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Placental FKBP51 mediates a link between second trimester maternal anxiety and birthweight in female infants

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Prenatal distress is associated with adverse outcomes in affected offspring. Alterations in placental glucocorticoid signalling and subsequent foetal overexposure to glucocorticoids have been implicated as an underlying mechanism. Infant sex is emerging as an important factor in disease susceptibility. This study aimed to examine the effects of maternal distress across pregnancy on birth outcomes and placental glucocorticoid genes in a sex-dependent manner. Participants completed psychological distress questionnaires throughout pregnancy. Placental HSD11B2, NR3C1 and FKBP51 were analysed by real time PCR and cortisol was measured in new-born hair. Second trimester stress was negatively correlated with birthweight in males and positively correlated with placental NR3C1 mRNA in females. Second trimester anxiety was negatively correlated with birthweight and placental FKBP51 mRNA in females. In mediation analysis, placental FKBP51 mRNA expression was found to mediate the link between prenatal anxiety and birthweight. New-born cortisol was negatively correlated with second trimester anxiety and positively correlated with female placental FKBP51 mRNA levels. Again, FKBP51 mRNA was found to mediate the link between anxiety and new-born cortisol. These results highlight a role for FKBP51 in the placental response to prenatal distress in females. The precise role that placental FKBP51 has in foetal and infant development has not been extensively studied and warrants further investigations.

There is now a large body of evidence showing that the *in utero* experience is a critical determinant of future health[1–3]. One factor that has been extensively studied in this regard is the adverse effects of prenatal maternal psychological distress, which we define as the experience of significant levels of psychological stress, depression, and/or anxiety during pregnancy[4,5]. We have previously reported the incidence of this in pregnancy using the SCOPE (Screening for Pregnancy Endpoints) pregnancy cohort of nulliparous healthy pregnant women[6,7]. All participants completed a combination of validated questionnaires used to assess maternal psychological distress[5]. These included the 10-item Perceived Stress Scale (PSS) to measure psychological stress[8], the 6-item version of State Trait Anxiety Inventory (STAI) to measure maternal anxiety[9], and the Edinburgh Postnatal Depression Scale (EPDS) to measure maternal depressive symptoms in pregnancy[8,10]. We found that 15% of women experienced 'very high levels of perceived psychological stress ($\geq 90^{th}$ percentile score), 18% were classified as being as 'very highly anxious' ($\geq 90^{th}$ percentile score), while 15% were classified as being 'highly likely depressed' (EPDS score >9)[5]. Collectively these data have shown that approximately one in seven women experience clinically significant levels of prenatal maternal psychological distress during pregnancy.

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	Mean ± SD	Range	N
Maternal Age	30.80 ± 4.61	19–41	55
Maternal BMI	25.11 ± 4.15	19–39	55
Gestational Age (weeks)	39.75 ± 0.16	34–42	55
1 min Apgar Score	8.36 ± 0.18	3–10	55
5 min Apgar Score	9.51 ± 0.09	6–10	55
Birthweight	3623.45 ± 62.07	2170–4980	55
Birthweight Centiles	54.13 ± 3.48	9–100	55

Table 1. Descriptive statistics of continuous variables. Abbreviations: Standard Deviation (SD), Body Mass Index (BMI).

This is important as numerous epidemiological studies have reported that exposure to prenatal maternal psychological distress is a risk factor for a range of adverse short and long-term outcomes in affected offspring. These include an increased risk of adverse obstetric outcomes including caesarean delivery, preterm birth (PTB), low birth weight (LBW) and babies who are small for gestational age (SGA)[4,5,11–14]. Moreover prenatal maternal psychological distress has been proposed to be a risk factor for the development of immune[15,16], metabolic[17,18] and neuropsychiatric disorders[19–21] later in life, with the relative risk varying by offspring sex[20,22–24]. These studies highlight the importance of prenatal maternal psychological distress as a risk factor for adverse outcomes in exposed offspring; however, the causal pathways mediating these associations are unclear.

The glucocorticoid hypothesis is the most widely studied biological mechanism proposed to mediate the association between prenatal maternal psychological distress and adverse outcomes[25]. During pregnancy, changes in the maternal hypothalamic-pituitary-adrenal (HPA) axis leads to an exponential rise in cortisol in the maternal circulation[26,27]. This cortisol stimulates the release of corticotrophin releasing hormone (CRH) from the placenta that enters the maternal circulation and further increases the production of cortisol forming a feed forward loop. As a result maternal cortisol levels are up to ten-fold higher than foetal levels[28]. This progressive increase in maternal cortisol is necessary for foetal organogenesis, however excessive foetal exposure may alter developmental trajectories[29]. The maternal-foetal cortisol gradient is maintained by the expression of 11β-hydroxysteroid dehydrogenase type 2 (HSD11B2) in the placental trophoblast which converts active cortisol into inactive cortisone[29]. Additionally, the glucocorticoid receptor (NR3C1) and FKPB51, a chaperone protein which regulates nuclear transport of NR3C1[30], play an important role in the foetal response to cortisol. We and others have shown that maternal distress in late pregnancy reduces placental HSD11B2 expression[4,31,32]. We also found that the glucocorticoid receptor NR3C1 is upregulated by third trimester distress[4]. Increased methylation of placental FKBP51 has been reported following early third trimester stress[33], however we previously observed no change in FKBP51 expression following distress in the third trimester[4], indicating the need to examine other trimesters. Collectively these data suggest that prenatal maternal psychological distress may alter molecular mechanisms that regulate foetal exposure to maternal cortisol. Importantly alterations in the expression and regulation of HSD11B2, NR3C1 and/or FKBP51 has been linked to poor birth outcomes[34–36] as well as neurobehavioral problems in infants[37–40], suggesting that these may play a causal role in mediating the association between maternal distress and adverse outcomes.

In this study we sought to examine the relationships between psychological prenatal distress in the second and third trimester of pregnancy with birth outcomes and placental HSD11B2, NR3C1 and FKBP51 expression, as three key mediators of placental cortisol signalling. Moreover, we undertook causal mediation analysis to determine whether any changes in the placental expression of these genes were associated with birth outcomes using gender-sensitive methodology.

