The Neurostructural Phenotypes of CACNA1C rs1006737 in Adolescents with Bipolar Disorder and Healthy Controls of Caucasian Race

by

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Institute of Medical Science

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Abstract:
Background: CACNA1C rs1006737 has been implicated in structural brain differences in adults with bipolar disorder (BD) and/or healthy controls (HCs). No prior study has examined associations between rs1006737 and brain structure in adolescents.

Methods: Seventy-one adolescents (14-20 years; 38BD, 33HC) underwent 3-Tesla Magnetic Resonance Imaging (MRI). ROI and whole-brain vertex-wise analyses examined cortical and subcortical volume, surface area (SA), and/or thickness. General linear models included main effects of diagnosis and rs1006737, and an interaction term, controlling for age, sex, and total intracranial volume.

Results: Vertex-wise analysis found significant diagnosis-by-rs1006737 interactions for prefrontal and occipital brain structure. Main effects of rs1006737 were found on anterior cingulate cortex SA from ROI analysis, and occipital SA from vertex-wise analysis.

Conclusion: The current study identified neurostructural intermediate phenotypes relevant to the impact of CACNA1C rs1006737 on adolescent BD. Further investigation is warranted into the relevance of rs1006737 associations with BD-specific elevations in regional SA.
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**Abbreviations:**

3T- 3 Tesla  
ACC- Anterior Cingulate Cortex  
ADHD- Attention Deficit Hyperactivity Disorder  
ANCOVA- Analysis of Covariance  
BD- Bipolar Disorder  
BD-I- Bipolar Disorder Type I  
BD-II- Bipolar Disorder Type II  
BD-NOS- Bipolar Disorder Not Otherwise Specified  
BMI- Body Mass Index  
C-GAS- Children’s Global Assessment Scale  
CACNA1C- calcium channel, voltage dependent L-type alpha-1C subunit  
DODS- Different Offset, Different Slope  
DOSS- Different Offset, Same Slope  
DSM- Diagnostic and Statistical Manual of Mental Disorders  
FDR- False Discover Rate  
GLM- General Linear Model  
GM- Grey Matter  
GWAS- Genome-wide Association Study  
GUI- Graphical User Interface  
HC- Healthy Control  
ICV- Intracranial Volume  
K-SADS-PL- Kiddie-Schedule for Affective Disorder and Schizophrenia- Present and Lifetime Version  
LH- Left Hemisphere  
LOC- Lateral Occipital Cortex  
RH- Right Hemisphere  
MRI- Magnetic Resonance Imaging  
ROI- Region of Interest
SNP- Single Nucleotide Polymorphism
SSRI- Selective Serotonin Reuptake Inhibitor
SUD- Substance Use Disorder
vlPFC- Ventrolateral Prefrontal Cortex
vmPFC- Ventromedial Prefrontal Cortex
WM- White Matter
1.0 Review of Literature

1.1 BD in Adults and Adolescents

Bipolar Disorder (BD) is a severely impairing chronic psychiatric condition characterized by fluctuating episodes of depression and/or mania/hypomania, interspersed with periods of euthymia. BD is thought to be one of the most debilitating psychiatric conditions, having a profound impact on the lives of patients as well as their caregivers (Jann, 2014; Peele et al., 2004; Freeman et al., 2009). This condition is thought to affect between 2-5% of people worldwide (Jann, 2014; Kessler et al., 2009). BD typically manifests in adolescence or early adulthood, having life-long adverse effects on many parts of a patient’s life, including mental and physical well-being, occupation, recreation, education, interpersonal relationships, and daily functioning (McCormick et al., 2015). BD is thought to be one of the most economically costly psychiatric conditions, with an economic burden estimated to total about $120 billion USD in the United States for 2009 (Jann, 2014). Economic costs of BD can be attributed to numerous interrelating factors, including treatment costs, attenuated employment, lost productivity, and reduced functioning (McCormick et al., 2015). Due to its heavy social and economic burdens, BD is a heavily researched field of study.

1.1.1 Diagnosis and Subtypes
BD is characterized by fluctuating mood states of depression, mania/hypomania, and relatively normal mood (but often not completely symptom free) (Figure 1; Vieta and Goikolea, 2005). Formal definitions of manic and depressive episodes can be found in the *Diagnostics and Statistical Manual of Mental Disorder, Fourth Edition (DSM-IV)* (American Psychiatric Association, 2013).

A manic episode is described as a period of abnormal chronically elevated and/or irritable mood, lasting at least one week. A manic episode must also include three or more of the following symptoms: low self-esteem, decreased sleep, pressured speech, racing thoughts, activity at heightened levels, goal agitation, and/or risky behaviors. The symptoms of a manic episode cause a significant impairment in daily functioning. A hypomanic episode is defined by a period of abnormally and persistently elevated and/or irritable mood and increased energy and activity for at least four days. In a hypomanic episode, symptoms are not severe enough to lead to significant
impairment in daily functioning. A depressive episode is defined by five or more of the following symptoms over a period of two weeks: decreased mood, anhedonia, significant weight changes, sleep disturbances, agitation, fatigue, feelings of worthlessness, inability to focus, and/or suicidal thoughts. A depressive episode must also cause significant distress and/or impairment in daily functioning. There are two main types of BD subtypes: BD-I and BD-II (McCormick et al., 2015).

BD-I diagnosis requires the patient to have had at least one episode of mania. Episodes of depression are also commonly found in BD-I, but are not required for diagnosis. Without intervening treatment, periods of mania and depression often repeat over time, with time spent in depressive episodes outnumbering time spent in manic episodes, at a ratio of about 3:1 (Price and Marzani-Nissen, 2012).

BD-II diagnosis cannot occur if there is the presence of even a single manic episode. It is characterized by episodes of hypomania and depression. Due to the fact that episodes of hypomania may appear as periods of normal functioning or regular happiness, BD-II is commonly misdiagnosed as unipolar depression (Price and Marzani-Nissen, 2012).

1.1.2 Epidemiology

BD has been found to have a global population prevalence of about 1%, with about 5% being affected by bipolar spectrum conditions. Studies have found differences in the lifetime prevalence rates for BD-I (1%), BD-II (1.1%) and sub threshold BD (2.44%). BD-I has been found to affect women and men equally, while BD-II has been found to be more common in women than men. There is no clear indication of an association between BD diagnosis and ethnicity, socioeconomic status, and living environment (urban vs. rural). However, BD has been found to be more common in unmarried individuals (Ayano, 2016).
The average onset of BD-I is about 18 years of age, while the average onset of BD-II is 22 years of age. It has been found that, on average, people do not seek help in treating BD-related symptoms for about five years, and do not receive BD diagnosis until about 8 years after initially seeking treatment. BD is commonly found in primary care environments. Between 21-26 percent of patients being treated for unipolar depression or an anxiety disorder will meet the diagnostic criteria for BD (Ayano, 2016; Hilty et al., 2006).

Untreated BD has been associated with significantly higher chances of death from cardiovascular disorders. In addition, suicide rates are about 20 fold higher in the BD population when compared to the general population. BD has been found to have the highest rates of suicide attempts for any psychiatric disorder, with about one-third of BD patients attempting suicide (Price and Marzani-Nissen, 2012).

1.1.3 Co-morbidities

BD patients are often found to have other accompanying co-morbid medical conditions, with about 66 percent of patients having at least one other psychiatric diagnosis, and about 60 percent having at least 2 other non-psychiatric medical conditions (Jann, 2014). The patient burden of BD is often increased by comorbid conditions, often as a result of increased symptom burden, worse outcome, diminished treatment response, longer duration of illness, increased costs, increased requirements for medical assistance, shorter periods of euthymia, and related factors such as poor treatment compliance and increased suicidal ideation. The most common BD co-morbid conditions are anxiety, attention hyperactivity deficit disorder (ADHD), substance use disorder, and general non-psychiatric medical conditions (Jann, 2014; Hilty et al., 2006; McCormick et al., 2015; Birmaher et al., 2006; Klassen et al., 2010).
Anxiety disorders, including generalized anxiety, social anxiety, post-traumatic stress, and obsessive compulsive disorder, have been found to worsen the psychiatric outcome of BD patients. Treating BD and anxiety concurrently can pose challenges, as medications that are typically used to treat anxiety confer risk for precipitating mania in BD (Yatham et al., 2013).

ADHD is very commonly found in patients with BD. In addition to comorbidity there is a challenge of differential diagnosis, particularly in young persons, given that they both share many symptoms, including poor concentration, distractibility, impulsivity, restlessness, and agitation. Therefore, it is important to pay special attention to the presence of BD-specific symptoms, such as elevated mood, grandiosity, increased sexuality, and lower requirements for sleep, when concurrently treating patients who suffer from both BD and ADHD. Avoiding certain drugs, such as stimulants, which can precipitate mania, is paramount to effectively treating ADHD in BD patients (Klassen et al., 2010).

Substance use has been found to have about 60 percent co-morbidity with BD-I, and 48 percent co-morbidity with BD-II, with a lifetime prevalence of over 90 percent. The substances most frequently abused by BD patients are alcohol and marijuana, with alcohol abuse being higher in female BD patients. Diagnosis of BD becomes difficult when the patient already suffers from substance use disorder because the short-term and long-term drug effects can often mimic BD symptoms. Successful treatment of BD with substance use co-morbidity often requires the involvement of the patient in drug rehabilitation, such as the use of psychosocial rehabilitation, cognitive-behavioral therapy, and/or group therapy (Hilty et al., 2006; Najt et al., 2011).

General medical BD co-morbidities often include conditions such as diabetes, cardiovascular diseases, obesity, migraines, hepatitis C virus (HCV) infection, and obstructive
sleep apnea. Various reasons may potentially account for BD medical co-morbidities, including shared genetic predisposition, substance use co-morbidity, and/or detrimental effects of treatment (Jann, 2014).

1.1.4 Treatments

Timely and consistent treatment is important for the effective attenuation of BD symptom burden. The updated World Federation of Societies of Biological Psychiatry (WFSBP) Guidelines for the Biological Treatment of Bipolar Disorder (Grunze et al., 2013) highlights the importance of educating BD patients about illness progression, treatment options, and the potential impact of the illness on factors such as social relationships, employment, and finances. A primary psychiatrist should continue to monitor treatment, because of a risk of relapse, treatment resistance, and/or development of co-morbidities. Treatment selection is most often based on efficacy rather than tolerability. The most common treatment options are pharmacological and/or psychosocial in nature.

1.1.4.1 Pharmacological Treatment

Pharmacotherapy is a crucial aspect of effectively treating and managing BD progression and symptomatology. Drug choice depends on the exhibited symptoms and short-term and long-term goals of the pharmacological intervention. For acute episodes, the short-term goal is the easing of symptom burden, with the long-term goal being full remission of BD symptomatology. For pharmacological maintenance therapy, the main focus is to prevent relapse into mood episodes. The main classes of drugs used in treating BD are mood stabilizers, atypical antipsychotics, and conventional antidepressants. Some pharmacotherapies display greater efficacy in treating acute
episodes, while others are more effective as maintenance therapies (Jann, 2014; McCormick et al., 2015).

Mood stabilizers are the most commonly used drugs for treating BD and are used for treating both manic and depressive symptoms. They require between one to four weeks of consistent therapeutic dose intake for the full effects to first be seen. About 50 to 60 percent of BD patients will respond sufficiently to pharmacological therapy from a single mood stabilizer, while others require a combination of BD treatment drugs (Hilty et al., 2006; Geddes et al., 2013). Lithium has historically been the most common mood stabilizer used in the treatment of BD (McCormick et al., 2015). It is effective in the treatment of and prevention of recurrent manic and depressive episodes. An effective therapeutic dose of lithium is between 600-2400mg/day, with a blood serum level of between 0.8-1.2µg/mL (Hilty et al., 2006). Although lithium has many limitations, including harmful side effects, delayed onset of action, a narrow therapeutic window, and limited efficacy in treating bipolar depression, it still has an important role in BD pharmacotherapy today. Another commonly used BD mood stabilizer is sodium valproate. Relative to lithium, sodium valproate has a more rapid onset of action, and offers broader coverage in treating mixed mania and rapid cycling episodes. A therapeutic dosage of this mood stabilizer can range from between 500 and 3500mg/day, with a blood serum level of between 75 and 125µg/mL (Hilty et al., 2006). There is little evidence for the efficacy of sodium valproate in maintenance therapy (McCormick et al., 2015). Studies have found that combination pharmacotherapy which involves both lithium and valproate is superior to either alone (Jann, 2014). Other mood stabilizers that are not as commonly used as lithium and valproate include lamotrigine and carbamazepine (Jann, 2014).
Atypical antipsychotic (also known as second generation antipsychotics) are commonly used in the treatment of bipolar mania. The most common atypical antipsychotics used in treating BD include: olanzapine, quetiapine, ziprasidone, aripiprazole and risperidone (McCormick et al., 2015). The most common side effects of atypical antipsychotics are metabolic in nature, such as weight gain, increased lipid levels, and hyperglycemia. As such, continuous monitoring of metabolic health is important when using atypical antipsychotics in a BD treatment regimen (Jann, 2014; McCormick et al., 2015; Geddes et al., 2013).

The use of conventional antidepressants in treating BD has long been a controversial idea. The main reasoning behind this controversy is the ability of antidepressants to precipitate manic episodes in BD patients, which is estimated to occur in 3 to 5 percent of cases in which antidepressants are used as a monotherapy for treating BD depression (McCormick et al., 2015). It is recommended that if antidepressants are used on BD pharmacotherapy, they should be used alongside a mood stabilizer or antipsychotic. Additionally, as the role of antidepressants in maintenance therapy is unclear, antidepressant usage should be stopped once full remission of depressive symptoms has occurred, or if manic symptoms begin to precipitate. Antidepressants commonly used in the treatment of BD include selective serotonin reuptake inhibitors and bupropion (Jann, 2014; McCormick et al., 2015; Geddes et al., 2013).

1.1.4.2 Psychosocial Treatment

Most BD patients experience psychosocial stressors throughout the course of their illness (Hilty et al., 2006). Psychosocial therapies can be provided individually for patients, or as group therapies that provide educational and/or supportive assistance to patients and often time their families as well (McCormick et al., 2015). The use of psychosocial therapies, when used in
conjunction with pharmacotherapies, has consistently shown evidence of better outcomes relative to either alone (Hilty et al., 2006). Psychosocial treatments focus on educating patients about the illness, including an emphasis on the importance of adhering to treatment regimens.

Two common forms of psychosocial interventions include family-focused therapy and interpersonal and social rhythm therapy. Family-focused therapy incorporates elements such as psychoeducation, communication enhancement and problem solving training, with a focus on reducing familial conflict and hostility. Studies find that family-focused therapy in combination with medication confers greater symptom improvement relative to various other forms of psychoeducation in combination with medication (Miklowitz and Chung, 2016). Interpersonal and social rhythm therapy involves stabilizing of a patient’s circadian rhythm through improvement of affective symptom management techniques, medication adherence, and resolution of interpersonal problems. Studies indicate that interpersonal and social rhythm therapy may be beneficial during acute phases of BD (Miziou et al., 2015; Chatterton et al., 2017).

Psychosocial treatment often requires intensive regimens in order to obtain significant effects on patient outcomes. Miklowitz and colleagues (2007) found that intensive family-focused therapy, interpersonal and social rhythm therapy, and cognitive behavior therapy were more beneficial in enhancing stabilization from bipolar disorder episodes than 3 sessions of collaborative care. In addition to symptom improvement, long term psychosocial interventions have also been associated with patient functional outcomes, such as life satisfaction and relationship functioning (Miklowitz, 2007).

1.2 BD and Genetics
BD is thought to be one of the most heritable medical conditions. Many family studies have found that BD is more common among relatives of persons with BD as compared to the general population. Studies suggest that an individual has between a 45 and 75 percent chance of developing BD if they have a monozygotic twin with BD. They have a 4 to 9 percent chance of developing BD if any of their first-degree relatives suffers from BD. In comparison, an individual has between a 0.5 to 1.5 percent chance of developing BD if they do not have any close relatives with BD. Genetics is thought to account for between 60 to 85 percent of the risk for developing BD within one’s lifetime. Genetics may not be the sole factor responsible for the development of BD, but it seems to be an important factor (Birmaher et al, 2009; Birmaher et al, 2013). However, there are no single genetic mutations that are directly responsible for the development of BD. Instead, it seems that a multitude of different genes interact in a complex manner, and along with environmental influences, work to express BD-related pathophysiology. As such, certain genetic factors have been found to increase the probability of developing BD. The use of genome-wide association studies (GWAS) is one of the most common manners by which genetic factors have been linked to BD (Barnett and Smoller, 2009; Berk et al. 2011; Birmaher et al, 2009; Birmaher et al, 2013).

