PERINATAL OBSESSIVE-COMPULSIVE DISORDER

THE ASSOCIATION OF THE 5-HTTLPR POLYMORPHISM WITH PERINATAL ONSET OBSESSIVE-COMPULSIVE DISORDER AND DISTINCT BRAIN ACTIVATION PATTERNS: A GENETIC NEUROIMAGING STUDY

By:

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A Thesis Submitted to the School of Graduate Studies In Partial

Fulfillment of the Requirements for the Degree Master of Science

MASTER OF SCIENCE (2014) McMASTER UNIVERISTY
(Neuroscience) Hamilton, Ontario, Canada

TITLE: The Association of the 5-HTTLPR Polymorphism with Perinatal Onset of Obsessive-Compulsive Disorder and Distinct Brain Activation Patterns: A Genetic-Neuroimaging Study

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NO. OF PAGES: xv, 90

ABSTRACT

Obsessive-compulsive disorder (OCD) is heterogeneous. Clinical presentation of OCD differs by sex and age-of-onset and evidence supports classification based on these subtypes. The prevalence of OCD in the general population is 2%. However, it has been established that women tend to experience onset and exacerbation of OCD during reproductive milestones. In particular, the prevalence of postpartum OCD is between 4 to 9%. This study seeks to examine the effects of past childhood maltreatment and S/L_g-allele status of the 5-HTTLPR polymorphism on perinatal obsessive-compulsive symptoms and aberrant resting state functional connectivity in the postpartum period. Forty women participated in the first visit and sixteen women have been followed up with in the postpartum period. 5-HTTLPR genotype was determined from whole blood samples via polymerase chain reaction and a restriction fragment length digest. We used the Yale-Brown Obsessive-Compulsive Scale and Perinatal Obsessive-Compulsive scale to measure symptom severity. Resting state functional connectivity was determined from functional magnetic resonance imaging data. Obsessive-compulsive symptoms during late pregnancy are significantly predicted by 5-HTTLPR genotype, past history of total childhood maltreatment or childhood emotional neglect and trait anxiety symptoms. Whereas obsessive-compulsive symptoms during the postpartum period are predicted by poor sleep quality and childhood emotional maltreatment or 5-HTTLPR genotype, childhood emotional maltreatment and trait anxiety symptoms. Seed to region-of-interest analysis was employed to evaluate resting state functional connectivity differences between OCD patients and healthy controls in the postpartum period. Compared to

healthy controls, OCD patients show greater connectivity between the caudate nucleus with the orbitofrontal cortex, the pars triangularis and the cingulate area. The insular cortex shows decreased connectivity between the right and left, the dorsal anterior cingulate area and the pars opercularis. The amygdala has increased connectivity with the cingulate area, the calcarine fissure, the supramarginal gyrus and decreased connectivity with the gyrus rectus. The above clinical and neuroimaging findings are in line with past work. However, this is the first study to show both 5-HTTLPR genotype and history of childhood maltreatment predict obsessive-compulsive symptoms in a perinatal population. Further, the resting state data replicates findings in the OCD literature but the study is the first to show this in postpartum women. This study serves as a platform for future work to further investigate both gene-environment interactions and distinct neuroimaging correlates in perinatal OCD.

ACKNOWLEDGEMENTS

I would like to thank my mentor and supervisor Dr. Meir Steiner for providing me the opportunity to be his student and to complete my Master's thesis under his supervision. I appreciate every single conversation we have had and every lesson you have taught me. My experience with you has fostered a passion in me and you have played a pivotal role in my career path. Thank you for believing in me when I did not believe in myself – your support has allowed me to succeed both academically and personally. I am leaving this experience changed for the better and I hope to make you proud in the next chapter of my academic life.

I would like to thank Dr. Luciano Minuzzi for not only being an excellent teacher and making me realize that statistics can be enjoyable but also having the patience to teach such a wonderful statistics course. Your guidance and last minute help before committee meetings is greatly appreciated. I have learned so much from you and I will take your lessons with me.

I would like to thank my committee members Dr. Geoffrey Hall and Dr. Zena Samaan for their support, encouragement, insight and advice throughout my thesis. Despite having an ambitious amount of work I wanted to accomplish for my Master's, your faith in my ability did not waiver. I would like to give a special thank you to Dr. Hall for all the marathon neuroimaging analysis sessions. Even though our sessions were few, I have learned so much from you and I hope to represent McMaster University well next year.

I would like to thank Dr. Jens Pruessner for opening his lab and welcoming me to Montreal. I appreciate the training that your lab provided me with the imaging stress task.

I would like to thank Robyn Mackenzie for allowing me to shadow her in the lab and teaching me all the skills I needed to genotype my samples. I would also like to thank Marg Coote for being such a wonderful lab coordinator and advocating for my success. Without you, I would not have had my materials on time and would not have been able to troubleshoot all the issues that arose. Your support is greatly appreciated.

I would like to thank all the lovely MRI technologists: Carol, Cheryl and Julie. I came into my Master's with no neuroimaging experience and the three of you were so patient with my protocol. I would also like to thank Dr. Norman Koyner for working so closely with my project in the beginning to troubleshoot all the kinks and allowing my protocol to run smoothly.

I would like to thank all the WHCC staff and students for making my day-to-day life so wonderful. It was a pleasure to come to work every day. I have learned so much from all of you.

Last but not least, I would like to thank my family (my mom, my dad, Sarah, Gary, Cathy and my grandma) and friends for their unconditional love and support. I truly have the greatest support system in the world and I attribute my success to them.

ABBREVIATIONS

5-HTTLPR	Serotonin transporter linked polymorphic region
BOLD	Blood oxygen level dependent
CIDI-Venus	Composite International Diagnostic Interview for women
COMT	Catechol-O-methyl transferase
CTQ	Childhood Trauma Questionnaire
DMN	Default mode network
DRN	Dorsal raphe nucleus
DTI	Diffusion tensor imaging
EPDS	Edinburgh Postnatal Depression Scale
FA	Fractional anisotropy
fMRI	Functional Magnetic Resonance Imaging
IPC	Inferior parietal cortex
L_a-allele	Long (adenosine) allele
L_g-allele	Long (guanosine) allele
MAO-A	Monoamine oxidase-A
MIST	Montreal Imaging Stress Task
MRI	Magnetic Resonance Imaging
OCD	Obsessive-Compulsive Disorder
OFC	Orbitofrontal cortex
POCS	Perinatal Obsessive-Compulsive Scale
PCC	Precuneus/posterior cingulate cortex

(dl/m/vm)PFC	(Dorsolateral/Medial/Ventromedial) prefrontal cortex
ppOCD	Postpartum Obsessive-Compulsive Disorder
PSQI	Pittsburg Sleep Quality Index
rs-FC	Resting state functional connectivity
S-allele	Short allele
SMA	Supplementary motor area
SSRI	Selective serotonin reuptake inhibitor
STAI	State Trait Anxiety Inventory
YBOCS	Yale-Brown Obsessive-Compulsive Scale

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STATEMENT OF CONTRIBUTORS

I would like to acknowledge Julie Mahoney and Tina Li for helping with the neuroimaging stress task, Dr. Geoffrey Hall with assisting me with the neuroimaging analysis, Dr. Luciano Minuzzi for the statistical analysis support, the doctor's offices and midwifery clinics that allowed me to post my recruitment flyer and Dr. Meir Steiner for his vigilant recruitment support. Experimental design and protocol, study visits, blood collection, genotyping, analysis and interpretation of the data were all conducted by myself in fulfillment of my thesis requirements.

1. INTRODUCTION

1.1 Introduction to Obsessive-Compulsive Disorder

According to the American Psychiatric Association, obsessive-compulsive disorder (OCD) is characterized by the presence of obsessions and compulsions, which are distressing and time-consuming to the afflicted individual (APA 2013). Epidemiological studies conducted in several countries report a current OCD prevalence of 1% and lifetime prevalence between 2 and 3% (Ruscio, Stein, Chiu, & Kessler, 2008; Torres & Lima, 2005). Obsessions have four core features: (1) recurrent and persistent thoughts, impulses, or images experienced as intrusive and cause great anxiety; (2) not merely excessive worries about real life issues; (3) the affected individual attempts to ignore, suppress, or neutralize them with some other thoughts or actions; (4) and the affected individual recognizes that these thoughts are self-produced. Compulsions are the repetitive behaviours or mental acts that the affected individual feels obliged to do in response to an obsession. The goals of compulsions are to prevent or reduce distress but they are excessive and are not realistically connected to what the individual intends to prevent (APA 2013).

Family studies of OCD indicate that the prevalence of OCD is significantly higher in relatives, especially in the presence of comorbid tics and early age of onset (Hollander, Kim, Braun, Simeon, & Zohar, 2009; Nicolini, Arnold, Nestadt, Lanzagorta, & Kennedy, 2009). Family studies on the whole support the heterogeneity of OCD and the identification of subgroups based on time of onset, sex, symptoms, clustering and treatment response (Hollander et al., 2009). In particular, cluster analyses have identified

three clusters of OCD spectrum symptoms: [1] Reward deficiency (including trichotillomania, pathological gambling, hypersexual disorder and Tourette's disorder), [2] Impulsivity (including compulsive shopping, kleptomania, eating disorders, self-injury and intermittent explosive disorder), and [3] Somatic complaints (including body dysmorphic disorder and hypochondriasis) (Lochner & Stein, 2006; Lochner et al., 2005).

1.2 Genetics of Obsessive-Compulsive Disorder

Several reviews and studies have looked at genetic associations in OCD but findings have been inconsistent (M. T. Pato, Pato, & Pauls, 2002). A recent meta-analysis conducted on the molecular genetics of OCD yielded conflicting and weak findings (Taylor, 2012b). OCD is a heterogeneous disorder and recent evidence supports subtype classification. Subtypes based on sex and age-of-onset may explain the observed diversity (Taylor, 2011; 2012a). For example, polymorphisms involved in catecholamine modulation are only associated with OCD in males (Taylor, 2012b). It has been well replicated that the genes of monoamine oxidase-A (MAO-A) and catechol-O-methyl transferase (COMT) demonstrate significant linkage in the families of male probands (Nicolini et al., 2009). Furthermore, 70% of OCD pediatric cases are seen in males, suggesting that males tend to experience early-onset (Mathis et al., 2011). This has also been replicated in a Chinese population (Wang et al., 2012). Janowitz et al. (2009) found that subjects with early-onset; defined as onset before 10 years of age, were more like to have tic/Tourette's disorders than late-onset (Janowitz et al., 2009). This lends support for the genetic

makeup of OCD in males and suggests that females may be different (Arnold et al., 2004; Dickel et al., 2007; Stewart et al., 2007).

1.3 Serotonergic Dysfunction and Obsessive-Compulsive Disorder

The role of serotoninergic dysfunction in OCD has been widely implicated. Specifically, it has been reported that OCD patients have reduced serotonin transporter binding in the insular cortex compared to healthy controls (Matsumoto et al., 2010). Furthermore, it has been shown that serotonin transporter availability is reduced significantly in drug naïve patients with late-onset but not early-onset OCD (Hesse et al., 2011). In addition, antidepressants such as serotonin re-uptake inhibitors have been moderately efficacious in treating OCD (Stahl, 1998; Vaswani, Linda, & Ramesh, 2003). However, doses used in OCD exceed those needed for depression and other anxiety disorders (Bloch, McGuire, Landeros-Weisenberger, Leckman, & Pittenger, 2009). These doses are also substantially higher than what is necessary to completely inhibit the serotonin transporter (Pampaloni et al., 2010). Thus, mounting evidence supports serotonin involvement in OCD and has led for the search of serotonergic candidate genes.

1.4 The 5-HTTLPR Polymorphism

One gene of interest is the serotonin transporter (5-HTT) gene. The serotonin transporter protein is localized on the presynaptic membrane of serotoninergic neurons. It plays an important role regulating serotonin homeostasis. The transporter facilitates the reuptake of released serotonin in the synaptic cleft following neurotransmission (Bengel et al.,

1999). The human 5-HTT protein is encoded by a single gene, which is mapped to chromosome 17q11.1–q12 (Nakamura, Ueno, Sano, & Tanabe, 2000). The 5-HTT gene-linked polymorphic region (5-HTTLPR) is a common functional polymorphism of the transporter in the 5' promoter region. The 5-HTTLPR involves two common alleles, which correspond to a 44-base pair insertion, long allele (L-allele) or deletion, short allele (S-allele). The S-allele is associated with reduced transcription efficiency of the 5-HTT gene and thus decreased

5-HTT expression and serotonin re-uptake (Avshalom Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Lesch et al., 1996). Notably, a previous meta-analysis found that upon stratification, the L-allele is only associated with OCD status in Caucasians and studies that included primarily children, providing further support for OCD subtypes (Bloch et al., 2008). More recently, it has been established that there are three rather than two biologically active alleles: L_a, L_g and S, where the L_g-allele behaves biologically like the S-allele (Hu et al., 2006). This is caused by a single-base substitution (Adenosine → Guanosine) occurring at the sixth nucleotide within the first of two extra 20-23 base pair repeats in the L-allele. The G substitution creates a functional AP2 transcription factor-binding site and upon binding, AP2 suppresses transcription (Hu et al., 2006). Studies that fail to acknowledge the triallelic nature of the polymorphism and group the L_g- and L_a-alleles together are at risk of skewing their results.

1.4.1 Prevalence of 5-HTTLPR Alleles

The prevalence of the S-allele varies among different ethnic groups. The frequency of the S-allele is lowest in individuals of African descent (25%), intermediate in whites

(35-40%), and highest in American Indians (64-66%) (Hu et al., 2006). Moreover, in a typical East Asian sample, 70-80% of individuals are S-allele carriers compared to a typical European sample where 40-45% of individuals are S carriers (Gelernter, Kranzler, & Cubells, 1997). The differing frequency may be accounted for by a culture-gene co-evolution, where the S-allele was selected for because carriers had qualities that were beneficial (Chiao & Blizinsky, 2010) or simply by neutral evolutionary processes (Eisenberg & Hayes, 2010). The frequency of the L_g-allele also varies by ethnic groups where the frequency is lowest in Plains and Southwest Indians (1%) and highest in African Americans (24%) (Hu et al., 2006).

1.5 General Effects of the S-allele

Healthy carriers of the S-allele exhibit different phenotypes compared to the L/L homozygotes. S-allele carriers tend to have increased anxiety related temperamental traits (Schinka, Busch, & Robichaux-Keene, 2004). Moreover, healthy individuals carrying the S-allele show an exaggerated amygdala response to threatening visual stimuli compared to L/L homozygotes (Schinka et al., 2004). Suggesting that there may be a possible link between variation of the gene and normal brain mechanisms involved in negative emotion processing (Hariri et al., 2005; 2002). Healthy S-allele carriers also exhibit differing brain volumes and connectivity of certain brain regions. S-allele carriers show significantly reduced perigenual anterior cingulate cortex (pACC) and amygdala volumes compared to L/L homozygotes, with these volumes co-varying with each other. Furthermore, not only are these two areas shown to be functionally connected, but S-allele carriers exhibit a

highly significant reduction of amygdala-pACC connectivity (Pezawas et al., 2005). To compound the effect, the S-allele adversely affects SSRI treatment outcomes in depressed subjects and this effect is greater in females (Smits et al., 2008).

1.6 Environmental Factors and the S-allele

Caspi et al. (2003) first proposed that the 5-HTTLPR polymorphism acts as a moderator of psychiatric disease development. They found that individuals with one or two copies of the S-allele moderated the influence of stressful life events on the development of depressive symptoms, depression, and risk of suicide compared to individuals homozygous for the L-allele (Avshalom Caspi et al., 2003). Zalsman et al. (2006) found that the S-allele independently predicted greater depression severity, but also that the S/S genotype is associated with more severe depression in individuals with high versus low stressful life events scores (Zalsman et al., 2006). Evidence currently indicates that childhood trauma plays a significant role in the development of maladaptive obsessive-compulsive symptoms (Mathews, Kaur, & Stein, 2008). Specifically, it has been found that sexual abuse is significantly associated with OCD in adults (Asaf Caspi et al., 2008). Moreover, a recent meta-analysis has suggested that the S-allele may actually be a marker of differential susceptibility rather than vulnerability. Suggesting that S-allele carriers are more sensitive to both adverse and positive environments and may benefit significantly more from positive environmental input than L/L individuals (van IJzendoorn, Belsky, & Bakermans-Kranenburg, 2012). To date, studies have not examined if the S-allele acts as a moderator between childhood maltreatment and the development of OCD later in life.

1.7 Sex Differences and the S-allele

We recently conducted a meta-analysis that investigated the association of the S-allele of the 5-HTTLPR polymorphism with OCD and OCD subtypes (sex and late-onset). The polymorphism was investigated as biallelic since not enough studies acknowledged the polymorphism as triallelic. The primary outcome measures were allele frequency and OCD diagnosis. A full meta-analysis was completed comparing the L- and S-alleles using a random effects model in RevMan 5.2.1. A secondary meta-analysis stratified by sex and onset was conducted for S- versus L-allele frequency. In the primary meta-analysis, OCD was not associated with the S-allele of the 5-HTTLPR polymorphism [Z=0.07, p=0.94]. Moreover late-onset OCD was not associated with the S-allele [Z=1.45, p=0.15]. However, when stratified by sex, there was an emerging sex-specific relationship. There was a trending association between the S-allele and OCD status in females [Z=1.62, p=0.10] but not in males [Z=0.69, p=0.49]. The results are in line with a recent review suggesting that OCD may have a different prognosis and development depending on sex (Mathis et al., 2011). In particular, co-morbidities and type of obsessions present in females differ from males. For example, more females than males will present with co-morbid anxiety, eating disorders and depression (Labad et al., 2008). Furthermore, females are twice as likely to have contamination-centered obsessions (Labad et al., 2009). Whereas sexual obsessions are more associated with males (Karadaĝ, Oguzhanoglu, Özdel, Ateşci, & Amuk, 2006).

