

Running head: MULTIGENERATIONAL DEPRESSION AND ANXIETY & MATERNAL MEASURES OF STRESS

Multigenerational depression and anxiety influence maternal measures of stress during pregnancy

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Abstract

Objective: The strongest risk factor for depression during pregnancy is a history of depression, and depression and anxiety during pregnancy can lead to adverse maternal, birth, and early childhood outcomes. There is a research gap in multigenerational studies that examine the influence of heredity on the development of maternal mood disorders during pregnancy and the postpartum period. The purpose of our research was to determine if a history of major depressive disorder (MDD) and/or anxiety in the pregnant woman's mother (maternal grandmother, or MGM) predicts the same psychiatric illness in the pregnant woman and if a family history of depression and/or anxiety correlates with maternal and fetal hypothalamic-pituitary-adrenocortical (HPA) axis function, as represented by hair cortisol. We also sought to determine if a family history of psychiatric illness correlates with maternal and fetal hair cortisol concentration and maternal scores on the Center for Epidemiological Studies of Depression Scale (CES-D) and the State-Trait Anxiety Inventory (STAI).

Methods: Pregnant participants ≥ 18 years old without major psychiatric or physical comorbidities were enrolled in a prospective longitudinal study at ≤ 15 weeks gestation. Family history of psychiatric illness was abstracted from a Structured Clinical Interview for DSM-IV Disorders (SCID), conducted during a scheduled study visit. Maternal diagnoses of MDD and anxiety were compared with the MGM's psychiatric history. CES-D and STAI scores for participants who had a MGM with depression and/or anxiety were compared with participants who had no family history of psychiatric illness. Maternal hair was collected at 16 and 28 weeks and maternal and fetal (neonatal) hair was collected following delivery for cortisol analysis.

Results: 153 participants in the study had data for analysis. Of these, 40% met criteria for MDD and 24% for an anxiety disorder (including PTSD) as determined by SCID. CES-D and STAI scores for participants with a history of MDD and/or anxiety in the MGM were higher compared to participants with no family history of psychiatric illness ($p=0.02$ and $p=0.005$), and these scores corresponded with the respective scales (CES-D for MDD and STAI for anxiety). A history of depression and/or anxiety in the MGM was not significantly correlated with maternal HPA axis function as represented by hair cortisol.

Conclusion: History of MDD and/or anxiety in the MGM is associated with maternal scores on the CES-D and STAI that are consistent with depression and anxiety. A history of depression and/or anxiety in the MGM also predicts the same psychiatric illness in the pregnant participant. Healthcare providers must consider family history of psychiatric illness to improve recognition, diagnosis, and treatment of depression and anxiety during prenatal and postpartum care.

Introduction and Literature Review

Maternal stress during pregnancy can lead to adverse maternal, fetal, and neurodevelopmental childhood outcomes. According to Kalra et al. (2007), stress is defined as “a state of bodily or mental tension resulting from factors that tend to alter an existent equilibrium,” and is clearly present in conditions of depression and anxiety. Therefore, presence of maternal depression and/or anxiety during pregnancy is linked to negative impacts on maternal mental health, pregnancy outcomes, fetal development, and neurodevelopmental childhood outcomes (Bergman, Sarkar, Glover, & O’Connor, 2010; Dunkel Schetter & Tanner, 2012; Glover, 2014; Kalra, Einarson, Karaskov, Van Uum, & Koren, 2007). More specifically, maternal anxiety during pregnancy is linked with preterm delivery and maternal depression during pregnancy is linked with lower fetal birth weight, and both psychiatric illnesses are linked with learning, behavior, and motor development problems during childhood (Bergman et al., 2010; Dunkel Schetter & Tanner, 2012; Field & Diego, 2008; Glover, 2014; Hoffman, Mazzoni, Wagner, Laudenslager, & Ross, 2016; O’Donnell, O’Connor, & Glover, 2009). These adverse effects could be due to changes in functioning of the maternal hypothalamic-pituitary-adrenocortical (HPA) axis and to consequent changes in development of the fetal nervous system.

Defining MDD and Anxiety

MDD is defined by many symptoms, often working in concert, that will interfere with an affected patient’s ability to work, sleep, eat, and experience activities that they once enjoyed (National Institute of Mental Health, 2007). Anxiety disorders are characterized by both mental and physiological overreactions to what patients believe to be stressful (Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013). Patients who are diagnosed with MDD are often also

diagnosed with an anxiety disorder, such as generalized anxiety disorder (GAD), post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), and certain phobias (National Institute of Mental Health, 2007). PTSD is diagnosed as a specific anxiety disorder under the DSM-IV, but is a stand-alone psychiatric illness according to the recently released DSM-V. For this study, PTSD is treated as an anxiety disorder with this recent change in mind, and is defined as an anxiety disorder that arises after a patient experiences a traumatic event; examples of which could be a car accident, abuse, or exposure to military combat (National Institute of Mental Health, 2007).

The “Depression Gene”

It is important to clarify that no studies have yet found a so-called “depression gene.” It is possible that the genotype for a specific serotonin transporter (5-HTTLPR) could be associated with the heredity of depression. Risch et al. (2009) performed a meta-analysis of literature on serotonin transporter 5-HTTLPR and concluded that while the number of stressful life events was significantly associated with depression, there is no association between the serotonin transporter 5-HTTLPR genotype and depression in any of the individual studies or in the weighted average of studies used for the meta-analysis. Overall, the meta-analysis resulted in no evidence that the 5-HTTLPR genotype, whether analyzed alone or in conjunction with stressful life events, is associated with an elevated risk of depression in men alone, women alone, or in both sexes (Risch et al., 2009).

Despite the lack of association in genetic studies, there is a consensus that mood disorders are hereditary in some way even though the biological mechanisms are not yet understood. There does seem to be a hereditary influence on the development of certain

psychiatric illnesses, as Weissman et al. (2005, 2006) found that children of depressed parents were about three times as likely to develop MDD, anxiety disorders, and/or substance use disorders.