GWAS provide researchers a method to find candidate genes associated with medical condition, without have a predefined hypothesis as to which genes will be found. Using DNA microarrays, an entire genome can be scanned for up to one million single nucleotide polymorphisms (SNPs). GWAS have associated several genes with BD including CACNA1C, ANK3, DGHK, PALB2, NDUFA1, DCTN5, JAM3, and SLC39A3. Most of these genes have a low degree of association with BD, with odds ratios less than 1.4. This low degree of association provides credence to the idea that BD is highly polygenic in nature. From all of the genes
associated with BD in GWAS, the two genes which have been most consistently strongly associated with BD are CACNA1C and ANK3 (Barnett and Smoller, 2009; Berk et al. 2011; Birmaher et al, 2009; Birmaher et al, 2013; Zhang et al., 2013; Fiorentino et al., 2014).

1.3 Genome-wide Association Studies linking CACNA1C rs1006737 to BD

Several genome-wide association studies have linked multiple SNPs on the CACNA1C gene to various psychiatric conditions, including BD. In 2008, Ferriera and colleagues, looking at a mix of 10,000 BDs and HCs, and Sklar and colleagues, looking at over 1400 BD-I cases, were the first two groups to publish results of significant associations between rs1006737 on the CACNA1C gene and BD. Multiple studies have replicated findings of associations between rs1006737 and BD (Green et al., 2013, Zhang et al., 2013; Fiorentino et al., 2014). Taken together, these findings provide the strongest evidence to date of associations between a single given polymorphism and BD. The odd ratio of this association is on average about 1.2 (Green et al., 2010). Given that similar odds ratios are found in studies linking rs1006737 to other psychiatric conditions, including schizophrenia and unipolar depression (Green et al., 2010; Nyegaard et al., 2010; Williams et al., 2011), it seem likely that this SNP may be associated with intermediate symptomatic phenotypes expressed in BD, rather than BD as a monolithic construct.

1.4 CACNA1C rs1006737

1.4.1 CACNA1C gene
CACNA1C (calcium channel, voltage dependent L-type alpha-1C subunit) gene is found on the short arm of chromosome 12p13.3. It contains about 6.45Mb of genetic information, of which 740kb are represented as the gene’s 55 exons. As exons are the expressed portions of a gene, it seems that the majority of the CACNA1C is made up of unexpressed introns, which do not directly affect the gene product, but can have indirect effects through regulation of transcription and translation processes. (Bhat et al., 2012)

CACNA1C encodes the α1-C subunit of the L-type voltage-gated calcium channel (also known as Ca_v1.2) (Soeiro-de-Souza et al., 2017). The gene is expressed in numerous different types of tissues in the human body during various stages of development, including embryonic, fetal, infant and adult stages. CACNA1C has been found to be expressed in the brain, cardiac muscle, cerebral arteries, heart, and fibroblasts (Bhat et al., 2012).

**Figure 2.** Genomic information of CACNA1C beginning at exon 1. Exons are indicated by the vertical bars and are separated by introns. SNPs associated with mental disorders (BD, schizophrenia, and depression) are displayed as colored circles at their approximate location on CACNA1C. (Bhat, 2012)

Various SNPs on CACNA1C have been associated with various psychiatric conditions. (Figure 2; Bhat, 2012). All of these SNPs are located on intron 3 of CACNA1C, a region spanning
a size of approximately 328.5kb. As introns are unexpressed regions of a gene, these SNPs are not thought to directly affect the structure and/or function of Ca_v.1.2. Rather, it is more likely that the polymorphisms play a role in regulating the expression of these calcium channels (Bhat et al., 2012).

1.4.2 Ca_v.1.2 calcium channel

![Figure 3](image_url)

**Figure 3.** An illustration of Ca_v.1.2, a voltage-gated L-type calcium channel (Khosravani and Zamponi, 2006)

Ca_v.1.2, a voltage-gated L-type calcium channel, is a multsubunit transmembrane protein complex (**Figure 3;** Khosravani and Zamponi, 2006). It is composed of three subunits: α_1_, α_2δ_, and β. The α_1_ subunit forms the Ca^{2+} selective pore, houses the voltage sensor as well as bindings sites for endogenous and exogenous binding elements, such as modulatory regulators and drugs. It is made up of 24 transmembrane segments, split up into four repeating domains (I-IV), with each domain consisting of six α-helical segments which are connected by intra-cellular and extra-cellular loops. The α_1_ subunit also consists of a long intracellular C-terminus and short intracellular N-tail. The α_2δ_ and β subunits are involved in anchoring the protein complex to the membrane, trafficking
elements to and away from the channel, and various regulatory functions (Bhat et al., 2012; Hoffman et al., 2014).

Ca, 1.2 increases intracellular calcium concentrations by allowing an influx of Ca$^{2+}$ into the cell following membrane depolarization. The channel’s $\alpha_1$ subunit voltage sensor detects strong membrane depolarization and accordingly signals the opening of the Ca$^{2+}$ selective channel pore. Once the channel pore is open, Ca$^{2+}$ moves freely from the extracellular to the intracellular environment. Inside the cell, Ca$^{2+}$ acts as an intracellular messenger, regulating and mediating numerous cellular functions and processes, such as genetic transcription/translation, synaptic plasticity, neuronal transmission, muscle contraction, and hormone secretion (Hoffman et al., 2014). All of these calcium-linked cellular functions are dependent on calcium pathways involving numerous other intracellular elements.

Examples of two major types of pathways which studies have linked to Ca,1.2-mediated increases in intracellular calcium concentrations include the CaM-dependent protein kinases (CaMK) pathways, and the mitogen-activated protein kinase (MAPK) pathways. CaMKII, an essential protein in the CaMK pathway, has been found to be directly connected to the C-terminus of Ca,1.2. This proximity is integral for the coupling of calcium increases to cyclic adenosine monophosphate response element binding protein (CREB)-dependent genetic transcription. In addition, CaMKII has been found to activate the MAPK calcium-dependent pathway, highlighting the cross-talk that occurs between independent Ca,1.2-associated calcium pathways (Bhat et al., 2012).

1.4.3 rs1006737
rs1006737 is a SNP found on intron 3 of the \textit{CACNA1C} gene. As it is found on an intron, a non-coding and unexpressed region of the gene, this SNP is believed to affect the \textit{CACNA1C} gene indirectly through the regulation of its transcription and/or translation. The two alleles for this SNP are the A allele, and the G allele (Bhat et al., 2012). The A allele is thought to be the rs1006737 risk allele, as it has been associated with higher chances of developing various psychiatric conditions, including bipolar disorder, schizophrenia and unipolar depression (Ou et al., 2015). As rs1006737 is found on a non-coding region of \textit{CACNA1C}, it is thought to confer risk for the development of psychopathologies through interferences in gene expression and cellular signaling pathways (Bhat et al., 2012). For example, increased intracellular calcium concentrations associated with the A allele are thought to affect calcium signaling pathways, which in turn are thought to alter the normal development of neuroimaging phenotypes (Bigos et al., 2010). Gaining a more comprehensive understanding for the role of rs1006737 in the development of psychiatric conditions will allow for a better understanding of the physiological mechanisms involved in these conditions, as well as how such mechanisms can be better targeted through treatment initiatives.

The association between rs1006737 and BD is thought to be a result of the role of calcium signaling in the pathophysiology of BD. Alterations in calcium homeostasis and signaling have consistently been found in BD. Cultured cells from BD patients have provided evidence of disturbances in the flow of calcium into the intracellular environment through both transmembrane calcium channels, and intracellular storage sites such as the endoplasmic reticulum (Warsh et al., 2004). Cells from patients with BD also display aberrant calcium signaling pathways which can be ameliorated by BD therapeutics, such as lithium (Mertens et al., 2015; Alda, 2015). Thus far, studies have not examined cell models in relation to rs1006737 in humans.
1.5 Neuroimaging Findings in BD

Improved imaging technologies and techniques over the past few decades have allowed for the availability of high quality neuroimaging tools. Neuroimaging research in BD has made significant advances due this availability. Numerous structural and functional brain imaging studies have been undertaken to examine the neuroanatomical correlates of BD. Investigating the neuroimaging phenotypes of BD allows for a better understanding of the role brain dysfunction plays in BD psychopathology. Although results sometimes contradict one another, the vast majority of BD neuroimaging findings point to abnormalities in the structure and functioning of emotional control centers of the brain. (Phillips et al., 2014; Berk et al., 2011; Frey et al. 2013)

1.5.1 Brain Structure

Numerous studies have reported structural brain differences between BD and HC groups in both adults and adolescents. Most early studies found that BD groups had greater grey matter volume in the amygdala, pointing towards a neurostructural basis for the emotional dysfunction displayed in BD (Altshuler et al., 1998). Recent studies looking at neurostructural abnormalities in BD have found brain regions involved in emotional processing and emotional regulation to be most commonly implicated (Wise et al., 2017; Sun et al., 2018; Ganzola & Duchesne, 2017).

Prefrontal regions of the brain and the anterior cingulate cortex (ACC), two regions involved in emotional processing and cognitive functioning, are most commonly associated with BD neurostructural abnormalities. Findings predominantly indicate reduced grey matter volume and thickness for these regions in both adults and adolescents with BD, as well as for individuals at familial risk for BD, relative to HCs (Phillips and Swartz, 2014). The ventrolateral prefrontal cortex (vIPFC) is the specific region of the prefrontal cortex which seems to have the strongest
negative association with BD, relative to other prefrontal regions of the brain. It has been reported that vlPFC structure is also negatively associated with duration of BD diagnosis. Individuals who have been living with BD for a long period of time have been found to have smaller vlPFC volumes compared to HCs, while larger vlPFC volumes were found in individual in their early stages of BD diagnosis (Hajek et al., 2013). Various studies have also found widespread BD-associated bilateral prefrontal thickness reductions not focalized in the vlPFC, in addition to bilateral thickness reductions in temporal and parietal regions of the brain. However, these reductions have been reported to be normalized by lithium treatment (Phillips and Swartz, 2014).

In both adult and adolescent BDs compared to HCs, differences in subcortical volumes, primarily in the amygdala and hippocampus, have been reported, indicating a neurostructural basis for BD-associated deficits in emotional processing. These differences have been reported to be normalized with lithium treatment, although to varying extents (Foland et al., 2008). One meta-analysis found reduced amygdala volume in youth, but not adults with BD, indicating the potential for volumetric normalization of amygdala neurostructural deficits over the course of BD, perhaps due to medication (Pfeifer et al., 2008). A similar finding was found in the hippocampus, by a study which reported hippocampal abnormalities in BD can attenuate with illness duration (Javadapour et al., 2010). Various studies have also reported BD-associated structural abnormalities in other subcortical regions, including reduced volumes in the caudate (Ong et al., 2012) and putamen (Almeida et al., 2009).

1.5.2 Brain Function

Neurostructural abnormalities are often accompanied by neurofunctional abnormalities. Not surprisingly, many of the regions structurally implicated in BD are also reported to exhibit
abnormalities in studies examining neurofunctional correlates of BD. The literature on BD functional neuroimaging points toward deficits in neural circuitry involving emotional processing (Figure 4) and/or reward processing (Figure 5). (Phillips and Swartz, 2014)

As emotional dysregulation is a characteristic symptom of BD, it is not surprising that brain regions involved in emotional processing and regulation are neurofunctionally implicated in BD pathophysiology. The amygdala and prefrontal regions of the brain are major components of the brain’s emotional processing and regulation network. As such, the amygdala and prefrontal cortex are widely reported in BD neuroimaging studies examining neurofunctional deficits in emotional processing and regulation (Phillips and Swartz, 2014). Most early BD neurofunctional imaging studies have predominantly reported increased amygdala activity (Blumberg et al., 2005) and reduced medial and lateral prefrontal activity (Phillips et al., 2003) during emotional processing. Various recent studies have reported decreased vlPFC activity and decreased vlPFC-amgydala functional connectivity during emotional processing tasks, in BD groups relative to HC groups. In addition, BD groups have been reported to exhibit increased amygdala activity during cognitive tasks, indicating a heightened perception of emotional labeling in non-emotional contexts (Gruber et al., 2010; Fleck et al., 2012).

Beyond emotional dysregulation, another characteristic feature of BD is increased reward sensitivity. The ventral striatum and prefrontal regions of the brain are key regions involved in reward processing. As such, these regions exhibit reduced activity during rewarding processing tasks in BD groups. Studies that have examined the functional neurocorrelates of BD during reward processing tasks have found increased activity in the ventral striatum and various prefrontal regions of the brain (Phillips and Swartz, 2014). Compared the HC groups, BD groups have exhibited increased vlPFC and ventral striatum activity during reward anticipation (Nusslock at
al., 2012; Chase et al., 2013). Increased orbitofrontal and amygdala activity has been reported in BD in response to reward reception (Linke et al., 2012). In addition, increased ventral striatum activity has been reported in BD in response to reward cues (O’sullivan et al., 2011).

**Figure 4.** A diagram highlighting the key nodes involved in the brain’s emotion processing network. Panel A showcases all the nodes functioning properly in a healthy individual. Panel B highlights dysfunction in the brain’s emotional processing network in the form of abnormal activity as noted by the red nodes. The sizes of the nodes reflect their relative functional activity levels. (Phillips and Swartz, 2014)
**Figure 5.** A diagram highlighting the key nodes involved in the brain’s reward processing network. Panel A showcases all the nodes functioning properly in a healthy individual. Panel B highlights dysfunction in the brain’s reward processing network in the form of abnormal activity as noted by the red nodes. The sizes of the nodes reflect their relative functional activity levels. (Phillips and Swartz, 2014)

### 1.6 Neuroimaging findings in CACNA1C rs1006737

As *CACNA1C* rs1006737 is one of the SNPs most commonly associated with various psychiatric disorders, including BD, associations between rs1006737 and brain imaging phenotypes has garnered interest over the past decade. As most psychiatric conditions, including BD, are heterogeneous in terms of pathophysiology, studying intermediate phenotypes, such as brain structure and/or function, provides a means of elucidating the contributions of genetics to psychiatric illnesses. Numerous studies have examined the neurostructural and neurofunctional correlates of rs1006737 with BD groups, as well as with healthy individuals without any psychiatric diagnosis. Most of these studies have examined associations between rs1006737 and
imaging phenotypes specifically in adult populations, although a select few have examined adolescent populations. Although some studies have reported contradictory, and sometimes null, findings, the current literature on neurostructural and neurofunctional correlates of rs1006737 point to abnormalities primarily in prefrontal brain regions, as well as subcortical limbic brain regions. (Ou et al., 2015)

1.6.1 Brain Structure

When examining healthy adults, several studies have reported findings of associations between brain structure and rs1006737, while other have found null findings. Wang and colleagues (2011) found significantly greater total grey matter volume in risk allele carriers (AA/AG), relative to non-carriers (GG). When examining risk allele dosage effects (AA vs. AG vs. GG), Kempton and colleagues (2009) found the rs1006737 risk allele to be positively correlated with total grey matter volume, with increasing number of risk alleles associated with increasing total grey matter volume. Conversely, Franke and colleagues (2010) found increasing number of risk alleles to be associated with decreasing total grey matter volume. Various studies have also found association between rs1006737 and regional grey matter. Franke and colleagues (2010) found a negative relationship between number of rs1006737 risk alleles and brain stem volume, but no relationship was found when examining associations with subcortical regions of interest, including the amygdala and hippocampus. Contradictorily, when examining associations between rs1006737 and subcortical brain structure, Lancaster and colleagues (2016) found increased amygdala grey matter volume in risk allele carriers, a finding also supported by Perrier and colleagues (2011). Additionally, Wang and colleagues (2011) found rs1006737 risk allele carriers had greater grey matter volume in prefrontal regions of the brain, the anterior cingulate, as well as in temporal, parietal, and occipital regions of the brain. Findings of rs1006737 associations with prefrontal
brain structure was replicated by Soeiro-de-Souza and colleagues (2017), who found increased prefrontal thickness in risk allele carriers relative to non-carriers.