OCD age-of-onset extends into another difference where females tend to either experience onset or exacerbation of symptoms during reproductive milestones (Guglielmi

et al., 2014). Patients with obsessions of contamination were more likely to report onset of their disorder during menarche or the perinatal period (Labad et al., 2009). Moreover, the perinatal period has been found to be associated with both worsening and new onset of OCD symptoms (Forray, Focseneanu, Pittman, McDougle, & Epperson, 2010). This sex specific effect may be due to the interaction of estrogen with the serotoninergic system (Labad et al., 2009; Rubinow, Schmidt, & Roca, 1998). Compared to the general prevalence, the prevalence of postpartum OCD (ppOCD) is between 4 to 9%, which is significantly greater than the general prevalence of 2% (Ruscio et al., 2008; Uguz, Akman, Kaya, & Cilli, 2007a; Uguz et al., 2007b). In particular, pregnant and postpartum women are 1.5 to 2 times more likely to experience OCD compared to the general population (Russell, Fawcett, & Mazmanian, 2012). It is adaptive for new mothers to develop worries, concerns and to manifest preoccupations about the newborn's well being to meet the demands of parenthood. However, when obsessions and compulsions develop, the process becomes maladaptive.

1.8 Estrogen and the Serotonergic System

The interaction between estrogen and its regulatory effect on the serotoninergic system have been well documented. The animal literature has provided robust support for ovarian steroids and its effect on affect via estrogen's modulatory effect on serotonin activity (Lokuge, Frey, Foster, Soares, & Steiner, 2011). Early studies found that surgical menopause and hormone replacement therapy of estrogen ± progesterone in rhesus macaques caused a significant reduction of serotonin transporter mRNA in the dorsal

raphe nucleus (DRN) (Lu, Eshleman, Janowsky, & Bethea, 2003; Pecins-Thompson, Brown, Kohama, & Bethea, 1996). Estrogen may regulate serotonin transporter expression and activity by increasing the concentration of extracellular serotonin. The increased concentration will cause the transporter to continually pump serotonin into the cell. The translocation prevents phosphorylation and subsequent internalization of the transporter (Ramamoorthy & Blakely, 1999). Chronic administration of estrogen results in centrally decreased levels of the serotonin transporter, decreased MAO-A and increased levels of tryptophan hydroxylase 2 (L. J. Smith, Henderson, Abell, & Bethea, 2004). The net effect is an increase in the amount of central serotonin available for neurotransmission (Lokuge et al., 2011). Additionally, in macaques, ovarian hormones promote increased cellular resilience of serotonin neurons, which may prevent their cell death. In particular, in the DRN, estrogen decreases the gene expression of c-jun n-terminal kinase and kynurenine mono-oxygenase, which promotes cell death and increases gene expression of superoxide dismutase, vascular endothelial growth factor, and caspase inhibitory proteins that promote cellular resilience (Bethea, Reddy, Tokuyama, Henderson, & Lima, 2009). In humans, women in the postpartum period have significantly increased global levels of MAO-A, suggesting that the sudden drop in estrogen during the immediate early postpartum period may result in elevated levels of MAO-A (Sacher et al., 2010). Together, the evidence in the literature supports estrogens role on serotonin production. Therefore during reproductive milestones that are characterized by decreased levels of estrogen, such as the postpartum period and

menopause, women are more susceptible to mood disorders and skewed modulation of affective processing (Epperson, Amin, Ruparel, Gur, & Loughead, 2012).

1.9 Functional Neuroimaging

1.9.1 Resting State Functional Connectivity

Functional connectivity is defined to be the temporal association between anatomically distinct regions in the brain (Salvador, Suckling, Schwarzbauer, & Bullmore, 2005; Whitfield-Gabrieli & Nieto-Castanon, 2012). Since there are no behavioural demands during rest, resting state functional connectivity (rs-FC) is of great interest to characterize baseline functional brain network differences between "healthy" individuals and a clinical population. There are two main approaches to analyze rs-FC – independent component analysis (Beckmann, DeLuca, Devlin, & Smith, 2005; Greicius et al., 2007) and seed-driven rs-FC (Fox et al., 2005; Greicius, Krasnow, Reiss, & Menon, 2003). Independent component analysis assumes that there is an archetypal spatial pattern seen in resting-state allowing it to be reliably detected using a template-matching procedure for each subject (Greicius et al., 2007). Seed-driven analysis calculates the Pearson's correlation coefficients between the seed brain region of interest and all other voxels in the brain, thus producing correlation maps (Fox et al., 2005). The correlation coefficients are then converted to normally distributed Z-scores using a Fisher's transform to allow for second level general linear model analysis (Fox et al., 2005; Whitfield-Gabrieli & Nieto-Castanon, 2012).

1.9.2 Default Mode Network

Functional neuroimaging studies of task-related activity observed a consistent pattern of deactivation across a network of brain regions that includes the precuneus/posterior cingulate cortex (PCC), medial prefrontal cortex (mPFC) and medial, lateral and inferior parietal cortex (Greicius et al., 2003). This observation led to a hypothesis that these regions make up a region that supports a default mode of brain function during rest. The network was coined the default mode network (DMN), which was responsible for a task negative self-referential and introspective state (Greicius et al., 2003; Long et al., 2008). Further, it has been observed that there is a temporally anti-correlated network coined the task positive network that is responsible for an extrospective state that ensures the individual is alert and is related to response preparation (Fox et al., 2005; Long et al., 2008). The two brain networks are diametrically opposed and characterized by both spontaneous correlations within each network and anticorrelations between the two networks. The task positive network includes the dorsolateral prefrontal cortex (DLPFC), inferior parietal cortex (IPC) and supplementary motor area (SMA) (Fox et al., 2005). Low frequency toggling between the two anti-correlated networks ensures the individual is alert and attentive to unexpected or novel environmental events (Broyd et al., 2009; Fox et al., 2005). Sex and age differences of the DMN are currently unclear (Bluhm et al., 2008).

1.9.3 Resting State and Activation Patterns in Mental Disorders

Evidence suggests that provocation of cleaning symptoms in female OCD patients is correlated with greater activation of the ventromedial prefrontal regions and the right

caudate nucleus (Mataix-Cols & Wooderson, 2004). These areas are sexually dimorphic, with women having larger volumes and the areas containing a higher level of sex hormone receptors (Goldstein et al., 2001). Moreover, it has been seen that highly anxious individuals exhibit reduced amygdala-ventromedial prefrontal cortex (vmPFC) rs-FC. This circuit is of particular interest to anxiety disorders since this pathway is normally postulated to downregulate threat or fear related anxiety in the amygdala (M. J. Kim et al., 2011). Furthermore, recent work done by Burghy et al. (2012) found that higher early life stress (ELS) within the first year of life predicts higher cortisol levels at four years of age. At 18 years, the childhood cortisol levels were significantly inversely correlated with amygdala-vmPFC rs-FC. It is important to note that for females only, high levels of ELS predicted increased levels of childhood afternoon basal cortisol, which in turn predicted lower amygdala-vmPFC rs-FC. Moreover, amygdala-vmPFC rs-FC was negatively correlated with concurrent symptoms of anxiety at 18 years of age in females only (Burghy et al., 2012).

An aberrant DMN and resting state are proposed to underlie or result from mental disorders. In particular, there are four proposed mechanisms: (1) the DMN may interfere during a task performance, these would be seen as attentional lapses during goal-directed tasks as a result of interference arising from spontaneous self-referential thought; (2) altered patterns of antagonism between the task-positive network and DMN, this is based on the idea that the reciprocal relationship is of more clinical relevance than the DMN alone; (3) Altered connectivity suggesting altered integrity of the default- mode functional network, decreased connectivity of the DMN may underlie deficits in self-

referential processing, attentional control and working memory. In contrast, over zealous connectivity within the DMN and task-positive network may instigate excessive introspective and extrospective processing; (4) altered patterns of DMN activity, for example, in patients with anxiety disorders, reduced deactivation of mPFC and increased deactivation of the PCC is thought to be indicative of increased levels of anxiety (mPFC) and emotional processing (PCC) of task-specific threat words (Broyd et al., 2009).

1.9.4 Functional Neuroimaging and OCD

The finding of increased regional metabolic activity in the orbitofrontal cortex and caudate in OCD patients versus controls, during both resting and obsessive-compulsive symptomatic states, has been one of the most replicable findings in psychiatric imaging (Rasmussen, Eisen, & Greenberg, 2013). Functional connectivity studies highlight the importance of complex network connections as well as the significance of synchrony versus desynchrony (Ramussen, Eisen and Greenberg 2013). Specifically, it has been well replicated that OCD patients demonstrate a common connectivity alteration involving the ventral striatum and orbitofrontal cortex that predicted overall illness severity levels (Harrison et al., 2013; 2009).

1.10 Thesis Objectives

1.10.1 Hypothesis

Women with the S/Lg-allele who experience childhood maltreatment may be more susceptible to develop psychiatric disorders. In particular, we hypothesize that these

women may develop OCD during the perinatal period and exhibit an aberrant resting-state functional connectivity during the postpartum period compared to healthy controls.

1.10.2 Main Objective

This study examines the effects of past childhood maltreatment and S/L_g-allele status on the development of aberrant brain functional connectivity in the postpartum period and perinatal onset/exacerbation of obsessive-compulsive symptoms. We analyzed if the 5-HTTLPR genotype acts a moderator for childhood maltreatment to lead to the development of obsessive-compulsive symptoms in the perinatal period and the consequences on activation patterns in the brain.

2. METHODS

2.1 Ethics

All experimental procedures followed the mandate of and were approved by the Hamilton Integrated Research Ethics Board. All participants provided informed consent and signed a consent form. Information was kept private and confidential. Participant identifying information was not placed on any of the study materials and all participants were assigned a unique alphanumeric code.

2.2 Experimental Design

2.2.1 Study Population

Pregnant women in their third trimester (28-40 weeks gestation) were recruited through the Women's Health Concerns Clinic (WHCC) at St. Joseph's Health Hamilton, the community via recruitment flyers and medical offices. Upon registration, patients in the WHCC indicate whether they are willing to be contacted about research studies being conducted in the clinic.

2.2.2 Inclusion and Exclusion Criteria

Women in the clinic who have given consent to be contacted or women who express interest in the study are then screened to assess eligibility. Participants must be at least 21 years of age, can be any ethnicity, must present with no comorbid psychiatric disorders other than anxiety and/or depression. Participants must be overall in good physical health and could not of had a concussion within the past 10 years.

2.2.3 Participant Grouping

Participants were grouped by three criteria: healthy controls versus not healthy, current diagnosis of OCD versus no current diagnosis and 5-HTTLPR genotype status. Participants were considered healthy controls if they presented with no current psychiatric diagnosis, had no past history of a psychiatric disorder and had no first-degree relative with a confirmed psychiatric diagnosis. Participants were also categorized by current diagnosis. Participants that presented with no current OCD diagnosis but may have had a past diagnosis of depression and/or an anxiety disorder were placed in the no current diagnosis group. Participants that presented with a primary OCD diagnosis and may or may not have a secondary present or past diagnosis of depression and/or an anxiety disorder were placed in the current diagnosis group. Genotype was determined by the below genotyping methodology.

2.3 Behavioural Measures

One investigator-administered questionnaire and six self-administered were used in this study.

2.3.1 Childhood Trauma Questionnaire

The short form Childhood Trauma Questionnaire (CTQ) is a retrospective scale that assesses childhood trauma via 25 Likert-type items (Bernstein et al., 2003). This enhances the reliability and maximizes statistical power. The main components of the CTQ are five rotated factors that are labeled emotional abuse, physical abuse, sexual abuse, emotional neglect and physical neglect (Bernstein et al., 1994). Scores in each category can range

from 5 to 25 for a minimum total score of 25 and a maximum total score of 125. A sixth category is included as a validity scale where a score of 3 indicates that the individual may be minimizing their past childhood maltreatment (Bernstein et al., 2003).

2.3.2 Edinburgh Postnatal Depression Scale

The Edinburgh Postnatal Depression Scale (EPDS) is a 10-item self-reported questionnaire that assesses depressive and anxiety symptoms during the perinatal period. The EPDS exhibits high sensitivity and specificity to depression in relation to standardized psychiatric interviews (Cox, Holden, & Sagovsky, 1987). Scores can range from 0 to 30.

2.3.3 Montgomery-Asberg Depression Scale

The Montgomery-Asberg Depression Scale (MADRS) is a clinician rated 10-item semi-structured interview used to establish the clinical severity of depressive symptoms. When compared the gold standard Hamilton Depression Rating Scale, the MADRS shows higher inter-rater reliability and an increased sensitivity to change in depressive symptoms (Montgomery & Åsberg, 1979). Scores can range from 0 to 60.

2.3.4 State Trait Anxiety Inventory

The State Trait Anxiety Inventory (STAI) is a psychological inventory based on a 4-point Likert scale. It is a reliable and sensitive tool to assess anxiety symptoms. The STAI measures both state anxiety and trait anxiety by 20 items per category with higher scores being positively correlated with higher levels of anxiety. State anxiety describes how the individual is feeling at that moment whereas trait anxiety describes how the individual

feels in general. Scores can range from 20 to 80 for each state and trait anxiety (Spielberger & Gorsuch, 1970).

2.3.5 Yale-Brown Obsessive-Compulsive Scale

The Yale-Brown Obsessive-Compulsive Scale (YBOCS) measures the severity of obsessive-compulsive symptoms. The scale has 10-items each rated from 0 (no symptoms) to 4 (extreme symptoms), with separate subtotals for severity of obsessions and compulsions (Goodman & Price, 1989). The YBOCS is considered the gold standard for OCD symptom severity assessment due to its strong psychometric properties, high internal consistency, good inter-rater reliability and construct validity. Furthermore, it has been seen that the self-report version proves reliable and shows strong convergent validity with the interview (Steketee, Frost, & Bogart, 1996). Scores range from 0 to 40.

2.3.6 Perinatal Obsessive-Compulsive Scale

Perinatal obsessions and compulsions are very specific in content and are frequently directed towards the baby's health, well-being and environment. The Perinatal Obsessive-Compulsive Scale (POCS) is a self-report scale that assesses the unique content, context, severity and onset of obsessions and compulsions during the perinatal period. The POCS has good construct validity, reflected by representative items, high internal consistency, good concurrent validity and discriminative capacity (Lord, Rieder, Hall, Soares, & Steiner, 2011). Scores range from 0 to 40.

2.3.7 Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) was administered to participants during the second visit to assess overall sleep quality. The PSQI is a self-administered test that

generates seven "component" scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications and daytime dysfunction. The sum of all these seven components yield a global score that indicates either good or poor sleep quality (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). The scores range from 0 to 21.

2.4 Genotyping

2.4.1. DNA Extraction

Participant blood samples were stored at -80°C until they were ready to be genotyped. Genomic DNA was extracted from EDTA anti-coagulated whole blood using the QIAamp DNA blood mini-kit (QIAGEN, Mississauga, ON). The NanoDrop 2000c was used to determine the concentration of DNA in ng/μL.

2.4.2 Polymerase Chain Reaction and Restriction Fragment Length Polymorphism

The polymorphism in the promoter region of the serotonin transporter gene on chromosome 17q11.2 was assessed to identify a 44 base pair insertion (L-allele) or deletion (S-allele). Genotyping for 5-HTTLPR was done in a two-step process. First, the gene was amplified using polymerase chain reaction (PCR) using a forward primer 5'-GGCGTTGCCGCTCTGAATGC and reverse primer 5'-GAGGGACTGAGCTGGACAACCAC (Lesch et al., 1996). AccuPrime GC-Rich DNA polymerase (Invitrogen 12337-016) was used to amplify the gene. The 25 μL amplification mixture contained 100 ng of genomic DNA and a master mix of 0.5 μL of each primer (forward and reverse), 0.5 μL 5x GC-Rich Buffer A and 5.0 μL of

Accuprime GC-Rich DNA polymerase. Conditions for cycling were: initial denaturation at 95°C for 3 minutes followed by 7 cycles of 95°C for 30 seconds, 68°C for 30 seconds and 72°C for 1 minute, then 7 cycles of 95°C for 30 seconds, 68°C for 30 seconds and 72°C for 1 minute and another 7 cycles of of 95°C for 30 seconds, 68°C for 30 seconds and 72°C for 1 minute. Final extension took place at 72°C for 10 minutes. 13 μL of the uncut portion was run on a 1.5% agarose gel at 50V for 90 minutes. The resulting band patterns for the PCR mixture are as follows: 528 base pairs for the L-allele and 484 base pairs for the S-allele (Appendix 1) (Lesch et al., 1996).

Second, 12 μL of the PCR product underwent restriction fragment length polymorphism analysis. 0.1 μL of restriction enzyme MSPI and 1.3 μL of CutSmart buffer were used to digest the DNA into fragments to visualize the L_a-allele and L_g-allele (New England Biolabs). The mixture was then incubated for 3 hours at 37°C (Praschak-Rieder et al., 2007). The cut product was run on a 4-20% gradient polyacramide gel (Invitrogen EC6225BOX) at 14 mA for 60 minutes to separate the bands. The gel was then soaked in a 100 mL water and 10 μL SybrSafe (Life Technologies S33102) solution for 3 hours. The resulting band patterns for the cut PCR product was 340 base pairs for the L_a-allele, 297 base pairs for the S-allele and 166+174 base pairs for the L_g-allele (Appendix 2) (Praschak-Rieder et al., 2007).

Both the agarose and gradient polyacramide gel was visualized in a UV transilluminator (UVP Bioimaging Systems). 13 (32.5%) samples were randomly selected to re-genotype in order to evaluate genotyping accuracy – there was 100% re-testing accuracy.

2.5 Functional Magnetic Resonance Imaging

2.5.1 Resting State Functional Connectivity

Participants were told to remain motionless with their eyes opened and to not think of anything in particular. They were given a fixation point to look at during the task. The scan lasted for 5 minutes and 24 seconds (Appendix 3).