Results

Study Population. As part of a longitudinal cohort study at Cork University Maternity Hospital, 121 nulliparous pregnant women were recruited in their first or early second trimester of pregnancy. Placenta samples were available for 56 women. Detailed medical records were available for 55 of these women. 51 participants completed the PSS, STAI and EPDS in the second trimester (mean = 20.37 ± 0.85 gestational weeks (GW)). 46 participants completed these questionnaires in the third trimester (mean = 32.62 ± 1.03 GW) (Supplementary Fig. S1). New-born hair samples were available for 29 infants (51.8%). The mean ± SD PSS, STAI and EPDS scores were 14.41 ± 5.19, 4.87 ± 3.49 and 6.31 ± 4.16 in the second trimester and 12.13 ± 5.62, 5.27 ± 3.13 and 6.30 ± 4.63 in the third trimester respectively (Supplementary Fig. S2). Descriptive statistics for this cohort are presented in Tables 1 and 2.

Exposure to second trimester maternal anxiety negatively affects female birth weight. We first sought to determine whether maternal psychological distress scores affected infant birth weight. To do this we examined the associations between PSS, STAI and EPDS scores in the second and/or third trimester with birth weight (mean = 3623 ± 460.3 g) (n = 55). We found that PSS scores in the second trimester were negatively correlated with male ($p < 0.05$), but not female birth weight (Fig. 1a,b). We found no associations between PSS scores measured in the third trimester or combined across pregnancy with male or female birth weight (Table 3). In contrast, second trimester STAI scores were negatively correlated with female ($p < 0.05$) but not male birth weight (Fig. 1c,d). We found no associations between STAI scores in the third trimester and birth weight in male or female infants (Table 3). When combined across pregnancy, STAI scores were negatively correlated with female

		Frequency (%)	N
Marital Status	*Single*	12.7	7
	Married	52.7	29
	Defacto	34.5	19
Employment	*Full-time*	87.3	48
	Part-time	5.5	3
	Unemployed	7.3	4
Country of Birth	*Ireland*	83.6	46
	United Kingdom	5.5	3
	Poland	5.5	3
	Brazil	1.8	1
	Spain	1.8	1
	Romania	1.8	1
Parity	*Nulliparous*	100	55
	Multiparous	0	0
Mode of Delivery	*Unassisted vaginal*	41.8	23
	Operative vaginal	36.4	20
	Prelabour LSCS	9.1	5
	LSCS in labour	12.7	7
Infant sex	*Male*	45.5	25
	Female	54.5	30
Gestational Age	*Term (>38 wks)*	98.2	54
	Preterm (<37 wks)	1.8	1
Gestational Size	*SGA*	1.8	1
	AGA	90.9	50
	LGA	7.3	4

Table 2. Descriptive statistics of categorical variables. Abbreviations: Lower Segment Caesarean Section (LSCS), SGA (Small for Gestational Age (SGA), Average for Gestational Age (AGA), Large for Gestational Age (LGA).

birthweight (Table 3). We observed no association between EPDS scores in the second and/or third trimester with birth-weight of infants of either sex (Table 3). As infant birth weight was significantly altered by maternal BMI (Supplementary Table 1), we adjusted our regression model to examine the potential confounding effects of maternal BMI. When BMI was included in the analyses, the relationship between second trimester PSS scores and male birth weight disappeared ($a\beta = -0.32$, $t(23) = -1.72$, $p = 0.099$). In contrast, second trimester anxiety remained correlated with female birth weight when BMI was included in the regression model ($a\beta = -0.46$, $t(26) = -2.71$, $p = 0.012$). These data revealed a gender specific effect of maternal anxiety on birth weight in female infants.

Placental FKBP51 mediates the association between second trimester maternal anxiety and female birth weight. As second trimester anxiety was associated with female birth weight, we next examined the relationship between second trimester STAI scores and three key genes involved in glucocorticoid signalling in the placenta, HSD11B2, NR3C1 and FKBP51. In agreement with our findings on female birth weight (Fig. 1), we found a significant negative correlation between second trimester STAI scores and placental FKBP51 expression in females ($\beta = -0.64$, $t(25) = -4.10$, $p < 0.0001$), but not males ($\beta = -0.53$, $t(23) = -1.78$, $p = 0.09$). We found no significant associations between STAI scores in the second and/or third trimester and HSD11B2 or NR3C1 expression in males or females (Table 4). Additionally, no associations were observed between PSS and EPDS scores in the second or/and third trimester and placental expression of HSD11B2, NR3C1 and FKBP51 (Table 4), indicating that this effect is specific to heightened anxiety levels. We subsequently examined if placental FKBP51 expression was an independent predictor of infant birth weight. There was a significant association between placental FKBP51 with birth weight in female ($\beta = 0.54$, $t(29) = 3.38$, $p = 0.002$) but not male ($\beta = -0.16$, $t(24) = -0.78$, $p = 0.44$) infants (Table 5). These data show that second trimester anxiety (STAI scores) negatively correlates with both birth weight and placental FKBP51 in females, and that FKBP51 positively correlates with birth weight in females. Given these findings, we hypothesised that placental FKBP51 may be mediating the relationship between maternal anxiety and female birth weight. In support of this hypothesis when FKBP51 was included into the regression model the association between maternal anxiety and female birth weight was reduced ($\beta = 0.19$, $t(25) = -0.816$, $p = 0.423$) (Fig. 2a). These data show that the association between second trimester maternal anxiety and female birth weight is mediated by placental FKBP51 (Figs 2 and 3).