HPA Axis Functioning in MDD and Anxiety

Both hyper- and hypo activity of the HPA axis are seen in populations of patients who are diagnosed with psychiatric illnesses such as MDD and anxiety, and this hyper or hypo activity may actually lead to the development of certain pathologies (S. M. Smith & Vale, 2006; Staufenbiel et al., 2013). It is uncertain whether hyper or hypo activity of the HPA axis is seen in PTSD. There is some consensus that there is dysregulation and hypoactivity of the HPA axis, but several studies conclude that more research much be done to study the HPA axis in those diagnosed with PTSD (de Kloet et al., 2006; Meewisse, Reitsma, Vries, Berthold, & Olff, 2007; Shea, Walsh, MacMillan, & Steiner, 2005).

HPA Axis Functioning in Pregnancy

Cortisol is a stress hormone that is regulated by the HPA axis. When a stressor is introduced, the hypothalamus produces corticotropin-releasing hormone (CRH), which then triggers the pituitary to release adrenocorticotrophic hormone (ACTH). This stimulates the adrenal cortex to secrete cortisol, which is released in response to chronic stress (Osborne & Monk, 2013; Staufenbiel et al., 2013).

Cortisol levels fluctuate greatly throughout the day and tend to be highest in the morning. Cortisol levels as measured in the morning are 6 to 23 micrograms per deciliter (mcg/dL) (U.S. National Library of Medicine, 2015). This is a wide range because cortisol levels vary between

patients and between measurements; however, this is the standard cortisol range when cortisol measurements are taken in the morning.

Due to hyperactivity of the HPA axis, pregnancy is a state of hypercortisolemia, with cortisol levels increasing during the third trimester of pregnancy especially (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011; Hoffman et al., 2016; Kalra et al., 2007). Therefore, maternal cortisol levels increase during pregnancy, but significant increases in maternal cortisol, in particular during the second trimester of pregnancy, are linked with the aforementioned adverse maternal, fetal, and childhood neurodevelopmental effects (Hoffman et al., 2016; Kalra et al., 2007).

Moreover, while cortisol is absolutely necessary during pregnancy to facilitate normal fetal development, elevated maternal cortisol levels tend to accompany pathological diagnoses (S. M. Smith & Vale, 2006; Wadhwa et al., 2004). Diagnoses of depression and anxiety are one of the most common complications of pregnancy. Anxious mothers have higher cortisol levels during both the prenatal and postpartum periods, and depressed mothers show either increased or decreased cortisol levels during the prenatal and postpartum periods, implying that more research regarding cortisol levels in participants with depression are needed (Staufenbiel et al., 2013). The hypercortisolemia seen in pregnancy suggests that different mechanisms regulate cortisol release during pregnancy and outside of pregnancy.

Outside of pregnancy, glucocorticoids such as cortisol are the downstream effectors of the HPA axis. They regulate physiological changes through intracellular receptors that respond to inadequate or excessive activation of the HPA axis via negative feedback (S. M. Smith & Vale, 2006). As soon as levels of circulating glucocorticoids increase past a threshold value, the same

circulating glucocorticoids inhibit HPA axis activation through the hypothalamus and the pituitary gland (S. M. Smith & Vale, 2006).

There is a different mechanism at work in regards to the release of placental corticotropin-releasing hormone (pCRH) during pregnancy. Cortisol follows a positive feedback mechanism during pregnancy and pCRH production is increased. pCRH then stimulates the maternal HPA axis to increase ACTH secretion, which directly stimulates maternal cortisol secretion and therefore contributes to hypercortisolemia during pregnancy (Majzoub & Karalis, 1999). Since 10-20% of maternal cortisol crosses the placenta and enters the fetus, one possible mechanism could be that elevated maternal cortisol levels (as seen during pregnancy) can stimulate an increase in fetal cortisol through increasing the release of pCRH through hyperactivity of the HPA axis (Field & Diego, 2008). This can influence timing of delivery, which helps explain the high preterm delivery rates of pregnant women who have depression and/or anxiety (Field & Diego, 2008).

While hypercortisolemia during pregnancy is associated with adverse maternal, fetal, and neurodevelopmental childhood outcomes, ample glucocorticoid levels are actually vital for fetal development. Glucocorticoids promote brain development by stimulating neurons to mature, remodeling synaptic connections, and pruning unnecessary synaptic connections (Glover, 2014; Harris & Seckl, 2011). Sufficient glucocorticoid levels are essential for normal maturation of the central nervous system.

Possible Cortisol Release Mechanisms During Pregnancy

First trimester maternal cortisol levels represent the mother's baseline cortisol status and maternal cortisol levels increase during the second and third trimesters of pregnancy. A decrease

in cortisol binding globulin, the carrier protein for cortisol, and increases in pCRH during the last four to six weeks of pregnancy could explain why there is a cortisol increase during pregnancy (Kalra et al., 2007).

Another mechanism for the cortisol increase seen in pregnancy could be due to an increase in the expression of the protein 11-beta-hydroxysteroid dehydrogenase type 2 (11β HSD2), which is an enzyme that shields the fetus from the mother's glucocorticoid levels by converting the active steroid cortisol into inactive cortisone (Glover, Bergman, Sarkar, & O'Connor, 2009; Harris & Seckl, 2011). High levels of 11β -HSD2 are expressed in the placenta, but these levels are shown to decrease throughout pregnancy, which would explain the increased levels of cortisol that reach the developing fetus later in gestation. It has been hypothesized that 11β -HSD2 levels lead to increased glucocorticoid signaling within the placenta, and that these levels are affected by maternal stress, dietary protein restriction, and possibly oxygen levels (Harris & Seckl, 2011).

The physiological mechanism of cortisol release during pregnancy involves placental corticotropin-releasing hormone (pCRH); at around 32 weeks gestation pCRH begins to regulate maternal cortisol production rather than maternal CRH (Field & Diego, 2008; Majzoub & Karalis, 1999). The mother's CRH levels are the highest near delivery, and CRH levels decrease to prenatal levels within 24 hours of delivery (Majzoub & Karalis, 1999).

Due to findings by Glover et al. (2009) that there is a stronger correlation between maternal and fetal cortisol among more anxious pregnant women, it is suggested that the mother's emotional state can affect the function of the placenta. Thus, elevated maternal cortisol levels may result in elevated fetal cortisol levels by directly crossing the placenta into the fetus.

Or, the placenta developed by an anxious mother may have increased pCRH production, accelerating the normal physiologic up regulation of the maternal-fetal HPA axes.