A few studies have examined associations between brain structure and BD diagnosis-by-rs1006737 interactions. When comparing between BD and HC groups, Tesli and colleagues (2013) found no significant differences in effect of rs1006737 on brain total and regional grey matter volume. On the other hand, while investigating diagnosis-by-rs1006737 interactions on regional subcortical volume, Perrier and colleagues (2011) found an interaction such that BD risk allele carriers had decreased putamen volume relative to HC risk allele carriers.

To date, only one study has looked at associations between rs1006737 and brain structure in adolescents. When examining associations between rs1006737 and amygdala structure and function, Sumner and colleagues (2015) found no significant difference in amygdala volume between risk allele carriers and non-carriers.

1.6.2 Brain Function

Various studies have examined the neurofunctional phenotypes of rs1006737 in healthy adult groups and/or BD adult groups. All of these studies have examined brain functioning during tasks which can be broken down into two main groupings: emotional processing tasks and cognitive functioning tasks (Ou et al., 2015).

1.6.2.1 Emotional Processing Tasks

Erk and colleagues (2010) had 110 healthy adults perform an emotional memory task whole undergoing functional magnetic resonance imaging (fMRI). They found significantly reduced activity in the hippocampus and ACC in rs1006737 risk allele carriers, relative to non-
carriers. These findings were replicated by Erk and colleagues (2014) in a different sample of 179 healthy adults. Additionally, they found reduced functional connectivity between the left and right hemisphere of the hippocampus in risk allele carriers when compared to non-carriers. These functional brain differences were not significantly associated with any difference in task scoring outcome.

Several facial emotional processing studies have examined associations between rs1006737 and brain functioning during such tasks. In a study of 131 healthy adults, Bigos and colleagues (2010) found no significant results, but a trend towards greater amygdala activity in rs1006737 risk allele homozygotes (AA) relative to HCs during a facial emotional processing task. Jogia and colleagues (2011) found risk allele carriers had significantly greater activity in the amygdala, relative to non-carriers. These findings were replicated by Tesli and colleagues (2013), who after breaking down the analysis by diagnosis, found that this effect was only significant within the BD group, and not the HC group. Using an fMRI facial emotional processing task, Wang and colleagues (2011) found decreased functional connectivity between the amygdala and the ventral PFC in risk allele carriers. During a similar task, Dima and colleagues (2013) found a BD diagnosis-by-rs1006737 interaction such that HC risk allele carriers displayed increased connectivity between the occipital cortex and the ventral prefrontal cortex relative to HC non-carriers, while the opposite was found within the BD group.

1.6.2.2 Cognitive Functioning Tasks

Previous studies with adults highlight the idea that mostly the hippocampus and prefrontal brain regions display an effect of rs1006737 on brain functioning during various working memory tasks. Paulus and colleagues (2014), using fMRI during a working memory task, found decreased
activity in the dorsal lateral prefrontal cortex in rs1006737 healthy risk allele carriers, relative to non-carriers. They also found increased functional connectivity between the dorsal prefrontal cortex and hippocampus in risk allele carriers. During a different working memory task, Krug and colleagues (2014) found decreased hippocampal activity in the hippocampus in healthy risk allele carriers. Similarly, when examining the brain functioning of relatives of individuals suffering from psychiatric conditions including BD, schizophrenia, and major depression, Erk and colleagues (2014) found decreased hippocampal and ACC activity in risk allele carriers relative to non-carriers. Bigos and colleagues (2010) had healthy participants perform a 2-back style working memory task while undergoing fMRI. They found that rs1006737 risk allele homozygotes (AA) displayed greater activity in PFC clusters, relative to non-carriers.

A few studies have also examined the effect of rs1006737 on brain functioning in healthy adults during various executive functioning tasks, including verbal fluency, reward reversal learning, and attention. Krug and colleagues (2010) examined males who performed a verbal fluency task while undergoing fMRI. They found significantly greater precuneus and inferior frontal activity in risk allele carriers, relative to non-carriers. When examining fMRI-derived brain functioning during a reward reversal learning task, Wessa and colleagues (2010) found significantly greater amygdala activity during reception of the reward, in risk carriers relative to non-carriers. Further analyses revealed that this effect was dependent on the dosage of the risk allele, with more risk alleles being associated with greater activity. During an attention task, Thimm and colleagues (2011) found that, relative to non-carriers, risk carriers undergoing fMRI exhibited significantly reduced activity in the inferior parietal region of the brain during attention orienting, and reduced activity in the medial frontal area of the brain during executive control of
attention. There was also found to be significantly diminished behavioral performance on the attention task in risk carriers when compared to non-carriers.

1.7 Neuropsychology

1.7.1 CACNA1C rs1006737 and Neuropsychology

Given that rs1006737 has been associated with brain structure and functioning, it follows that it may have an effect on neuropsychology. As such, studies have examined the effects of rs1006737 on performance during an array of numerous different types of cognitive neuropsychological outcomes. Examining performance on a battery of different tests, including attention, spatial memory, executive functioning, and personality/temperament, Roussos and colleagues (2011) found significantly reduced extraversion scores, and higher harm avoidance, anxiety, paranoia in healthy risk allele carriers relative to non-carriers. They found no difference on performance during any of the cognitive tasks.

Various studies have also looked at the association between rs1006737 and neuropsychological performance within psychiatric groups, including individuals suffering from BD and those suffering from schizophrenia. While examining performance during a 1-back style working memory task, Zhang and colleagues (2012) found BD risk carriers were prone to a greater number of error relative to risk carriers suffering from schizophrenia or who were HCs. Arts and colleagues (2013) examined the performance of BDs, their first-degree relatives, and HCs during a variety of cognitive tasks, including domains such as verbal memory, attention, and working memory. They found BD risk allele homozygotes (AA) had significantly reduced scores on the cognitive composite measure, which combined scores from all the tasks in the experiment. These
results were not found in the first-degree relatives or HCs. A study by Soeiro-de-Souza and colleagues (2012), examining BD patients and HCs, found that the BD risk allele homozygotes group was associated with reduced cognitive task performance. These findings were not seen in the HC group. Another study by Soeiro-de-Souza and colleagues (2013), examining executive functioning task performance in BDs and HCs, found reduced executive functioning among the BD risk allele homozygote group, when compared to non-carriers. However, no significant difference was found in the HC group.

1.7.2 BD and Neuropsychology

BD has been associated with impairments in a wide range of cognitive domains. Cullen and colleagues (2016), having conducted a systematic review including 30 articles, found the following prevalence rates of cognitive impairment in BD populations: 5.3-57.7% for executive functioning, 9.6-51.9% for attention/working memory, 23.3-44.2% for speed/reaction time, 8.2-42.1% for verbal memory, and 11.5-32.9% for visual memory. These impairments have been found to worsen over the course of BD. There is evidence that cognitive functioning in BD is negatively associated with symptoms of illness progress, such as numbers of affective episodes, illness duration, antipsychotic medicate dosage and/or length of use, and instances of BD-related hospitalization. However, significant interactions between age and BD on cognitive functioning have not been found, indicating aging does not seem to affect cognition to a greater extent in BDs than in HCs. Cognitive decline is BD can be interpreted based on a theory of neuroprogression which links pathology-related brain changes with cognitive impairment (Cardoso et al., 2015). In fact, relative to HCs, BD is often associated with differences in brain regions implicated in various cognitive functions found to be impaired in BD (Wise et al., 2017; Sun et al., 2018; Ganzola & Duchesne, 2017).
1.8 MRI Measures of Brain Structure

Brain cortical and subcortical structure can be measured using T1-weighted images obtained using MRI. T1-weighted MRI involves the use of magnetic pulses to excite brain tissue, such that relaxation of the brain tissue back to equilibrium can be measured. This relaxation of brain tissue can then be used to create anatomical images of the brain. During image processing, the created anatomical images can be segmented into cortical and subcortical brain structure (Georgeto et al., 2016).

Cortical brain structure can be measured using three cortical metrics (volume, SA, and thickness) (Figure 6; Winkler et al., 2010). Cortical volume is measured in mm$^3$ and is a composite metric of SA and thickness. Cortical SA is measured in mm$^2$ and is determined by number of cortical columns, while thickness is measured in mm and is determined by numbers of cells with a cortical column (Rimol et al., 2012; Sanabria-Diaz et al., 2010; Winkler et al., 2010). Previous literature provide evidence that cortical SA and thickness are phenotypically distinct cortical measures, and are affected differently during adolescent development (Panizzon et al., 2009; Winkler et al., 2010; Wierenga et al., 2013; Vijayakumar et al., 2016).
Panizzon and colleagues (2009) conducted a twin study to examine the genetic relationship between cortical SA and thickness. The heritability of SA and thickness was estimated, as well as their degree of overlapping heritability. Although they found that each cortical measure was highly heritable (0.89 for SA and .081 for thickness), they were found to be genetically unrelated (genetic correlation of about 0.08). A similar but independent study conducted by Winkler and colleagues (2010) yielded similar results. These findings demonstrate that cortical SA and thickness are genetically and phenotypically distinct measures of cortical brain structure. As such, imaging-genetic studies should be careful to examine all three metrics of cortical measurements.

Cortical SA and thickness, as well as their composite measure, cortical volume, have been reported to be differentially affected by adolescent brain development. Wierenga and colleagues
(2013) used a longitudinal design MRI study to examine the development of adolescent cortical volume, SA, and thickness (Figure 7; Wierenga et al., 2013). They found that the developmental trajectory of cortical volume was best described by a quadratic curve, such that volume is slowly decreasing throughout adolescence and into early adulthood. The developmental trajectory of cortical SA was best described by a cubic curve, such that SA is slowly increasing during early adolescence, until it hits a peak and then slowly starts to decline into later adolescence. The developmental trajectory of cortical thickness was best described as linear, such that thickness is linearly decreasing throughout adolescence. They also found that the maximum SA occurred later in development relative to maximum thickness and maximum volume. A similar but independent study by Vijayakumar and colleagues (2016) found similar results. These findings suggest that the adolescent development of cortical volume, SA, and thickness differ in their pattern and timing, providing evidence for a tri-metric approach when examining cortical brain structure in adolescents.

**Figure 7.** The average late childhood/adolescent/early adulthood developmental trajectories of cortical thickness (measured in mm), cortical surface area (measured in mm$^2$), and cortical volume (measured in mm$^3$). The curves in blue represent trajectories for males, while the curves in red represent trajectories for females. Arrows indicate peak values. (Wierenga et al., 2013)
2.0 Research Aims

2.1 Statement of Problem

BD is a highly debilitating mood disorder characterized by fluctuating mood episodes of depression and/or mania/hypomania. BD is estimated to affect between 2% and 5% of people worldwide (Jann, 2014). BD typically develops during adolescence or early adulthood, making this period of time important for initial diagnosis and treatment initiatives. In the adolescent population, BD has a population prevalence of about 1%, with an additional 5% being affected by BD-spectrum conditions (Kessler et al., 2009). Compared to adults with BD, adolescents with BD have been found to spend a greater proportion of their time suffering from BD symptoms, as well as co-morbid symptoms such as anxiety and substance abuse (Birmaher et al., 2006). Compared to HCs, adolescents suffering from psychiatric conditions, such as BD, exhibit greater functional and psychological impairment, psychiatric co-morbidities, hospitalizations, and suicide attempts (Peele et al., 2004). As such, BD adolescents have been found to report a lower overall quality of life relative to HCs (Freeman et al., 2009). BD detection and treatment during adolescence is imperative for long-term psychiatric and physical well-being (Price and Marzani-Nissen, 2012).

Along with mood symptoms and co-morbidities, BD individuals also suffer from multi-domain impairments, including cognitive impairments (e.g. working memory, attention, processing speed), psychiatric co-morbidities (e.g. anxiety, SUD, ADHD), and general medical co-morbidities (e.g. cardiovascular disease, diabetes, obesity, sleep apnea) (Jann, 2014; Hilty et al., 2006; McCormick et al., 2015). Although there is not known to be any direct cause, BD has been associated with particular biological phenotypes, including structural and functional neuroimaging phenotypes, inflammation, oxidative stress, and neurotropic factors (Berk et al.,
Many of these BD-associated phenotypes have been associated with specific genetic polymorphisms. Consistent with this idea of a genetic link to the development of BD, studies have consistently found that there is a high degree of heritability for BD. It is believed that genetics accounts for between 60 and 85 percent of one’s chance for developing BD (Barnett and Smoller, 2009; Berk et al. 2011; Birmaher et al, 2009; Birmaher et al, 2013).

Several GWAS have found associations between CACNA1C rs1006737 and BD (Green et al., 2013, Zhang et al., 2013; Fiorentino et al., 2014). With an odds ratio of approximately 1.2, this association is modest in effect, suggesting that rs1006737 is likely involved with in BD intermediate phenotypes, rather than with BD as a monolithic cluster (Ou et al., 2015). Not surprisingly, rs1006737 has been associated with various neuroimaging phenotypes in BD and HC adult populations. Several studies have found that the CACNA1C rs1006737 risk (A) allele is associated with increased volume in several regions of interest (ROIs) implicated in BD psychopathology, including the amygdala, ACC, and prefrontal regions of the brain (Ou et al., 2015; Franke et al., 2010; Kempton et al., 2009; Wang et al., 2011; Perrier et al., 2011; Soeiro de Souza et al., 2012; Tesli et al., 2013). However, interactions between BD diagnosis and rs1006737 is an area of research very few studies have examined, with significant interactions only having been found in the putamen, such that reduced putamen volume was found in BD A-carriers relative to HC A-carriers (Perrier et al., 2011). In addition, only one study has examined association between rs1006737 and brain structure in adolescents. Sumner and colleagues (2015) found no significant difference in amygdala volume between adolescent risk allele carriers and non-carriers in a study which examined associations between rs1006737 and amygdala structure and function.

Given the severity of BD in adolescence, and the importance of early BD detection and treatment in long-term well-being, research on adolescent BD is a crucial area of research (Price
and Marzani-Nissen, 2012). As BD has a strong genetic epidemiology, understanding the role of specific genetic polymorphisms in BD intermediate phenotypes allows for a better understanding of BD pathophysiology and the possibility of developing new targeted treatment initiatives (Barnett and Smoller, 2009). The consistent associations found between CACNA1C rs1006737 and structural neuroimaging phenotypes (Ou et al., 2015) in adults begs the question of whether such findings are also prevalent in adolescence. Despite the rationale for doing so, based on multiple adult imaging studies, no prior study has examined the associations between CACNA1C rs1006737 and brain structure in BD and HC adolescents. Such an approach may yield insights regarding for the role of this genetic polymorphism in the development of BD at its early stages. Clinically, such insights may provide elucidate which patients could stand to benefit most from interventions that modify calcium signaling.

### 2.2 Purpose of Study and Objectives

The purpose of this study was to examine the associations between CACNA1C rs1006737 and structural neuroimaging phenotypes in BD and HC adolescents. The primary analysis examined this association in a priori selected ROIs. These ROIs were chosen based on their associations with BD and/or rs1006737 in the adult and adolescent literature. Three cortical ROIs (vIPFC, vmPFC, and ACC) and two subcortical ROIs (amygdala and putamen) were chosen for the ROI analyses. It is important to note that for cortical ROIs, volume, SA, and thickness were examined, each as separate ROI cortical measures, while only volume was examined for subcortical ROIs. A secondary analysis was also conducted, which examined the associations between CACNA1C rs1006737 in BD and HC adolescents using a vertex-wise whole-brain
exploratory analysis, in which there were no predefined ROIs. In both the primary and secondary analyses, examined effects include main effect of diagnosis, main effect of CACNA1C rs1006737, and diagnosis-by-rs1006737 interaction effects on cortical and subcortical brain structure.

The objectives of this study were to:

1. Determine if there is a main effect of CACNA1C rs1006737 on brain structure in adolescents.
2. Determine if there is an interaction between BD diagnosis and CACNA1C rs1006737 on brain structure in adolescents.
3. Determine if there is a main effect of BD diagnosis on brain structure in adolescents.

2.3 Hypotheses and Rationales

2.3.1 Primary Hypothesis: Main Effects of CACNA1C rs1006737

Studies in the adult population have found significantly greater cortical volume in risk allele (A) carriers relative to non-carriers in the vlPFC, vmPFC, ACC, and amygdala (Ou et al., 2015; Franke et al., 2010; Kempton et al., 2009; Wang et al., 2011; Perrier et al., 2011; Soeiro de Souza et al., 2012; Tesli et al., 2013). As such, main effects of CACNA1C rs1006737 on brain structure in this study’s adolescent sample were hypothesized. Specifically, it was hypothesized that there would be significantly greater volume and/or SA, and/or thickness in the vlPFC, vmPFC, ACC, and amygdala, in A-carriers relative to non-carriers.