2.5.2 Montreal Imaging Stress Task

In order to elicit a psychological stress, the MIST paradigm was employed. The MIST requires the participant to complete a challenging mental arithmetic task in the presence of negative social evaluation. The arithmetic equations are presented on a computer screen, and the participants will provide answers by choosing a 1-digit number from a current designs magnetic resonance compatible response pad (Appendix 4). There are 3 conditions to the MIST: the rest condition where the interface is presented without arithmetic equations to complete; the control condition, which consists of simple mental arithmetic challenges; and the stress–experimental condition (Appendix 5). During the stress condition, the difficulty of the arithmetic tasks will adapt to the user's performance to induce failure, preventing the participant from scoring higher than 50%–55%. To increase the socioevaluative threat, prior to the scan, participants are told that the task is usually completed with an average of 80%– 90% correct answers (Dedovic et al., 2005; Lord, Steiner, Soares, Carew, & Hall, 2012; Pruessner, Dedovic, Khalili-Mahani, & Engert, 2008). In addition, while performing the task in the scanner, participants are provide with negative feedback with a mock performance indicator suggesting their performance is significantly lower than the average participant. A time bar is also

displayed to increase time pressure (Dedovic et al., 2005). At the end of the experiment, the participants were debriefed about the true purpose of the arithmetic task and that the MIST requires deception. The participants were informed that a stress response was only possible if the goals of the MIST were not explained prior to the task. Participants were reassured that it was impossible for them to perform well since the program adapts to their arithmetic abilities to ensure they did not score above 55%. They were also told that the feedback bar was not real and purposely set high to provide negative feedback. They were asked to dismiss any negative feelings about their performance and were warmly thanked for their participation and understanding.

2.5.3 Imaging Procedure

Images were acquired using a GE short-bore 3-Tesla MRI scanner with an 8-channel array head coil (General Electric Healthcare). A T_1-weighted 3-dimensional axial mp rage anatomic scan was performed (repetition time [TR] 9 ms, echo time [TE] 3.2 ms, flip angle 12°, field of view [FOV] 240 mm, slice thickness 2.0 mm, 140-168 slices, matrix size 320 x 192). For the resting state scan, scanning parameters were: TR = 3000 ms, TE = 35 ms, flip angle = 90, matrix 64 x 64, FOV = 240 mm and 36 axial slices to ensure temporal lobes were covered. The functional images were acquired with a gradient-echo echo-planar imaging sequence covering 36 axial slices (4 mm thick, no gap), starting at the cerebral vertex and encompassing the entire cerebrum. The MIST fMRI scans were acquired with a TR = 2000 ms, TE = 35 ms, FOV 240 mm, flip angle 90°, matrix 128 x 64. A block design was employed with 2 runs consisting of rest, control and experimental blocks. This was completed 2 times randomly within a run and between participants.

Each run consisted of 198 acquisitions (32 rest, 104 control, 62 experimental) lasting 9 minutes and 56 seconds.

2.6 Timeline

The first visit took place when the participant was in their third trimester of pregnancy. During this visit, the study and study expectations were fully explained. Once all questions and concerns were answered, informed consent was obtained. Participants completed the EPDS, STAI, CTQ, POCS and YBOCS. A trained graduate student administered the MADRS. A blood sample was obtained to determine 5-HTTLPR genotype and the CIDI-Venus was used to determine psychiatric diagnosis.

The second visit took place when the participant was three months postpartum. During this visit participants re-completed the EPDS, STAI, POCS, YBOCS and completed the PSQI. The MADRS was re-administered by a trained graduate student. Women were scanned in a 3T magnetic resonance imaging scanner to visualize resting state functional connectivity and activation patterns while completing the MIST.

2.7 Data Analysis

2.7.1. Analysis of Clinical Measures

Clinical data and genotype status were analyzed using the open access statistical analysis program R (version 3.0.2 “Frisbee Sailing”, Lucent Technologies). The following packages were employed: bstats, car, ggplot2, gvlma, Hmisc, leaps, MASS, ply, psych, pwr, reshape2, Quantpsyc (Kabacoff, 2014). The mean and standard deviation were

calculated for the demographic data age and gestational week. A Shapiro test was used to determine if POCS and YBOCS scores were normally distributed. Scores were assessed at visit 1, visit 2 and changes were compared from visit 2 to visit 1. If the scores were normally distributed, a Bartlett test was used to assess the homogeneity of variance by genotype and by diagnosis. Either a parametric or non-parametric test was used to assess if there were significant group differences of age, gestational week, POCS or YBOCS scores by genotype, group and diagnosis at the first visit and POCS and YBOCS scores by genotype and group at the second visit. Multiple linear regression was used to estimate the effect of predictor variables on OCS during late pregnancy and the postpartum period. Further, multiple linear regression was used to estimate the effect of changes in predictor variables on changes on OCS from pregnancy to the postpartum period. Stepwise regression and Aikake information criterion determined the best models. Reported beta-values have been standardized to describe the predictor variable's effect on the standard deviation change of the outcome variable. Moderation analysis was employed to see if genotype influenced the strength of the predictor variable's effect on POCS or YBOCS scores at each time point.

2.7.2. Analysis of MRI Data

The MIST and anatomical MRI data were transferred to a computer to be preprocessed and analyzed using the program BrainVoyager Qx version 2.1 (Brain Innovation B.V.). The T_1-weighted 3-D anatomic MRI data was transformed into Talairach space. The functional MRI data was temporally corrected, 3-D motion corrected and normalized to the Talairach space. This transformation was applied to the aligned function data. A

random-effects general linear model was then used with experimental and control conditions as the predictor variables of blood-oxygen level-dependent (BOLD) signals between groups.

The resting state and anatomical MRI data were preprocessed using the Statistical Parametric Mapping software (SPM8; www.fil.ion.ucl.ac.uk/spm) and analyzed using the conn toolbox (http://www.nitrc.org/projects/conn/) in MATLAB. Functional data were sampled to 2 x 2 x 2 mm voxels. The first 2 volumes of the functional images were discarded. Preprocessing of the functional data included slice timing correction, motion correction, co-registration with the anatomical data, segmentation, spatial normalization to standard space and spatial smoothing. White matter, cerebrospinal fluid and the effect of movement were used as confounds for BOLD signals. White and grey matter were used as first level covariates and diagnosis of OCD and history of emotional maltreatment as a second level covariate. Differences in rs-FC between healthy controls and OCD patients were measured by a t-test. Further, we controlled for potential between group differences in rs-FC with childhood emotional maltreatment as a covariate via a one-way ANCOVA. Both hemispheres of the occipital frontal cortex (OFC), caudate, insula and amygdala were used as regions of interest.

3. RESULTS

3.1 Demographics

3.1.1 Group and Current Diagnosis

Forty women completed the pregnancy visit. 38 (95%) of women were Caucasian, 1 (2.5%) woman was of Chinese Han descent and 1 (2.5%) woman was Native American. The mean age of the participants was 30.5 ± 4.02 years and the mean gestational week was 30.55 ± 5.52 weeks. Demographic data summarized in table 1. Age (p=0.1221) and gestational week (p=0.2258) are normally distributed. Variances of age (p=0.5909) and gestational week (p=0.7799) are equal by group. There are no significant differences of age (p=0.4385) and gestational week (p=0.4857) by group as measured by an unpaired t-test (figure 1 and figure 2). Variances of age (p=0.2808) and gestational week (p=0.9264) are equal by current diagnosis. There were no significant group differences of age (p=0.1975) and gestational week (p=0.3795) by current diagnosis as measured by an unpaired t-test (figure 3 and figure 4).

3.1.2 5-HTTLPR Genotype

Genotype frequencies for the study population are as follows: 8 LL (20%), 17 LS (42.5%) and 15 SS (37.5%). Genotype frequency significantly differ from Hardy-Weinberg Equilibrium (X^2=16.455). Variances of age (p=0.583) and gestational week (p=0.3023) are equal by genotype. There are no significant group differences of age (p=0.793) and gestational week (p=0.312) by genotype as measured by a one-way ANOVA (figure 5 and figure 6). Chi-squared test shows that there is no relationship between genotype and group (X^2=0.1838, p=0.9122) or genotype and current diagnosis (X^2=2.0306, p=0.3623).

Table 1: Demographic and clinical characteristics of the study participants summarized by group, diagnosis and genotype.

	Group; mean (SD)		**Diagnosis; mean (SD)**	
Characteristic	**Healthy**, n=8	**Not Healthy**, n=32	**No Current OCD**, n=21	**Current OCD**, n=19
Age	29.50 (3.51)	30.75 (4.15)	29.71 (3.48)	31.37 (4.47)
Gestational Week	29.31 (5.18)	30.86 (5.64)	29.81 (5.48)	31.37 (5.60)
POCS Score	0.50 (1.07)	10.66 (8.60)	3.76 (5.44)	14 (8.57)
YBOCS Score	1.62 (3.46)	8.97 (8.04)	2.19 (4.01)	13.37 (6.94)
STAI Score – trait	26.29 (3.77)	46.31 (13.44)	36.95 (13.47)	48.79 (13.37)
STAI Score – state	23.12 (2.53)	40 (13.44)	31.62 (12.01)	42.16 (13.89)
CTQ total	30.25 (7.54)	39.12 (12.20)	35.71 (10.44)	39.16 (13.38)
CTQ – EA	6.12 (1.55)	9.84 (5.23)	8.33 (3.80)	9.95 (5.96)
CTQ – EN	6.75 (2.43)	10.16 (4.36)	8.95 (3.99)	10.05 (4.56)
EPDS Score	2.12 (1.13)	10.56 (5.66)	5.95 (5.36)	12.11 (5.29)
MADRS Score	1.75 (2.05)	14.53 (10.22)	8.48 (9.46)	15.84 (10.48)

	5-HTTLPR Genotype; mean (SD)		
Characteristic	**SS**, n=15	**LS**, n=17	**LL**, n=8
Age	30.80 (3.55)	30.65 (4.64)	29.62 (3.81)
Gestational Week	28.87 (6.66)	31.26 (4.79)	32.19 (4.28)
POCS Score	7.40 (8.05)	9.41 (9.51)	9.25 (9.00)
YBOCS Score	8.00 (8.12)	7.71 (8.08)	6.12 (7.97)
STAI Score – trait	39.71 (11.63)	42.74 (15.22)	47.94 (17.64)
STAI Score – state	32.27 (11.18)	37.82 (14.66)	42.25 (15.67)
CTQ total	40.47 (12.54)	34.71 (10.98)	37.12 (12.67)
CTQ – EA	9.60 (5.01)	8.76 (5.30)	8.88 (4.58)
CTQ – EN	10.80 (4.90)	8.76 (3.87)	8.50 (3.55)
EPDS Score	7.20 (4.84)	9.35 (6.52)	11.00 (7.23)
MADRS Score	10.93 (9.14)	11.41 (11.51)	15.12 (11.48)
Allele Frequency	S – 50%	Lg – 8.75%	La – 41.25%

CTQ = Childhood Trauma Questionnaire, EPDS = Edinburgh Postnatal Depression Scale, MADRS = Montgomery-Asberg Depression Scale, POCS = Perinatal Obsessive-Compulsive Scale, SD = standard deviation, STAI = State-Trait Anxiety Inventory, YBOCS = Yale-Brown Obsessive-Compulsive Scale
N.B. S=S or Lg; L=La

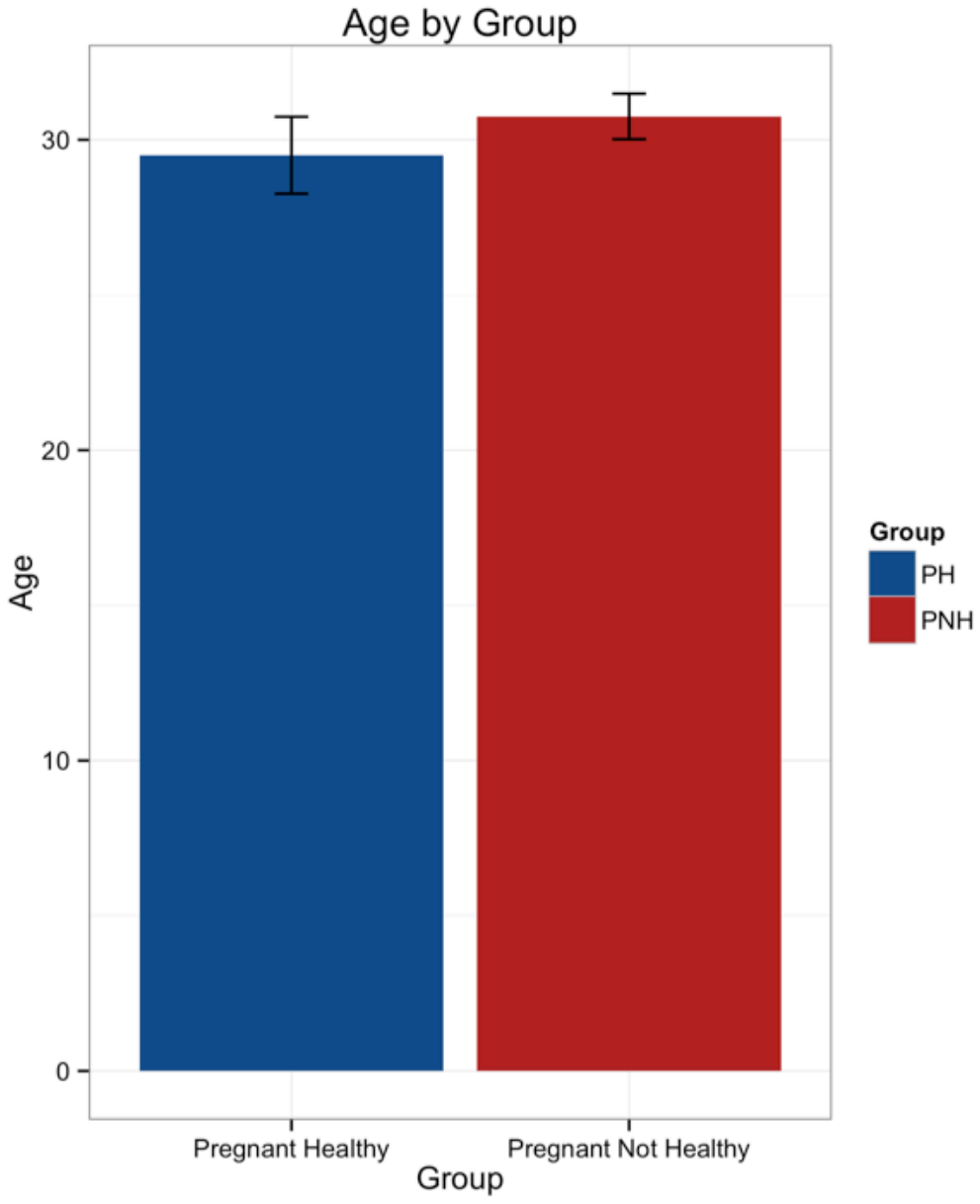

Figure 1. Age of participants is normally distributed and variances by group are equal. An unpaired t-test indicates that there is no significant difference of age by group p=0.4385

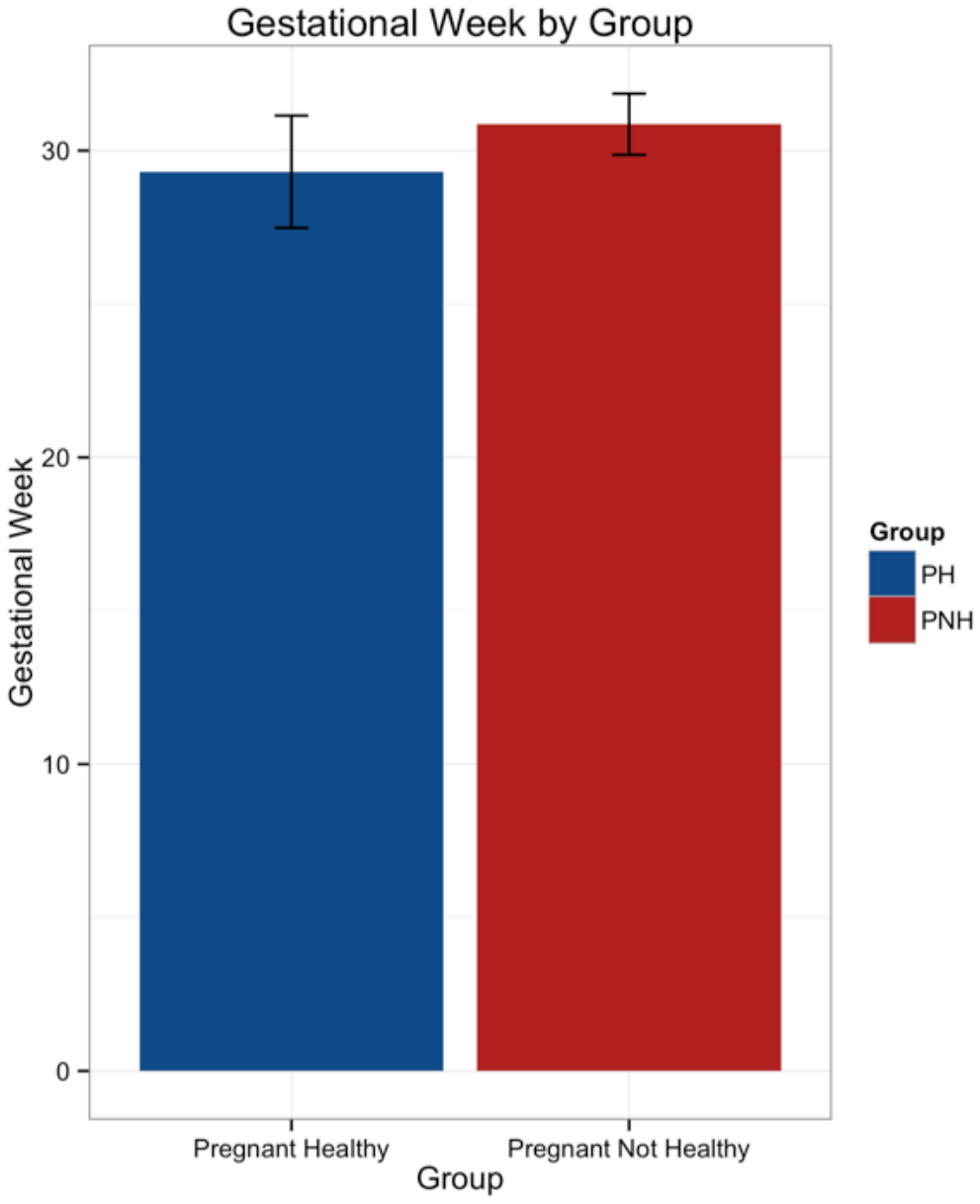

Figure 2. Gestational week of participants is normally distributed and variances by group are equal. An unpaired t-test indicates that there is no significant difference of gestational week by group p=0.4857