This relationship has been further studied through noting the moderating effect of maternal anxiety on the association between maternal plasma and amniotic fluid cortisol (O'Donnell et al., 2009). Depressed women also had higher cortisol levels during their last trimester of pregnancy, and this elevated cortisol pattern was reflected in their newborns' cortisol levels (Field & Diego, 2008). Cortisol release also seems to follow a positive feedback loop during pregnancy as opposed to the negative feedback loop that it follows outside of pregnancy (Kalra et al., 2007).

Fetal Programming Hypothesis

The fetal programming or fetal origins of adult disease hypothesis was created after the relationship between prenatal cortisol levels and the development of diseases later in life was illuminated. Since the maternal HPA axis does become more physiologically responsive to stress over pregnancy, it seems that this antenatal stress affects fetal development (Barker, Jaffee, Uher, & Maughan, 2011; O'Donnell et al., 2009; Osborne & Monk, 2013).

High levels of cortisol are inherent in cases of maternal psychiatric illnesses such as depression and anxiety, and such illnesses activate the maternal HPA axis and then potentially program the physiology of the fetal HPA axis (Dunkel Schetter & Tanner, 2012). Since the fetal HPA axis is not yet fully functional, maternal influence on fetal HPA axis development is highly influential. Harris & Seckl (2011) found that mothers who self report anxiety and/or depression during pregnancy give birth to offspring with higher basal HPA axis activity at three time markers: 6 months, 5 years of age and 10 years of age. Fetal cortisol, which can be measured in

amniotic fluid at delivery, reflects cortisol secreted by the fetus; these levels may be genetically determined and also a result of fetal stress due to maternal stress (Glover et al., 2009). Glover et al. (2009) and Field & Diego (2008) also demonstrated that the correlation between maternal and fetal (amniotic fluid) cortisol was greater in anxious mothers.

The fetal programming hypothesis builds off of the primary complication of unnaturally high levels of prenatal cortisol, which is low birth weight (LBW). LBW is characterized as a birth weight of ≤ 2500 g (Dunkel Schetter & Tanner, 2012; Field & Diego, 2008; Harris & Seckl, 2011). LBW leads to increased vulnerability for disease and neurodevelopmental complications later in life. Some examples of these complications are hypertension, type II diabetes, cardiovascular disease, schizophrenia, attention deficit hyperactivity disorder (ADHD), antisocial behavior, post-traumatic stress disorder (PTSD), depression, and anxiety disorders (Bergman et al., 2010; D'Anna-Hernandez et al., 2011; Glover, 2014; Harris & Seckl, 2011). These results suggest that the effects of maternal stress, depression, and anxiety in pregnancy can lead to adverse child neurodevelopmental outcomes through fetal programming.

Cortisol as a Biomarker

We can measure cortisol levels by extracting cortisol from hair and analyzing it as a retrospective biomarker (D'Anna-Hernandez et al., 2011; Hoffman et al., 2016; Kalra et al., 2007; Staufenbiel, Penninx, de Rijke, van den Akker, & van Rossum, 2015; Staufenbiel et al., 2013). Extracting cortisol from hair collected closest to the scalp can estimate cortisol production retrospectively for up to 6 months. Since hair cortisol levels are averaged over months (or even years, recently), hair cortisol profiles differ from salivary or blood cortisol measures, which show cortisol levels at exact, single time points. Therefore, it is important that psychological

questionnaires are also used as subjective measures of stress levels to provide further variables for analysis.

Psychological Questionnaires

The Center for Epidemiologic Studies-Depression Scale (CES-D) is used to screen for MDD or clinical depression through a 20-question survey. Each of these questions has possible scores between 0 and 60, and participants rate the frequency of 20 depressive symptoms during the past week (Hoffman et al., 2016). The Spielberg State-Trait Anxiety Inventory, State version (STAI) is used to screen for state anxiety through a 20-question survey as well. Each question has possible scores between 20 and 8025, and questions relate to state anxiety symptoms over the past month (Hoffman et al., 2016). While both of these questionnaires rely on self-report, these measures are validated in multiple languages and populations, and a higher score indicates a higher level of symptomatology (Hoffman et al., 2016).

Purpose

As mentioned previously, maternal psychiatric illnesses such as depression and anxiety are highly correlated with altered cortisol production and subsequent adverse maternal mental health, pregnancy outcomes, fetal development, and neurodevelopmental childhood outcomes. Since the risk for these adverse outcomes increases with a mood disorder diagnosis, clinical interventions for pregnant women who present with one of these mood disorders are absolutely necessary to help improve patient care (Barker et al., 2011; Glover, 2014; O'Donnell et al., 2009).

Therefore, the purpose of our research was to determine if a history of major depressive disorder (MDD) and/or anxiety in the pregnant woman's mother (MGM) predicted the same psychiatric illness in the pregnant mother and if family history of depression and/or anxiety

correlates with maternal and fetal HPA axis function as represented by hair cortisol. Since about 20% of mothers in the general obstetrics population are diagnosed with MDD, 15% are diagnosed with an anxiety disorder, and 7.9% are diagnosed with PTSD, it is important to examine the effects of these psychiatric illnesses on maternal and fetal HPA axis function (Dunkel Schetter & Tanner, 2012; Seng, Low, & Sperlich, 2009). While the strongest risk factor for depression during pregnancy is a history of depression (Rich-Edwards et al., 2006), there is a research gap in terms of multigenerational studies that examine the impact of heredity on the development of maternal psychiatric illnesses during pregnancy and postpartum periods. Early diagnosis of psychiatric illness, based on family history, may lead to clinical interventions that may benefit both maternal and child health.

Methods

This study was conducted at Denver Health Medical Center, a safety net hospital located in metro Denver, Colorado. This study is part of a longitudinal study that follows women through pregnancy, birth, and the postpartum period and their children from birth up to four years of age. This particular study in the larger longitudinal study seeks to understand how a pregnant mother's mental health influences both pregnancy outcomes and an infant's brain and behavior. Pregnant patients ≥ 18 years old without major mental or physical comorbidities were enrolled in a prospective longitudinal study at ≤ 15 weeks gestation during routine prenatal clinical visits. Participants were excluded from the study if they had a history of endocrine disorders or chronic steroid use or had an active infection.