Alterations in second trimester maternal anxiety and placental FKBP51 are associated with new-born cortisol levels. The placental and foetal response to glucocorticoids is crucial in determining foetal growth outcomes. This is highlighted by studies showing that exposure to synthetic glucocorticoids during

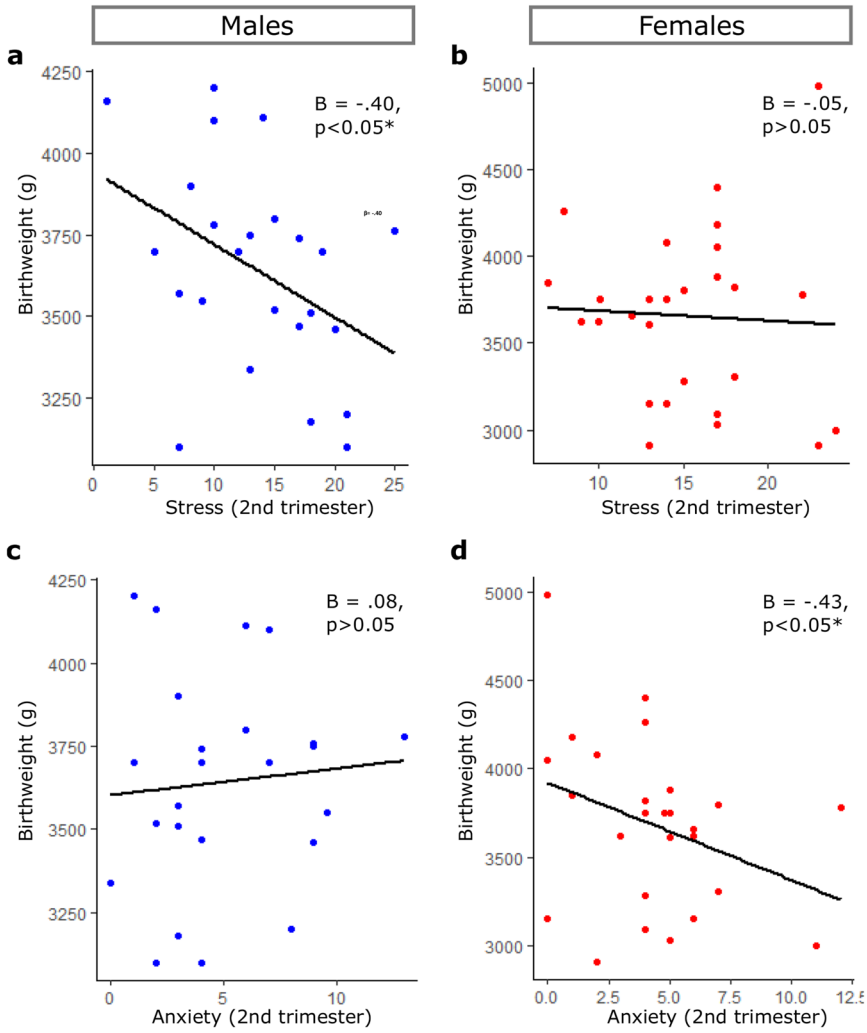

Figure 1. Second trimester distress correlates with reduced infant birthweight. Scatter plots of birthweight and (**a**,**b**) second trimester stress (PSS) and (**c**,**d**) second trimester anxiety (STAI) in males (blue) and females (red). Univariate linear regression analysis *$p < 0.05$.

pregnancy is associated with reductions in birth weight[41], with some sex-specific outcomes also observed[42]. As FKBP51, negatively regulates nuclear transport of NR3C1[30], we hypothesized that this may result in alterations in cortisol levels in infants. In an exploratory technique we measured cortisol levels in new-born hair as a potential retrospective measure of cortisol exposure *in utero*[43]. New-born hair samples were available for 29 infants in this cohort. We examined the relationship between maternal distress and infant cortisol levels. Maternal PSS or EPDS scores in the second and/or third trimester did not correlate with infant cortisol levels (Table 6). Surprisingly however, second trimester maternal anxiety (STAI) was negatively associated with infant hair cortisol levels ($\beta = -0.43$, t (25) $= -2.33$, $p = 0.028$). We next went on to examine the relationship between placental genes and birth outcomes with infant cortisol. Placental HSD11B2 ($\beta = -0.02$, t (28) $= -0.10$, $p = 0.920$) and NR3C1 ($\beta = -0.19$, t (28) $= -1.00$, $p = 0.325$) were not related to new-born cortisol. Intriguingly however, FKBP51 expression was positively correlated with infant cortisol levels in females only ($\beta = 0.54$, t (13) $= 2.23$, $p = 0.045$). As both maternal anxiety and FKBP51 were related to new-born cortisol levels, when we included both in a mediation model, the relationship between second trimester anxiety ($a\beta = -0.27$, t (11) $= -0.62$, $p = 0.550$) and FKBP51 ($a\beta = 0.30$, t (11) $= 0.68$, $p = 0.510$) with new-born cortisol levels was reduced. These data suggest that FKBP51 mediates the relationship between second trimester anxiety and new-born cortisol levels (Fig. 2b). As the relationship between second trimester anxiety and infant birthweight and cortisol levels were both independently mediated by placental FKBP51, we hypothesized that foetal cortisol may be the biological mediator behind these associations. However, there was no correlation observed between new-born cortisol levels and infant birth weight, suggesting the biological mediators linking placental FKBP51 with infant birthweight may not be related to foetal cortisol exposure.