2.3.2 Secondary Hypothesis: Diagnosis-by-rs1006737 Interaction Effects
Perrier and colleagues (2011) found evidence of significantly reduced adult putamen volume in BD A-carriers relative to HC A-carriers. As such, a diagnosis-by-rs1006737 interaction effects on brain structure in this study’s adolescent sample was hypothesized. Specifically, it was hypothesized that BD A-carriers would have significantly reduced putamen volume relative to HC A-carriers.

2.3.3 Tertiary Hypothesis: Main Effects of BD Diagnosis

Studies in the adult and adolescent literature have found significantly reduced volume in the vlPFC, vmPFC, ACC, amygdala, and putamen, for BDs relative to HCs (Phillips and Swartz, 2014; Pfeifer et al., 2008; Foland et al., 2008; Almeida et al., 2009). As such, main effects of BD diagnosis on brain structure in this study’s adolescent sample were hypothesized. Specifically, it was hypothesized that there would be significantly reduced volume and/or SA, and/or thickness in the vlPFC, vmPFC, ACC, amygdala, and putamen, for BDs relative to HCs.

3.0 Materials and Methods

3.1 Study Design

This study investigated the relationship between CACNA1C rs1006737 and brain structure in a group of BD and HC adolescents of European descent. All consenting participants completed anthropometric measures, an interview and self-reports for demographic and clinical information, saliva collection for genetic data, and a 3-Tesla (3T) structural magnetic resonance imaging (MRI)
scan for structural brain data. The entire study was completed on a single day in a period lasting
between three to five hours. Participants had the ability to end the study prematurely for any reason
at any point during the course of the study. The staff involved in data collection during the course
of the study included at least one study lead (a graduate student or research assistant), one MRI
technician, and one psychiatrist who was available for any medical concerns that could potentially
have risen.

3.2 Participants

3.2.1 Participant Recruitment

This study included data from 38 BD and 33 HC adolescent participants of European
descent. BD participants were recruited from the Centre for Youth Bipolar Disorder (CYBD), a
subspecialty clinical-research program at Sunnybrook Health Sciences Centre in Toronto, Ontario,
Canada. HC participants were recruited from the local Greater Toronto Area (GTA) using
advertisements in local print media, as well as on public transit and local public and private GTA
establishments.

This study was approved by the research ethics board at Sunnybrook Health Sciences
Centre before the start of this study (see Appendix 1 for ethics approval form). Each participant
and one participating parent/guardian completed and signed informed consent forms prior to study
commencement and was offered a copy of the signed consent forms (see Appendix 2 for consent
forms).

3.2.2 Inclusion Criteria
English-speaking male and female adolescents of European descent between the ages of 14 and 20 years of age were recruited for this study. BD participants met the DSM-5 diagnostic criteria for BD. HC participants had no history of a psychiatric disorder (i.e. no lifetime mood or psychotic disorder, no alcohol or drug dependence in the past 3 months, and no anxiety disorders in the past 3 months) and had no family history (first or second-degree relatives) of BD, or psychosis.

### 3.2.3 Exclusion Criteria

Data for this project was collected by two different studies encompassing various measurements in addition to those explicitly used in this project. As such, participants were excluded from this project if they met any of the exclusion criteria from either of these two studies, including:

1. Unable to provide informed consent (e.g. severe mania, psychosis, intellectual disability, or inability to communicate in English satisfactorily)

2. Had a pre-existing cardiac condition, auto-immune illness, or inflammatory illness

3. Taking any anti-inflammatory, anti-platelet, anti-lipidemic, anti-hypertensive or hyperglycemic agents (including insulin or metformin)

4. Had an infection illness within the past 14 days

5. Had any of the following MRI contraindications:
   - cardiac pacemaker
- tattoos
- non-removable body piercings
- braces/retainers
- claustrophobia
- any metal in the body (rods, pin, bullet, etc.)

6) Had a health condition of physiological impairment that prohibited exercise

7) Had a neurological or severe cognitive impairment (e.g. autism or intellectual disability)

8) Were unable to provide informed assent/consent

3.3 Study Chronology

New and existing adolescent participants were initially identified from the CYBD recruitment database. All eligible participants were contacted in-person or via phone and/or email. An overview of the study was presented to the adolescent participant as well as their parent/guardian. Prior to being invited to take part in this study, participants were screened for inclusion and exclusion criteria.

Study intake usually proceeded in two separate visits. During the first visit, the participant and at least one parent/guardian completed a semi-structured diagnosis interview, which took between approximately 1-4 hours to complete. During the second visit, each eligible participant completed a clinical research interview to evaluate current mood symptoms and collect family medical/psychiatric history, provided a saliva sample for genetic analysis, and underwent a 3T T1-
weighted MRI scan. Both study visits were entirely completed at Sunnybrook Health Science Centre. Figure 8 gives an overview of the study chronology.

![Figure 8. A flowchart describing the study chronology](image)

### 3.4 Demographic and Clinical Data

#### 3.4.1 Anthropometric

Collected anthropometric measures include height, weight, and waist circumference (WC). Height was collected using a standard tape measure in centimeters (cm). Participants stood up straight, feet together, with their back against a wall to which the tape measure was mounted. Weight was collected in kilograms (kg) using a standard digit weight scale. Collected weight figures were adjusted for the approximate weight of the clothing worn by the participant. Weights were subtracted by 1.3kg if the participant was wearing long pants and a long sleeve shirt, by 1.1kg if the participant was wearing one of either short pants or a short sleeve shirt, or by 0.9kg if the participant was wearing both short pants and a short sleeve shirt. Waist circumference in cm was
collected using a standard tape measure from a point in between the bottom of the ribcage and top of the hipbone. All anthropometric measures were obtained twice, and the average of the two readings was used. Participant body mass index (BMI) was calculated, using collected height and weight figures, as weight in kg divided by height in meters squared, for a BMI figure presented in kg/m².

### 3.4.2 Blood Pressure

Systolic and diastolic blood pressure measures were collected in millimeters of mercury (mmHg) using a standard automated digital blood pressure cuff and monitor. The cuff was wrapped around the participant’s right bicep. Two blood pressure readings were taken two minutes apart from each other. A third reading was taken if the two systolic and/or diastolic figures were 20 mmHg or more apart from each other. If the systolic reading was above 130 or below 80 and/or the diastolic reading was above 90 or below 60, the medical professional on-call was contacted for further instructions.

### 3.4.3 Interview

Demographic and clinical information was collected during a semi-structured clinical interview conducted by a trained research assistant or graduate student. The Hollingshead Four-Factor Index of Socio-economic Status (SES-Child) was used to measure socio-economic status (SES). Several clinically certified interview tools were used, including the Affective Disorder and Schizophrenia for School Aged Children, Present and Life Version – mania and depression scaled (K-SADS-PL/ MRS and DEP-P respectively), Medication Listing, Children’s Global Assessment Scale (C-GAS), family medical history screening form, CARDIA Medical and Family Medical History, and Demographics Form.
The Hollingshead Four Factor Index of Socio-economic Status is a survey designed to measure social status of an individual based on four domains: marital status, retired/employed status, educational attainment, and occupational prestige. The child participant’s parent’s education score is rated on a 7-point scale that lists highest grade completed. The child participant’s parent’s occupational score is rated on a 9-point scale. An SES score is calculated for a total parental SES score (Hollingshead, 1975).

The CARDIA Family Medical History is a questionnaire which collected clinical information pertaining to cardiovascular disease, stroke, and metabolic syndrome history regarding 1st and 2nd degree relatives (Burke et al., 1991). The Family History Screen is a tool used to detect psychiatric disorder in 1st and 2nd degree relatives (Weissman et al., 2000).

The Medication Listing form is used to collect information about the participant’s medication regimen, taken the day before and the day of the study. Information pertaining to the participant’s regular psychotropic medication status is collected during the initial clinical interview, and included average dose per week and type of psychotropic medication.

3.5 Genetic Data

3.5.1 European Descent

Participants were filtered such that all participants for this study were of European descent, noted as Caucasian ethnicity on the Demographics Form. As different ethnic groups are known to differ in their susceptibility to certain genetic variations, conducting this study with only participants of European descent allowed for a reduction of genetic variability in the findings.
Specifically, \textit{CACNA1C} rs1006737 has been found to have highly variable minor allele frequencies among different ethnic groups (Zheng et al., 2014). As such, using a racially homogeneous participant group in this study is important for the reduction of genetic variability specifically for \textit{CACNA1C} rs1006737.

\textbf{3.5.2 Saliva Collection}

Participants were asked to abstain from eating, drinking, smoking, or chewing gum 30 minutes prior to saliva collection. Saliva was collected using an Oragene DNA Self-Collection Kit (OG-500) for collection of human DNA. Participants were asked to salivate in the saliva collection tube up to a line indicated on the tube. All saliva samples were stored at room temperature prior to genetic DNA extraction and genotyping.

\textbf{3.5.3 Genotyping}

Genetic DNA extraction and genotyping occurred in the Neurogenetics Laboratory at Dr. James Kennedy’s Lab at the Centre for Mental Health and Addiction (CAMH) in Toronto, Ontario, Canada. DNA was extracted from the collected saliva sample on a chemagen MSM-I DNA extraction (Perkin-Elmer, Waltham, MA) as per manufacturer’s instructions. The extracted DNA was quantified using a Nanodrop 8000 spectrophotometre (ThermoFisher Scientific, Waltham, MA) and an aliquot diluted to 20ng/µL for use in standard downstream genotyping analysis.

\textit{CACNA1C} rs1006737 SNP was genotyped using the TaqMan® Open Array® Format32 method (ThermoFisher Scientific, Waltham, MA) as per manufacturer’s directions on the QuantStudio™ 12K Flex Real-Time PCR System (ThermoFisher Scientific, Waltham, MA). A custom assay for the Amelogenin regions was included for purposes of quality control. In a brief description, genotyping and analysis procedures involved manually combining a 2µL sample of
DNA at a concentration at 20ng/µL and 2µL of 2X TaqMan® Open Array® Master Mix in 384-well plates loaded onto the Open Array® genotypingplates using the AccuFill System (ThermoFisher Scientific, Waltham, MA). Prepared arrays were then amplified, visualized, and analyzed on the QuantStudio™ system. Genotyping of about 10 percent of samples from each run were replicated for purposes of quality control for each marker. Finally, genotyped data was imported into the TaqMan® Genotyper software v1.3 and confirmed manually by two independent personnel from Dr. James Kennedy’s Lab. This finalized genotyped data was then sent to CYBD and merged into their existing subject database.

3.5.4 Hardy-Weinberg Equilibrium

Hardy-Weinberg Equilibrium (HWE) is a principle which states that, in the absence of evolutionary influences, allele and genotype frequencies in a population will remain constant from generation to generation. Evolutionary influences which can disturb HWE include migration, mutation, natural selection, and mate choice, among various other forces (Wigginton et al., 2005). Testing for HWE is commonly used in genetic studies to determine whether there are any problems with genotyping for the sample, or in the population structure in general. Deviations from HWE can compromise key inferences in genetic studies (Salanti et al., 2005; Wigginton et al., 2005). The current study tested for HWE using the HWE-expected proportions ($p^2$, $2pq$, and $q^2$) and the goodness-of-fit $X^2$ test (Salanti et al., 2005).

3.6 Structural Imaging Data

3.6.1 Patient Preparation
Prior to entering the MRI room, all participants were asked to wear a provided sterile scrub and/or a hospital gown. All jewelry was removed from their body, along with any other metal item attached to their body. Participants were asked to remove any glasses they were wearing, and were provided with MRI-compatible lenses if required for the session. A licensed MRI technologist screened each participant for MRI safety before signing off the eligibility of the participant to enter the MRI room. Participants had their ears covered with earmuffs or MRI-compatible headphones while in the MRI machine. Physiological data, including heart rate and respiratory rate, were collected using a finger pulse collector and a respiratory bellow, respectively. Participants were told to press a specific button nearby their hand at any given time during the MRI scan if, for any reason, they felt the need to get out of the MRI machine. The MRI technologist conducting the scan maintained periodic communication with the participant throughout the scan, ensuring physical and psychological well-being.

3.6.2 Imaging Acquisition

MRI data was collected a 3T Phillips Achieva MRI system at Sunnybrook Health Sciences Centre in Toronto, Ontario, Canada. This scanner utilizes a body coil transmitter and 8-channel head receiver coil. The MRI consisted of localizer scans, used to plan subsequent images, and anatomical structural scans of the brain. Anatomical images, used to quantify GM and white matter (WM) in the brain, were collected using a standard T1-weighted high resolution fast-field echo imaging (repetition time (TR)/echo time (TE)/inversion time (TI) = 9.5/2.3/1400 ms, spatial resolution 0.94 × 1.17 × 1.2 mm, 256 × 164 × 140 matrix, scan duration 8 min and 56s).

3.6.3 Imaging Data Pre-processing
T1-weighted structural images were processed using FreeSurfer version 5.3.0 on a MAC desktop computer. Images were processed into surface-based morphometric data using the fully automated reconstruction function found in the FreeSurfer software package (Fischl, 2012). Automated pre-processing included re-sampling of 3D coronal images (1mm isotropic voxels), intensity normalization, registration to MNI space, and additional intensity normalization with automated skull stripping (Fischl, 2012). Next, automated parcellation was undertaken, with cortical surface reconstruction which included generation of binary white matter masks in two hemispheres which were used to produce a triangle-based mesh of white matter surface and smoothed to remove voxel-based effects and corrected for topological effects. White matter and pial surfaces were then estimated with a deformation and surfaces were spherically inflated to be registered to a canonical template. Finally, a parcellation algorithm used the FreeSurfer default Desikan-Killiany (DK) cortical atlas, spatial landmarks, curvatures, and sulcal depth to assign 34 gyral regions of interest (ROIs) (Fischl et al., 2002; Fischl et al., 2004). Each individual participant’s volume, SA, and thickness measurements were extracted for each of these 34 regions. Subcortical volumes were extracted using the default FreeSurfer subcortical parcellation algorithm.

### 3.6.4 Imaging Data Quality Control

Of 77 initial participants, 6 were removed due to deficiencies noted following T1 and/or parcellation quality ratings, providing a final dataset of 71 participants. The quality control rating system is discussed in further detail below.

#### 3.6.4.1 T1 Rating
Two independent raters visually inspected all structural T1-weighted images using Freeview, a graphical user interface (GUI) found in the FreeSurfer v5.3 software package (Fischl, 2012). Each rater scored each subject’s brain images with a score between 0 and 3 (Figure 9). Scores were given based on image quality, taking into account factors such as graininess, WM-GM contrast, and image artifacts. Each rater scrolls through T1-weighted slices in the axial plane and flags abnormalities in image quality. In cases where incongruences were found in the ratings between the two different raters, these images were discussed and rated again in a consensus fashion. All images that were rated 3 were re-processed. If, after re-processing, images still had a quality of 3, these images were removed from the dataset.

**Figure 9.** Scoring system for quality control of T1-weighted images

### 3.6.4.2 Parcellation Rating

After T1-weighted images have been reviewed for image quality, the next step is reviewing parcellation quality in order to check the accuracy of the FreeSurfer-based parcellation algorithm. Using Freeview, two independent raters scored each image for parcellation quality
with a score of between 0 and 3. Parcellation quality was dependent on how well the WM and GM boundaries are delineated. Essentially, raters are looking to visually determine whether the FreeSurfer-created parcellation match WM and GM boundaries on the T1-weighted volume. Raters scroll through slices in the axial, sagittal and coronal slices, flagging any improper boundary delineations. A commonly example of improper parcellation is the under extension of the GM boundary such that GM is left out of the parcellation (Figure 10), and as such is not included in calculation of GM metrics. Other examples of improper segmentation include overextension of the GM boundary such that the skull is encapsulated, over extension of WM boundaries, and under extension of WM boundaries.

![Figure 10](image)

**Figure 10.** A commonly found example of improper segmentation. The blue arrow points to a region of the brain in which the GM boundary (red) does not fully encapsulate the underlying GM (regions in grey).