Family history of psychiatric illness was abstracted from maternal Structured Clinical Interview for DSM-IV Disorders (SCID) reports performed by a trained social worker, and

maternal diagnoses of MDD and anxiety were compared with the maternal grandmother's psychiatric history. History and current diagnoses of psychiatric history were collected at both obstetric and psychiatric visits (if they occurred during pregnancy), with the pregnant participants reporting family history of psychiatric illness. Through the SCID, maternal diagnoses of MDD and anxiety were collected.

Participants completed the Center for Epidemiological Scales of Depression (CES-D) and Spielberg State-Trait Anxiety Inventory (STAI) questionnaires at 5 study time points. Scores for participants who had an MGM with depression and/or anxiety were compared with participants who had no family history of psychiatric illness. Scores were obtained at 16 and 28 weeks and at delivery. Significant scores (i.e., scores that are implicated in diagnosis of anxiety and depression) are >16 (CES-D) and >39 (STAI).

Maternal hair was collected at 16 and 28 weeks and maternal and fetal hair was collected at delivery for cortisol analysis. This provided cortisol data for each trimester of gestation and at delivery. Hair was collected from the posterior vertex of the scalp in women using scissors as close to the scalp as possible. In newborns, hair was collected from the nape of the neck as close to the scalp as possible. Since hair grows approximately 1 cm per month, a length of approximately 3 cm of hair was collected for analysis to represent one trimester of hair cortisol status.

To extract cortisol from hair for analysis, hair was washed twice in isopropanol and then dried for 4 days (D'Anna-Hernandez et al., 2011; Hoffman et al., 2016). To prepare the hair for centrifugation, it was ground using a Retsch ball mill for ten minutes at 25 Hz until it was ground into a fine powder. This powder was weighed in glass tubes to a weight of 50 mg and then

extracted via slow rotation in 1ml of HPLC grade methanol at room temperature for 24 hours.

After extraction, the consequent methanol and powdered hair solution was transferred to a 2 ml micro-centrifuge tube and spun for 120 s in a micro-centrifuge at ~20,000 g.

Following centrifugation, the resulting supernatant was removed. The supernatant was portioned into a new micro-centrifuge tube and dried at 38 °C for 30 minutes under nitrogen (D'Anna-Hernandez et al., 2011). To determine cortisol levels, the supernatant was reconstituted with 400 µl of assay buffer, and then cortisol levels were measured with a commercial high sensitivity enzyme immunoassay kit from Salimetrics, LLC. Cortisol levels were recorded for data analysis. Statistical significance for both cortisol and questionnaire values was determined using the one-way ANOVA test and the Kruskal-Wallis test, with a p-value < 0.05 considered to be significant.

Results

Demographics

The mean birth weight (\pm standard deviation), gestational age (\pm standard deviation), and maternal age (\pm standard deviation) are compiled in Table 1 (Appendix A). The children of the 153 pregnant participants had a mean birth weight of 3151.18 ± 606.42 grams, a mean gestational age of 38.68 ± 2.77 weeks, and a mean maternal age of 29.38 ± 6.20 years.

Table 2 (Appendix A) shows the race and ethnicity breakdowns of the participant population. The participant population is predominantly White Hispanic, with 43.79% of participants identifying as White Hispanic.

Psychiatric Illness Prevalence

Table 3 (Appendix B) shows the breakdown of psychiatric illnesses that are present in the participant population. Although other psychiatric illnesses (bipolar disorder, schizophrenia, substance abuse, etc.) were identified in the participant population, for the purposes of this study the focus was on anxiety (including PTSD) and depressive disorders and so only the prevalence of those disorders is displayed below. With a prevalence rate of 33.99% for major depressive disorder (MDD) without psychosis and a prevalence rate of 18.3% for post-traumatic stress disorder (PTSD), this participant population had higher prevalence rates of these disorders than the general obstetrics population. PTSD is diagnosed as a specific anxiety disorder under the DSM-IV, but is a stand-alone psychiatric illness according to the recently released DSM-V. For this study, PTSD is treated as an anxiety disorder with this recent change in mind.

Table 4 (Appendix B) shows the percentage of pregnant participants with a family history of psychiatric illness when broken down into psychiatric illness groups. It is important to note that 24.84% of pregnant participants had a family member who was diagnosed with multiple psychiatric illnesses, and no MGM history of PTSD was recorded in this population.

Table 5 (Appendix B) shows the percentage of pregnant participants who were diagnosed with the same psychiatric illness as at least one of their family members. 69.7% of participants who had a family member with MDD were also diagnosed with MDD, and 7.69% of participants who had a family member with an anxiety disorder were also diagnosed with an anxiety disorder.

The one-way ANOVA statistical test and the Kruskal-Wallis statistical test were used to determine statistical significance across results. The Kruskal-Wallis statistical test is used for non-parametric populations and is best described as a one-way ANOVA test that is utilized between groups of independent variables. A p-value < 0.05 was considered significant.

Figure 1 (Appendix C) depicts the Spielberg State-Trait Anxiety Inventory (STAI) scores for pregnant participants as separated into those participants with a family history of anxiety (+FH) and those without a family history of anxiety (no FHx) as recorded at each trimester. A p-value of 0.005 shows statistical significance between the group of participants with a family history of anxiety and the group of participants without a family history of anxiety between all trimesters.

Figure 2 (Appendix C) depicts the Center for Epidemiological Scales of Depression (CES-D) scores for pregnant participants as separated into those participants with a family history of major depressive disorder (+FH) and those without a family history of major depressive disorder (no FHx) as recorded at each trimester. A p-value of 0.02 shows statistical significance between the group of participants with a family history of major depressive disorder and the group of participants without a family history of major depressive disorder over all trimesters.

Table 6 (Appendix C) shows the mean levels of maternal cortisol at each trimester as well as the mean level of fetal (neonatal) cortisol at delivery as extracted from hair. The standard deviations for each measure are also listed.

Figure 3 (Appendix D) depicts hair cortisol levels (pg/mg) separated by each trimester of pregnancy and grouped by race and ethnicity categories. A p-value of less than 0.0001 between the first trimester hair cortisol values and a p-value of 0.0004 between the second trimester hair cortisol values shows a statistically significant difference in hair cortisol levels between race and ethnicity categories. A p-value of 0.0796 between the third trimester hair cortisol values shows

no statistical difference in the hair cortisol levels between race and ethnicity categories during the third trimester of pregnancy.

