likely results in subsequent effects on HPA axis sensitivity, explaining the relationship with antenatal depression (raised EPDS scores). This will in turn increase the risk of postpartum depression, both indirectly via antenatal depressive risk, in addition to the noted association between IL-6/IL-10 and shorter gestational length and increased risk of emergency caesarean section.

As described in Chapter 1, the physiology of PND likely involves HPA variability and its role in the evoked inflammatory response. It is thought that some women possess an altered sensitivity to the inflammatory stimuli of pregnancy and childbirth which may contribute to the development of depression. The observed association between high circulating IL-10 and low risk of depression may represent the appropriate anti-inflammatory response taking place which is known to suppress inflammation[168]. On the other hand, in high risk women with low IL-10 levels, the inflammatory circuit may instead be disrupted, ultimately resulting in dysregulated cytokine production and cortisol secretion and ultimately contributing to PND[164 165].

Previous studies have suggested that people with a lower SES are less able to adapt to stress than higher SES counterparts, with evidence for stress-induced increased in IL-6[321]. A study involving both animal and human subjects reported that early social experience moderates the effects of adult social status on IL-6 levels[322]. The finding in this study that a lower social status is associated with raised IL-6 concentration in this study corroborate this, since lower social status is generally indicative of social disadvantages which often start in early life. A recent study investigating effects of perinatal stress also reports findings of raised IL-6 levels[323]. The activation of Toll-like receptor (TLR) 2 and 4 signalling and the NLRP3 inflammasome in placental immune cells is a proposed mechanism occurring during PND[33]. The resultant shift to a predominant Th1/Th17 inflammatory response is associated with increased secretion of pro-inflammatory cytokines. Overall, a

pathway involving neuroendocrine and immune system function is becoming increasingly implicated in perinatal stress.

Is there a difference observed in DNA methylation patterns? (Aim 4)

The sub-study investigating global DNA methylation finds an association between %5-mC and social status. Additionally, 5-mC% and EPDS are associated with statistical significance. It can be argued that this effect is indirect, and is mediated through social status. Methylation levels are also correlated with housing status, a further indicator of social status. Further work to detail the impact of social status and a larger study investigating global DNA methylation would be required to investigate this.

Of the biomarkers measured in this study, modelling demonstrates that IL-6/IL-10 ratio most improves the prediction of EPDS score. Including all psychosocial factors measured, this biomarker falls in the top 10 predictive variables. IL-6 alone also improves the model, yet IL-6/IL-10 provides the optimal predictive power. The inclusion of these two biomarkers improves the prediction of antenatal EPDS score. The variation in score, explained by R^2 in the regression models, rose from 14% to 20% with the addition of these biomarkers, meaning that 20% of the variability in EPDS scores is now explained by the model in this analysis. Overall this means that the concentration of IL-6 and the ratio of IL-6/IL-10 does help to predict risk of antenatal depression. The finding of these markers improving prediction is consistent across cohorts, with smaller and larger sample numbers. When IL-6/IL-10 is combined with a history of anxiety, the most important psychosocial risk factor identified, these two predictors alone can be used as a negative screen with 89% certainty. The inclusion of this biomarker raises prediction by 10%.

The analysis of circulating BDNF concentration and its association with depressive risk revealed an interesting relationship in this study. It was expected that BDNF levels would be reduced in those with high depressive risk due to the reported associations with depression[190 192]. On initial analysis of the data the opposite effect was seen with a mild positive association, and so a number of possible reasons for this were first considered. The majority of previous studies have used post-mortem brain samples, and measurement in patient blood samples, especially human plasma samples, is not well studied[188]. It is possible that this method of detecting BDNF is unsuitable for the sample type available. A second possibility is that patients with raised EPDS were treated with antidepressants and this was not accurately recorded, since BDNF levels are known to normalise following

antidepressant treatment[188]. On further analysis however, reference ranges were used to separate 'healthy controls' from 'patients'. This analysis then revealed an association that was expected, and an inverse relationship was reported between BDNF and depressive risk in 'patients'. The study was biased towards a normal EPDS score, due to the prospective design. The relationship may therefore not have been apparent in the whole study population due to the large number of participants scoring very low on the EPDS resulting in low mean EPDS, and the role of BDNF concentration may only come into effect when depressive risk becomes higher.