	Both	Males	Females
PSS (2nd trimester)			
Birthweight	$\beta = -0.18$, $t_{50} = -1.32$, $p = 0.19$	**$\beta = -0.40$, $t_{23} = -2.08$, $p = 0.04$**	$\beta = -0.05$, $t_{26} = -0.25$, $p = 0.79$
Birthweight Centiles	$\beta = -0.18$, $t_{50} = -1.29$, $p = 0.20$	$\beta = -0.30$, $t23 = 1.50$, $p = 0.14$	$\beta = -0.16$, $t_{26} = -0.82$, $p = 0.41$
PSS (3rd trimester)			
Birthweight	$\beta = -0.10$, $t_{45} = -0.71$, $p = 0.47$	$\beta = -0.24$, $t_{20} = -1.08$, $p = 0.29$	$\beta = -0.05$, $t24 = -0.25$, $p = 0.80$
Birthweight Centiles	$\beta = -0.05$, $t_{45} = -0.37$, $p = 0.70$	$\beta = -0.15$, $t_{20} = -0.67$, $p = 0.50$	$\beta = 0.00$, $t_{24} = 0.01$, $p = 0.99$
PSS (Combined)			
Birthweight	$\beta = -0.266$, $t_{41} = -1.75$, $p = 0.09$	$\beta = -0.40$, $t_{19} = -1.87$, $p = 0.08$	$\beta = -0.22$, $t_{21} = -0.22$, $p = 0.34$
Birthweight Centiles	$\beta = -0.20$, $t_{41} = -1.331$, $p = 0.19$	$\beta = -0.29$, $t_{19} = -1.32$, $p = 0.20$	$\beta = -0.19$, $t_{21} = -0.86$, $p = 0.40$
STAI (2nd trimester)			
Birthweight	*$\beta = -0.25$, $t_{50} = -1.85$, $p = 0.07$*	$\beta = 0.08$, $t_{23} = 0.39$, $p = 0.69$	**$\beta = -0.43$, $t_{26} = -2.42$, $p = 0.02$**
Birthweight Centiles	$\beta = -0.19$, $t_{50} = -1.41$, $p = 0.16$	$\beta = -0.00$, $t_{23} = -0.02$, $p = 0.97$	*$\beta = -0.34$, $t_{26} = -1.80$, $p = 0.08$*
STAI (3rd trimester)			
Birthweight	*$\beta = -0.28$, $t_{45} = -1.95$, $p = 0.05$*	$\beta = -0.18$, $t_{20} = -0.81$, $p = 0.42$	$\beta = -0.33$, $t_{24} = -1.70$, $p = 0.10$
Birthweight Centiles	$\beta = -0.19$, $t_{45} = -1.33$, $p = 0.18$	$\beta = -0.19$, $t_{20} = -0.84$, $p = 0.40$	$\beta = -0.23$, $t_{24} = -1.17$, $p = 0.25$
STAI (Combined)			
Birthweight	**$\beta = -0.36$, $t_{41} = -2.41$, $p = 0.02$**	$\beta = -0.05$, $t_{19} = -0.22$, $p = 0.83$	**$\beta = -0.50$, $t_{21} = -2.59$, $p = 0.02$**
Birthweight Centiles	$\beta = -0.26$, $t_{41} = -1.73$, $p = 0.09$	$\beta = -0.15$, $t_{19} = -0.64$, $p = 0.53$	$\beta = -0.37$, $t_{21} = -1.79$, $p = 0.09$
EPDS (2nd trimester)			
Birthweight	$\beta = -0.13$, $t_{50} = -0.95$, $p = 0.34$	$\beta = -0.15$, $t_{23} = -0.72$, $p = 0.47$	$\beta = -0.13$, $t_{26} = -0.69$, $p = 0.49$
Birthweight Centiles	$\beta = -0.09$, $t_{50} = -0.69$, $p = 0.49$	$\beta = -0.22$, $t_{23} = -1.06$, $p = 0.29$	$\beta = -0.10$, $t_{26} = -0.51$, $p = 0.60$
EPDS (3rd trimester)			
Birthweight	$\beta = -0.12$, $t_{45} = -0.85$, $p = 0.39$	$\beta = -0.08$, $t_{20} = -0.37$, $p = 0.70$	$\beta = -0.14$, $t_{24} = -0.72$, $p = 0.47$
Birthweight Centiles	$\beta = -0.06$, $t_{45} = -0.39$, $p = 0.69$	$\beta = -0.08$, $t_{20} = -0.35$, $p = 0.73$	$\beta = -0.03$, $t_{24} = -0.15$, $p = 0.87$
EPDS (Combined)			
Birthweight	$\beta = -0.21$, $t_{41} = -1.37$, $p = 0.18$	$\beta = -0.12$, $t_{19} = -0.53$, $p = 0.61$	$\beta = -0.27$, $t_{21} = -1.26$, $p = 0.22$
Birthweight Centiles	$\beta = -0.11$, $t_{41} = -0.72$, $p = 0.48$	$\beta = -0.20$, $t_{19} = -0.87$, $p = 0.39$	$\beta = -0.12$, $t_{21} = -0.56$, $p = 0.58$

Table 3. Maternal distress across pregnancy and birth outcomes. Linear regression analysis. Data shown are crude unstandardized betas with corresponding t-statistic and p-values. Abbreviations: Perceived Stress Scale (PSS), State Trait Anxiety Inventory (STAI) and Edinburgh Postnatal Depression Scale (EPDS).

Discussion

A large proportion of women report experiencing psychological distress throughout their pregnancy[5,32]. This is important as prenatal distress has been linked to a wide range of poor obstetric and neonatal outcomes as well as an increased risk of disease in childhood and adulthood for exposed offspring. Of particular importance prenatal distress is commonly linked to birthweight and birth size[44]. Reduced birthweight remains a significant clinical challenge as it is often associated with increased mortality and morbidity[45]. Additionally, infants of lower birthweights are at an increased risk of developmental impairments in childhood, particularity in relation to neurodevelopment[46,47]. Whilst the poor outcomes associated with being born low birthweight are well documented, the prenatal determinants linking psychological distress and birthweight are not very well understood.

Here we find the effect of maternal distress on birth outcomes to depend on the type of distress, timing of distress and sex of the infant. We initially observed a significant relationship between second trimester stress and reduced infant birthweight in males. However, this association disappeared after adjustment for maternal BMI. Most notably we observe a significant negative correlation between birthweight and second trimester anxiety, consistent with a recent report[48]. When stratified based on sex, this relationship was only observed in females. This sex difference pertaining to birthweight and prenatal anxiety has previously been reported where males born from anxious pregnancies had increased birthweight compared to male controls, and females born from anxious mothers had reduced birthweights compared to female controls[49]. Anxious mothers of females are more likely to develop obstetric complications, whereas anxious mothers of males are not[50]. Male fetuses are generally more vulnerable to the effects of maternal distress[51]. It has been postulated that under conditions of adversities the male fetus favors growth at the expense of other developmental processes, whereas the female fetus conserves growth, thus being born at lower weights but with fewer morbidities in later life[50]. In support of this, mid pregnancy exposure to dexamethasone, a synthetic glucocorticoid, was found to decrease maternal blood in sinusoids of the female but not male placenta, restricted blood flow may mechanistically explain restricted growth in female fetuses[52].