A score of 0 was given to images where no problems were found in parcellation quality. A score of 1 was given to images which contained a few small localized regions of improper parcellation. A score of 2 was given to images which contained multi-focal regions of improper segmentation in which the problems persisted across multiple slices. A score of 3 was given to images which contained major distortions in parcellation which were deemed impossible to fix.
Inter-rater incongruences were discussed between the raters and a scoring consensus was reached for these select cases. Any score of 3 was removed from the dataset. Any score of 1 or 2 was edited and re-processed. Edits were undertaken using the cortical segmentation editing tools found on Freeview, including the Voxel Edit tool and Control Points on the brainmask volume as well as the WMmask volume. Edits were consolidated into a new image through re-processing.

3.7 Statistical Analyses

3.7.1 Demographic and Clinical Characteristics

Group comparisons were made for various demographic and clinical characteristics in order to test for effects of BD diagnosis status, CACNA1C rs1006737, and diagnosis-by-rs1006737 interactions. Continuous variables were tested using a univariate analysis of covariance (ANCOVA) GLM design matrix, whereas categorical variables were tested using Chi-squared $\chi^2$ tests. Statistical significance was defined as $p < .05$.

3.7.2 ROI Analysis

3.7.2.1 Defining ROIs

ROIs were determined from the previous literature on CACNA1C rs1006737 and BD. They were defined by summing up extracted volume, SA, and/or thickness measurements. Cortical as well as subcortical ROIs were analyzed, with cortical ROI measurements in volume, SA, and thickness, while subcortical ROI measurements were only available for volume, as FreeSurfer only extracts volume measurements from subcortical regions. Cortical ROIs included the ventro-lateral prefrontal cortex (vlPFC), ventro-medial prefrontal cortex (vmPFC), and the anterior cingulate cortex (ACC). Subcortical ROIs included the amygdala and the putamen.
The vlPFC was defined as the DK cortical atlas bilateral pars triangularis and pars opercularis (Figure 11).

Figure 11. Lateral view of the DK brain atlas. The red oval highlights the DK-defined vlPFC, including the pars triangularis and pars opercularis.

The vmPFC was defined as the DK cortical atlas bilateral medial orbitofrontal area (Figure 12).
Figure 12. Lateral view of the DK brain atlas. The red oval highlights the DK-defined vmPFC, including the medial orbitofrontal area.

The ACC was defined as the DK cortical atlas bilateral caudal and rostral ACC (Figure 13).

Figure 13. Lateral view of the DK brain atlas. The red oval highlights the DK-defined ACC, including the caudal and rostral ACC.
The amygdala was defined as the bilateral amygdala derived from the default FreeSurfer v5.3 subcortical parcellation algorithm.

The putamen was defined as the bilateral putamen derived from the default FreeSurfer v5.3 subcortical parcellation algorithm.

### 3.7.2.2 SPSS GLM Design

ROI analyses were performed using SPSS version 22 (SPSS, 2013) on a MAC desktop. A univariate ANCOVA GLM design matrix was used for this analysis. This model was used to determine whether the means of the dependent variable were different across different levels of independent variables, while statistically controlling for other continuous variables not of primary interest. For the purposes of this study, the dependent variables were the volume, SA, and/or thickness measurements for each ROI, and the independent variables were BD diagnosis status and \textit{CACNA1C} rs1006737 allele subtype. The covariates in the ROI analyses were age, sex, and intracranial volume (ICV), variables that have all each been found to have effects on brain structure. ICV was not used for thickness analyses.

ROI analyses tested for a main effect of diagnosis, main effect of rs1006737, and diagnosis-by-rs1006737 interaction on cortical and subcortical brain structural measurements. Bonferroni corrections were applied to correct for running multiple tests with each ROI. Statistical significance was defined as $p < .05$.

### 3.7.2.3 Assumption Testing
ANCOVA assumption testing included testing for normality and testing for homogeneity of regression slopes. Normality was tested by plotting the measurements for each individual ROI across all subjects in a histogram on SPSS. Normality testing revealed the data for all ROIs were normally distributed, precluding the necessity of non-parametric testing. Testing for homogeneity of regression slopes was undertaken by testing the interaction of each covariate with each independent variable in the ANCOVA GLM SPSS model design for each ROI dependent variable. Significant interactions were kept in the final model for each relevant ROI in which significance for these interactions were found.

3.7.2.4 Post-hoc Analyses

Post-hoc analyses included sensitivity analyses and breaking down significant interaction results.

Sensitivity analyses were performed for all relevant demographic/clinical variables which had a significant main effect of BD diagnosis, main effect of rs1006737, or interaction effect, in this study’s sample. Variables were tested only within BDs if the variable was predominantly more prevalent in BDs relative to HCs (e.g. psychotropic medications). Sensitivity analyses were conducted in a two-step approach. First, correlation analyses were run for each ROI measure and each of the relevant significant demographic/clinical variables. Secondly, variables found to be significantly correlated with a given ROI were then individually added to the ANCOVA GLM design model for this ROI. Essentially, sensitivity analyses examined whether any of the various demographic/clinical factors could have had an effect on the ROI findings for this study.

Post-hoc analyses also included breaking down significant diagnosis-by-rs1006737 interaction results using SPSS. The interactions were broken down to determine whether the
interaction was driven by an effect of diagnosis within rs100673 groups, or an effect of rs1006737 within diagnosis groups.

3.7.2.5 Benjamini-Hochberg FDR corrections

The Benjamini-Hochberg False Discovery Rate (FDR) method was used to correct for testing multiple ROIs. This method was used to control for Type I errors, which involves incorrectly rejecting a true null hypothesis. Corrections were done within family groups, such that separate corrections were performed for each of the cortical measurements (i.e. all cortical ROI volumes grouped together, all cortical ROI SAs grouped together, and all cortical ROI thicknesses grouped together), as well as separate corrections for each all the subcortical ROIs. In this FDR method, FDR-adjusted $p$-values were expressed as $q$-values (Benjamini and Hochberg, 1995).

3.7.3 Whole-brain Vertex-wise Exploratory Analysis

3.7.3.1 FreeSurfer GLM Design

The whole-brain vertex-wise exploratory analysis was performed using a FreeSurfer-based GLM script with a custom model design. The whole-brain analysis used a similar design matrix as that used in the ROI analysis, with the independent variables being BD diagnosis status and CACNA1C rs1006737 allele subtype, and age, sex, and ICV as covariates. However, in this case, the dependent variable was the vertex-wise cortical measurement. Each analysis was run separately for each cortical measurement (volume, SA, thickness) in each hemisphere, for a total of 6 separate vertex-wise analyses. Given that previous literature has consistently shown an effect of age on brain structure, including in the developing adolescent brain, this analysis incorporates an examination of age-by-group interactions into the final results.
3.7.3.2 Age-by-group interactions

In order to account for age-by-group interactions in this study’s subjects, two models were run in each case: the different onset same slope (DOSS) model, and the different onset different slope (DODS) model. The model used for the final results depended on age-by-group interaction findings. For both models, both independent variables were paired such that all possible groupings were created.

The four possible categorical groups were: BDs with the A-allele, HCs with the A-allele, BDs homozygous for the G-allele, and HCs homozygous for the G-allele. In the DOSS model design matrix, one regressor was created for each of the four categorical groups, and one regressor was created for each of the three covariates. As only one regressor is present for the covariate of age, age-by-group interactions are not accounted for in the DOSS model. In the DODS model design matrix, one regressor was created for each of the four categorical groups, while one regressor was used for each of the two covariates of sex and ICV, but four regressors were used for age. Four regressors for age were required in the DODS model such that one age regressor was available for interaction with each categorical group, allowing for a testing of age-by-group interactions. Accounting for age-by-effect interactions also required a design matrix to be created for age-by-effect interactions. In each of the three design matrices, weighting were applied to test for the relevant contrast.

For each of the DOSS and DODS models, an .fsgd file containing a list of all the independent variables and covariate classifications for each subject was created. The variables are listed in the same order in which they are listed in the design matrix. For the DOSS model, sex and ICV are mean-centered, while age is not. For the DODS model, all three covariates are mean-
centered. It should be noted that separate .fsgd files were also created for each of the DOSS and DODS models for cortical thickness, as ICV is not accounted for in the thickness analyses.

3.7.3.3 Processing and analysis

Initial processing involved the generation of an average control brain, known as the FreeSurfer fsaverage, with the volume, SA, and thickness measurements from each subject being mapped onto this average surface, and smoothed using a standard Gaussian filter at a full width and half maximum of 15mm. This initial processing was performed separately for each of the DOSS and DODs models, as different .fsgd files are used for each model. Separate analyses were then run for each of the DOSS, DODS, and age-by-group interaction design matrices. Vertex-to-vertex contrasts were performed with a surface-wide primary threshold of $p < .05$. Finally, DOSS and DODS results were corrected for multiple comparisons using a Monte-Carlo z-simulation cluster analysis at a threshold of $p < .05$.

3.7.3.4 Monte-Carlo z-simulation cluster analysis

Monte-Carlo simulations are a large group of computational algorithms which use probability statistics to correct for multiple comparisons. Essentially, these simulations create probability distributions which are then used to determine the probability that a certain outcome would occur by chance. Specifically for the Monte-Carlo z-simulation cluster analysis, a multiple comparison tool found on the FreeSurfer v5.3 package, a simulation is run to get a measure of the distribution of the maximum cluster sizes under the null hypothesis.

The first step in performing Monte-Carlo z-simulation cluster analysis is the creation of a probability distribution as a reference point for multiple comparisons. This is accomplished by performing the following steps over several 5000 iterations (Forman et al., 1995):
1) Synthesize a z map

2) Smooth z map (using residual FWHM)

3) Threshold z map (level and sign)

4) Find clusters in thresholded map

5) Record area of maximum cluster size

The above steps create data regarding the probability distribution of maximum cluster size. This information is stored by FreeSurfer as a text file called a CSD (Cluster Simulation Data). Once this information has been created and stored, corrections for multiple comparisons can be performed using the following steps:

1. Going back to the original data
2. Thresholding using same level and sign
3. Finding clusters in thresholded map
4. For each cluster, \( p \) = probability of seeing a maximum cluster that size or larger during simulation.

3.7.3.5 DOSS, DODS, and age-interactions

Deciding whether a DOSS or DODS significant result represent a true finding depends on the relevant age-by-effect interactions. A comparison of significant DOSS and DODS clusters to their paired age-by-effect interaction brain map was undertaken in order to decide whether to include the clusters in the final results. Clusters in age-by-effect interaction brain maps signify age-by-effect interactions on the regions of the brain in which the cluster is located. The DOSS model did not take into account age-by-effect interactions. As such, a significant DOSS cluster was used in the final results only if the location of the cluster on the brain did not overlap with
clusters found on the brain map of the interaction between age and effect for which the cluster was found. For example, if a significant main effect of rs1006737 DOSS cluster was found, the cluster was accepted in the final results only if the cluster did not overlap with any cluster in the same region of the brain on the age-by-rs1006737 brain map. On the other hand, the DODS model intrinsically takes into account age-by-effect interactions. Therefore, a DODS cluster was used in the final results only if the cluster overlapped with the location of any cluster found on the brain map of the interaction between age and effect for which the cluster was found. For example, a significant DODS diagnosis-by-rs1006737 interaction cluster was accepted in the final results only if the cluster overlapped with any cluster in the same region of the brain on the age-by-interaction interaction brain map.

**3.7.3.6 Mean-centering**

Covariates were manually mean-centered in the whole-brain exploratory analysis, as this is not automatically done by FreeSurfer, as it is by SPSS. Mean-centering involves making the mean of a variable for your sample equal to zero. This is done by subtracting the variable’s mean from each subject’s value for this variable. This process redefines the zero point for the predictor variable, in this case each covariate, shifting the scale, but retaining the units. Mean-centering does not affect the slope between the predictor and outcome variables, but changes the interpretation of the intercept. The intercept is the mean of the outcome variable when the predictor is equal to zero. By mean-centering covariates, interaction effects are better interpretable (Enders et al., 2007).

It is important to note that age is not mean-centered in the DOSS model, but it is in the DODS model. This is done because age-interactions are accounted for in the DODS model, while the same is not done in the DOSS model.
3.7.3.7 Cluster Extraction

Once whole-brain vertex-wise analysis has been completed, cluster stats are extracted using the FreeSurfer-based cluster extraction script. Cluster extraction yields a .txt file which contains the volume, and/or SA, and/or thickness measurement values for the extracted cluster in each subject. These values are put into SPSS and are later used in post-hoc analyses.

3.7.3.8 Post-hoc Analyses

Post-hoc analyses for whole-brain vertex-wise analysis involved breaking down diagnosis-by-rs1006737 interactions using an SPSS ANCOVA GLM design model, similar to that used in the ROI analysis. In this manner, interactions are examined to determine whether they are driven by an effect of diagnosis within rs1006737 groups, and/or an effect of rs1006737 in diagnosis groups.

3.7.4 Power Calculations

Post-hoc power calculations were made using a software known as G*Power Statistical Power Analysis for Mac Version 3.1.9.3 (Faul et al., 2007). Using an $\alpha=0.05$ and power=0.80 with total sample size of 71 and 4 groups with 3 covariates, it was determined that this study had the power to detect effect sizes of Cohen’s $d=0.67$.

3.7.5 Covariates

3.7.5.1 Age

The idea that the brain structure changes with age has been a widely agreed upon fact for many decades (Fjell and Walhovd, 2010). As the brain is developing, its morphology is undergoing significant changes, especially during adolescence. Therefore, it is important to add age as a
covariate in a statistical model examining brain structure, in order to account for potential age-related confounds.

### 3.7.5.2 Sex

Sex-specific differences in brain structure are consistently reported. Although there are more similarities than differences between the biological male and female brain, there are also significant differences, such as differences in total brain volume, as well as regional volumetric differences (Cosgrove et al., 2007). Given these differences, having sex as a covariate in a statistical model examining brain structure is important, in order to account for potential sex-related confounds.

### 3.7.5.3 ICV

Differences in head size, measured using intracranial volume (ICV), are a well-known source of inter-subject variability in total and regional grey matter volume (Malone et al., 2015). If there is a significant imbalance in head sizes between subjects/groups, results can be significantly affected. In order to account for potential confounds due to head size, it is important to account for ICV in any statistical model examining cortical GM volume or SA. However, when examining cortical thickness, ICV is not needed as a covariate because volume scales with head size due to changes in SA, whereas thickness alone scales to a non-significant degree. Adding ICV to a statistical model examining cortical thickness would just add noise to the results (Buckner et al., 2004).

### 4.0 Results
4.1 Demographic and Clinical Characteristics

Relative to the HC adolescent group, the BD adolescent group was significantly older ($p<.001$), had significantly greater body mass indices ($p=.002$), and higher rates of ADHD ($p=.04$) and anxiety ($p<.001$).

No significant effects of CACNA1C rs1006737 was found on any of the demographic and clinical variables.

Significant interactions between BD diagnosis status and CACNA1C rs1006737 were found for rates of ADHD ($p=.04$) and anxiety ($p<.001$).

Refer to Table 1 for a complete list of demographic and clinical characteristics and the effects of diagnosis, rs1006737 and diagnosis-by-rs1006737 interactions on these variables.