Does HPA axis sensitivity provide a marker for PND by genotyping target SNPs? (Aim 5)

Genetics

Both previous findings and the results of the current study suggest that demographic and psychosocial risk factors have a reduced predictive power for postpartum depression compared to antenatal depression[14]. This suggests a diminished contribution from environmental factors and hint at additional biological-genetic components for the development of PPD. A possible genetic component, as hinted at by the contribution of covariates indicating a family history of PND, was explored to assess whether this could lead to an improvement in prediction. This was designed to test at the clinical setting the potential use of SNPs in prospectively identifying women with high EPDS scores and thus increased risk of PND. This analysis also aimed to test whether findings from a smaller pilot study were replicated in this larger cohort.

In the analysis of the whole study population, no significant associations between the selected three SNPs and depressive symptoms were observed, but associations were found when the study group was split by their family history. The Bcl1 minor allele of the glucocorticoid receptor was found to be associated with an increased risk of PND in patients with a family history of PND. Both the pilot study and a previous study in a non-pregnant population also found a significant association between the Bcl1 minor allele and high risk of PND, which appears to be due to a known association with glucocorticoid (GC) hypersensitivity[131 324 325]. In women without a family history, the reverse relationship was identified, and a mildly protective and significant effect was found for those testing positive for the minor allele. A relationship between the IL-6/IL-10 ratio and Bcl1 has also been identified in the whole study population. The relationship suggests that the presence of the

Bcl1 minor allele is associated with a lower IL-6/IL-10 ratio which may be in turn be related to GC hypersensitivity, induced by Bcl1, and its anti-inflammatory effects.

The rs242924 SNP of the CRH-R1 was found to have no direct relationship with increased EPDS score. A relationship was instead observed between a family history and a personal history of PND in women positive for the minor allele. This therefore suggests that although a direct correlation between family history and EPDS score was not observed, a family history has had a previous effect on a former pregnancy depressive risk, which is in turn a risk factor for a future pregnancy, and can therefore be considered linked with PND risk. This SNP was not measured in the earlier pilot study and therefore results cannot be compared.

The rs242939 SNP of the CRH-R1 was found to be significantly associated with the easing of depressive symptoms postpartum in women with a family history of PND. This SNP was associated with a DPA score of -1.6, meaning symptoms are likely to ease by an average of 1.6 points as measured on the EPDS in the postpartum period. This indicates that women with this SNP who also have a family history of PND may have an increased risk of depression during pregnancy, but symptoms are likely to ease postpartum. When considering the group analysis conducted in this study, these women are most aligned with those in Group 1.

Overall, the genetic analysis did not reveal any strong associations with current depressive symptoms. This could partly be due to the small number of participants reporting a family history of PND, and future work should examine a large population of women with a family history of PND in order to accurately determine any genetic component to its intergenerational transmission. Women with a family history of PND represent an especially vulnerable group and the elucidation of the related factors, whether directly genetic or acting through psycho-sociodemographic covariates, requires further investigation. Future genetic studies with a focus on women with a family history of PND would improve our understanding of this element of heritability. This genetic study has also identified the relative ease of the method used in genotyping specific SNPs within a hospital laboratory setting.

Gestational length/birth weight

Seemingly inconsequential effects on birth weight can have long-term consequences for cognitive development and responses to stress, as well as general psychological wellbeing in later life[52-54]. PND is thought to be a risk factor for low birth weight, with more than half of publications finding a significant association

between prenatal depression and birth weight[48]. Much of the evidence has however been inconclusive. An association with gestational length has also been hypothesised although results are less consistent. Low infant birth weights pose risks for health and development, with an increased risk of mortality. This study finds a significant inverse association between gestational length and both antenatal and postpartum EPDS, with a shorter gestational length in women at higher risk of PND. This finding adds weight to the hypothesis that PND compromises gestational length and therefore birth weights and child development.