At a biological level, sex specific responses in the placenta to maternal perturbations may explain why one sex is more vulnerable over the other[53]. Sex-specific responses to maternal glucocorticoids, or more specifically how the placenta regulates glucocorticoids differentially may play a role[54,55]. In this study we focused on three genes in the placenta involved in glucocorticoid regulation; HSD11B2, NR3C1 and FKBP51. Inconsistent with our previous work[4] and work of others[31], we do not observe a reduction in HSD11B2 following prenatal distress, however

	Both	Males	Females
PSS (2nd trimester)			
HSD11B2	$\beta = 0.26, t_{49} = 1.89, p = 0.07$	$\beta = 0.39, t_{22} = 1.99, p = 0.06$	$\beta = 0.09, t_{26} = 0.47, p = 0.64$
NR3C1	$\beta = -0.11, t_{50} = 0.80, p = 0.43$	$\beta = -0.15, t_{23} = -0.71, p = 0.49$	**$\beta = 0.42, t_{26} = 2.33, p = 0.03$**
FKBP5	$\beta = -0.05, t_{49} = -0.32, p = 0.75$	$\beta = -0.04, t_{23} = -0.17, p = 0.87$	$\beta = -0.09, t_{25} = -0.42, p = 0.68$
PSS (3rd trimester)			
HSD11B2	$\beta = -0.11, t_{45} = -0.71, p = 0.48$	$\beta = -0.20, t_{20} = -0.90, p = 0.38$	$\beta = -0.02, t_{24} = -0.11, p = 0.92$
NR3C1	$\beta = -0.21, t_{45} = -1.39, p = 0.17$	$\beta = -0.31, t_{20} = -1.44, p = 0.17$	$\beta = -0.21, t_{24} = -0.59, p = 0.55$
FKBP5	$\beta = -0.16, t_{44} = -1.05, p = 0.29$	$\beta = -0.12, t_{20} = -0.53, p = 0.60$	$\beta = -0.21, t_{23} = -1.04, p = 0.32$
PSS (Combined)			
HSD11B2	$\beta = 0.13, t_{41} = 0.81, p = 0.42$	$\beta = 0.15, t_{19} = 0.64, p = 0.53$	$\beta = 0.08, t_{21} = 0.34, p = 0.74$
NR3C1	$\beta = -0.10, t_{41} = -0.65, p = 0.52$	$\beta = -0.39, t_{19} = -1.84, p = 0.08$	$\beta = 0.17, t_{21} = 0.81, p = 0.43$
FKBP5	$\beta = -0.14, t_{40} = -0.89, p = 0.38$	$\beta = -0.05, t_{19} = -0.23, p = 0.82$	$\beta = -0.28, t_{20} = -1.31, p = 0.20$
STAI (2nd trimester)			
HSD11B2	$\beta = -0.12, t_{49} = -0.81, p = 0.42$	$\beta = -0.12, t_{22} = -0.54, p = 0.59$	$\beta = -0.11, t_{26} = -0.56, p = 0.58$
NR3C1	$\beta = 0.15, t_{50} = 1.04, p = 0.31$	$\beta = 0.17, t_{23} = 0.78, p = 0.44$	$\beta = 0.13, t_{26} = 0.66, p = 0.52$
FKBP5	**$\beta = -0.46, t_{49} = -3.59, p = 0.001$**	$\beta = -0.36, t_{23} = -1.79, p = 0.08$	**$\beta = -0.64, t_{25} = -4.10, p = 0.000$**
STAI (3rd trimester)			
HSD11B2	$\beta = -0.00, t_{45} = -0.02, p = 0.98$	$\beta = -0.00, t_{20} = -0.03, p = 0.98$	$\beta = -0.01, t_{24} = -0.06, p = 0.95$
NR3C1	$\beta = -0.02, t_{45} = -0.14, p = 0.89$	$\beta = -0.01, t_{20} = -0.08, p = 0.94$	$\beta = -0.02, t_{24} = -0.09, p = 0.92$
FKBP5	$\beta = -0.21, t_{44} = -1.40, p = 0.17$	$\beta = -0.14, t_{20} = -0.61, p = 0.55$	$\beta = -0.31, t_{23} = -1.51, p = 0.15$
STAI (Combined)			
HSD11B2	$\beta = -0.04, t_{41} = -0.23, p = 0.82$	$\beta = -0.01, t_{19} = -0.05, p = 0.96$	$\beta = -0.06, t_{21} = -0.27, p = 0.79$
NR3C1	$\beta = 0.06, t_{41} = -0.40, p = 0.68$	$\beta = 0.06, t_{19} = 0.26, p = 0.79$	$\beta = 0.07, t_{21} = -0.30, p = 0.77$
FKBP5	**$\beta = -0.36, t_{40} = -2.43, p = 0.019$**	$\beta = -0.26, t_{19} = -1.17, p = 0.26$	$\beta = -0.53, t_{20} = -2.70, p = 0.01$
EPDS (2nd trimester)			
HSD11B2	$\beta = 0.09, t_{49} = 0.67, p = 0.50$	$\beta = 0.06, t_{22} = 0.27, p = 0.79$	$\beta = 0.08, t_{26} = 0.39, p = 0.69$
NR3C1	$\beta = 0.03, t_{50} = 0.19, p = 0.85$	$\beta = -0.13, t_{23} = -0.63, p = 0.54$	$\beta = 0.16, t_{26} = 0.82, p = 0.42$
FKBP5	$\beta = -0.26, t_{49} = -1.83, p = 0.07$	$\beta = -0.24, t_{23} = -1.16, p = 0.26$	$\beta = -0.34, t_{25} = -1.74, p = 0.09$
EPDS (3rd trimester)			
HSD11B2	$\beta = -0.05, t_{45} = -0.34, p = 0.74$	$\beta = 0.08, t_{20} = 0.33, p = 0.75$	$\beta = -0.15, t_{24} = -0.72, p = 0.48$
NR3C1	$B = -0.19, t_{45} = -1.25, p = 0.22$	$\beta = -0.24, t_{20} = -1.08, p = 0.29$	$B = -0.16, t_{24} = -0.76, p = 0.46$
FKBP5	$\beta = -0.14, t_{44} = -0.92, p = 0.36$	$\beta = 0.07, t_{20} = 0.35, p = 0.73$	$\beta = -0.40, t_{23} = -2.09, p = 0.051$
EPDS (Combined)			
HSD11B2	$\beta = 0.06, t_{41} = 0.36, p = 0.72$	$\beta = 0.16, t_{19} = 0.67, p = 0.51$	$\beta = -0.06, t_{21} = -0.28, p = 0.78$
NR3C1	$B = -0.12, t_{41} = -0.75, p = 0.46$	$\beta = -0.26, t_{19} = -1.17, p = 0.26$	$\beta = -0.00, t_{21} = -0.00, p = 0.99$
FKBP5	$\beta = -0.19, t_{40} = -1.27, p = 0.21$	$\beta = -0.02, t_{19} = -0.10, p = 0.92$	**$\beta = -0.44, t_{20} = -2.16, p = 0.04$**