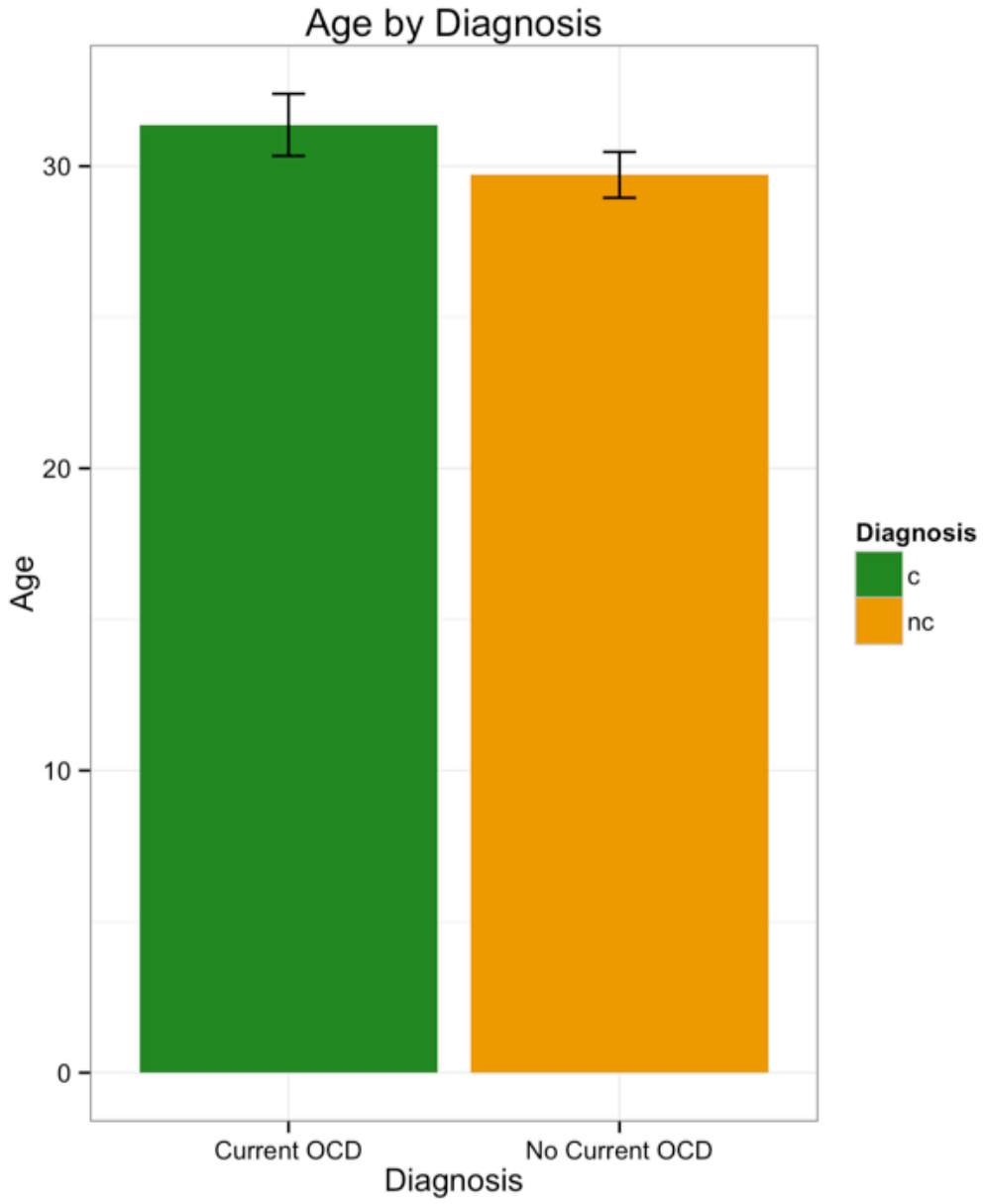

Figure 3. Age of participants is normally distributed and variances by diagnosis are equal. An unpaired t-test indicates that there is no significant difference of age by diagnosis p=0.2808

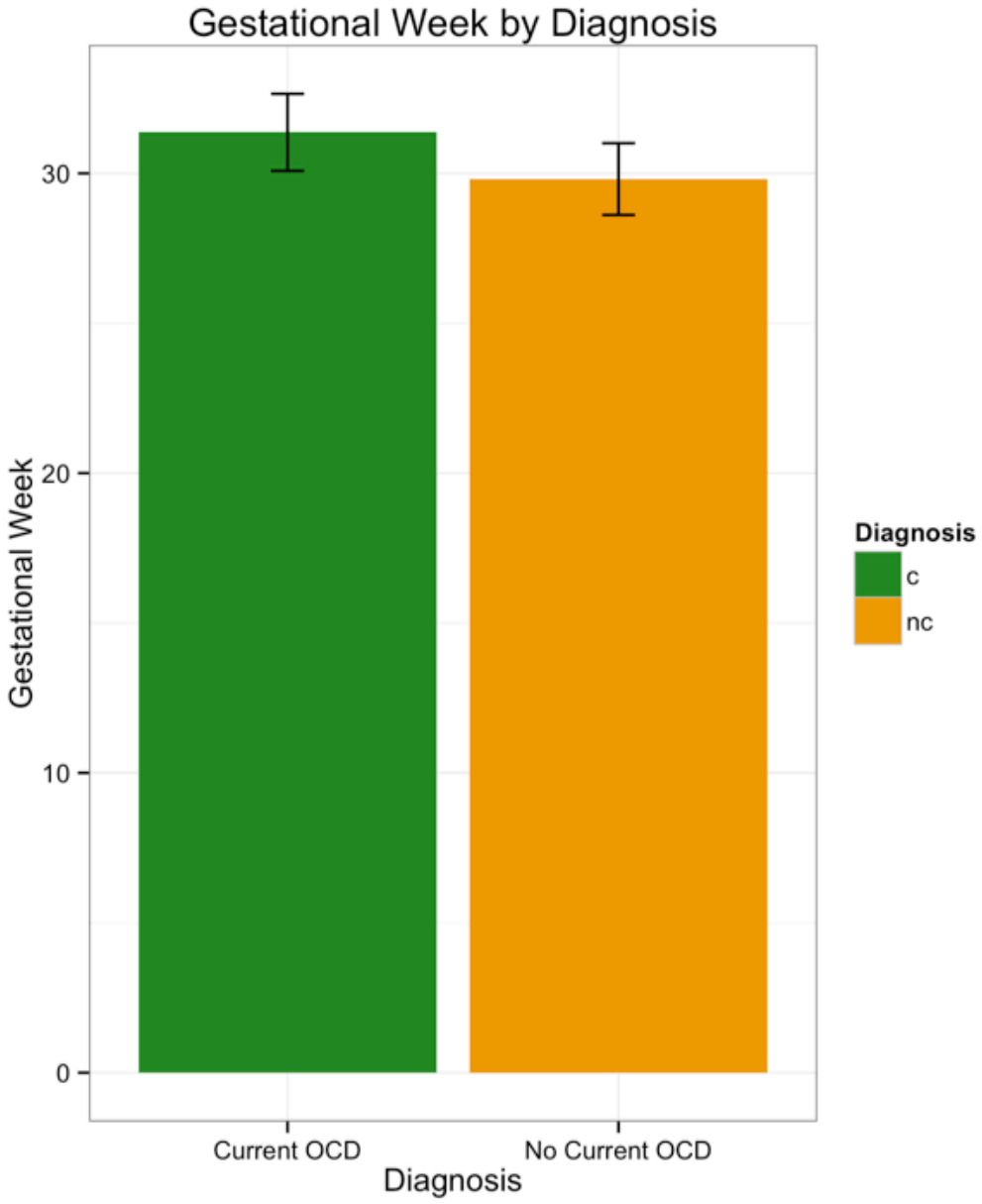

Figure 4. Gestational week of participants is normally distributed and variances by diagnosis are equal. An unpaired t-test indicates that there is no significant difference of gestational week by diagnosis p=0.9264

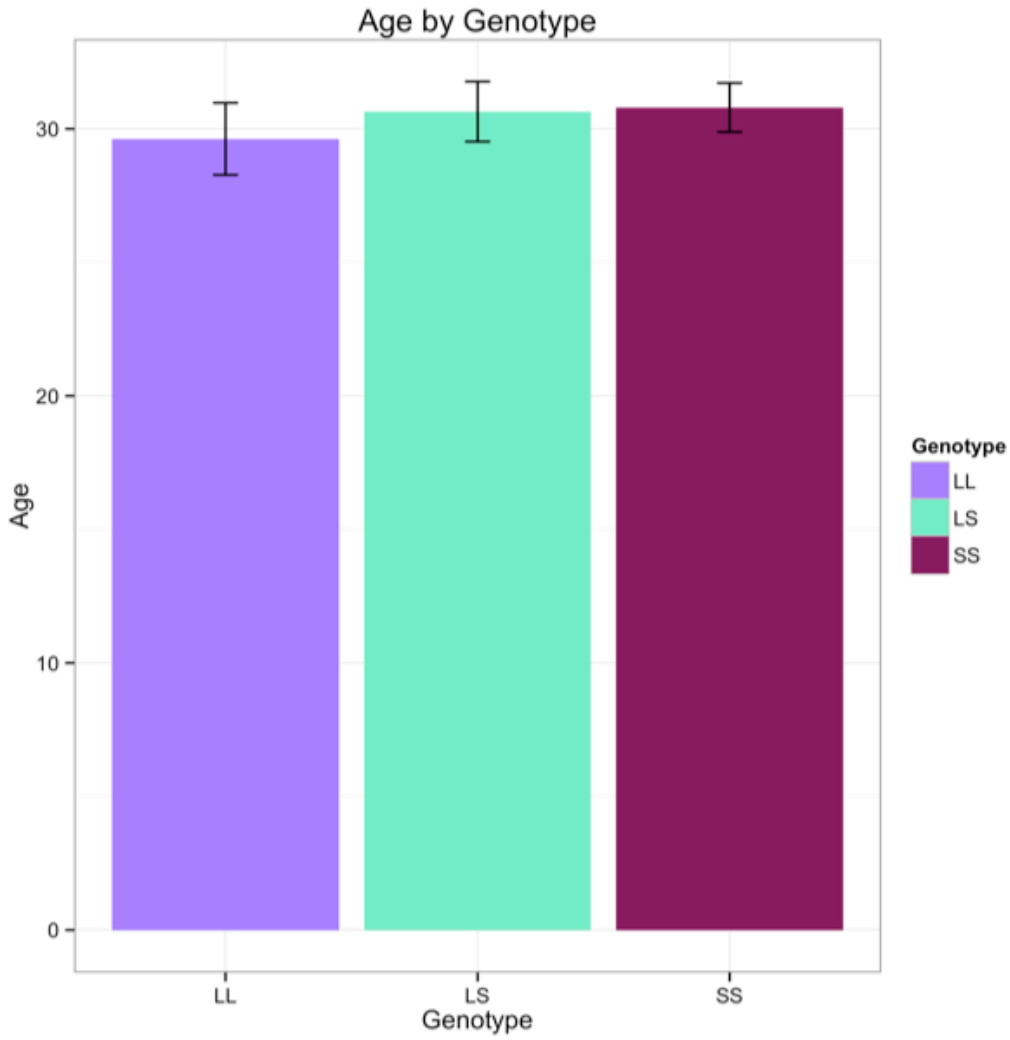

Figure 5. Age of participants is normally distributed and variances by genotype are equal. A one-way ANOVA indicates that there is no significant difference of age by genotype p=0.7930

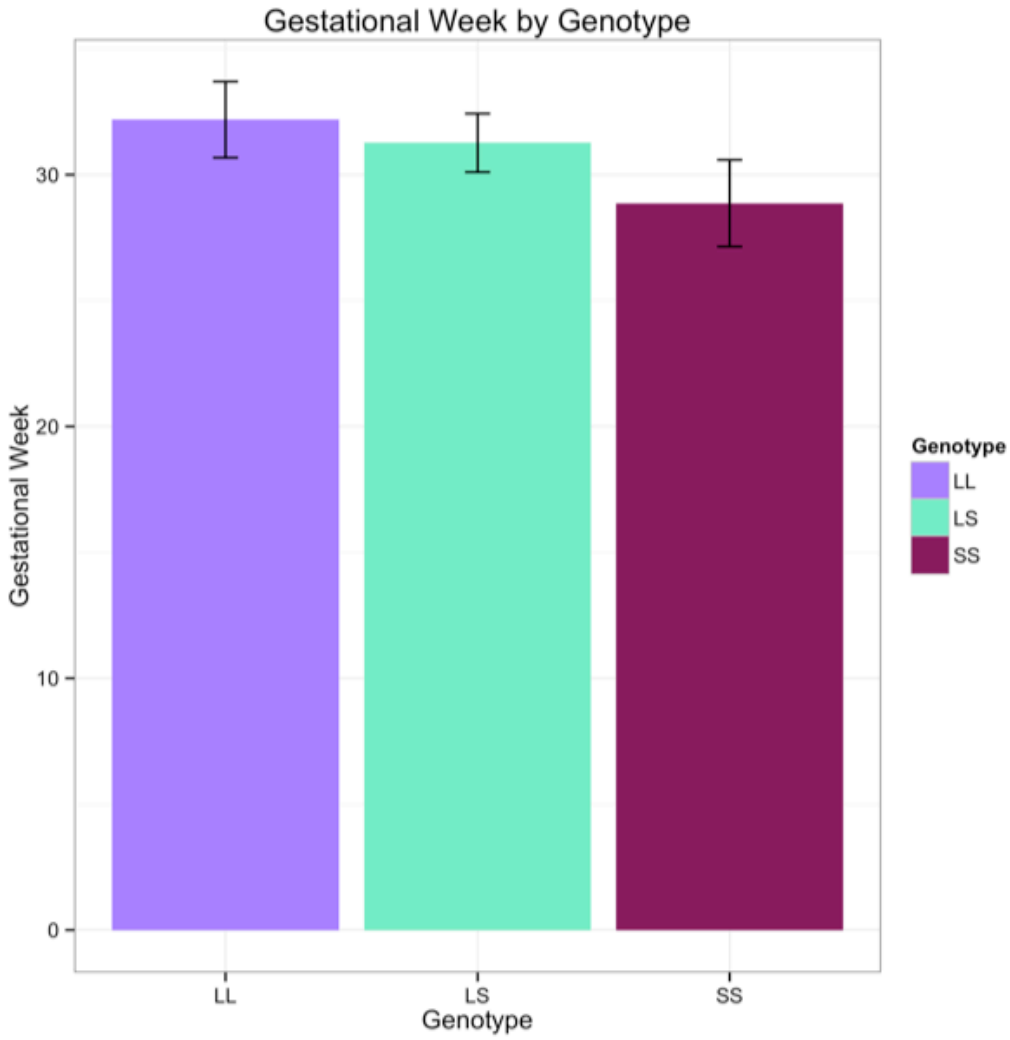

Figure 6. Gestational week of participants is normally distributed and variances by genotype are equal. A one-way ANOVA indicates that there is no significant difference of gestational week by genotype p=0.3120

3.2 First Visit (Pregnancy)

POCS (p=0.0005931) and YBOCS (p=0.0001145) scores are not normally distributed. This is expected due to the two discrete groups of the sample population, so measurements of obsessive-compulsive symptoms would be expected to not be present or to present as clinically significant.

3.2.1 POCS (Pregnancy)

As expected, there are significant differences of POCS scores by group (p=0.000879***) and diagnosis (p=0.000127***) as measured by a Mann-Whitney *U* (figure 7 and 8). However, there is no significant difference of POCS scores by genotype (p=0.7496) as measured by a Kruskal-Wallis test (figure 9). Therefore, individual genotypes could not be used as predictors but rather genotype was used a group predictor. Multiple linear regression indicates that genotype, total childhood maltreatment and trait anxiety significantly predict POCS scores during pregnancy (R^2=0.637, p=6.213e-8***), with both trait anxiety (β=0.5662, t=6.424, p=2.43e-7***) and total childhood maltreatment (β=0.01798, t=2.646, p=0.0123*) as significant predictors. Further, the genotype SS was set as the factor that LS and LL are compared to in the genotype group predictor variable. Compared to SS individuals, LS individuals will have higher POCS (β=0.1496, t=-0.831, p=0.4118) scores and LL individuals will have lower POCS scores (β=-1.9390, t=-0.478, p=0.6359). Genotype was not a significant moderator for POCS scores during pregnancy.