The regression model predicting birth weight finds that two of the measured biomarkers, IL-6 and BDNF, are both influential factors, playing statistically significant roles in birth weight. The observed relationship is inverse, with higher circulating concentrations of both IL-6 and BDNF associated with a smaller birth weight. The most influential factor in birth weight in this study is gestational length, but other important factors include maternal BMI and age, instrument assisted delivery, low social status and parity, as well as smoking and alcohol. The inclusion of two biomarkers in the optimal prediction model points to a highly physiological mechanism involving the neuro-inflammatory system, possibly related to depression.

Due to the clear association between gestational length and birth weight, the model next controlled for gestational length to assess whether the associated variables remained significant predictors in the model. The influence of biomarkers IL-6 and IL-10 were no longer seen in this model, indicating that the observed association was instead with gestational length rather than birth weight. This was next investigated by prediction of gestational length in exploratory regression modelling.

In predicting gestational length, circulating BDNF concentration is included in the optimal model and therefore improves its prediction. BDNF improves the prediction of gestational length both with and without the inclusion of birth weight in the model. A relationship between postpartum EPDS score and gestational length was also identified, with a shorter gestational length associated with raised EPDS scores following birth. This may represent an underlying level of stress during pregnancy which was not detected by the EPDS, since prenatal stress and associated glucocorticoid exposure is related to shorter gestation[49], or alternatively could be a postpartum outcome of delivering baby earlier than expected. The final

model finds that 40% of the variability in gestational length is accounted for by the included covariates. Aside from birth weight, other predictive factors for gestational length were mode of delivery, social status, maternal BMI and parity. Since all these variables are also risk factors for PND, they are clearly interlinked and demonstrate the importance of monitoring these factors during pregnancy.

The factors discussed above (age, past anxiety/depression, social status, education, smoking, alcohol, mode of delivery, and biomarkers) were the most consistent risk factors for PND coming out of the analysis. Other factors which did show some level of significant association but not consistently throughout the analyses were BMI, support, past PND, family history of PND and ethnicity. Women who identified as 'unsupported' were at increased risk of AND in both the Warwick and Coventry cohorts, and this is fitting with the literature demonstrating a lack of social support as a predictor of PND[55]. Increased support from the mother's partner, family and social environment is thought to act as a buffering effect against parental stress and protect maternal mental health[98 326].

Past PND was an additional risk factor for postpartum symptoms in the Coventry cohort. Past PND was a risk factor across the main Warwick cohort for both antenatal and postpartum depressive risk, which is in line with the literature finding that recurrence of PND is common in future pregnancies[23]. A family history was not a risk factor in the main cohort, which may indicate why genetic factors were not found to be associated, but in the smaller sub-study family history was a significant risk factor for both antenatal and postpartum depressive risk. Family history was additionally risk factor for antenatal depressive symptoms in the Coventry cohort. Finally, ethnicity was identified as a risk factor in the Coventry cohort, with women of Asian or Mixed ethnicity at the highest risk of depressive symptoms, which is in fitting with the finding that Black Asian and minority ethnic (BAME) women are at increased risk of PND[122 327].

7.5 Conclusions

Overall, the findings of this study fit well with the conceptual framework describing stress vulnerability and depression in women[14]. Previous history of relevant illness might contribute to individual chronic and acute burdens and accumulation of life stressors that prevent effective adjustment of regulatory mechanisms. Previous studies that employed structural equation modelling to integrate variables such as stressful life events, social support, personality traits, anxiety and coping strategies with postpartum depressive symptoms in a conceptual model of vulnerability to PPD, suggest that women with specific personality traits are more sensitive to the depressogenic effects of adversity and stress events[328].