Table 4. Maternal distress across pregnancy and placental HSD11B2, NR3C1 and FKBP51 expression. Linear regression analysis. Data shown are crude unstandardized betas with corresponding t-statistic and p-values. Abbreviations: Perceived Stress Scale (PSS), State Trait Anxiety Inventory (STAI) and Edinburgh Postnatal Depression Scale (EPDS).

the mean stress score in this population was relatively low and the effect of maternal stress on HSD11B2 expression has been shown to be dependent on severity[56]. Second trimester maternal stress increased NR3C1 expression in female placentae. This increase in NR3C1 we observe among females but not males could again represent an adaptive response of the female placenta in response to maternal distress.

FKBP51 is a chaperone protein that interacts with steroid hormone receptors through heat shock protein 90 (HsP90), inhibiting the activation of the glucocorticoid receptor and the progesterone receptor (PR) and increasing the activation of the androgen receptor[57]. Consistent with previous reports[33,58] we find prenatal distress to reduce placental FKBP51 expression. Importantly we find placental FKBP51 to mediate a relationship between second trimester anxiety and infant birth weight in females only, suggesting a critical role for this chaperone protein in the female foetal response to maternal anxiety. As FKBP51 regulates the glucocorticoid receptor we hypothesised that placental changes in FKBP51 would result in alterations in foetal cortisol. Indeed, we observed a positive correlation between placental FKBP51 and new-born cortisol levels in female infants. Of interest, second trimester anxiety was the only distress variable that influenced new-born cortisol levels. Following our mediation analysis, we showed second trimester maternal anxiety decreases new-born cortisol levels by reducing FKBP51 in female placentae (Fig. 3). However, no association between new-born cortisol levels and birthweight were found. Alternatively, the inhibitory action of FKBP51 on the PR may underlie the relationship between maternal anxiety and infant birthweight. Progesterone supplementation is commonly administered to women at risk of preterm birth and women who receive progesterone are less likely to deliver a preterm or deliver low birthweight

	Both	Males	Females
HSD11B2			
Birthweight	$\beta = 0.05, t_{53} = 0.36, p = 0.72$	$\beta = -0.17, t_{23} = -0.81, p = 0.42$	$\beta = 0.14, t_{29} = 0.76, p = 0.45$
Birthweight Centiles	$\beta = 0.14, t_{53} = 1.04, p = 0.30$	$\beta = -0.07, t_{23} = -0.36, p = 0.71$	$\beta = 0.21, t_{29} = 1.17, p = 0.24$
NR3C1			
Birthweight	$\beta = -0.07, t_{54} = -0.55, p = 0.58$	$\beta = -0.05, t_{24} = -0.24, p = 0.80$	$\beta = 0.09, t_{29} = -0.47, p = 0.63$
Birthweight Centiles	$\beta = -0.16, t_{54} = -1.24, p = 0.21$	$\beta = -0.03, t_{24} = -0.15, p = 0.87$	$\beta = -0.26, t_{29} = -1.43, p = 0.16$
FKBP51			
Birthweight	$\beta = 0.21, t_{53} = 1.58, p = 0.11$	$\beta = -0.16, t_{24} = -0.78, p = 0.44$	$\boldsymbol{\beta = 0.54, t_{29} = 3.38, p = 0.002}$
Birthweight Centiles	$\beta = 0.21, t_{53} = 1.57, p = 0.122$	$\beta = -0.09, {}_{t24} = -0.43, p = 0.67$	$\boldsymbol{\beta = 0.56, t_{29} = 3.51, p = 0.002}$

Table 5. Placental HSD11B2, NR3C1 and FKBP51 expression and neonatal outcomes. Linear regression analysis. Data shown are crude unstandardized betas with corresponding t-statistic and p-values.

infants[59]. As the relationship we observed between placental FKBP51 and birthweight was specific to females, it is of particular interest that increased maternal serum placental progesterone in the first trimester has been found to be associated with increased birthweight in females, with no significant effect on males[60]. Therefore, it may be possible that the inhibitory actions of FKBP51 on the PR may underlie the link between maternal anxiety and female birthweight. Future studies into this relationship should help to elucidate these mechanisms. None the less our finding, together with the previously reported relationship between placental FKBP51 methylation and neurobehavioral problems in infants[40], suggests placental FKBP51 as a novel player in foetal programming.

Identifying vulnerable periods of development where the foetus (and placenta) are most susceptible to environmental perturbations is a growing area of research. By prospectively examining women in the second and third trimester we have been able to identify the second trimester as a critical window where the foetus might be most susceptible to the effects of maternal anxiety. This is consistent with a number of similar studies that have demonstrated alterations in the mRNA expression and/or methylation levels of glucocorticoid regulating genes, following second trimester maternal distress[31,33,61]. Similarly, mid-gestation exposure to severe life events, particularly in months 5 and 6 of pregnancy have been shown to heighten the risk of adverse neonatal outcomes[62]. The vulnerability of the second trimester is further evident by a number of studies that have shown second trimester stress to predict poor neurodevelopment in infants[63–66]. The second trimester is a period of rapid foetal growth, particularly for the foetal brain[67]. Further, the foetal HPA response becomes active from 20 weeks of pregnancy[68], therefore maternal stress arising in this period may have a more detrimental impact on development.