Figure 4 (Appendix D) shows the statistically significant increase ($p = 0.0063$) in hair cortisol levels (pg/mg) within all subjects throughout pregnancy.

Figure 5 (Appendix D) depicts hair cortisol levels (pg/mg) for each trimester as separated into family history of psychiatric illness groups. A p-value of 0.6827 for the first trimester hair cortisol levels showed no statistical significance between groups. P-values of 0.9327 and 0.3442 for the second and third trimester hair cortisol levels, respectively, also showed no statistical significance between groups. Additionally, there is no increase in cortisol levels throughout pregnancy in the separate family history of psychiatric illness or no family history of psychiatric illness groups, which is contrary to what is expected through the literature.

Figure 6 (Appendix E) shows the percent change in hair cortisol levels (pg/mg) between the first and second, the first and third, and second and third trimesters for each family history of psychiatric illness group. A p-value of 0.02 between groups dictates statistical significance between hair cortisol levels for each trimester amongst family history of psychiatric illness groups.

Discussion

The purpose of this study was to determine if a history of major depressive disorder (MDD) and/or anxiety in the pregnant woman's mother (MGM) predicts the same psychiatric illness in the pregnant mother and if family history of depression and/or anxiety correlates with maternal HPA axis function as represented by hair cortisol. Our data suggest that family history of psychiatric illness in the MGM influences the appearance of symptoms of the same

psychiatric illness in the pregnant mother as seen through CES-D and STAI scores and psychiatric diagnosis. We did not find significant influences of family history in the MGM on maternal HPA axis function as represented by hair cortisol.

To begin, it is important to note that there are higher rates of diagnosed MDD and anxiety (including PTSD) in this subject population as compared to the general obstetrics population (Table 3, Appendix B). This population, however, is representative of the patient population seen at Denver Health Medical Center and is consistent with findings in other vulnerable populations. 37% percent of pregnant participants in this study had a family member whose diagnosis was comorbid with at least one other psychiatric illness.

Figures 1 and 2 (Appendix C) show that participants with psychiatric illness in the MGM have higher CES-D and STAI scores at all study time points, regardless of the pregnant participant's diagnosis of anxiety or MDD. This was a statistically significant result, and is very important, because it shows that even if the pregnant participant does not have a psychiatric illness diagnosis, her scores on subjective measure questionnaires are significantly higher during pregnancy, and higher scores mean increased likelihood of diagnosis of MDD (CES-D) and anxiety (STAI). This result could be due to a hereditary influence on the development of depression and anxiety symptomatology and ultimately on development of these psychiatric illnesses. Although a particular gene has not yet been identified that could prove this hereditary relationship, these results suggest that there could be a mechanism that is involved in a predisposition to development of psychiatric illnesses if one has a family history of psychiatric illness.

As shown by Figure 3 (Appendix D), there are statistically significant differences in hair cortisol levels between participants in race and ethnicity categories during the first and second trimesters of pregnancy, but not during the third trimester of pregnancy. It could be important to continue to examine differences in antenatal care based on these results, as participants in the Black Non-Hispanic group had consistently higher hair cortisol levels throughout the first and second trimesters as compared to participants in the other race and ethnicity categories, and this result is seen often. These results suggest that there may be different baseline cortisol reactivity levels between race and ethnicity groups and/or that there is an effect of race and ethnicity on the HPA axis during pregnancy (and possibly outside of pregnancy).

Figure 4 (Appendix D) shows that the means levels of hair cortisol increased during pregnancy across all 153 participants in the study, which is consistent with the expectation of hypercortisolemia throughout pregnancy. However, when participants are separated into groups based on their family history of psychiatric illness diagnosis, this significance is no longer present, as discussed below and shown by Figure 5.

Figure 5 (Appendix D) shows that participants with a family history of anxiety and MDD do not show a significantly different increase or decrease in cortisol over pregnancy when compared with participants that have no family history of psychiatric illness. This result reinforces the conclusion reached through the literature review that there is not yet a consensus on whether MDD leads to an increase or decrease in cortisol levels. We expected an increase in cortisol levels in those participants with a family history of anxiety, but our statistical results do not support this increase. This unexpected result could be due to sample size, since there were 13 participants with a family history in the MGM of anxiety (including PTSD), but only 7 of these participants had complete hair cortisol data for analysis. Further, we would expect the

group of participants with no family history of psychiatric illness to show a steady increase in hair cortisol levels over pregnancy, however, that is not seen in our results, most likely due to data outliers, sample size and errors with cortisol extraction from hair samples.

Although utilizing hair cortisol as a retrospective biomarker is a relatively new method, studies have found that both salivary and hair cortisol are reliable measures of cortisol levels. The lack of expected hypercortisolemia in the group of participants with no history of psychiatric illness resulted in a non-significant statistical result in hair cortisol levels over pregnancy between participants with a family history of psychiatric illness and those participants without a family history of psychiatric illness.

An important note regarding this result is that the use of hair cortisol to measure HPA axis activity measures a different aspect of the HPA axis than the use of single time point cortisol measures using saliva or blood. Salivary cortisol provides a snapshot of a participants' same-day cortisol status while hair cortisol provides a long-term profile of a participants' (chronic) cortisol levels (D'Anna-Hernandez et al., 2011). Hair cortisol is averaged over months and measures long-term cortisol levels and stress reactivity, while saliva and blood measure short-term cortisol levels and activity and is particularly valuable with providing information about cortisol dynamics and stress reactivity (Staufenbiel et al., 2013). To study the role of cortisol in psychiatric illnesses, it may be beneficial to look at both measures: salivary and blood cortisol levels to determine baseline cortisol reactivity and hair cortisol levels to examine long-term chronic cortisol status.

There was a non-significant statistical result in hair cortisol levels over pregnancy between participants with a family history of psychiatric illness and those participants without a

family history of psychiatric illness. Even when not significantly represented by cortisol, there seems to be a cross-generational prediction of MDD, as 69.7% of mothers with a family history of MDD were also diagnosed with MDD. There does not seem to be as much of a cross-generational diagnostic prediction of anxiety, as only 7.69% of mothers with a family history of anxiety were also diagnosed with anxiety, but this is also inherently due to the small sample size of participants who were diagnosed with anxiety.