Similarly, socioeconomic deprivation is another factor clearly linked to stress vulnerability, as observed in low-income populations that exhibit higher levels of stress and impaired coping and depression. Therefore, our findings support and expand the biopsychosocial model of perinatal depression as proposed by Leigh and Milgrom[329]; a modified version of the model incorporating our findings is presented in Figure 7.4. The complex interplay of stress vulnerability factors, precipitating factors associated with adjustment in pregnancy and antenatal stressors, personal resources and coping behaviours and predisposing factors result

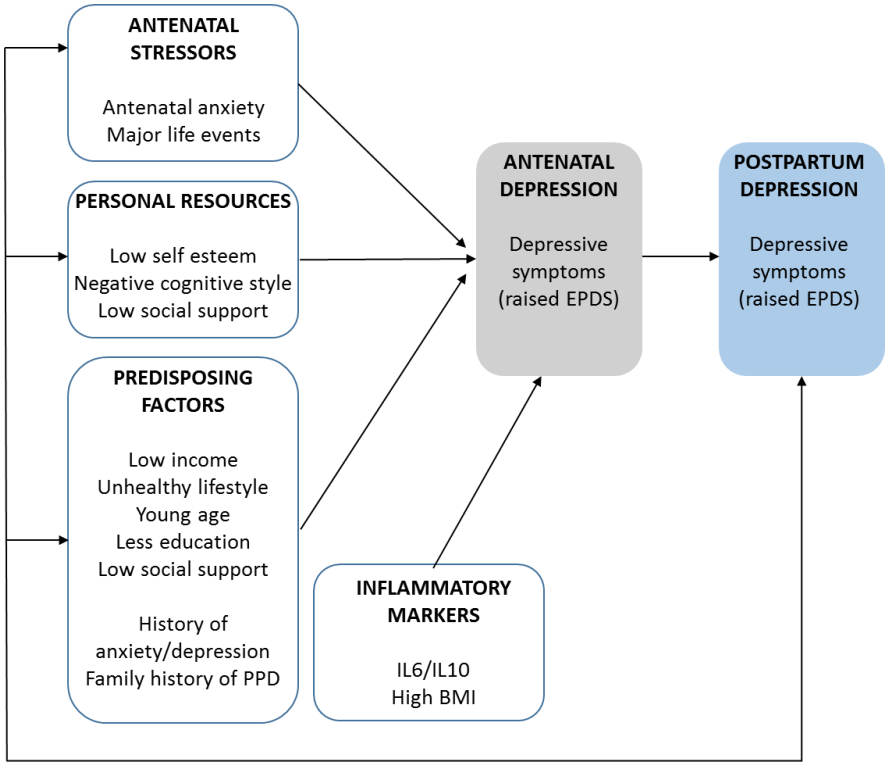

Figure 7.4. A modified version of the Leigh and Milgrom biopsychosocial model of perinatal depression, incorporating findings from the analysis of this study.

in a heterogeneous presentation of PND with affected women experiencing distinct patterns of onset of symptoms, severity, duration and recurrence.

In light of the previous state-of-the-art of the field, this research study introduces valuable findings, with noted implications which upon further refinement could be directly transferrable to the clinic. Key risk factors have been identified and validated, and a novel approach shows quantifiable contributions to EPDS scores. Information regarding a woman's risk of PND would benefit the complex decision making process surrounding choice of intervention, such as whether pharmacological intervention is required despite any risks. Ease of prediction in the clinic is an important outcome underpinning this study, however future work could also utilise this data in the generation of a prediction algorithm.

Future research could include a qualitative study consisting of interviews with stakeholders, health professionals and patients relevant to perinatal mental health services. Increased patient and public involvement (PPI) would help to ensure that any changes to the service provision are suitable for all involved and any barriers for using this type of approach in the clinic are identified. Additionally future work could collect more detailed information for key variables, for example the number of cigarettes smoked and units of alcohol consumed. Inclusion of additional variables such as a history of trauma and initiation of breastfeeding would also be useful to collect.