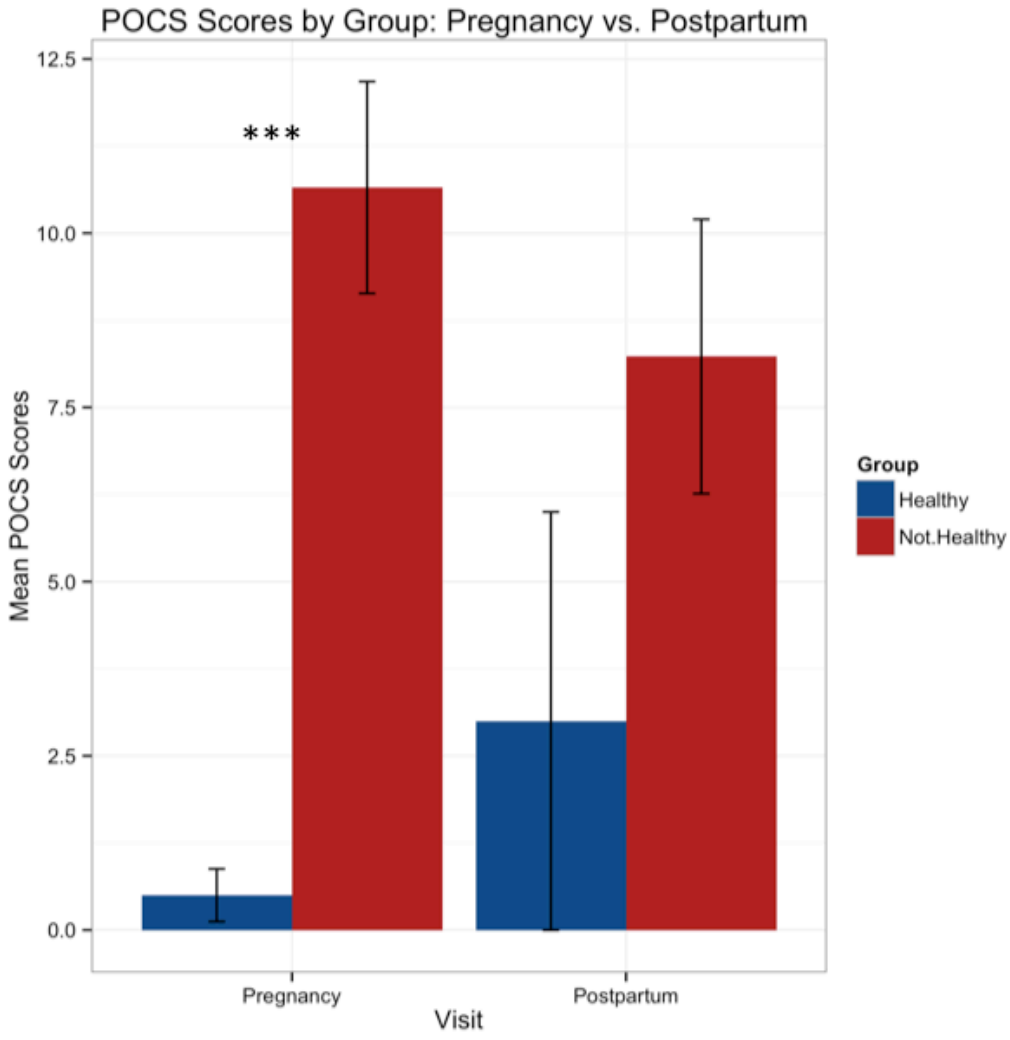

Figure 7. POCS scores of participants during pregnancy and postpartum are not normally distributed. A Mann-Whitney *U* indicates that there is a significant difference of mean POCS scores by group during pregnancy p=0.000879*** but not in the postpartum period p=0.2215

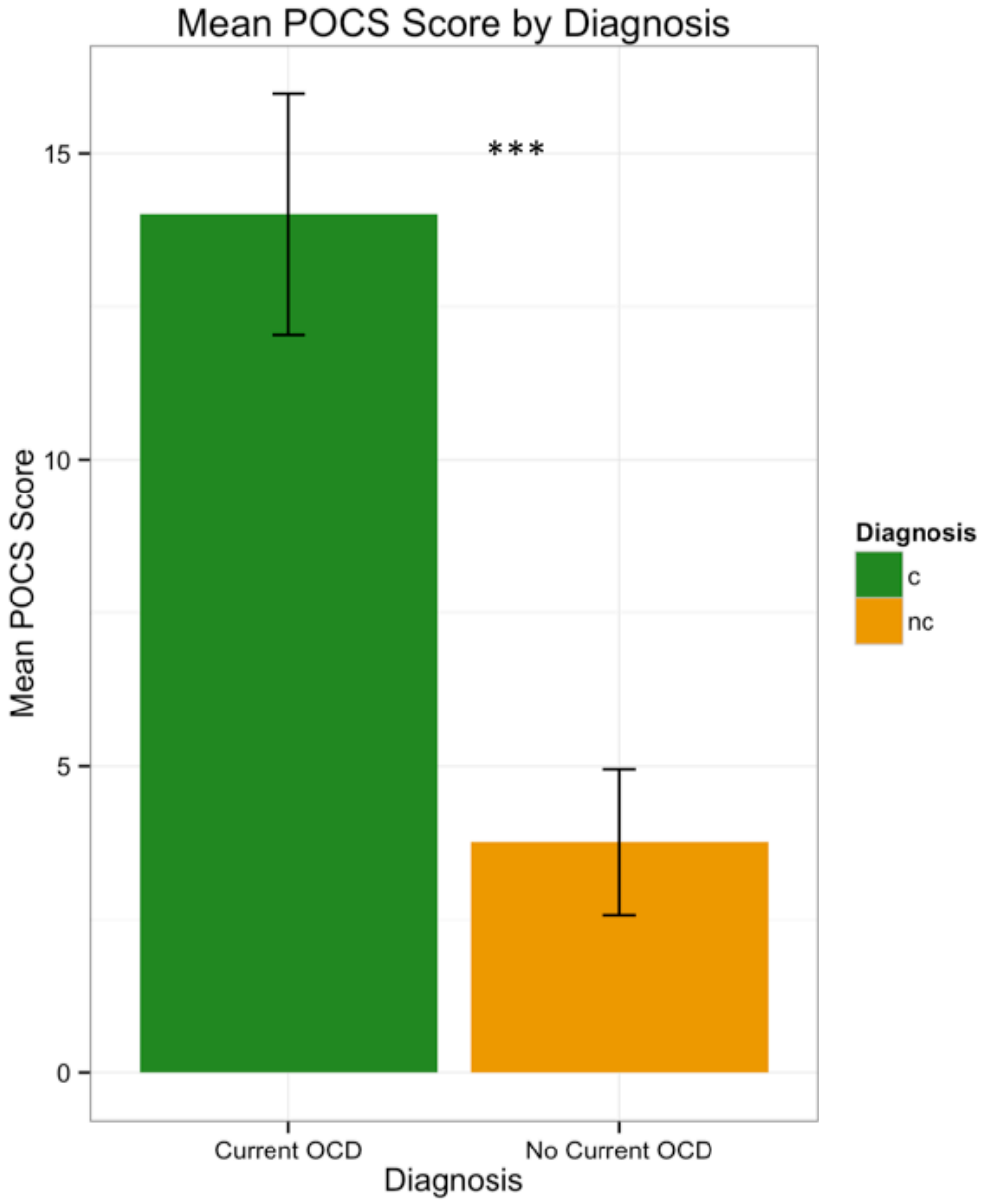

Figure 8. POCS scores of participants during pregnancy are not normally distributed. A Mann-Whitney *U* indicates that there is a significant difference of mean POCS scores by diagnosis during pregnancy p=0.000127***

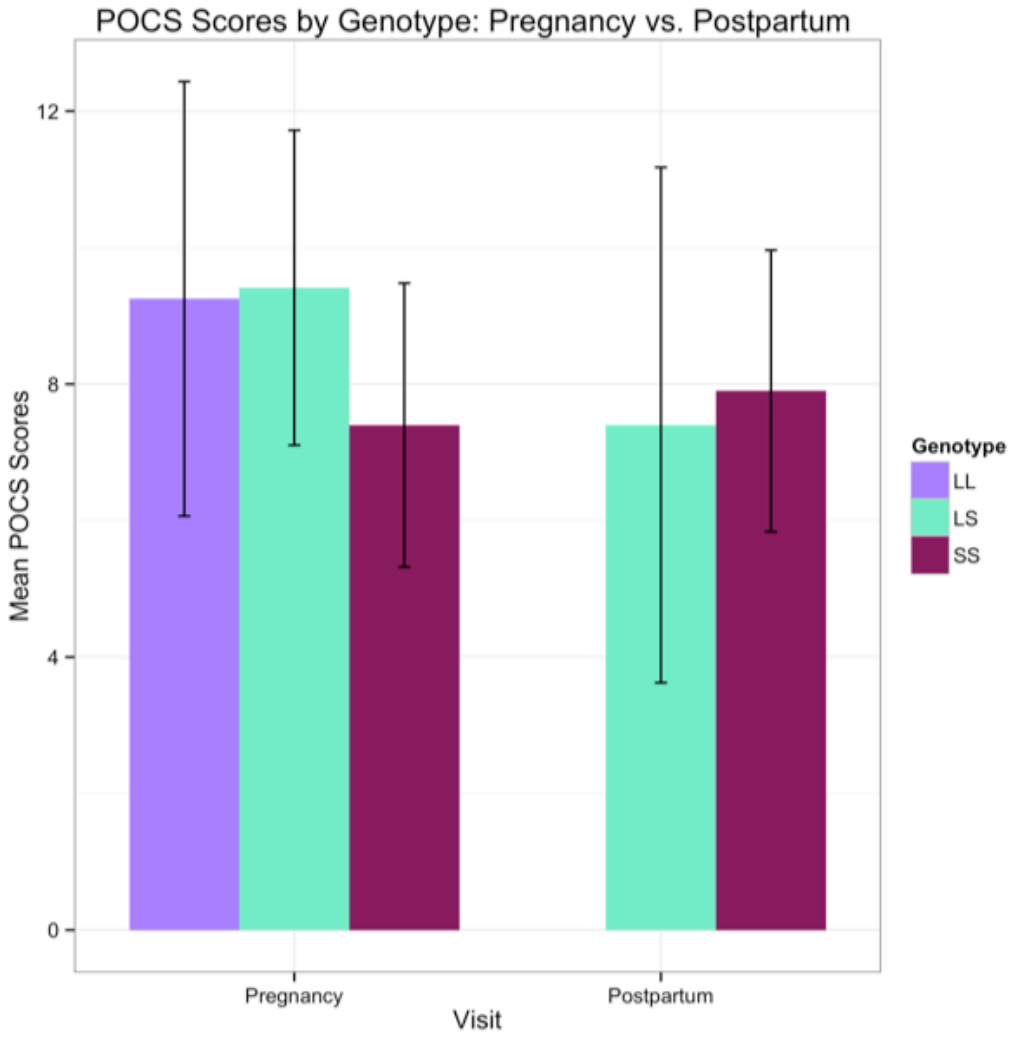

Figure 9. POCS scores of participants during pregnancy and postpartum are not normally distributed. A Kruskal-Wallis test indicates that there is not a significant difference of mean POCS scores by genotype during pregnancy p=0.7496 and in the postpartum period p=0.4153

3.2.2 YBOCS (Pregnancy)

YBOCS scores significantly differed by group (p=0.01958*) and diagnosis (p=5.927e-6***) as measured by a Mann-Whitney *U* (figure 10 and 11). In contrast, there is no significant difference of YBOCS scores by genotype as measured by a Kruskal-Wallis test (p=0.7746) (figure 12). Multiple linear regression indicates that genotype, childhood emotional neglect and trait anxiety significantly predict YBOCS scores during pregnancy (R^2=0.393, p=0.000273***), with trait anxiety as a significant predictor (β=0.1673, t=4.115, p=0/000232***). Compared to SS individuals, LS (β=-0.0745, t=-0.337, p=0.7384) and LL (β=-7.1180, t=-1.318, p=0.1964) individuals will have lower YBOCS scores. Genotype was not a significant moderator for YBOCS scores during pregnancy.

3.3 Second Visit (Postpartum)

Sixteen women have completed the follow-up visit. POCS (p=0.04175) and YBOCS (p=0.01396) scores are not normally distributed. Genotype frequencies for participants followed-up in the postpartum period are as follows: 1 LL (6.25%), 5 LS (31.25%) and 10 SS (62.5%). Therefore, group calculations in the postpartum period could not be calculated for the LL genotype since only one participant with the genotype has been followed-up with to date.

3.3.1 POCS (Postpartum)

There are no significant differences of POCS scores by group (p=0.2215) or by genotype (p=0.4153) as measured by a Mann-Whitney *U* and Kruskal-Wallis test respectively

(figure 7 and 9). Multiple linear regression indicates that childhood emotional maltreatment and poor sleep quality significantly predict postpartum POCS scores (R^2=0.53, p=0.002916**), with both childhood emotional maltreatment (β=0.5267, t=2.973, p=0.01078*) and poor sleep quality (β=0.5819, t=3.285, p=0.00592**) as significant predictors. Genotype was not a significant moderator for POCS scores during the postpartum period.

3.3.2 YBOCS (Postpartum)

There are no significant differences of YBOCS scores by group (p=0.09998) or by genotype (p=0.6098) as measured by a Mann-Whitney *U* and a Kruskal-Wallis test respectively (figure 10 and 12). Multiple linear regression indicates that the model that best predicts postpartum YBOCS scores include genotype, childhood emotional maltreatment and trait anxiety as predictors (R^2=0.7268, p=0.0007799***) with trait anxiety as a significant predictor (β=0.03808, t=4.980, p=0.000416***). Childhood emotional maltreatment is bordering significance (β=0.3601, t=2.135, p=0.05612). The genotype SS was set as the factor that LS and LL are compared to in the genotype group predictor variable. Compared to SS individuals, LS individuals will have lower YBOCS (β=-0.1496, t=-0.822, p=0.4287) scores and LL individuals will have higher YBOCS scores (β=4.0903, t=0.661, p=0.5221). Genotype was not a significant moderator for YBOCS scores during the postpartum period

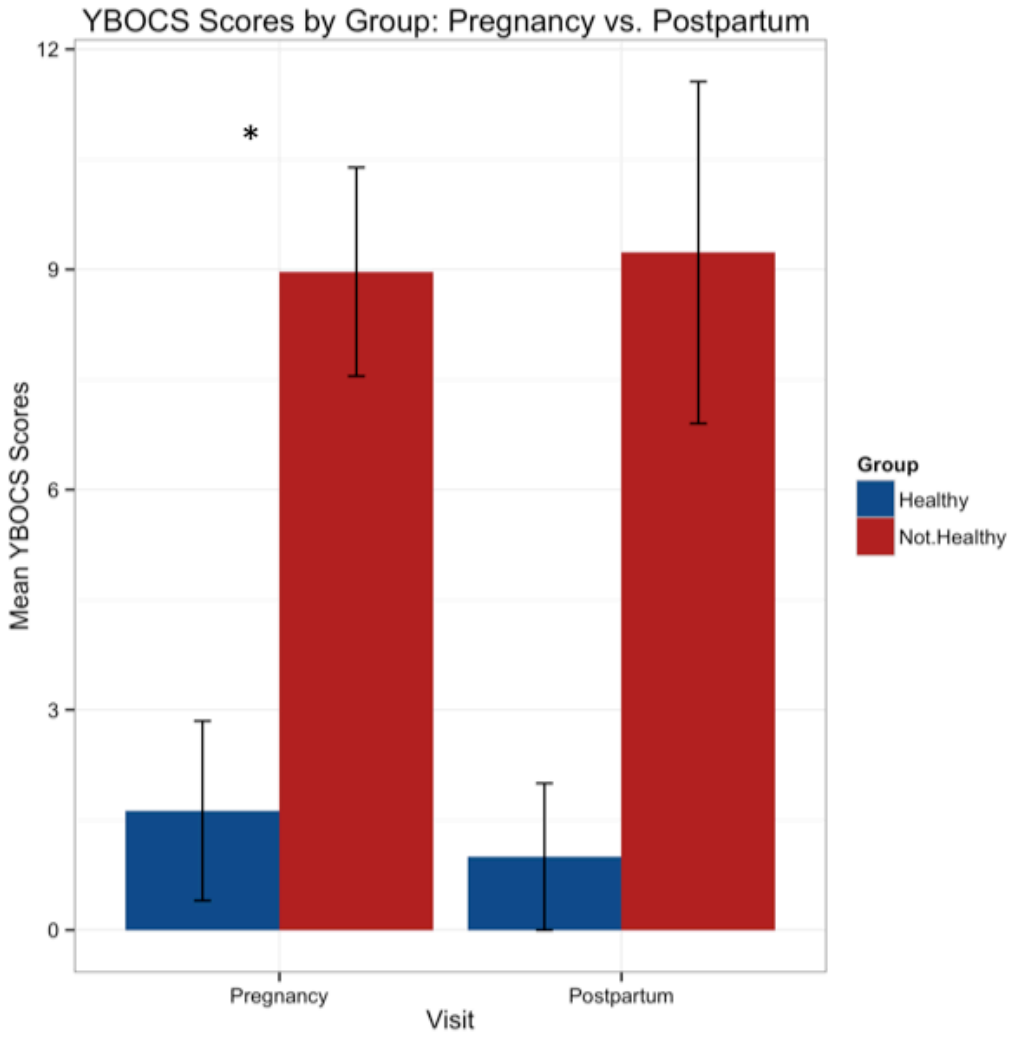

Figure 10. YBOCS scores of participants during pregnancy and postpartum are not normally distributed. A Mann-Whitney *U* indicates that there is a significant difference of mean POCS scores by group during pregnancy p=0.01958* but not in the postpartum period p=0.09998