The current study has several strengths and limitations. Although the sample size used in this study is comparable to that of previously published work examining maternal distress and placental gene expression[31,32], we acknowledge the small sample size and suggest this research be carried out on a larger scale, although appreciate the difficulties in running large scale placental collection studies for mRNA analysis[69]. Due to the limited sample size in this cohort, we did not routinely adjust for potential confounders outside of maternal age and BMI, therefore it may be possible that maternal lifestyle factors and socioeconomic status may be further influencing this relationship. We report significant associations with prenatal distress and birthweight we would like to highlight that only one (1.8%) infant in this cohort was born <2500 g, the WHO estimate for clinically defined low birthweight[70]. Validating this work in a cohort of clinically defined low birthweight infants will be an important next step in unravelling the role of FKBP51 in the foetal response to maternal anxiety. By prospectively examining maternal distress in the second and third trimester of pregnancy we have been able to identify the second trimester as a crucial period during development whereby the foetus may be most susceptible to the effects of maternal distress. This will add to the growing body of literature examining critical windows of foetal development.

Overall this study is important as it identifies a crucial role for the timing of distress, the type of distress and foetal sex in the relationship between prenatal distress and placental gene expression. This adds to the existing literature supporting a role for alterations in placental glucocorticoid signalling following prenatal distress. To our knowledge this is the first study to identify an association between placental FKBP51 and infant birthweight. Importantly we identify this gene to be a key mediator underlying a relationship between prenatal anxiety and birthweight in females, which highlights the crucial role placental signalling has in terms of exposure to maternal distress and infant development. The identification of this relationship warrants further investigation into the precise role that FKBP51 has in foetal development.

Methods

Participants. This study received full ethical approval from the Clinical Research Ethics Committee of Cork Teaching Hospitals and was carried out in accordance with the guidelines and regulations outlined in the ethics. Nulliparous pregnant women enrolled in the IMPROvED study[71] at Cork University Maternity Hospital were invited to participate in this study. After giving informed consent, participants completed the PSS, STAI, and EPDS in the second and/or third trimesters of pregnancy. Detailed demographic and medical information was acquired from the participants' medical records.

New-born Hair Collection and Processing. New-born hair was acquired from the posterior vortex of the new-borns head within 24 h of birth and stored at room temperature until processing. 1 mg of hair was incubated in 1 ml of methanol at 50 °C for 24 h. Samples were sonicated for 30 min at 37 °C followed by another incubation

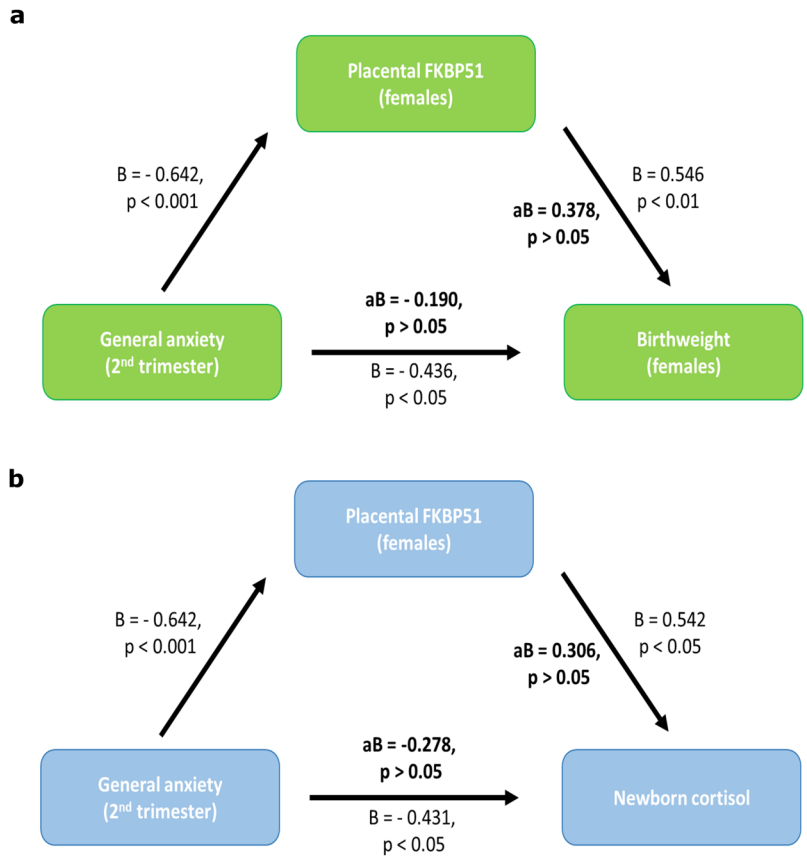

Figure 2. Placental FKBP51 mediates the relationship between prenatal anxiety and birthweight in females. Mediation Plots (**a**) Placental FKBP51 mediates the relationship between second trimester maternal anxiety and infant birthweight in females. (**b**) Placental FKBP51 mediates the relationship between second trimester maternal anxiety and new-born hair cortisol in females. Linear regression analysis.

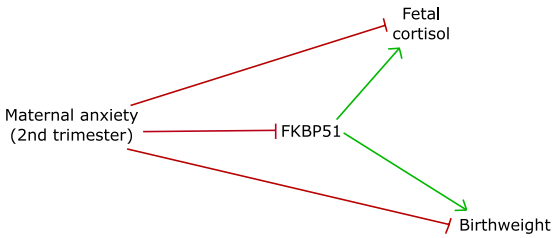

Figure 3. Summary Figure. Second trimester maternal anxiety decreases infant birthweight in female offspring by inhibiting placental FKBP51. Similarly second trimester maternal anxiety reduced foetal cortisol exposure by inhibiting FKBP51 in female offspring.

for 24 h at 50 °C. The supernatant was removed and evaporated under nitrogen and the pellet was resuspended in Phosphate Buffered Solution. Cortisol concentration was determined by ELISA as per the manufacturer's instructions (Enzo life Sciences).