Already in progress is work to explore the diets of participants of this study, and possible relationships between nutrient intake and the EPDS as well as biomarkers, in particular DNA methylation levels due to an association with B12 and folate. Additional data on nutritional supplements taken, especially folic acid, will be recorded. As described earlier, a larger cohort of women with a relevant family history should be recruited to further investigate the genetic of PND. A larger and more diverse study population is now necessary to further investigate these promising findings. Biomarker studies could be improved by adding a longitudinal element, utilising a study design with multiple sampling points, in particular with the inclusion of a postpartum sample. It would also be beneficial to assess how the biomarkers might predict other disorders of maternal mental health such as anxiety and OCD.

The risk factors identified in this study such as low social status, lower educational level, and housing status point to the prioritisation of the most disadvantaged women in society when it comes to PND. Socioeconomic depravation is an

established risk factor which we also find to increase the prevalence of PND in agreement with other studies[56 99]. At a time when the National Health Service is extremely stretched, the appropriate prioritisation of resources is crucial. With this in mind, this study has demonstrated that data which is routinely collected in the NHS can be utilised in prediction of depression, limiting the impact on resources if a new PND patient pathway is to be introduced. The cost of intervention and most importantly prevention, is far less than the cost of treating the myriad of health, social and economic consequences brought about by the impact of maternal depression[16]. The findings of this study galvanise the need to prioritise women's mental health and promote the awareness of key predictors in order to reduce depression and the associated outcomes for mothers and children.

This study provides novel evidence for distinct profiles of psycho-socio-demographic characteristics associated with within-person heterogeneity of perinatal depressive symptoms severity, timing of onset and remission. These results identified key predisposing factors that affect individual's chronic/acute burden and stress vulnerability and influence depressive risk trajectories during pregnancy and postpartum. The findings here indicate that as women move from the antenatal to the postpartum period, sociodemographic and lifestyle risk factors appear to play a smaller role in risk, and a personal and family history of mental health become increasingly important. Inclusion of covariates, shown to be most predictive of PND as demonstrated in this study, in a predictive screening system could prioritise resources such as specialist assessment for women most at-risk.

Do investigated biomarkers improve prediction of PND symptoms when compared with psychosocial risk factors? (Aim 6)

Previous studies suggested that despite acceptable overall discriminatory power, the EPDS has limited predictive accuracy for absolute PPD risk stratification, regardless of the cut-off value used or the trimester of administration[276]. This appears to be related to the presence of multiple risk factors that are not currently included in antenatal screening instruments[110]. To address this, one approach could be to combine screening for specific psychosocial or biological risk factors alongside EPDS administration. For example, the addition of prior history of anxiety and IL-6/IL-10 ratio to antenatal EPDS seems to yield a more accurate overall prediction of PPD.

This study provides a unique insight into the combination of biological and psycho-sociodemographic predictors of PND. This novel approach has combined

biomarkers with psychosocial risk factors in order to explore the contributions to PND (Figure 7.5). The findings of this thesis are clinically relevant and can be directly translated into the clinic in order to improve the currently inadequate detection of perinatal depression and improve its prediction.