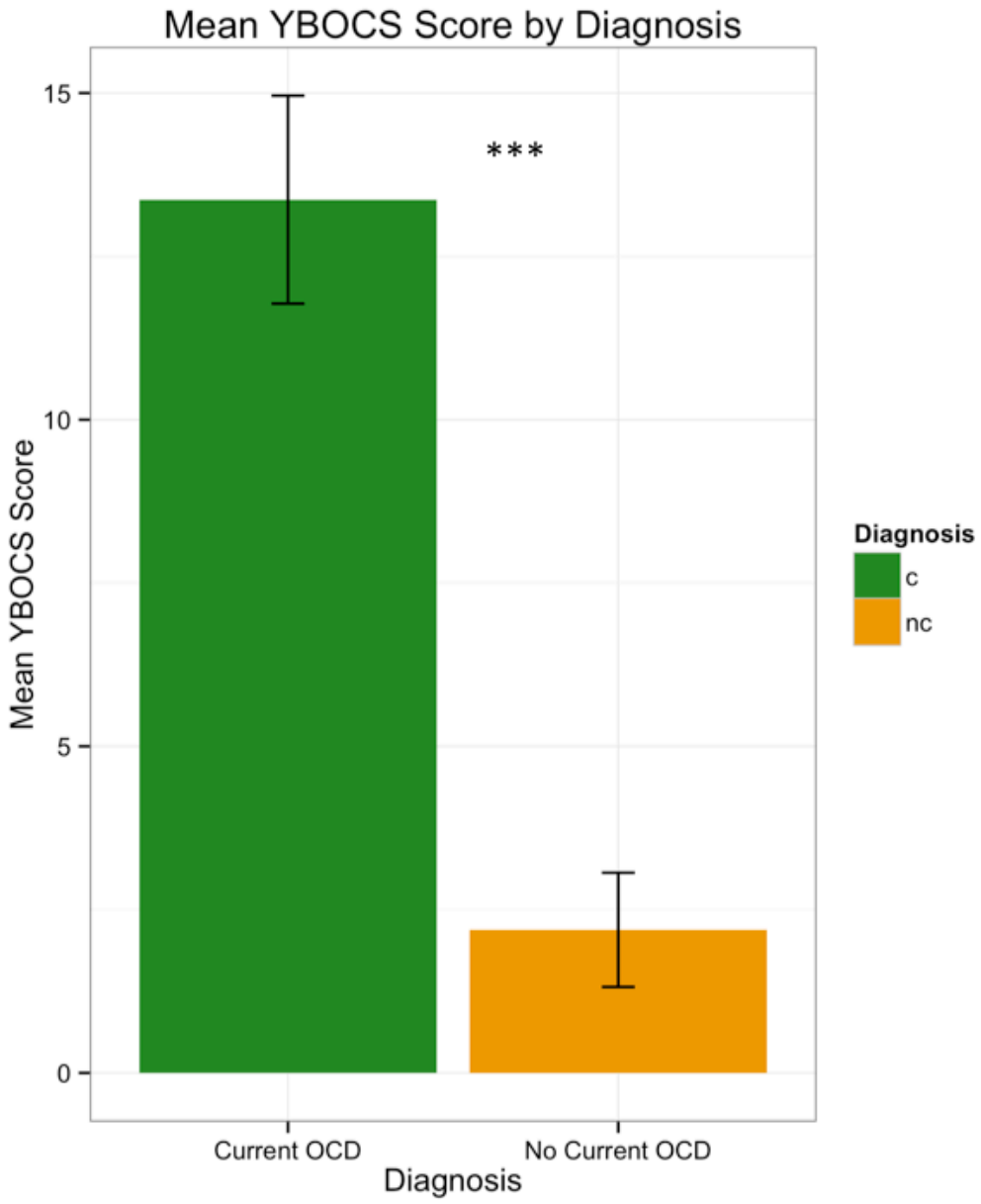

Figure 11. YBOCS scores of participants during pregnancy are not normally distributed. A Mann-Whitney *U* indicates that there is a significant difference of mean POCS scores by diagnosis during pregnancy $p=5.927e^{-6}$ ***

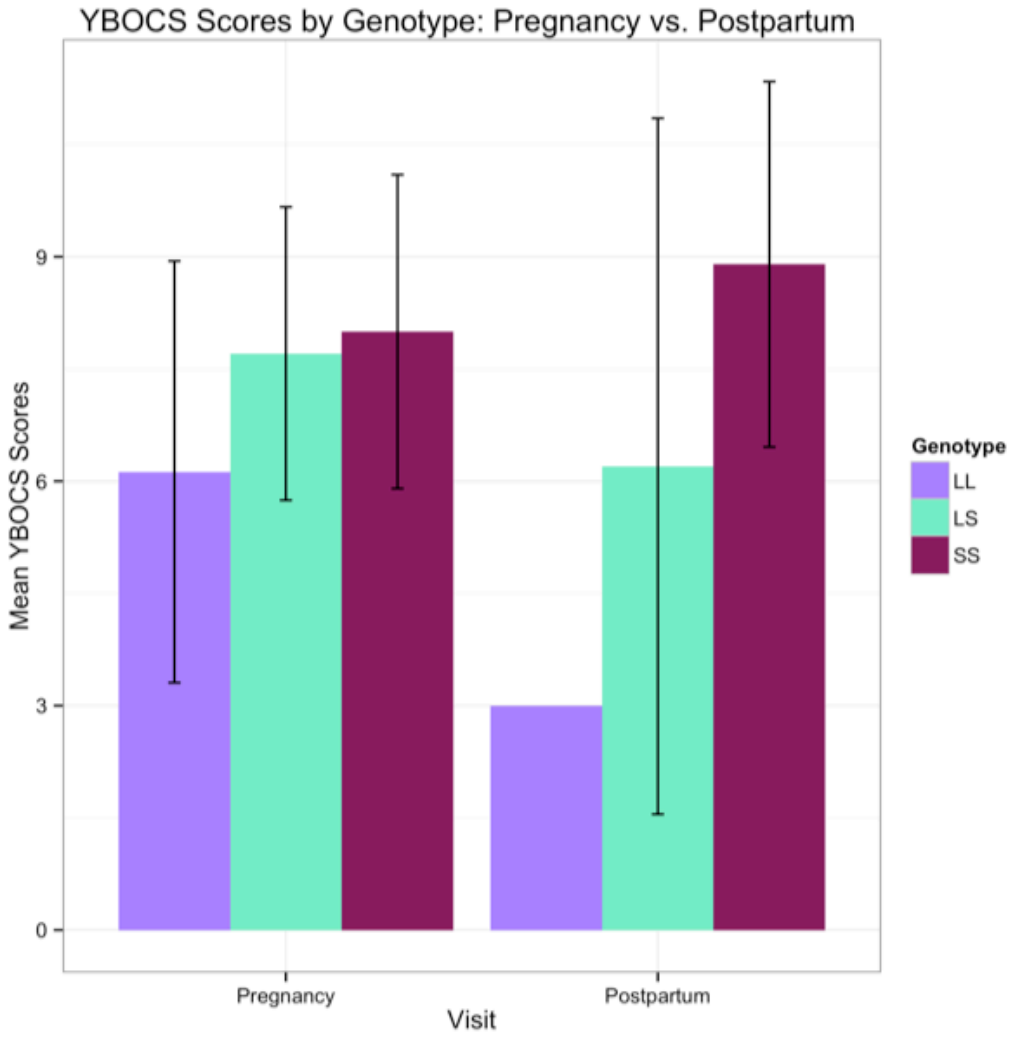

Figure 12. YBOCS scores of participants during pregnancy and postpartum are not normally distributed. A Kruskal-Wallis test indicates that there is not a significant difference of mean YBOCS scores by genotype during pregnancy p=0.7746 and in the postpartum period p=0.6098

3.4 Changes from Pregnancy to Postpartum

Multiple linear regression indicates that changes in POCS scores from pregnancy to the postpartum are predicted by genotype, state anxiety and total childhood maltreatment (R^2=0.5014, p=0.01775), with total childhood maltreatment as a significant predictor (β=-0.02728, t=-2.555, p=0.0267*). Such that individuals with higher total CTQ scores will have higher POCS scores during pregnancy compared to the postpartum period. Furthermore, compared to SS individuals, LS individuals will have higher POCS scores during pregnancy (β=-0.2506, t=-0.954, p=0.3604) and LL individuals will have higher POCS scores during the postpartum period (β=0.0211, t=0.004, p=0.9968). The moderation model explains more variance than the multiple linear regression model as measured by the adjusted R^2 value (R^2=0.3994 versus R^2=0.7783) and the two models are significantly different (p=0.02311*). However, the interaction between genotype and total childhood maltreatment was not significant, indicating that the moderation model is not significant. There was no significant model to predict changes in YBOCS scores.

3.5 Resting State Functional Connectivity

In total, 15 follow-up scans were included in the analysis. One scan was discarded because the file was corrupted. Due to the small number of scans, the uncorrected p-values are reported and do not account for multiple comparisons using the false discovery rate. Thus findings are of an exploratory nature and the findings allude to emerging patterns.

3.5.1 Caudate Nucleus

The right caudate nucleus as a seed point, (figure 13A) has greater connectivity at rest with the left ventral posterior cingulate cortex (β=-0.23, T-value=-2.31, p-unc=0.03811) and the right orbitofrontal cortex (β=-0.20, T-value=-2.20, p-unc=0.04645) in OCD patients compared to healthy controls. The left caudate nucleus as a seed point (figure 13B) has greater connectivity with the right (β=-0.28, T-value=-5.05, p-unc=0.000223) and left (β=-0.22, T-value=-2.35, p-unc=0.03538) orbitofrontal cortex, the right ventral anterior cingulate area (β=-0.27, T-value=-2.27, p-unc=0.04081) and the right pars triangularis (of the inferior frontal gyrus) (β=-0.18, T-value=-2.20, p-unc=0.04643) in OCD patients versus healthy controls.

3.5.2 Insular Cortex

The right insular cortex as a seed point (figure 14A) has lower resting connectivity with both the right (β=0.29, T-value=2.59, p-unc=0.02224) and left (β=0.27, T-value=2.40, p-unc=0.03183) pars opercularis in OCD patients compared to healthy controls. The left insular cortex as a seed point (figure 14B) has lower resting connectivity with connectivity with the right insular cortex (β=0.25, T-value=2.34, p-unc=0.03570), the right dorsal anterior cingulate area (β=0.27, T-value=2.35, p-unc=0.03537) and the right (β=0.29, T-value=2.72, p-unc=0.01739) and left (β=0.28, T-value=2.88, p-unc=0.01294) pars opercularis in OCD patients compared to healthy controls.

3.5.3 Amygdala

The right amygdala as a seed point (figure 15A) has lower resting connectivity with the right gyrus rectus, which is a portion of the frontal lobe medial to the medial orbital gyrus

(β=0.27, T-value=2.40, p-unc=0.03183) and higher resting connectivity with the inferior parietal lobule, in particular the supramarginal gyrus (β=-0.23, T-value=-2.23, p-unc=0.04411) in OCD patients compared to healthy controls. The left amygdala as a seed point (figure 15B) has greater resting connectivity with the right dorsal (β=-0.40, T-value=-3.30, p-unc=0.005729) and right ventral posteriot cingulate area (β=-0.31, T-value=-3,17, p-unc=0.007432) and the right calcarine fissure (β=-0.30, T-value=-2.40, p-unc=0.03187) in OCD patients compared to healthy controls.

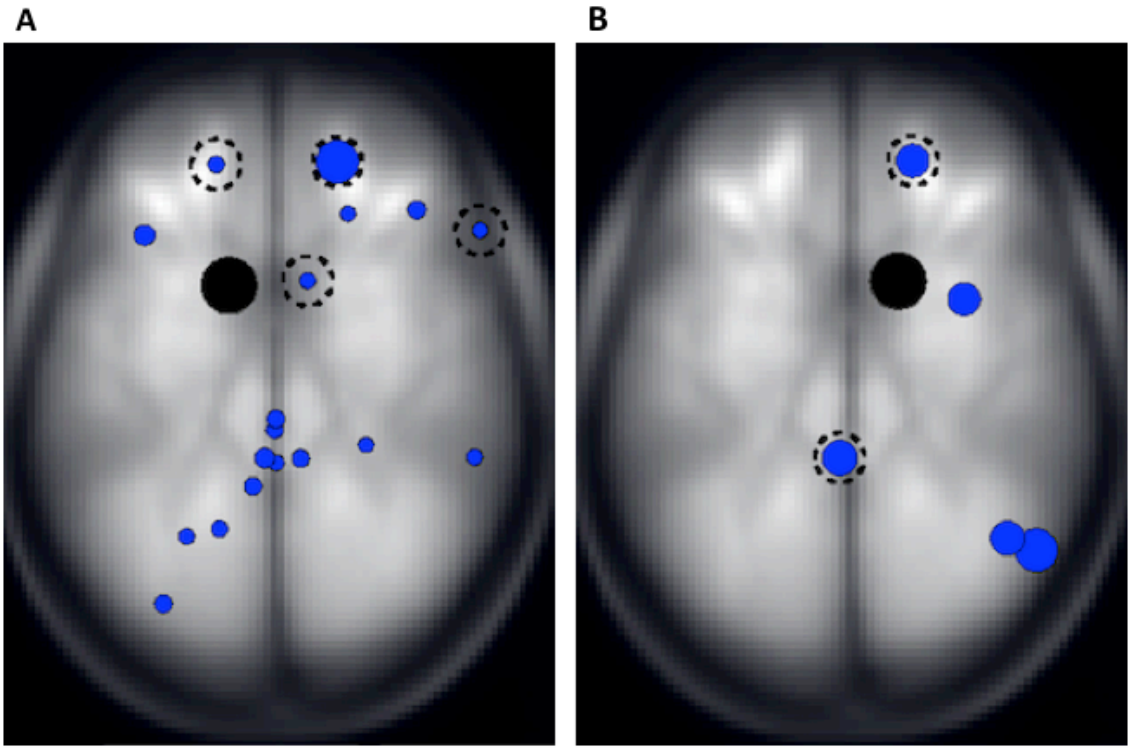

Figure 13. (A) Left caudate nucleus. Has greater connectivity with the right (p=0.000223) and left (p=0.03538) orbitofrontal cortex, right ventral anterior cingulate area (p=0.04081) and the right pars triangularis (p=0.04643) in OCD patients vs. healthy controls **(B)** Right caudate nucleus. Has greater connectivity with the left ventral posterior cingulate cortex (p=0.03811) and the right orbitofrontal cortex (p=0.04645) in OCD patients vs. healthy controls.

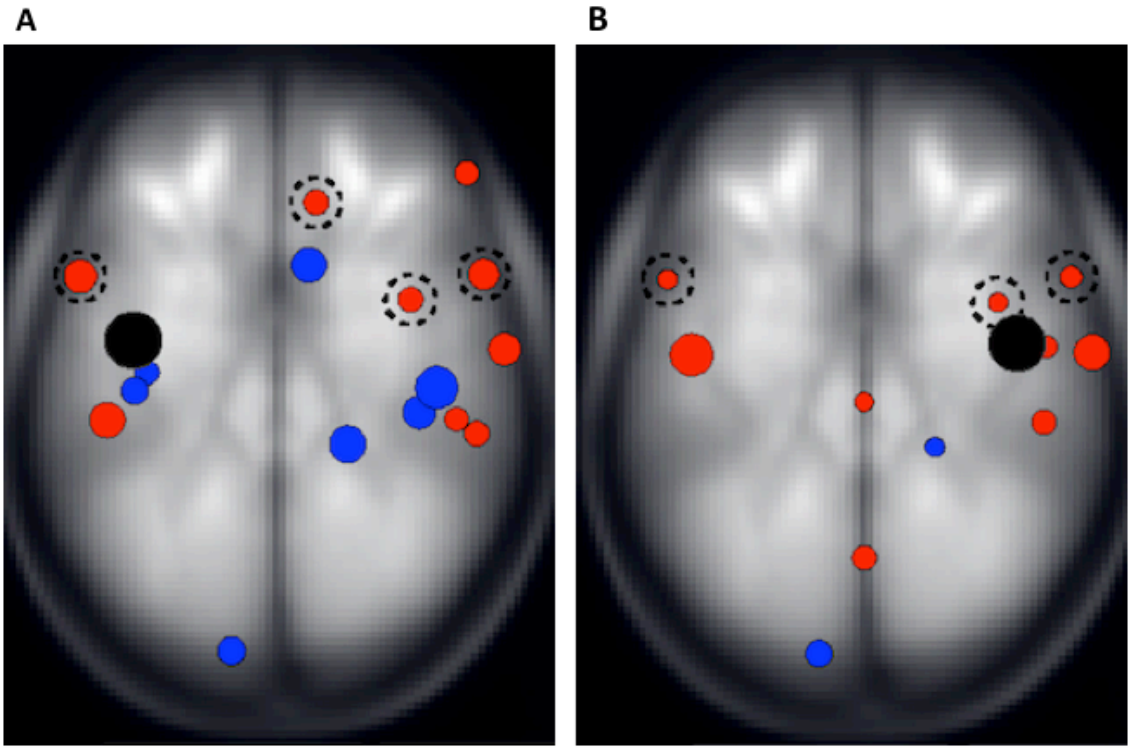

Figure 14. (A) Left insular cortex. Has decreased connectivity with the right insular cortex (p=0.03570), the right dorsal anterior cingulate area (p=0.03537) and the right (p=0.01739) and left (p=0.01294) pars opercularis in OCD patients vs. healthy controls **(B)** Right insular cortex. Has decreased connectivity with both the right (p=0.0224) and left (p=0.03183) pars opercularis in OCD patients vs. healthy controls.

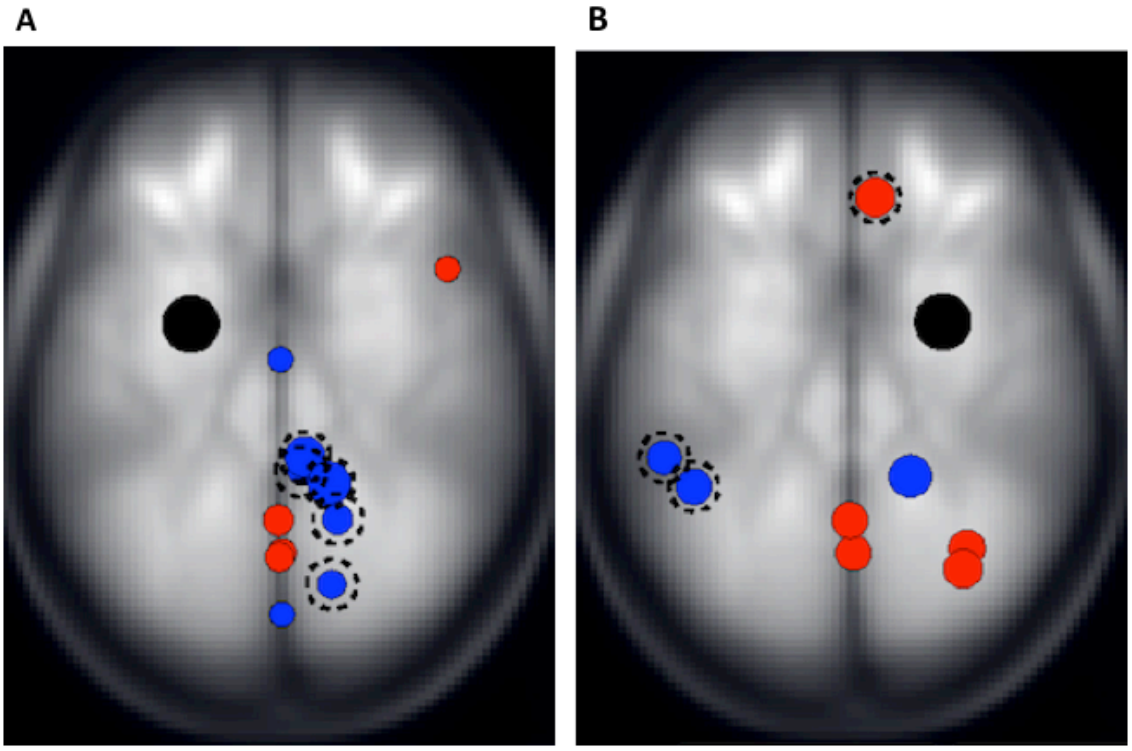

Figure 15. (A) Left amygdala. Has greater connectivity with the right dorsal (p=0.005729) and right ventral cingulate area (p=0.007432) and the right calcarine fissure (p=0.03187) in OCD patients vs. healthy controls **(B)** Right amygdala. Has decreased connectivity with the right gyrus rectus (p=0.03183) and greater connectivity with the supramarginal gyrus (p=0.04411) in OCD patients vs. healthy controls.