<p>| Table 1. Demographic and Clinical Characteristics of the Study Participants by BD Diagnosis, CACNA1C rs1006737, and Diagnosis x rs1006737 Interaction |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Characteristic | Main Effect of Diagnosis | Main Effect of rs1006737 | Effect of Diagnosis x rs1006737 Interaction |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| BD (n=38)       | HC (n=33)       | A allele present (n=34) | Only G allele present (n=37) | BD with A allele (n=14) | HC with A allele (n=20) | BD with G allele only (n=24) | HC with G allele only (n=13) |
| Age, year, mean (SE)a | 17.66 (0.22) | 16.41 (0.23) | 17.27 (0.22) | 16.8 (0.22) | 18.17 (0.34) | 16.38 (0.29) | 17.16 (0.26) | 16.44 (0.36) |
| Sex, n          | Male            | 15              | 14              | 12              | 17              | 4               | 8               | 11              | 6               |
|                 | Female           | 23              | 19              | 22              | 20              | 10              | 12              | 13              | 7               |</p>
<table>
<thead>
<tr>
<th>Socioeconomic status total score (SE)</th>
<th>4.31 (0.13)</th>
<th>4.58 (0.14)</th>
<th>4.53 (0.14)</th>
<th>4.37 (0.14)</th>
<th>4.5 (0.21)</th>
<th>4.55 (0.18)</th>
<th>4.13 (0.16)</th>
<th>4.62 (0.22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Subtypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD-I</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD-II</td>
<td>14</td>
<td>5</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD-NOS</td>
<td>14</td>
<td>6</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (SE)a</td>
<td>23.73 (0.61)</td>
<td>20.49 (0.74)</td>
<td>22.12 (0.63)</td>
<td>22.09 (0.73)</td>
<td>23.22 (0.97)</td>
<td>21.03 (0.79)</td>
<td>24.24 (0.75)</td>
<td>19.95 (1.26)</td>
</tr>
<tr>
<td>Systolic Blood Pressure (SE)</td>
<td>110.36 (2.48)</td>
<td>109.65 (2.62)</td>
<td>111.81 (2.57)</td>
<td>108.2 (2.54)</td>
<td>111.46 (3.94)</td>
<td>112.15 (3.29)</td>
<td>109.25 (3.01)</td>
<td>107.15 (4.08)</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (SE)</td>
<td>68.73 (1.53)</td>
<td>67.52 (1.62)</td>
<td>69.51 (1.58)</td>
<td>66.74 (1.57)</td>
<td>70.21 (2.43)</td>
<td>68.8 (2.03)</td>
<td>67.25 (1.86)</td>
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<tr>
<td>ADHD, nace</td>
<td>16</td>
<td>6</td>
<td>8</td>
<td>14</td>
<td>6</td>
<td>2</td>
<td>10</td>
<td>4</td>
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<tr>
<td>Anxiety, nace</td>
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<td>21</td>
<td>11</td>
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<td>1</td>
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<tr>
<td>SUD, n</td>
<td>6</td>
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<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>ODD, n</td>
<td>14</td>
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<td>4</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Second-generation antipsychotics use, n</td>
<td>21</td>
<td>0</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>SSRI antidepressant use, n</td>
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<td>0</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Non-SSRI antidepressants use, n</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Lithium use, n</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>0</td>
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<tr>
<td>Stimulants, n</td>
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<td>2</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: BD = Bipolar Disorder; HC = healthy control; CACNA1C = calcium voltage-gated channel subunit alpha1 C; SE = standard error; n = number of participants; NOS = not otherwise specified; BMI = body mass index; RHI = Reactive Hyperemia Index; IQ = intelligence quotient; ADHD = attention deficit hyperactivity disorder; SUD = substance use disorder; ODD = oppositional defiant disorder; SSRI = selective serotonin reuptake inhibitor; LPI = Life Problems Inventory

a Significant main effect of diagnosis (p<0.05)
b Significant main effect of CACNA1C rs1006737 (p<0.05)
c Significant diagnosis x CACNA1C rs1006737 interaction (p<0.05)

### 4.2 Hardy-Weinberg Calculations
This study’s sample was found to meet Hardy-Weinberg equilibrium. There were 104 G-alleles and 38 A-alleles. Using these numbers, $\chi^2 = .369$ with a $p = .831$.

### 4.3 ROI Analysis

#### 4.3.1 Main Effects of Diagnosis

Relative to the HC adolescent group, BD adolescents had significantly greater ACC volume ($p = .022; \eta^2_p = .08$; **Figure 14**), vmPFC SA ($p = .029; \eta^2_p = .075$; **Figure 15**), and ACC SA ($p = .008; \eta^2_p = .105$; **Figure 16**), and significantly reduced vlPFC thickness ($p = .025; \eta^2_p = .075$; **Figure 17**). Refer to **Table 2** to see complete statistical information for ROI main effect of diagnosis results.

It is important to note that none of the ROIs with significant main effects of diagnosis survived FDR corrections for multiple comparisons using the Benjamini-Hochberg method.

<table>
<thead>
<tr>
<th>Table 2. ROI Differences between BD and HC Adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ROI EMM (SE)</strong></td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td><strong>vmPFC volume</strong></td>
</tr>
<tr>
<td><strong>vlPFC volume</strong></td>
</tr>
<tr>
<td><strong>Amygdala volume</strong></td>
</tr>
<tr>
<td>ROI</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Putamen volume</td>
</tr>
<tr>
<td>(175.52)</td>
</tr>
<tr>
<td>ACC volume*</td>
</tr>
<tr>
<td>(232.17)</td>
</tr>
<tr>
<td>vmPFC SA*</td>
</tr>
<tr>
<td>(64.75)</td>
</tr>
<tr>
<td>vlPFC SA</td>
</tr>
<tr>
<td>(101.16)</td>
</tr>
<tr>
<td>ACC SA*</td>
</tr>
<tr>
<td>(67.76)</td>
</tr>
<tr>
<td>vmPFC thickness</td>
</tr>
<tr>
<td>(0.03)</td>
</tr>
<tr>
<td>vlPFC thickness*</td>
</tr>
<tr>
<td>(0.024)</td>
</tr>
<tr>
<td>ACC thickness</td>
</tr>
<tr>
<td>(0.03)</td>
</tr>
</tbody>
</table>

Abbreviations: ROI = regions of interest, BD = bipolar disorder, HC = healthy control, EMM = estimated marginal mean, SE = standard error, FDR = false discovery rate, SA = surface area, vmPFC = ventromedial prefrontal cortex, vlPFC = ventrolateral prefrontal cortex, ACC = anterior cingulate cortex

*Significant main effect of diagnosis
**Figure 14.** Main effect of diagnosis on ACC volume ($p<0.05$). The BD group had significantly larger ACC volume relative to the HC group.

**Figure 15.** Main effect of diagnosis on vmPFC SA ($p<0.05$). The BD group had significantly larger vmPFC SA relative to the HC group.
Figure 16. Main effect of diagnosis on ACC SA ($p<0.05$). The BD group had significantly larger ACC SA relative to the HC group.

Figure 17. Main effect of diagnosis on vlPFC thickness ($p<0.05$). The BD group had significantly smaller vlPFC SA relative to the HC group.

4.3.2 Main Effects of CACNA1C rs1006737
<table>
<thead>
<tr>
<th>ROI</th>
<th>A allele (n=34)</th>
<th>G allele (n=37)</th>
<th>Main Effect of rs1006737</th>
<th>Diagnosis x rs1006737 Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated Marginal Mean (SE)</td>
<td>Bipolar disorder (n=14)</td>
<td>Healthy control (n=20)</td>
<td>Total (n=34)</td>
</tr>
<tr>
<td>Amygdala volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3357.64 (84.68)</td>
<td>3221.16 (67.25)</td>
<td>3289.4 (50.52)</td>
<td>3338.59 (58.55)</td>
</tr>
<tr>
<td>Putamen volume</td>
<td>12017.18 (285.88)</td>
<td>12021.81 (227.05)</td>
<td>12019.5 (170.57)</td>
<td>12150.25 (197.68)</td>
</tr>
<tr>
<td>ACC volume$^a$</td>
<td>11192.14 (381.44)</td>
<td>9854.49 (296.27)</td>
<td>10523.32 (237.87)</td>
<td>9988.67 (249.32)</td>
</tr>
<tr>
<td>vmPFC volume</td>
<td>12302.78 (330.67)</td>
<td>11830.35 (256.83)</td>
<td>12066.56 (206.21)</td>
<td>11828.06 (216.13)</td>
</tr>
<tr>
<td>vIPFC volume</td>
<td>20292.32 (540.18)</td>
<td>19251.16 (429.02)</td>
<td>19771.74 (322.3)</td>
<td>19897.17 (373.53)</td>
</tr>
<tr>
<td>ACC SA$^ab$</td>
<td>3595.02 (111.32)</td>
<td>3136.8 (86.46)</td>
<td>3365.91 (69.42)</td>
<td>3197.39 (72.76)</td>
</tr>
<tr>
<td>vmPFC SA</td>
<td>4108.06 (107.31)</td>
<td>3801.4 (82.47)</td>
<td>3954.73 (67.41)</td>
<td>3890.64 (68.96)</td>
</tr>
<tr>
<td>vIPFC SA</td>
<td>6457.6 (166.02)</td>
<td>6019.84 (128.92)</td>
<td>6238.72 (96.44)</td>
<td>6332.91 (111.01)</td>
</tr>
<tr>
<td>ACC thickness</td>
<td>2.79 (0.04)</td>
<td>2.77 (0.03)</td>
<td>2.78 (0.03)</td>
<td>2.76 (0.03)</td>
</tr>
<tr>
<td>vmPFC thickness</td>
<td>2.58 (0.05)</td>
<td>2.68 (0.04)</td>
<td>2.63 (0.03)</td>
<td>2.64 (0.03)</td>
</tr>
<tr>
<td>vIPFC thickness</td>
<td>2.71 (0.04)</td>
<td>2.79 (0.03)</td>
<td>2.75 (0.02)</td>
<td>2.73 (0.03)</td>
</tr>
</tbody>
</table>

Abbreviations: SE = standard error, ROI = region of interest, ACC = anterior cingulate cortex, vmPFC = ventromedial prefrontal cortex, vIPFC = ventrolateral prefrontal cortex, SA = SA, FDR = false discovery rate

$^a$ Significant main of effect of CACNA1C rs1006737 ($p<0.05$)

$^b$ Significant BD diagnosis x CACNA1C rs1006737 interaction ($p<0.05$)
Relative to the non-carriers adolescent group, A-carrier adolescents had significantly greater ACC volume ($p=.039; \eta^2_p=.066)$; **Figure 18** and ACC SA ($p=.026; \eta^2_p=.076); **Figure 19**. Refer to Table 3 to see complete statistical information for ROI main effect of rs1006737 results.

It is important to note that none of the ROIs with significant main effects of rs1006737 survived FDR corrections for multiple comparisons using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995).

![Figure 18](image)

**Figure 18.** Main effect of CACNA1C rs1006737 on ACC volume ($p<0.05$). The A-carrier group had significantly larger ACC volume relative to the non-carrier group.
Figure 19. Main effect of CACNA1C rs1006737 on ACC SA ($p<0.05$). The A-carrier group had significantly larger ACC SA relative to the non-carrier group.

4.3.3 Diagnosis-by-rs1006737 Interaction Effects

A diagnosis-by-rs1006737 interaction effect was found in ACC SA ($p=.041$; $\eta^2_p=.064$; Figure 20). Post-hoc analysis revealed that BD-A-carriers had significantly larger SA relative to both BD non-carriers and HC A-carriers. Refer to Table 3 to see complete statistical information for ROI diagnosis-by-rs1006737 interaction effect results.

It is important to note that none of the ROIs with significant diagnosis-by-rs1006737 interactions effects survived FDR corrections for multiple comparisons using the Benjamini-Hochberg method.
Figure 20. Diagnosis-by-rs1006737 interaction effects on ACC SA (p<0.05). The BD A-carrier groups had significantly larger SA relative to both the BD non-carriers group and the HC A-carrier group.

4.3.4 Sensitivity Analysis

The following demographic/clinical variables had a significant main effect of diagnosis, rs1006737, and/or diagnosis-by-rs1006737 interaction: BMI, ADHD, Anxiety, SUD, ODD, second generation antipsychotic use, SSRI antidepressant use, non-SSRI antidepressant use, and lithium use. In the whole sample, BMI was significantly correlated with vmPFC thickness (p<.001) and vlPFC thickness (p=.003). Adding BMI to the GLM model revealed no significant difference in results for vmPFC thickness or vlPFC thickness. Within the BD sample, lifetime non-SSRI antidepressant use was significantly correlated with vmPFC thickness, whereas lifetime SUD was significant associated with ACC thickness. Adding lifetime non-SSRI antidepressant use to the GLM model for vmPFC thickness revealed no significant difference in results; the same was also true for
adding lifetime SUD to the GLM model for ACC thickness. In sum, no demographic/clinical characteristic had any significant effect on results from this study.

4.4 Vertex-wise Whole-brain Exploratory Analysis

4.4.1 Main Effects of Diagnosis

Relative to the HC adolescent group, BD adolescents were found to have a significantly larger LH occipital/parietal SA cluster ($p=.0112$; Figure 21; Figure 22), RH frontal/ACC SA cluster ($p=.0083$; Figure 23; Figure 24), RH insula/prefrontal SA cluster ($p=.0126$; Figure 25; Figure 26), and RH fusiform/occipital SA cluster ($p=.01340$; Figure 27; Figure 28).

See Table 4 for a complete list of statistics on vertex-wise whole-brain main effect of diagnosis results.

<table>
<thead>
<tr>
<th>Peak Vertex Label</th>
<th>Encapsulated Regions</th>
<th>Cortical Measure</th>
<th>Hemisphere</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>inferior parietal</td>
<td>inferior parietal, lateral occipital cortex, supramarginal gyrus</td>
<td>surface area</td>
<td>left</td>
<td>0.01120</td>
</tr>
<tr>
<td>medial orbital frontal</td>
<td>medial orbital frontal, rostral anterior cingulate, superior frontal, paracentral, postcentral</td>
<td>surface area</td>
<td>right</td>
<td>0.00830</td>
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<tr>
<td>Postcentral</td>
<td>Insula, opercularis, rostral middle frontal, precentral, postcentral</td>
<td>Surface area</td>
<td>Right</td>
<td>0.01260</td>
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<tr>
<td>------------</td>
<td>-------------------------------------------------</td>
<td>--------------</td>
<td>-------</td>
<td>---------</td>
</tr>
<tr>
<td>Fusiform</td>
<td>Fusiform, lateral occipital</td>
<td>Surface area</td>
<td>Right</td>
<td>0.01340</td>
</tr>
</tbody>
</table>

**Figure 21.** Main effect of diagnosis on a LH parietal/occipital SA cluster ($p<0.05$). The BD group had significantly larger cluster SA relative to the HC group.

**Figure 22.** LH SA lateral and medial uncorrected (left), corrected (middle) brain maps
(p<.05) from vertex-wise whole-brain analysis for main effect of diagnosis on a parietal/occipital cluster, as well as the comparison age-by-rs1006737 interaction brain map (right; p<.05). The DODS model was chosen in this instance, as an overlap was found between the LH parietal/occipital SA cluster and the accompanying age-by-diagnosis interaction brain maps.

**Figure 23.** Main effect of diagnosis on a RH frontal/ACC SA cluster (p<0.05). The BD group had significantly larger cluster SA relative to the HC group.

**Figure 24.** RH SA lateral and medial uncorrected (left), corrected (middle) brain maps (p<.05) from vertex-wise whole-brain analysis for main effect of diagnosis on a
frontal/ACC cluster, as well as the comparison age-by-rs1006737 interaction brain map (right; \(p<0.05\)). The DODS model was chosen in this instance, as an overlap was found between the RH frontal/ACC SA cluster and the accompanying age-by-diagnosis interaction brain maps.

**Figure 25.** Main effect of diagnosis on a RH insula/prefrontal SA cluster \((p<0.05)\). The BD group had significantly larger cluster SA relative to the HC group.

**Figure 26.** RH SA lateral and medial uncorrected (left), corrected (middle) brain maps \((p<0.05)\) from vertex-wise whole-brain analysis for main effect of diagnosis on an insula/prefrontal cluster, as well as the comparison age-by-rs1006737 interaction brain
map (right; $p<.05$). The DODS model was chosen in this instance, as an overlap was found between the RH insula/prefrontal SA cluster and the accompanying age-by-diagnosis interaction brain maps.

**Figure 27.** Main effect of diagnosis on a RH fusiform/occipital SA cluster ($p<0.05$). The BD group had significantly larger cluster SA relative to the HC group.

**Figure 28.** RH SA lateral and medial uncorrected (left), corrected (middle) brain maps ($p<.05$) from vertex-wise whole-brain analysis for main effect of diagnosis on a fusiform/occipital cluster, as well as the comparison age-by-rs1006737 interaction brain map (right; $p<.05$). The DODS model was chosen in this instance, as an overlap was
found between the RH fusiform/occipital SA cluster and the accompanying age-by-diagnosis interaction brain maps.

4.4.2 Main Effects of CACNA1C rs1006737

Relative to non-carriers, A-carriers were found to have significantly larger SA in a LH cuneus/pericalcarine cluster ($p=0.0068$; Figure 29; Figure 30), a LH fusiform/LOC cluster ($p=0.0205$; Figures 31 and Figure 32), and a RH fusiform/occipital cluster ($p=0.0324$; Figures 33 and Figure 34).