Figure 6 (Appendix E) shows the changes in hair cortisol levels throughout pregnancy between trimesters and across groups of participants with no family history of psychiatric illness and those with family history of anxiety and MDD. The bars for the family history of anxiety and MDD groups show the blunted cortisol change between trimesters, and the bars for the no family history of psychiatric illness group show a 200% increase in hair cortisol levels between the first and second trimesters and a 200% decrease in hair cortisol levels between the second and third trimesters. This result is largely due to an outlier in the no family history of psychiatric illness group. When this outlier's data was removed from the statistical test, a statistically significant result was still observed over all trimesters between groups since the hair cortisol data is natural log transformed to create a more normal distribution, so we retained her data values.

Overall, these data suggest a multigenerational effect of mental illness on mood symptomatology as qualified through similarities in psychiatric diagnosis between pregnant women and their mothers, and the influences of family history on CES-D and STAI scores. These data also suggest that patients diagnosed with anxiety or MDD may have a different regulatory mechanism for cortisol during pregnancy, which supports the need for further research into the mechanism of cortisol release during pregnancy.

It is important to note that the stigma surrounding psychiatric illnesses has decreased in recent years, but that this stigma used to be enormously prevalent (and still is quite prevalent). It is possible that some maternal grandmothers were not diagnosed with a psychiatric illness due to this stigma, and therefore that these psychiatric illness statistics were not reflected in the family history of psychiatric illness data collection.

Finally, the implications of this study suggest that we should consider assessment of both maternal mental health and psychiatric family history during antenatal care. Family history of psychiatric illness could offer an avenue into early recognition and treatment of psychiatric illnesses during antenatal care and, since approximately 60% of female patients only see a doctor regularly during pregnancy (M. V Smith et al., 2004), utilizing antenatal care as a way to intercede with mental health could be a useful and successful patient care approach.

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Appendix A

Demographic	Mothers (N=153)
Birth Weight (g) \pm std	3151.18 \pm 606.42
Gestational Age (wks) \pm std	38.68 \pm 2.77
Maternal Age (yrs) \pm std	29.38 \pm 6.20

Table 1. Maternal demographics.

Race & Ethnicity	Mothers (N=153)
White Non-Hispanic	50 (32.68%)
White Hispanic	67 (43.79%)
African American Non-Hispanic	14 (9.15%)
African American Hispanic	0 (0%)
AIAN Non-Hispanic	4 (2.61%)
AIAN Hispanic	7 (4.58%)
Mixed Non-Hispanic	6 (3.92%)
Mixed Hispanic	5 (3.27%)

Table 1. Maternal race and ethnicity breakdown.

Appendix B

Psychiatric Illness	Mothers with Psychiatric Illness Diagnosis (N=153)
Anxiety disorder (including PTSD)	37 (24.18%)
MDD	52 (33.99%)
Minor DD	11 (7.19%)
No diagnosis	24 (15.69%)
None listed	25 (9.80%)

Table 3. Maternal psychiatric illness diagnoses according to SCID reports (MDD = major depressive disorder, DD = depressive disorder, PTSD = post-traumatic stress disorder).

Psychiatric Illness	Mothers with a MGM History of Psychiatric Illness (N=153)
Alcohol abuse	43 (28.10%)
Anxiety disorder	13 (8.50%)
MDD	33 (21.57%)
Substance abuse	15 (9.80%)
2 or more illnesses	38 (24.84%)
None listed	83 (54.24%)

Table 4. Percentage of pregnant participants with a family history of psychiatric illness diagnoses in the maternal grandmother as obtained through SCID reports (MDD = major depressive disorder).

Psychiatric Illness	Mothers with Same Psychiatric Diagnosis as MGM
MDD	23/33 (69.7%)
Anxiety disorder	1/13 (7.69%)

Table 5. Percentage of pregnant participants who were diagnosed with the same psychiatric illness as their mother.

Appendix C

Time Period	Cortisol Means (pg/mg) \pm std
1 st trimester cortisol	9.3 \pm 10.53
2 nd trimester cortisol	10.8 \pm 13.27
3 rd trimester cortisol	15.6 \pm 18.84
Fetal cortisol at delivery	291.2 \pm 637.65

Table 6. Mean maternal levels of cortisol at each trimester and mean level of fetal cortisol at delivery.

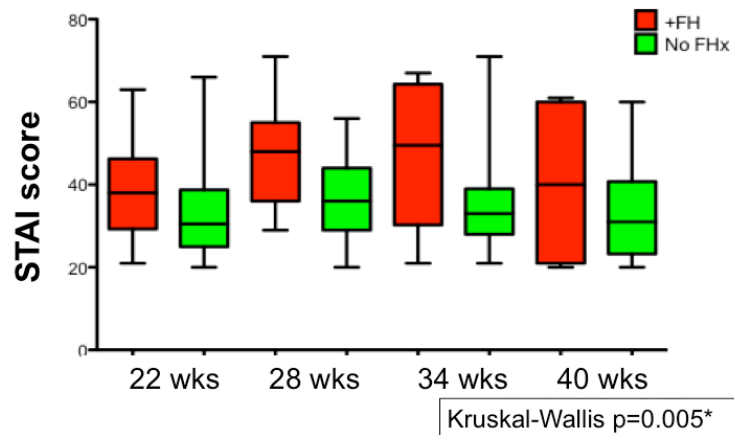


Figure 1. Maternal STAI scores at each trimester (n=102), separated by those participants with a family history of anxiety (+FH) and participants without a family history of anxiety (no FHx). A p-value of 0.005 showed significant results between +FH and no FHx subject groups under the Kruskal-Wallis test.