3.5.4 Orbitofrontal Cortex

The right orbitofrontal cortex (figure 16A) has greater resting connectivity with both the right (β=-0.20, T-value=-2.20, p-unc=0.04645) and left (β=-0.28, T-value=-5.05, p-unc=0.000223) caudate nucleus. The left orbitofrontal cortex (figure 16B) has greater resting connectivity with the left caudate nucleus (β=-0.22, T-value=-2.35, p-unc=0.03538) in OCD patients compared to healthy controls.

3.5.5 Childhood Emotional Maltreatment as a Covariate

There were no significant group differences between OCD patients and healthy controls when controlling for past history of childhood emotional maltreatment.

3.6 Montreal Imaging Stress Task

The MIST functional and anatomical data were preprocessed using the BrainVoyager Qx version 2.1. Successful preprocessing of the functional data included 3-dimensional head motion correction, linear trend removal, high-pass temporal filtering and spatial smoothing. Anatomic images were successfully transformed in Talairach stereotaxic space and this transformation was coregistered with the aligned functional data. Upon completion of all preprocessing steps, a general linear model must be built to specify conditions (rest, control and experimental) and the blocks of time associated with each condition. However, an issue arose with the experimental2 condition appearing as a control condition in the actual paradigm. The issue was brought up to the MIST's program developer. However, a timely response from the developer has yet to be received

and for the purposes of this thesis the data was not analyzed. The MIST data is considered to be a work-in-progress.

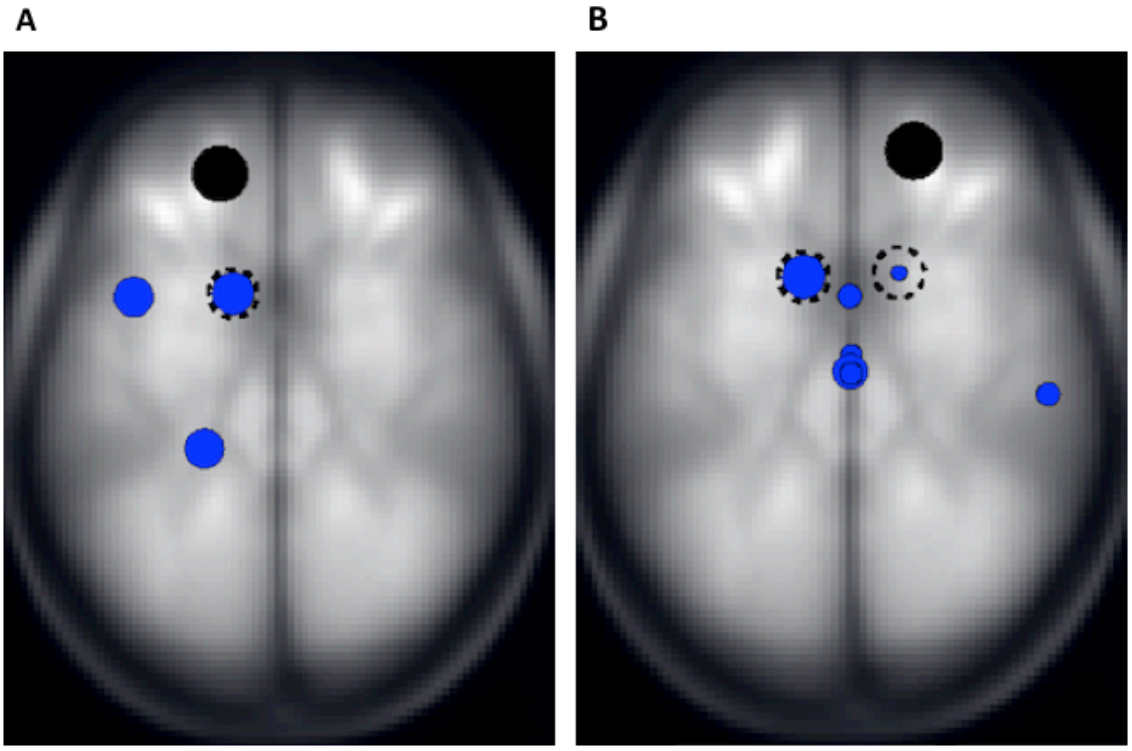

Figure 16. (A) Left orbitofrontal cortex. Has greater connectivity with the left caudate nucleus (p=0.03538) in OCD patients vs. healthy controls **(B)** Right orbitofrontal cortex. Has greater connectivity with both the right (p=0.04645) and left (p=0.000223) caudate nucleus OCD patients vs. healthy controls.

4. DISCUSSION

4.1 Thesis Impact and Summary of Findings

The perinatal period is recognized as a time of increased vulnerability to affective disorders. Traditionally, postpartum depression has been the most heavily researched (Bobo & Yawn, 2014; Marcus, 2008; O'hara & Swain, 1996). In contrast, the prevalence and clinical presentation of anxiety disorders and obsessive-compulsive disorder during pregnancy and the postpartum period have received relatively less research attention (Ross, McLean, & Psych, 2006). Postpartum OCD is often overlooked or inappropriately grouped with postpartum depression (Speisman, Storch, & Abramowitz, 2011). Even though 40% of women with postpartum OCD will have comorbid depression, the presentation of postpartum OCD and postpartum depression differ. For example, women with postpartum OCD have obsessions that trigger themes of catastrophic consequences whereas women with postpartum depression tend to have ruminations with more of a melancholic theme. In addition, obsessions in women with postpartum OCD tend to be bizarre in content but are generally focused/stable whereas women with postpartum depression tend to focus on actual circumstances but ruminations tend to drift from topic to topic (Speisman et al., 2011). Despite the data stressing that women tend to experience onset or exacerbation of OCD during reproductive milestones (Guglielmi et al., 2014), the presentation of OCD in females being unique (Labad et al., 2008; 2009) and the prevalence of perinatal OCD being 1.5-2x greater than the general population (Russell et al., 2012; Uguz et al., 2007b), very little research has been conducted to investigate potential risk factors, characterizing the presentation of perinatal OCD and testing

treatment strategies. It is adaptive for new mothers to develop fixations and manifest preoccupations about the newborn's well-being but when processes controlling this become deregulated and obsessions develop, the process becomes maladaptive. Therefore it is of great interest to understand why some women in the perinatal period develop OCD and others do not. This is the first study to investigate a genetic susceptibility and exploration of resting-state functional connectivity differences in women with perinatal obsessive-compulsive symptoms.

4.1.1 Predictors of Obsessive-Compulsive Symptom Severity

When conducting a stepwise multiple linear regression, it was shown that MADRS scores were not a significant predictor or involved as predictor in the most significant model. These findings do align with past work showing that co-morbid depression predicts poor quality of life but does not necessarily predict obsessive-compulsive symptom severity (Masellis, Rector, & Richter, 2003). During pregnancy, POCS scores are predicted by 5-HTTLPR genotype, total childhood maltreatment and trait anxiety, with total childhood maltreatment and trait anxiety as significant predictors. In comparison, YBOCS scores are predicted by 5-HTTLPR genotype, childhood emotional neglect and trait anxiety, with trait anxiety as a significant predictor. In the postpartum period, POCS scores are significantly predicted by both total childhood emotional maltreatment and poor sleep quality. Postpartum YBOCS scores are predicted 5-HTTLPR genotype, total childhood maltreatment and trait anxiety, with trait anxiety as the significant predictor. Further, we were interested to see what would predict changes of both YBOCS scores and POCS scores from pregnancy to the postpartum period. Changes in POCS scores from

pregnancy to the postpartum period are predicted by 5-HTTLPR genotype, state anxiety and total childhood maltreatment, such that individuals with higher total CTQ scores have greater POCS scores during pregnancy versus the postpartum period. No model was able to significantly predict changes in YBOCS scores in the perinatal period. Further, in all five regression models, the 5-HTTLPR genotype was not a significant moderator variable.

OCD and the search for candidate genes have been the topic of numerous studies. Findings have been inconsistent, which may be due to varying samples and methodology. It is important to note that a heterogeneous disorder such as OCD cannot fully be explained by a simple one-gene cause and effect model. This lends support to not only a polygenic model, but also to the possible role of environment-gene interactions.

Early life stress and in particular childhood maltreatment has been extensively researched as a risk factor for later development of psychiatric disorders (Avshalom Caspi et al., 2003; Cromer, Schmidt, & Murphy, 2007; McKeon, Roa, & Mann, 1984; Murphy et al., 2013). However, the gene-environment relationships in this population have not been thoroughly addressed. Environmental factors such as childhood maltreatment or general life stress may be a risk factor for the later development of OCD. Evidence currently indicates that childhood trauma plays a significant role in the development of maladaptive obsessive-compulsive symptoms later in life (Mathews et al., 2008). In particular, not only are OCD patients are more likely to experience greater childhood trauma but specifically they are more likely to experience childhood emotional neglect compared to healthy controls (Lochner et al., 2002).

OCD is no longer classified as an anxiety disorder in the DSM-V (APA 2013). However, both trait and state anxiety are significant predictors of obsessive-compulsive symptoms. Interestingly, it has been found that subjective anxiety questionnaire scores are correlated with increased activity in various neural systems seen in OCD (Leckman, Bloch, & King, 2009; Mataix-Cols & Wooderson, 2004). Further, it has been found that certain temperamental traits measured by the STAI such as internalizing problems and negative emotionality are significantly associated with OCD patients versus healthy controls (Grisham et al., 2011). Therefore despite OCD's new classification, past evidence supports anxiety temperamental traits as a predictor for obsessive-compulsive symptoms. Sleep disturbance and poor sleep quality is common among mental disorders, occurring on average in 12% of individuals (Cui Lijun, 2012). Further, it has been found that 38% of patients suffering from OCD have poor sleep quality as measured by the PSQI (Cui Lijun, 2012; Ramsawh, Stein, Belik, Jacobi, & Sareen, 2009). Therefore, the results from the postpartum period that overall sleep quality is a significant predictor of POCS scores is in line with previous work.

Despite no significant group differences of POCS or YBOCS scores by genotype, the 5-HTTLPR genotype was often included as a group predictor in the above significant models following a stepwise regression. The SS genotype was always set as the factor in the genotype group predictor that LS and LL genotypes were compared to in order to generate the standardized beta-values. During pregnancy, compared to LL individuals, carriers of the S-allele will have higher POCS and YBOCS scores. These findings are in line with past research indicating that S-allele carriers will have increased anxiety related

temperamental traits (Schinka et al., 2004) and women with the S-allele report greater obsessive-compulsive symptoms (Maurex, Zaboli, Öhman, Åsberg, & Leopardi, 2010). It should be cautioned that there is only one LL individual who has been followed-up with in the postpartum period, thus findings from the second visit should be seen as exploratory.

4.1.2 Resting-State Functional Connectivity

There are certain core brain structures that are classically involved with obsessive-compulsive disorder. Increased metabolic activity in the orbitofrontal cortex and caudate (Mataix-Cols & Wooderson, 2004; Rasmussen et al., 2013) and increased functional connectivity between the two structures (Harrison et al., 2009) in OCD patients has been one of the most replicable findings in OCD neuroimaging. Further, exposure to adverse childhood experiences has been shown to increase the grey matter volume of the caudate in OCD patients (Benedetti et al., 2012). Areas such as the insula have been associated with reduced serotonin transporter binding in OCD patients (Matsumoto et al., 2010) and greater activation in the right insula is associated with washing/contamination-centered symptoms (Shapira, Liu, He, Bradley, & Lessig, 2003) which females are twice as likely to have (Labad et al., 2009). The insula is implicated in emotion perception and processing of personally rewarding stimuli (Enzi, De Greck, Prösch, & Tempelmann, 2009). The amygdala has dense projections to the posterior orbitofrontal cortex in pathways that are critical for processing emotional content (Timbie & Barbas, 2014). In particular, the amygdala is hyperactive during OCD symptom provocation but this pattern is not seen when individuals are distracted (Simon, Adler, Kaufmann, & Kathmann,

2014). Further, in the functional neuroanatomy of OCD, a critical role has been assigned to the orbitofrontal cortex which is a brain region crucially involved in reward-guided learning and decision-making (Beucke, Sepulcre, & Talukdar, 2013a). The 5-HTTLPR polymorphism has been shown to determine both the size and functional connectivity between the amygdala and frontal cortical circuits in OCD. Moreover, the S-allele is associated with reduced gray matter volume in limbic regions and is associated with disrupted amygdala-cingulate coupling following emotional stimuli (Heinz, Braus, Smolka, Wrase, & Puls, 2005; Pezawas et al., 2005). The anterior cingulate cortex in particular has been postulated to have a role in regulating emotional behaviour and in treatment resistant OCD, bilateral cingulotomy have been efficacious (Gasquoine, 2013). The right caudate nucleus has greater resting connectivity with the left ventral posterior cingulate cortex and the right orbitofrontal cortex in OCD patients. The left caudate nucleus has greater resting connectivity in OCD patients with the right and left orbitofrontal cortex, the right ventral anterior cingulate area and the right pars triangularis. The pars triangularis along with brodmann area 44 (pars opercularis) comprise Broca's area. The greater connectivity between both the right and left caudate with the orbitofrontal cortex replicates past findings in the OCD literature (Beucke et al., 2013b). Past research has shown that the strength of the connectivity between the ventral caudate and orbitofrontal cortex predicts YBOCS scores, indicating a direct relationship to illness severity (Harrison et al., 2009). However, this study is the first to show this increased connectivity in postpartum women with OCD. A greater sample size is required to investigate if the connectivity also predicts obsessive-compulsive symptom severity in

this population. The posterior cingulate cortex is a component of the default mode network (Greicius et al., 2003). The PCC has been hypothesized to have a regulatory role in focusing internal and external attention. Specifically, abnormalities with the PCC are often associated with cognitive impairments including memory function, attention and problems maintaining balance between internal and external thought – like integrating memories of experience and initiating a signal to change behavioural strategies (Leech & Sharp, 2014). The significantly higher connectivity between the right caudate and left ventral PCC in OCD subjects may reflect a discrepancy in reward-processing networks in these subjects and could be involved with increased motivation. Interestingly, these findings are in direct contrast to the depression literature that finds a decreased connectivity between the structures, which is hypothesized to be involved with the presenting anhedonia in depressed patients (Bluhm et al., 2009). It has been seen that for OCD patients, there is a significant decrease in glutamate in the anterior cingulate (Pittenger, Bloch, Wegner, & Teitelbaum, 2006), with anterior cingulate regions hypothesized to play an important role in producing obsessive-compulsive symptoms due to its role in impulse control (C. M. Adler et al., 2000; Szeszko, Robinson, & Alvir, 1999).

Both the right and left insular cortex have lower connectivity with the pars opercularis in OCD patients. Further, compared to healthy controls there is lower resting connectivity between the left and right insular cortex in OCD patients.

The right amygdala has lower resting connectivity with the right gyrus rectus and higher connectivity with the supramaginal gyrus (parietal lobe) in OCD patients. The left

amygdala, which is larger in females (Cahill, 2006), has greater connectivity at rest with the calcarine fissure and the right dorsal posterior and ventral posterior cingulate area in OCD patients. The calcarine fissure is where the primary visual cortex is concentrated. However, it has been seen that there is a decrease in activation between the amygdala and visual cortex in OCD patients during an emotional face-matching task (Via et al., 2014). It is interesting that the relationship between the amygdala and visual cortex differs between rest and during a task.

The preliminary findings from the resting state functional connectivity analysis show promising findings that clearly distinguish healthy controls and OCD patients in the postpartum period. Future tests should remain significant after controlling for multiple comparisons via the p-false discovery rate. Further, it will be of interest to compare the differing brain regions involved during the resting state and stress task between healthy controls and OCD patients.

4.2 Limitations and Future Directions

4.2.1 Sample Size and Characteristics

There are many limitations to the current study and many stem from the relatively small sample size. Primarily, the non-significant group differences by genotype may be accounted for by the small amount of participants in each genotype. An ANOVA power analysis with a medium effect size and 95% significance level indicates that the study as 16% power. In order to achieve 80% power, each genotype must have a minimum of 53 participants. Once achieved, significant group differences in POCS and YBOCS scores

by genotype at the two time points may arise. This would allow each individual genotype to be used as a unique predictor and/or moderator variable in future analysis. Further, there were only eight healthy controls in the study. Recruitment of participants that fit the very strict criteria for healthy controls was a challenge. However, an independent t-test power analysis with a large effect size does indicate 51% power. Therefore, despite the small sample size, findings from this study do provide reason for further exploration. Generalizability of our findings is limited to all ethnicities since 95% of our sample is of Caucasian descent. Future work should continue to collect demographic data such as ethnicity of their sample. Since it is known that allele frequency varies by ethnic group (Hu et al., 2006); it would be interesting to see if our findings hold true for different populations.