See Table 5 for a complete list of statistics on vertex-wise whole-brain main effect of CACNA1C rs1006737 results.

<table>
<thead>
<tr>
<th>Peak Vertex Label</th>
<th>Encapsulated Regions</th>
<th>Cortical Measure</th>
<th>Hemisphere</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td>pericalcarine</td>
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<td>surface area</td>
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</tr>
<tr>
<td>lateral occipital</td>
<td>fusiform, lateral occipital</td>
<td>surface area</td>
<td>left</td>
<td>0.0205</td>
</tr>
<tr>
<td>fusiform</td>
<td>fusiform, lateral occipital</td>
<td>surface area</td>
<td>right</td>
<td>0.0324</td>
</tr>
</tbody>
</table>
Figure 29. Main effect of \textit{CACNA1C} rs1006737 on a LH cuneus/pericalcarine SA cluster ($p<0.05$). The A-carrier group had significantly larger cluster SA relative to the non-carrier group.

Figure 30. LH SA lateral and medial uncorrected (left), corrected (middle) brain maps ($p<0.05$) from vertex-wise whole-brain analysis for main effect of \textit{CACNA1C} rs1006737 on a cuneus/pericalcarine cluster, as well as the comparison age-by-rs1006737 interaction brain map (right; $p<.05$). The DODS model was chosen in this instance, as an overlap was found between the LH cuneus/pericalcarine SA cluster and the accompanying age-by-rs1006737 interaction brain maps.
**Figure 31.** Main effect of CACNA1C rs1006737 on a LH fusiform/LOC SA cluster ($p<0.05$). The A-carrier group had significantly larger cluster SA relative to the non-carrier group.

**Figure 32.** LH SA lateral and medial uncorrected (left), corrected (middle) brain maps ($p<.05$) from vertex-wise whole-brain analysis for main effect of CACNA1C rs1006737 on a LOC cluster, as well as the comparison age-by-rs1006737 interaction brain map.
The DODS model was chosen in this instance, as an overlap was found between the LH LOC SA cluster and the accompanying age-by-rs1006737 interaction brain maps.

**Figure 33.** Main effect of *CACNA1C* rs1006737 on a RH fusiform/occipital SA cluster (*p*<0.05). The A-carrier group had significantly larger cluster SA relative to the non-carrier group.

**Figure 34.** RH SA lateral and medial uncorrected (left), corrected (middle) brain maps
(p<.05) from vertex-wise whole-brain analysis for main effect of CACNA1C rs1006737 on a fusiform/occipital cluster, as well as the comparison age-by-rs1006737 interaction brain map (right; p<.05). The DOSS model was chosen in this instance, as no overlap was found between the RH fusiform/occipital SA cluster and the accompanying age-by-rs1006737 interaction brain maps.

4.4.3 Diagnosis-by-rs1006737 Interaction Effects

Significant diagnosis-by-rs100673 interactions were found on a LH prefrontal volume cluster (p=.0342; Figure 35; Figure 36), a LH prefrontal SA cluster (p=.0203; Figure 37; Figure 38), and a RH occipital/parietal SA cluster (p=.0432; Figure 39; Figure 40). Post-hoc analyses were performed to break down these interactions. The LH prefrontal volume cluster interaction was such that BD A-carriers had larger volume relative to BD non-carriers (p=.012) and HC A-carriers (p=.003), while HC A-carriers had smaller volume relative to HC non-carriers (p=.016). The LH prefrontal SA cluster interaction was such that BD A-carriers had larger SA relative to BD non-carriers (p=.001) and HC A-carriers (p=.001). The RH occipital/parietal SA cluster interaction was such that BD A-carriers had large SA relative to BD non-carriers (p=.038) and HC A-carriers (p=.001), while HC A-carriers had smaller volume relative to HC non-carriers (p=.008).

See Table 6 for a complete list of statistics on vertex-wise whole-brain diagnosis-by-rs1006737 interaction effect results.

<table>
<thead>
<tr>
<th>Table 6. Significant Cluster-Wise Diagnosis-by-rs1006737 Interaction Results from Vertex-Wise Whole-Brain Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Vertex Label</td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>pars opercularis</td>
</tr>
<tr>
<td>lateral orbitofrontal</td>
</tr>
<tr>
<td>lateral occipital</td>
</tr>
</tbody>
</table>

**Figure 35.** Vertex-wise whole-brain diagnosis-by-rs1006737 interaction effects on a LH prefrontal volume cluster (*p<0.05). BD A-carriers had significantly larger cluster volume relative to the BD non-carriers and HC A-carriers. HC A-carriers had significantly smaller cluster volume relative to the HC non-carriers.
Figure 36. LH volume lateral and medial uncorrected (left), corrected (middle) brain maps ($p<.05$) from vertex-wise whole-brain analysis for diagnosis-by-rs1006737 interaction effects on a prefrontal cluster, as well as the comparison age-by-rs1006737 interaction brain map (right; $p<.05$). The DODS model was chosen in this instance, as an overlap was found between the LH prefrontal volume cluster and the accompanying age-by-interaction interaction brain maps.

Figure 37. Vertex-wise whole-brain diagnosis-by-rs1006737 interaction effects on a LH prefrontal SA cluster ($*p<0.05$). BD A-carriers had significantly larger cluster SA relative to the BD non-carriers and HC A-carriers.
Figure 38. LH SA lateral and medial uncorrected (left), corrected (middle) brain maps ($p<.05$) from vertex-wise whole-brain analysis for diagnosis-by-rs1006737 interaction effects on a prefrontal cluster, as well as the comparison age-by-rs1006737 interaction brain map (right; $p<.05$). The DOSS model was chosen in this instance, as no overlap was found between the LH prefrontal SA cluster and the accompanying age-by-interaction interaction brain maps.

Figure 39. Vertex-wise whole-brain diagnosis-by-rs1006737 interaction effects on a RH occipital/parietal SA cluster (*=$p<0.05$). BD A-carriers had significantly larger cluster SA relative to the BD non-carriers and HC A-carriers. HC A-carriers had significantly smaller cluster SA relative to the HC non-carriers.
Figure 40. RH SA lateral and medial uncorrected (left), corrected (middle) brain maps ($p<.05$) from vertex-wise whole-brain analysis for diagnosis-by-rs1006737 interaction effects on a occipital/parietal cluster, as well as the comparison age-by-rs1006737 interaction brain map (right; $p<.05$). The DODS model was chosen in this instance, as an overlap was found between the RH occipital/parietal SA cluster and the accompanying age-by-interaction interaction brain maps.

5.0 Discussion

This study examined the relationship between CACNA1C rs10006737 and brain structure, as measured by cortical/subcortical volume and/or SA and/or thickness, in adolescents with BD and HCs. As of this writing, this study is the first of its kind to conduct such an examination in an adolescent population. Effects tested include main effects of BD diagnosis, main effects of CACNA1C rs10006737, and diagnosis-by-rs10006737 interaction effects on brain structure measurements.
5.1 Summary of Findings

Refer to Table 7 for a summary of findings for main effects of BD diagnosis from ROI and vertex-wise whole-brain exploratory analyses.

Refer to Table 8 for a summary of findings for main effects of CACNA1C rs1006737 from ROI and vertex-wise whole-brain exploratory analyses.

Refer to Table 9 for a summary of findings for diagnosis-by-rs1006737 interaction effects from ROI and vertex-wise whole-brain exploratory analyses.

5.1.1 ROI Analysis Findings

It is important to note that no ROI findings remained significant after performing Benjamini-Hochberg FDR corrections for the testing of multiple ROIs. However, due to the exploratory nature of this study, findings which were found to be significant after Bonferroni corrections, for running multiple tests with each ROI, are discussed below.

From this study’s ROI analysis main effects of diagnosis were found, such that, relative to HCs, BD had greater volume and SA in the ACC, greater vmPFC SA, and reduced vlPFC thickness. This study’s tertiary hypothesis had hypothesized reduced volume and/or SA and/or thickness in the vlPFC, vmPFC, ACC, amygdala, and putamen, for BDs relative to HCs. As a results, findings of reduced vlPFC thickness in BDs relative to HCs is consistent with this study’s tertiary hypothesis, while findings of greater volume and/or SA in the ACC and vmPFC are inconsistent with this study’s tertiary hypothesis.
There was also found to be main effects of CACNA1C rs1006737 such that, relative to non-carriers, A-carriers had greater ACC volume and SA. This study’s primary hypothesis had hypothesized greater volume and/or SA and/or thickness in the vlPFC, vmPFC, ACC, and amygdala, for A-carriers relative to non-carriers. As a result, findings of greater ACC SA in A-carriers relative to non-carriers are consistent with this study’s primary hypothesis.

There was found to be a diagnosis-by-rs1006737 interaction effect in ACC SA, such that BD A-carriers had greater SA relative to both the BD non-carrier group and the HC A-carrier group. This study’s secondary hypothesis had hypothesized an interaction effect only in the putamen. As such, the finding of an interaction effect in the ACC is inconsistent with this study’s secondary hypothesis.

Sensitivity analyses of demographic/clinical variables on ROIs revealed no significant differences in results. As such, it can be assumed that the ROI findings from this study were not significantly affected by any of the recorded demographic/clinical characteristics, accounting for potential confounds.

5.1.2 Vertex-wise Whole-brain Analysis Findings

From this study’s vertex-wise exploratory whole-brain analysis, main effects of diagnosis were found such that, relative to HCs, BDs had greater SA in a LH parietal/occipital cluster, RH frontal/ACC cluster, RH insula/prefrontal cluster, and a RH fusiform/occipital cluster.
There were also found to be main effects of CACNA1C rs1006737 such that, relative to non-carriers, A-carriers had greater SA in a LH cuneus/pericalcarine cluster, LH fusiform/LOC cluster, and a RH fusiform/occipital cluster.

Finally, there were also found to be diagnosis-by-rs1006737 interaction effects in LH prefrontal volume and SA clusters, and a RH occipital/parietal SA cluster. These interactions were such that BD A-carriers had greater volume/SA relative to BD non-carriers and HC A-carriers in all three of these clusters, while, relative to HC non-carriers, HC A-carriers had reduced volume/SA in the LH prefrontal volume cluster and RH occipital/parietal cluster.

| Table 7. Summary of Findings from ROI and Vertex-wise Whole-brain analyses examining main effects of BD diagnosis in an Adolescent Sample |
|---|---|---|---|
| Hemisphere | Brain Region(s) | Cortical Measure | Findings |
| Bilateral | ACC | Volume | BD > HC |
| Bilateral | ACC | SA | BD > HC |
| Bilateral | vmPFC | SA | BD > HC |
| Bilateral | vIPFC | Thickness | BD < HC |
| Left | Parietal & Occipital | SA | BD > HC |
| Right | Frontal & ACC | SA | BD > HC |
| Right | Insula & Prefrontal | SA | BD > HC |
### Table 8. Summary of Findings from ROI and Vertex-wise Whole-brain analyses examining main effects of CACNA1C rs1006737 in an Adolescent Sample

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Brain Region(s)</th>
<th>Cortical Measure</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral</td>
<td>ACC</td>
<td>Volume</td>
<td>A-carriers &gt; non-carriers</td>
</tr>
<tr>
<td>Bilateral</td>
<td>ACC</td>
<td>SA</td>
<td>A-carriers &gt; non-carriers</td>
</tr>
<tr>
<td>Left</td>
<td>Cuneus &amp; Pericalcarine</td>
<td>SA</td>
<td>A-carriers &gt; non-carriers</td>
</tr>
<tr>
<td>Left</td>
<td>Fusiform &amp; LOC</td>
<td>SA</td>
<td>A-carriers &gt; non-carriers</td>
</tr>
<tr>
<td>Right</td>
<td>Fusiform &amp; Occipital</td>
<td>SA</td>
<td>A-carriers &gt; non-carriers</td>
</tr>
</tbody>
</table>

### Table 9. Summary of Findings from ROI and Vertex-wise Whole-brain analyses examining diagnosis-by-rs1006737 Interaction Effects in an Adolescent Sample

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Brain Region(s)</th>
<th>Cortical Measure</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral</td>
<td>ACC</td>
<td>SA</td>
<td>BD A-carriers &gt; BD non-carriers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BD A-carriers &gt; HC A-carriers</td>
</tr>
<tr>
<td>Left</td>
<td>Prefrontal</td>
<td>Volume</td>
<td>BD A-carriers &gt; BD non-carriers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BD A-carriers &gt; HC A-carriers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC A-carriers &lt; HC non-carriers</td>
</tr>
</tbody>
</table>
5.2 Interpretation of Findings

5.2.1 Brain Regions Implicated

Most effects of CACNA1C rs1006737 in this study were found in the ACC, prefrontal brain regions, and occipital brain regions. It is important to note that the ACC is the only ROI for which there was a significant effect of rs1006737 and diagnosis-by-rs1006737 interaction. Most of this study’s results were found with the vertex-wise whole-brain analysis. In terms of why our ROIs did not generally return significant findings, this may relate to our choice of ROIs, which was guided by a limited literature. Alternatively, this may relate to how the ROIs are defined by the anatomic atlas, which may not align precisely with any potential gene effects.

5.2.1.1 ACC

This study’s findings of greater bilateral ACC SA in BD adolescents relative to HCs is inconsistent with findings from previous literature which have found reduced ACC volume in BD adolescents when compared to HCs (Kaur et al., 2005; Wilke et al., 2004). The ACC is part of the brain’s limbic system, a system which is believed to be...
largely responsible for emotional processing (Bush et al., 2000). The ACC has been linked to brain functions related to emotional and cognitive appraisal, such as conflict monitoring, error detection, and reward processing (Botvinick et al., 2004; Shenhav et al., 2013). As BD symptomology includes cognitive deficits and emotional dysfunction, structural abnormalities in the ACC have been thought to be linked to BD-related cognitive and emotional symptoms. Therefore, adolescent BD-HC differences in ACC structure may be related to the development of BD-related cognitive and emotion-related symptomology.

Findings of greater bilateral ACC SA in adolescent CACNA1C rs1006737 A-carriers relative to non-carriers is consistent with a study by Wang and colleagues (2011) which found healthy adult A-carriers had greater ACC volume when compared to non-carriers. In previous literature, adult A-carriers have been found to exhibit reduced ACC activity during emotional processing tasks (Erk et al., 2014A), as well as during cognitive functioning tasks (Erk et al., 2014B). Taken together, these results suggest that rs1006737 can have an effect on ACC structure and function. As the ACC is a region known to be implicated in BD dysfunction (Kaur et al., 2005; Wilke et al., 2004), it is possible that rs1006737 may be involved in BD intermediate symptomatic phenotypes, such as ACC neurostructural and/or neurofunctional dysfunction. This possibility is given credence with this study’s finding of a diagnosis-by-rs1006737 interaction effect in adolescents such that BD A-carriers had greater ACC SA relative to BD non-carriers and HC A-carriers. These results suggest an effect of rs1006737 within the BD group, as well as an effect of diagnosis within the A-carrier group.

5.2.1.2 Prefrontal Cortex
This study’s findings of greater prefrontal SA in BDs relative to HCs are inconsistent with findings in the adolescent and adult BD literature. However, this study’s finding of reduced prefrontal thickness (specifically in the vmPFC), is consistent with findings in the adolescent and adult BD literature which have found evidence of reduced prefrontal volume and thickness in BDs relative to HCs (Phillips and Swartz, 2014; Phillips et al., 2014; Berk et al., 2011; Frey et al. 2013). Prefrontal brain regions are involved in vast array of brain functions, including cognitive function, emotional appraisal, and reward processing. Abnormal BD prefrontal structure may be related to abnormalities in the functioning as these regions, as suggested by the BD literature (Gruber et al., 2010; Fleck et al., 2012; Phillips and Swartz, 2014). Findings from this study suggest adolescent BD prefrontal cortical pathophysiology may be dichotomous such that reduced prefrontal thickness, but increased prefrontal SA is symptomatic. These findings highlight the necessity for viewing all three cortical measures of volume, SA, and thickness when examining BD brain neurostructural dysfunction, especially in the case of prefrontal brain regions.