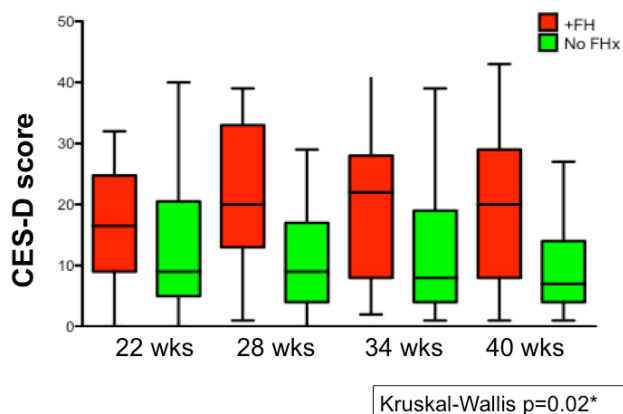


Figure 2. Maternal CES-D scores at each trimester (n=102), separated by those participants with a family history of major depressive disorder (+FH) and participants without a family history of major depressive disorder (no FHx). A p-value of 0.02 showed significant results between +FH and no FHx subject groups under the Kruskal-Wallis test.

Appendix D

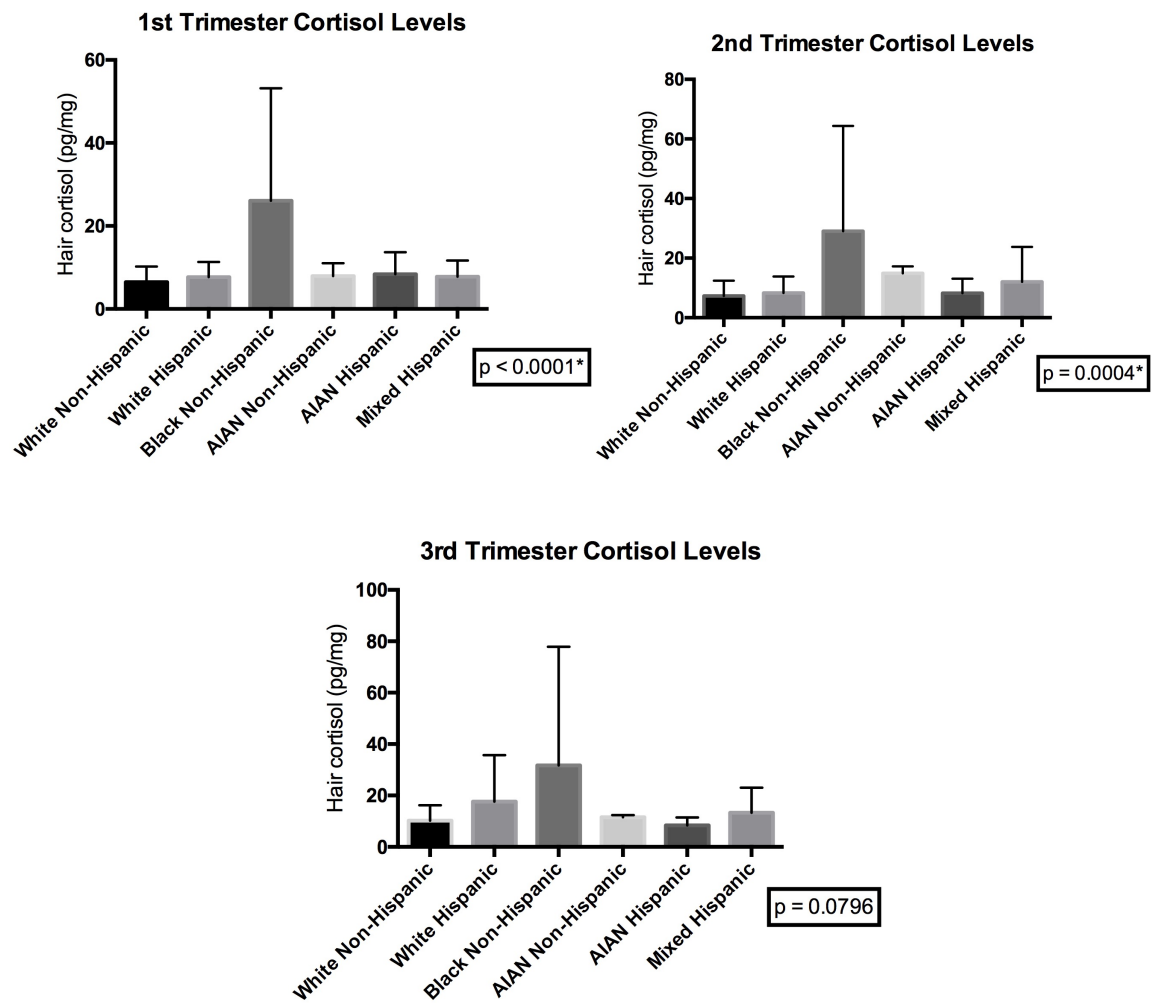


Figure 3. Maternal hair cortisol levels (pg/mg) grouped by race and ethnicity and separated by trimester of pregnancy (n=87). A one-way ANOVA test shows statistical significance between race and ethnicity groups during the first ($p < 0.0001$) and second ($p = 0.0004$) trimesters of pregnancy, while a one-way ANOVA test shows no statistical significance between race and ethnicity groups during the third trimester of pregnancy ($p = 0.0796$).

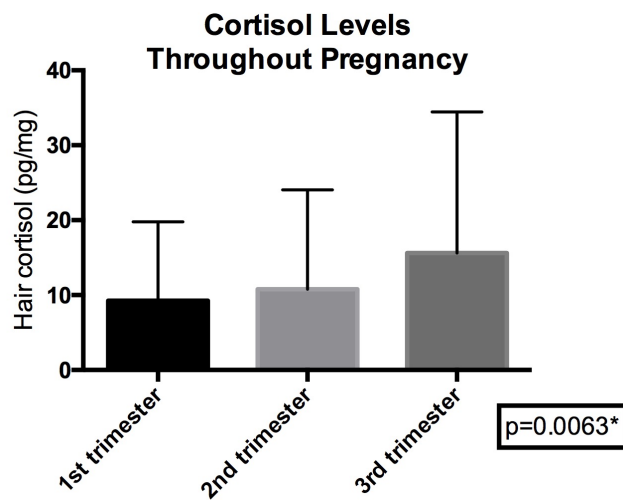


Figure 4. Hair cortisol levels (pg/mg) across all subjects in the study (n=87) throughout pregnancy. A one-way ANOVA statistical test shows significant differences between trimesters, as denoted by the p-value of 0.0063.