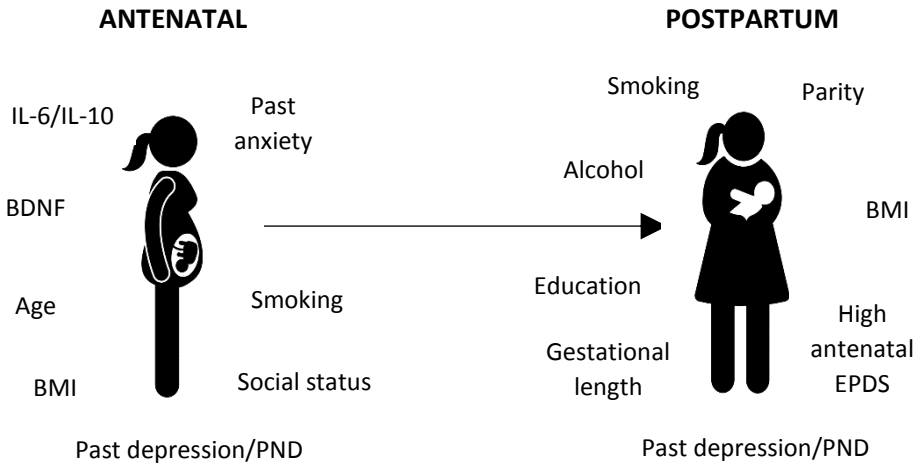

Figure 7.5. A summary of the key factors involved in antenatal and postpartum depressive risk as identified by this study.

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Appendix

Appendix A

From Cox, J.L., Holden, J.M., and Sagovsky, R. 1987. Detection of postnatal depression: Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry* 150:782-786.

Edinburgh Postnatal Depression Scale

As you are pregnant or have recently had a baby, we would like to know how you are feeling. Please check the answer that comes closest to how you have felt **IN THE PAST 7 DAYS**, not just how you feel today.

Here is an example, already completed.

I have felt happy:
- ☐ Yes, all the time
- ☒ Yes, most of the time — This would mean: "I have felt happy most of the time" during the past week.
- ☐ No, not very often — Please complete the other questions in the same way.
- ☐ No, not at all

In the past 7 days:

1. I have been able to laugh and see the funny side of things
   - ☐ As much as I always could
   - ☐ Not quite so much now
   - ☐ Definitely not so much now
   - ☐ Not at all

2. I have looked forward with enjoyment to things
   - ☐ As much as I ever did
   - ☐ Rather less than I used to
   - ☐ Definitely less than I used to
   - ☐ Hardly at all

*3. I have blamed myself unnecessarily when things went wrong
   - ☐ Yes, most of the time
   - ☐ Yes, some of the time
   - ☐ Not very often
   - ☐ No, never

4. I have been anxious or worried for no good reason
   - ☐ No, not at all
   - ☐ Hardly ever
   - ☐ Yes, sometimes
   - ☐ Yes, very often

*5 I have felt scared or panicky for no very good reason
   - ☐ Yes, quite a lot
   - ☐ Yes, sometimes
   - ☐ No, not much
   - ☐ No, not at all

*6. Things have been getting on top of me
   - ☐ Yes, most of the time I haven't been able to cope at all
   - ☐ Yes, sometimes I haven't been coping as well as usual
   - ☐ No, most of the time I have coped quite well
   - ☐ No, I have been coping as well as ever

*7 I have been so unhappy that I have had difficulty sleeping
   - ☐ Yes, most of the time
   - ☐ Yes, sometimes
   - ☐ Not very often
   - ☐ No, not at all

*8 I have felt sad or miserable
   - ☐ Yes, most of the time
   - ☐ Yes, quite often
   - ☐ Not very often
   - ☐ No, not at all

*9 I have been so unhappy that I have been crying
   - ☐ Yes, most of the time
   - ☐ Yes, quite often
   - ☐ Only occasionally
   - ☐ No, never

*10 The thought of harming myself has occurred to me
   - ☐ Yes, quite often
   - ☐ Sometimes
   - ☐ Hardly ever
   - ☐ Never

Administered/Reviewed by _____ Date _____

SCORING

QUESTIONS 1, 2, & 4 (without an *)
Are scored 0, 1, 2 or 3 with top box scored as 0 and the bottom box scored as 3.

QUESTIONS 3, 5-10 (marked with an *)
Are reverse scored, with the top box scored as a 3 and the bottom box scored as 0.

Maximum score: 30
Possible Depression: 10 or greater
Always look at item 10 (suicidal thoughts)