Placental collection and real-time PCR. Placenta biopsies were collected from 56 participants within 2 h of delivery, washed in dH_2O and immediately stored at −80 °C. RNA was extracted from placental samples using Trizol reagent as previously described[4]. Briefly, placental samples were homogenised in Trizol and left on ice for 10 min. Samples were centrifuged and the supernatant was incubated in chloroform at room temperature for 5 min followed by centrifugation for 15 min at 4 °C to remove the aqueous phase. RNA was isolated by incubation of the aqueous phase with propanol at room temperature for 10 min. Samples were centrifuged and the pellet washed in 70% ethanol before resuspension in RNAse free H_2O (Sigma). RNA quality and quantity were determined by the Nanodrop 1000. RNA was reverse transcribed into cDNA (400 ng/ml) using the high capacity cDNA reverse transcription kit (Applied Biosystems) under the following parameters: 25 °C for 10 min, 37 °C for

	Both	Males	Females
New-born Cortisol			
PSS (2nd trimester)	$\beta = -0.13$, $t_{25} = -0.68$, $p = 0.49$	$\beta = 0.12$, $t_{13} = 0.42$, $p = 0.68$	$\beta = -0.41$, $t_{25} = -1.44$, $p = 0.17$
PSS (3rd trimester)	$\beta = -0.10$, $t_{26} = -0.53$, $p = 0.59$	$\beta = 0.01$, $t_{13} = 0.03$, $p = 0.97$	$\beta = -0.17$, $t_{12} = -0.60$, $p = 0.55$
PSS (Combined)	$\beta = -0.22$, $t_{23} = -1.03$, $p = 0.31$	$\beta = 0.15$, $t_{12} = 0.52$, $p = 0.61$	$\mathbf{-\beta = -0.63, t_{10} = -2.45, p = 0.04}$
STAI (2nd trimester)	$\mathbf{\beta = -0.43, t_{25} = -2.33, p = 0.03}$	*$\beta = -0.51$, $t_{13} = -2.06$, $p = 0.06$*	*$\beta = -0.51$, $t_{11} = -1.91$, $p = 0.08$*
STAI (3rd trimester)	$\beta = -0.16$, $t_{26} = -0.81$, $p = 0.42$	$\beta = -0.23$, $t_{13} = -0.83$, $p = 0.42$	$\beta = -0.10$, $t_{12} = -0.36$, $p = 0.72$
STAI (Combined)	*$\beta = -0.38$, $t_{23} = -1.90$, $p = 0.07$*	$\beta = -0.42$, $t_{12} = -1.51$, $p = 0.16$	$\beta = -0.43$, $t_{10} = -1.43$, $p = 0.188$
EPDS (2nd trimester)	*$\beta = -0.36$, $t_{25} = -1.91$, $p = 0.067$*	$\beta = -0.15$, $t_{13} = -0.54$, $p = 0.59$	$\beta = -0.42$, $t_{11} = -1.46$, $p = 0.17$
EPDS (3rd trimester)	$\beta = -0.15$, $t_{26} = -0.80$, $p = 0.42$	$\beta = -0.04$, $t_{13} = -0.15$, $p = 0.87$	$\beta = -0.24$, $t_{12} = -0.84$, $p = 0.41$
EPDS (Combined)	$\beta = -0.23$, $t_{23} = -1.11$, $p = 0.28$	$\beta = -0.02$, $t_{12} = -0.08$, $p = 0.94$	$\beta = -0.36$, $t_{10} = -1.15$, $p = 0.28$

Table 6. Maternal distress across pregnancy and cortisol levels in new-born hair. Linear regression analysis. Data shown are crude unstandardized betas with corresponding t-statistic and p-values. Abbreviations: Perceived Stress Scale (PSS), State Trait Anxiety Inventory (STAI) and Edinburgh Postnatal Depression Scale (EPDS).

120 min, 85 °C for 5 min and 4 °C for at least 10 min. Real time PCR was performed with the following targets; GAPDH, HSD11B2, NR3C1 and FKBP51 (Integrated DNA Technologies; IDT) using the following parameters; 50 °C for 2 min, 95 °C for 10 min, 50 repetitions of 95 °C for 15 s and annealing/elongating at 60 °C, as previously described[4]. All samples were run in triplicate and gene expression was determined using the $2-\Delta\Delta$cycle threshold (2dCT) method[72] with GAPDH as the reference.

Statistical Analysis. Data analysis was performed on SPSS v22. Scatterplots were produced using R v3.4.2, library ggplot2. Normality of predictor and outcome variables were tested for using Kolmogorov-Smirnov tests. Questionnaire scores, birthweight and birthweight centiles were normally distributed. Placental gene expression and hair cortisol levels displayed a non-normal distribution and were log transformed prior to analysis (Supplementary Fig. S3). A cumulative stress, depression and anxiety score was determined for each participant by summing relevant questionnaire scores from the second and third trimester. This cumulative score is referred to as PSS, STAI or EPDS *combined* throughout the results section. Outliers were determined using a Grubbs test and removed if $p < 0.05$. Relationships were determined using linear regression analysis. Due to the limited sample size in this cohort, regression models were adjusted for maternal age and BMI, only when these demographics correlated with the predictor and/or outcome variables ($p < 0.05$) (Supplementary Table S1).

Data Availability

The raw data used to complete this work is available to readers in Supplementary Information, Supplementary Table S3.

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Author Contributions

K.L.T.: recruited study cohort, acquired data and samples, processed samples, analysed data and wrote manuscript. G.O.K.: provided reagents and reviewed/wrote the manuscript. A.S.K.: provided bioinformatics support, acquired data and reviewed the manuscript. G.C.: provided expertise in psychiatry, reviewed the manuscript and provided reagents. L.C.K.: access to the study cohort and subsequent medical records, provided support in obstetrics/pregnancy, provided reagents and reviewed the manuscript.

Additional Information

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