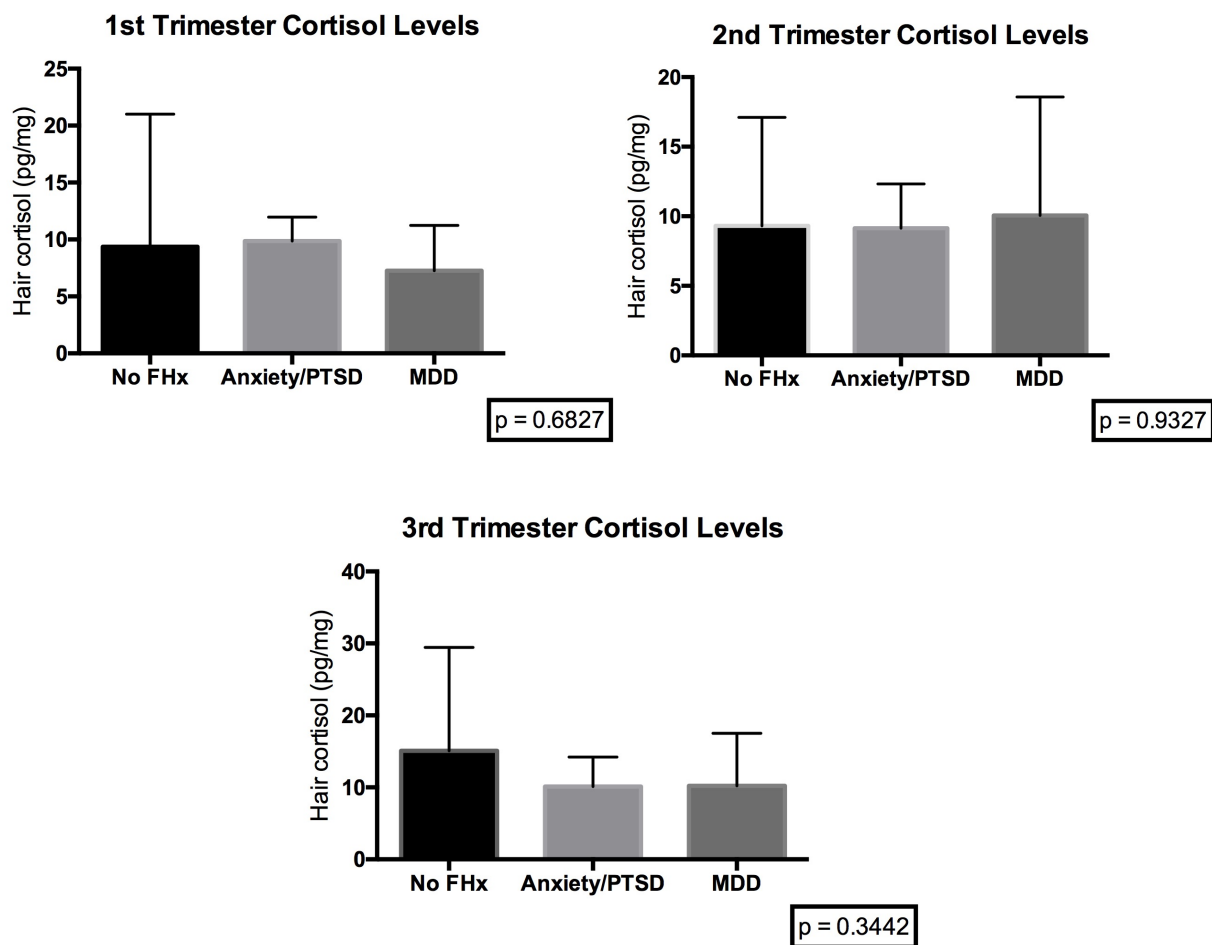


Figure 5. Maternal hair cortisol levels by trimester, separated by participants with no family history of psychiatric illness (no FHx), participants with family history of anxiety and PTSD (anxiety/PTSD), and participants with family history of major depressive disorder (MDD) (n=75). A one-way ANOVA test showed no statistical significance between subject groups.

Appendix E

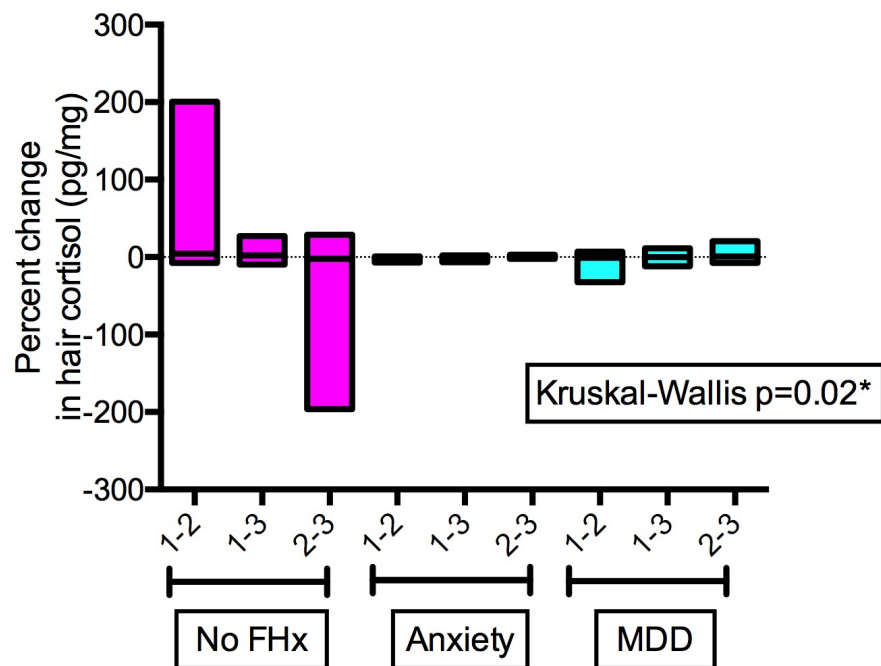


Figure 6. Percent change in maternal hair cortisol levels between trimesters, separated by participants with no family history of psychiatric illness (no FHx), participants with family history of anxiety (anxiety), and participants with family history of major depressive disorder (MDD) (n=75). A p-value of 0.02 showed significant results between +FH and no FHx subject groups under the Kruskal-Wallis test.