In this study, we did not exclude OCD participants if they were on medication. Moreover, if a participant was diagnosed with OCD as a part of the study, they were offered treatment through the clinic as per ethics guidelines. However, due to the small sample size, we were not able to group participants based on medication use or control for medication use. Medication has been shown to alter connectivity in OCD patients within the DMN with greater connectivity in ventral regions compared to unmedicated patients (Harrison et al., 2009; Jang et al., 2010). Thus, future work will need to take this factor into consideration.

Factors such as smoking and menstrual cycle are factors that influence BOLD signals. Tobacco smoking is fairly common and subjects who smoke may have appreciable levels of nicotine in their plasma and brain during screening. There are two primary concerns.

First, the concern is that the vascular effects of nicotine may alter the coupling between BOLD signals and neuronal activity. This is supported by both animal and human studies showing that acute administration of nicotine increases cerebral blood flow and decreases cerebral vascular resistance (G. H. Hall, 1972; Uchida, Kagitani, Nakayama, & Sato, 1997). In contrast, it has been shown that nicotine does not affect BOLD signal response to photic stimulation suggesting that nicotine does not alter coupling between BOLD signal and neuronal activity in the visual cortex. However, changes in BOLD signal may be attributed to direct neuronal effects of nicotine rather than its effect on cerebral vasculature (Jacobsen et al., 2002). Further, it has been found that nicotine improves attentional performance by downregulating resting brain function, such as the DMN in response to task-related cues. It has been postulated that is via a nicotine-induced potentiation of the alerting properties of external stimuli (Hahn et al., 2007). Therefore, future work must take into account smoking status of their subjects both during resting state and task analysis such as the MIST. The menstrual cycle has also been shown to influence brain activity. For example, there is a significantly greater BOLD signal in areas such as paraventricular nucleus, amygdala, anterior cingulate and OFC during the early follicular phase (Goldstein et al., 2005). Moreover, women who are anticipating a reward will have greater activation in the orbitofrontal cortex and amygdala during the mid-follicular phase in comparison to when they are in their luteal phase (Dreher et al., 2007).

Genotype significantly differed from Hardy-Weinberg Equilibrium and this may be due to genotyping error, small sample size and/or large proportion of a clinical population in sample.

4.2.2 Predictors and Characteristics of Perinatal Obsessive-Compulsive Symptoms

Perinatal onset and exacerbation of obsessive-compulsive disorder is a relatively new topic of research. The findings from the study do add to the existing preliminary findings in this new field. Due to the constraints of the study, only the 5-HTTLPR polymorphism association with obsessive-compulsive symptoms was analyzed. Polymorphisms involved in the glutamatergic system are also emerging candidates in the OCD literature and would be of interest in future studies. Resting state functional connectivity is different between OCD patients and healthy controls. However, we were not able to analyze if differences in connectivity predicted severity of obsessive-compulsive symptoms. Further, we were not able to analyze if genotype moderated the connectivity strength between brain regions. Larger sample sizes would facilitate these analyses.

4.2.3 Distinct Subtypes

Past work has demonstrated that age-of-onset may be a defining OCD subtype (Taylor, 2011). Further, evidence does support that males tend to experience earlier onset of OCD compared to females (Mathis et al., 2011; Wang et al., 2012). Taken together, sex and age-of-onset should be taken into account as subtypes that influence the presentation of obsessive-compulsive symptoms. It has also been shown that both onset and exacerbation of OCD occur during female reproductive milestones such as menarche, pregnancy, postpartum and menopause (Guglielmi et al., 2014). We did not take into account if the

current OCD diagnosis in our participants was a new onset or exacerbation of an earlier onset. It would be interesting to see if perinatal onset versus exacerbation presents differently.

4.3.4 Future Areas of Research

Due to unforeseen circumstances, we were not able to complete the analysis of the data from the MIST. Past work has shown that women with postpartum OCD in response to a stress task will have activation in the OFC, DLPFC, mPFC, and insular regions (Lord et al., 2012). It would of great interest to see if these results could be replicated and if the 5-HTTLPR polymorphism moderates any of these activation patterns.

Glutamate is the primary excitatory neurotransmitter in the adult brain. Certain overactive brain regions have been seen in OCD thus glutamatergic dysfunction in OCD has become an emerging field of interest. It has been found that glutamate levels in CSF of OCD patients is significantly higher than healthy controls (Chakrabarty, Bhattacharyya, Christopher, & Khanna, 2005). In addition, there has been substantial work researching the association of the glutamate transporter gene *SLC1A1* with OCD. This association has been investigated in 9 studies with the majority confirming the association (Pittenger, Bloch, & Williams, 2011). Interestingly three studies report a sex difference with the association only seen with male OCD probands (Arnold et al., 2004; Dickel et al., 2007; Stewart et al., 2007). In contrast, the ACC is often reported to be hyperactive in OCD, however a reduction in ACC glutamate is correlated with symptom severity in female OCD patients only (Yücel et al., 2008). No work to date has looked at the *SLC1A1* genotype and perinatal OCD. Further, glutmate-modulating drugs such as riluzole have

been shown to be beneficial in treating refractory OCD (Coric et al., 2003). Riluzole acts by inhibiting certain voltage-gated sodium channels which attenuates action potential near the axon terminal thus reducing transmitter release (Prakriya & Mennerick, 2000). Moreover, it has been shown to potentiate the reuptake of extrasynaptic glutamate by glial cells (Fumagalli, Funicello, Rauen, Gobbi, & Mennini, 2008). The role of glutamate concentrations and glutamatergic polymorphisms on perinatal OCD is a promising new area of research that needs to be explored.

Diffusion tensor imaging (DTI) measures the diffusion of water molecules. The extent and direction the water diffuses is dependent on the medium that it occurs. Diffusion can be isotropic where there are no cellular structures to restrict diffusion such as in cerebrospinal fluid or structures that restrict diffusion constantly such as in grey matter. In contrast, tissues such as white matter tracts are highly directional and diffusion will thus be anisotropic. Therefore DTI data calculates fractional anisotropy (FA), which describes the extent to which anisotropy occurs within certain voxels (Chamberlain & Menzies, 2009). Past work has shown a region of right parietal matter where FA was significantly reduced in both OCD patients and their relatives compared to healthy controls and region of right medial frontal matter where FA was significantly increased in both OCD patients and their relatives compared to healthy controls (Menzies, Chamberlain, & Laird, 2008). These findings provide preliminary evidence that familial and perhaps genetic risk for OCD is mediated by large-scale comprised white matter integrity. Future MRI work should include DTI as a part of their analysis arsenal.

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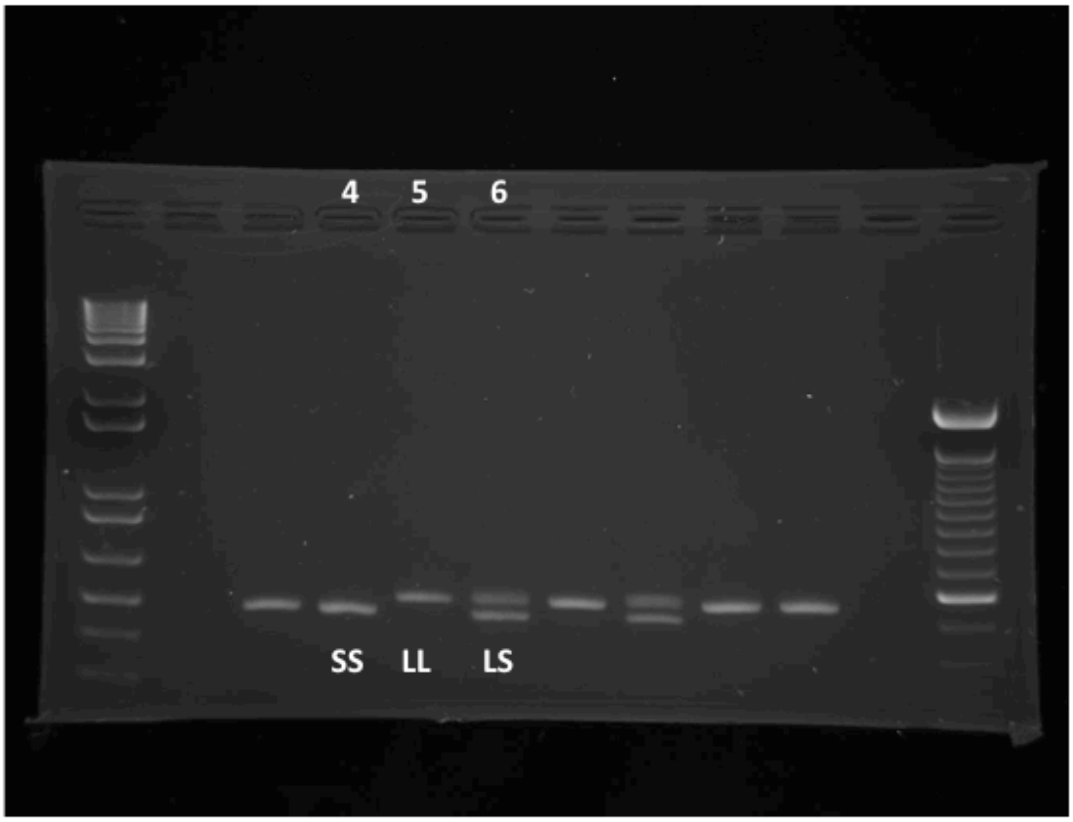
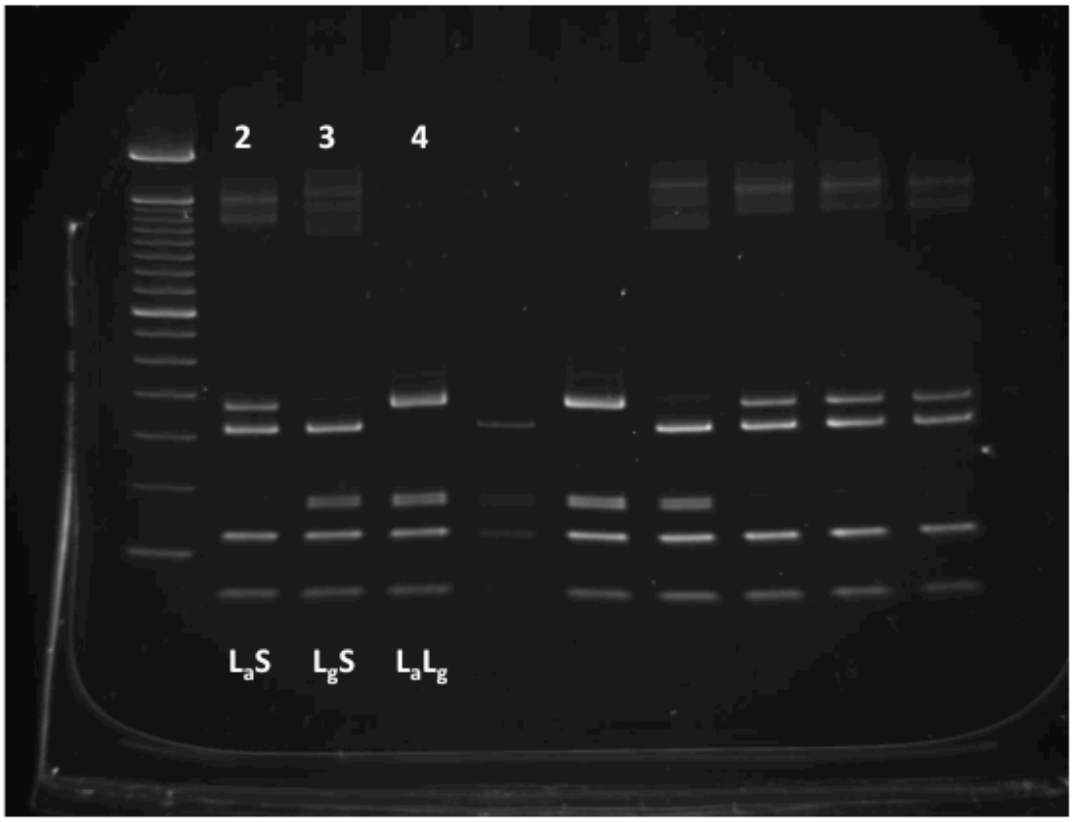
Appendix 1



Appendix 1. PCR (polymerase chain reaction) products run a 1.5% agarose gel at 50V for 90 minutes. Well 4 contains the genotype SS with both alleles at the 484 bp mark. Well 5 contains the genotype LL with both alleles at the 528 bp mark. Well 6 contains the genotype LS with one allele at the 484 bp mark and one allele at the 528 bp mark.

Appendix 2



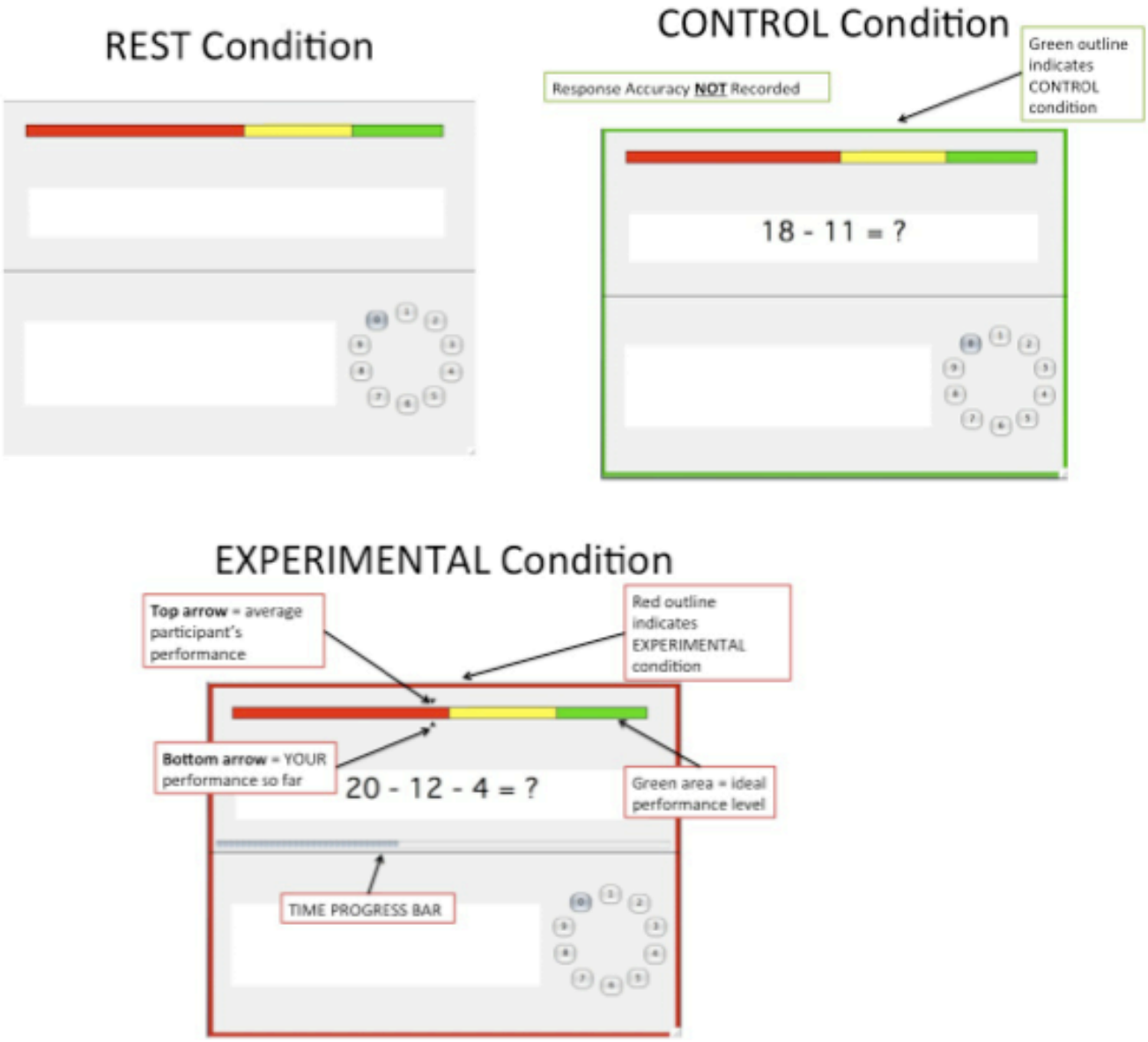
Appendix 2. Restriction digest products run a 4-20% polyacramide gel at14 mA for 60 minutes, followed by a 3 hour stain in a SyberSafe solution. Well 2 contains the genotype L_aS (originally LS). Well 3 contains the genotype L_gS (originally LS). Well 4 contains the genotype L_aL_g (originally LL).

L_a = 340 bp, S = 297 bp, L_g = 166 + 174 bp

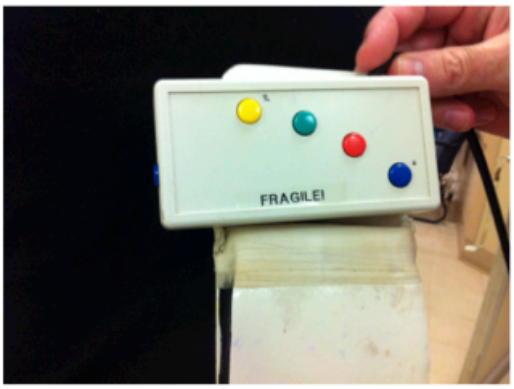
Appendix 3

Appendix 3. Fixation cross that participants would look at during the resting state.

Appendix 4


REST Condition
CONTROL Condition
Response Accuracy NOT Recorded
Green outline indicates CONTROL condition
18 - 11 = ?
EXPERIMENTAL Condition
Top arrow = average participant's performance
Red outline indicates EXPERIMENTAL condition
Bottom arrow = YOUR performance so far
20 - 12 - 4 = ?
Green area = ideal performance level
TIME PROGRESS BAR

Appendix 5



Yellow = moves cursor to the left (right index finger)
Green = moves cursor to the right (right middle finger)

Blue button on the side confirms response

Image of how the right hand should be placed on the response pad