This study’s findings of a diagnosis-by-rs1006737 interaction on LH prefrontal volume and SA are novel. Specifically, the interaction was such that BD A-carriers were found to have increased volume and SA relative to BD non-carriers and HC A-carriers. In addition HC A-carriers were found to have reduced volume relative to HC non-carriers for LH prefrontal volume only. These results suggest opposite effects of rs1006737 in the BD group relative to the HC group, and an effect of diagnosis within A-carriers. The adult CACNA1C rs1006737 literature has found evidence of greater prefrontal volume, as well as reduced prefrontal activity during cognitive functioning, in A-carriers relative to
non-carriers (Wang and colleagues, 2011; Soiero-de-Souza and colleagues, 2017). Taken together with findings from previous literature and findings from the current study of increased prefrontal SA in adolescents BDs relative to HCs, findings of a diagnosis-by-rs1006737 interaction suggest rs1006737 may be related to BD intermediate neuroimaging phenotypes. Specifically, rs1006737 may play a role in abnormal prefrontal volume/SA expansion during the development of BD.

Findings from this study suggest laterality in diagnosis-by-rs1006737 interactions on adolescent brain structure. Interactions effects were only found in the LH, suggesting this hemisphere is more vulnerable to these effects. The LH has been reported to have less regional interconnections compared to the RH, but more crucial regions hubs, suggesting LH specialization for more demanding processing tasks, such as cognitive functioning, language, and motor actions (Hervé et al., 2013). LH specific diagnosis-by-rs1006737 adolescent vulnerability suggests these effects may be related to BD-associated symptomology, such as cognitive dysfunction.

5.2.1.3 Occipital Cortex, Fusiform, Cuneus, and Pericalcarine

Findings of greater occipital and fusiform SA in BD adolescents relative to HCs are inconsistent with findings from previous adult and adolescent BD neurostructural literature. Several studies have found reduced cortical volume or thickness in the occipital and/or fusiform gyrus in BDs relative to HCs (Lyoo et al., 2006; Niu et al., 2017; Frazier et al., 2005). This study’s findings suggest occipital and fusiform SA may be affected differently, relative to cortical thickness, during BD adolescence. BD neurofunctional literature provides evidence of increased occipital and/or fusiform
activity during facial-emotional and emotional processing tasks (Pavuluri et al., 2008; Tesli et al., 2015). As occipital and fusiform brain regions are widely reported to be involved in various facial and facial-emotional processing, structural abnormalities in these brain regions may be related to dysfunction in these brain functions. BDs have been found to present with symptoms of emotional dysfunction and impairments in facial affect recognition (Getz et al., 2002), adding further credence to the possibility of BD adolescent occipital and/or fusiform abnormalities being linked to deficits in these two neuropsychological functions.

The fusiform gyrus, cuneus, and pericalcarine are all, for the most part, housed within the occipital cortex of the brain. As such, the current study’s findings of greater SA, for these brain regions, in rs1006737 A-carriers relative to non-carriers is consistent with findings from the CACNA1C rs1006737 adult neurostructural literature (Wang et al., 2011; Huang et al., 2016). Dima and colleagues (2013) found A-carriers exhibited increased activity in occipital brain regions during a facial affect processing tasks. Taken together, these findings suggest increased occipital SA in adolescents may be related to aberrant facial-emotional processing in A-carriers.

Findings of a diagnosis-by-rs1006737 interaction on RH occipital brain regions are novel to the current study. Specifically, the interaction was such that adolescent BD A-carriers were found to have increased SA relative to BD non-carriers and HC A-carriers. In addition adolescent HC A-carriers were found to have reduced SA relative to HC non-carriers. These results suggest opposite effects of rs1006737 on SA in BD adolescents relative to HCs. As both BD and rs1006737 have been independently associated with abnormalities in occipital structure, an interaction effects suggests that
rs1006737 is associated with BD intermediate occipital neurophenotypes, which may be further exasperated if an individual is both diagnosed with BD and is an A-carrier.

RH laterality was present in this study’s findings of a diagnosis-by-rs1006737 interaction on occipital SA. The RH is reportedly organized more efficiently than the LH, with greater interregional connections. As such, the RH is thought to be more specialized in broader functional processes involving the integration of input from several brain regions, such as visuospatial recognition (Hervé et al., 2013). RH laterality in these findings is in line with the possibility that rs1006737 affects BD intermediate functional neurophenotypes, such as facial-emotional processing, a broad-based brain function involving integration of input from multiple brain regions.

5.2.2 Surface Area

All of the main effect of CACNA1C rs1006737 results and diagnosis-by-rs1006737 interaction effect results from this study were found to be on cortical SA or volume. The same is true for most of this study’s main effect of BD diagnosis findings. For those results found for volume, a result was also found in the same region for SA, suggesting that the volume results were driven by the SA results, as cortical volume is a composite of cortical SA and thickness. Therefore, it seems that all of these findings were primarily on SA. The SA-specific findings for this study can potentially be attributed to the genetic distinctions between cortical SA and thickness, as well as the developmental trajectory of the adolescent brain.

Previous studies examining the genetic relationship between cortical SA and thickness had found a low genetic correlation between the two cortical measures,
indicating distinct genetic influences (Panizzon et al., 2009; Winkler et al., 2010). SA-specific findings in the present study may potentially be due to CACNA1C rs1006737 being primarily a genetic component of SA development, rather than thickness. Rs1006737 may affect SA by playing a role in one or multiple factors which impact SA development, including synaptogenesis, dendritic aborization, myelination, and neural connectivity (Rimol et al., 2012).

As the adolescent brain is developing, rs1006737 may be playing a role in guiding its developmental trajectory. Previous literature provides evidence that the adolescent brain is developing such that SA rapidly increases until it reaches a peak in late childhood/early adolescence, after which it beings to slowly decline (Wierenga et al., 2013; Vijayakumar et al., 2016). Given this information, it is possible that SA-specific findings from the present study may be due to rs1006737-driven aberrant overexpansion of the already expanding adolescent cortical SA. It is likely that rs1006737-driven SA overexpansion is occurring most strongly around the developmental time during which SA expansion is peaking, which previous studies have found occurs during late childhood/early adolescence (Wierenga et al., 2013). If this is true, it seems that these SA changes persist even after SA expansion transitions into slow decline during adolescence. This possibility is given credence by the fact that rs1006737 is associated with increased regional and total GM brain structure in adults as well (Ou et al., 2015). Another possibility is that rs1006737 affects SA in later adolescence and adulthood by leading to a milder decline, or even reversing the developmental trajectory of SA such that expansion continues.
5.3 CACNA1C rs1006737 and Brain Structure: Potential Physiological Underpinnings

The exact physiological mechanism(s) through which CACNA1C rs1006737 influences brain morphology has not yet been fully elucidated. However, there are various potential pathways through which this influence may take shape. The literature indicates that rs1006737 influences downstream calcium signaling pathways which can play roles in physiological functions linked to brain morphology, such as gene transcription/translation, neurogenesis, apoptosis, and neuroplasticity (Soeiro-de-Souza et al., 2017). As the rs1006737 risk allele has been linked to increased levels of intracellular calcium concentration (Uemura et al., 2016), aberrant calcium signaling is most likely due to high levels of intracellular calcium. High levels of intracellular calcium can lead to erratic activity of calcium-linked pathways. Additionally, calcium plays an important role in the excitability of neuronal membranes, a function which high levels of intracellular calcium can disrupt. As a result, in a theoretical model linking CACNA1C rs1006737 to brain structure, having the risk allele would make neurons more likely to have higher levels of intracellular calcium, higher activation of calcium-linked intracellular pathways, and higher membrane excitability, which in turn would have effects on brain morphology.

Aberrant calcium activity and intracellular concentration levels have been widely reported in BD. Excitatory-inhibitory imbalances are thought to play an important role in the fluctuations in mood between spectrums of depression and mania which characterizes BD, such that mania is characterized by overexcitation and depression is characterized by
overinhibition. The inositol depletion hypothesis states that overactivity in either the excitatory or inhibitory neurons may be driven by overactivity in the phosphoinositide signal pathway that in turns causes overactivity in calcium signaling pathways (Berridge, 2014). In turn, overactivity in calcium signaling pathways can have effects on brain morphology as mentioned in the previous paragraph. As CACNA1C rs1006737 is associated with increased calcium intracellular concentrations and signaling, its can interact with BD by exasperating BD-related aberrant signaling pathways and the excitatory-inhibitory imbalance. Such exasperation of BD cellular pathophysiology provides a potential explanation for the current study’s findings of diagnosis-by-rs1006737 interactions such that BD-A carriers were most severely affected.

5.4 The Potential of Calcium Channel Antagonists for BD Treatment

Given strong evidence of dysfunctional calcium signaling in BD, the use of calcium channel antagonists as a BD treatment drug class should be further investigated. At the time of this writing, there is a high level of clinical uncertainty as to the effectiveness of using calcium channel antagonists as a treatment drug for BD, as exemplified by the low quantity and quality of available data from randomized control trials (Cipriani et al., 2016). Several clinical trials have provided evidence of some therapeutic effects of calcium channel antagonists in BD patients, but these findings are reported as mixed results due to the poor lipophilicity of these drugs, a drug trait required for the effective crossing of the blood-brain-barrier (Bhat et al., 2012). Examples of calcium channel antagonists that have provided some evidence of BD therapeutic
efficacy include nimodipine, verapamil, and isradipine (Pazzaglia et al., 1993; Wisner et al., 2002; Ostacher et al., 2014). However, studies also report poor BD therapeutic efficacy for these same drugs (Cipriani et al., 2016). No calcium channel antagonist has been proved clinically effective as a treatment drug for BD, but this may be due to the small number of clinical research trials which have pursued this topic.

A potential reason for the low quantity of clinical research conducted regarding calcium channel antagonists as a potential drug treatment class for BD is the adverse effects of these drugs. Most of the reported side effects of calcium channel antagonists are a result of the peripheral actions of these drugs on the cardiovascular system. Given that calcium channel antagonists are often used in the treatment of cardiovascular conditions, such as hypertension and angina, the use of these drugs in individuals without cardiovascular conditions may lead to cardiovascular side effects, such as changes in blood pressure and vascular stiffening (Chobanian et al., 1996). Other potential side effects of the use of calcium channel antagonists in treating BD including increased risk of breast cancer, increased risk of suicide, precipitation of depression, and neurotoxicity (Holmes et al., 2013; Li et al., 2014; Lindberg et al., 1998; Hullet et al., 1988; Price et al., 1986).

Inconclusive findings as to the efficacy of calcium channel antagonists as a potential drug treatment class for BD, along with their wide side effect profile, suggests that these drugs hold very little or no promise for the treatment of BD. However, this conclusion would be premature because of the low quality and quantity of clinical trial data available. As calcium channel dysfunction is known to play an important role in BD pathophysiology, calcium channel antagonists as a potential drug therapy for BD should
not be discounted so quickly. Given the association of CACNA1C rs1006737 with BD intermediate phenotypes, future clinical drug trials should examine the potential for calcium channel antagonist BD drug treatment in targeted cases. For example, given its association with increased intracellular calcium concentrations, it may be possible that carriers of the rs1006737 risk allele would demonstrate greater therapeutic benefit from this class of drugs. In addition, in order to avoid various adverse peripheral side effects, special attention should be paid to individual cardiovascular profiles prior to inclusion in clinical trials. In sum, further clinical trials should be conducted examining the efficacy and acceptability of using calcium channel antagonists in BD drug treatment, with BD participants being chosen due to their individual potential suitability for this treatment.

5.5 Limitations

This study has several inherent limitations which warrant discussion. First, despite being among the larger adolescent BD neuroimaging studies, this study was only powered to detect an effect size of \( d = 0.67 \). As such, this study was not powered to detect small effect sizes for the selected two-way contrasts. An increased sample size could also potentially have improved power to detect effects and may have allowed some of the findings to survive Benjamini-Hochberg FDR corrections. In addition, due to the small number of participants specifically within the homozygous risk allele group (AA), this study lacked the statistical power to conduct a genotype grouping analysis (AA vs. AG vs. GG). A genotype analysis would have allowed for an examination of dose-dependent effects of CACNA1C rs1006737. As previous studies provide evidence of
dose-dependent effects of rs1006737 on brain structure (Kempton et al., 2009; Franke et al., 2010), such an analysis could potentially have yielded more noteworthy results. These power consideration indicate an increased potential for false negative findings. Also related to the risk of false negative findings is heterogeneity within the control group; several HC participants were on medications, such as stimulants, and/or had been diagnosed with psychiatric conditions, including anxiety and ADHD. Although these participants were a small minority, this may have impacted findings in concert with the aforementioned power limitations.

Second, the cross-sectional and observational design of this study precludes the application of directional causality to explain the relationship found between CACNA1C rs1006737 and neurostructure in adolescent BD.

Lastly, there are a number of measures which could potentially have been added to this study to provide a more holistic understanding for the role of CACNA1C rs1006737 in adolescent BD pathology. For example, the additional examination of peripheral biomarkers, such as proteins levels, inflammatory markers, triglyceride levels, and cortisol levels, could have been informative in forming a mechanistic understanding of the link between rs1006737 and adolescent BD neurostructure. The addition of cognitive measures would have potentially allowed this study to link neurostructural findings to neuropsychology, possibly providing a better understanding of how rs1006737 neurstructural effects can manifest as psychiatric symptoms. Other measures which could potentially have been added include Diffusion Tensor Imaging (DTI), for information regarding white matter tracts, and functional imaging, for imaging regarding brain function.
5.6 Future Studies

5.6.1 The Relationship between rs1006737 and Adolescent Brain White Matter

Future studies can build on findings from the current study by examining the relationship between \textit{CACNA1C} rs1006737 and white matter (WM) structure in adolescent BD. Using DTI, WM abnormalities can be identified, allowing for a more holistic understanding of neurostructure. The adolescent and adult BD literature provides evidence of WM dysfunction in BD neuropathology (Anderson et al., 2013; Nortje et al., 2013). In addition, the adult \textit{CACNA1C} rs1006737 literature provides evidence of WM abnormalities linked to this SNP (Dietche et al., 2014; Woon et al., 2014; Mallas et al., 2016). However, to date, no study has examined the link between rs1006737 and DTI-derived WM in adolescents with BD and HCs. Given evidence from previous literature and the fact that WM is undergoing rapid change during adolescence, it seems likely that the rs1006737 would be playing a role in BD intermediate WM phenotypes.

5.6.2 The Relationship between rs1006737 and Adolescent Brain Function

Findings from the current study can be built upon by examining the relationship between \textit{CACNA1C} rs100673 and brain function in adolescent with BD and HCs. Using fMRI, future studies can investigate whether there are abnormalities in brain functioning during specific tasks, or at rest. Such functional results can potentially be used to interpret findings from the current study by linking neurostructure with neurofunction. Both the adolescent and adult BD literature and \textit{CACNA1C} rs1006737 literature provides...
evidence of significant neurofunctional abnormalities due to BD and/or the rs1006737 risk allele (Phillips and Swartz, 2014; Ou et al., 2015). However, as of this writing, only one study has examined the relationship between CACNA1C rs1006737 and brain function in BD adolescents (Sumner et al., 2015). Specific neurofunctional studies that would be of interest would include cognitive and/or facial-emotional tasks. Examining brain function in BD and HC adolescents during these tasks would allow the current study’s interpretation of structural results in prefrontal and occipital brain regions to be respectively tested.

5.6.3 The Relationship between rs1006737 and Adolescent Neuropsychology

Examining the relationship between CACNA1C rs1006737 and neuropsychology in adolescents with BD and HCs could potentially add to findings from the current study. As of this writing, no study has examined this relationship in adolescents.

Neuropsychological tests which can be used include working memory, attention, facial-emotional recognition, inhibition, verbal fluency, memory and several other neuropsychological domains. The adolescent and adult BD literature has demonstrated BD neuropsychological dysfunction (Bora et al., 2008), while the adult rs1006737 literature has found evidence of similar dysfunction in risk allele carriers (Ou et al., 2015). Examining neuropsychology allows for an understanding of how BD and rs1006737 may interact in order to manifest adolescent BD intermediate psychiatric phenotypes which can be linked to neuropsychological dysfunction. Specifically, examining the relationship between rs1006737 and performance during cognitive and facial-emotional processing tasks would allow a testing of the interpretation for this study’s findings on brain structure in prefrontal and occipital brain regions.
5.6.4 Clinical Treatment Studies and Genotyping of rs1006737

Calcium signaling dysfunction is believed to play a significant role in BD cellular pathophysiology (Berridge, 2014). Future studies examining the efficacy of calcium-linked BD treatment drugs should consider rs1006737 during drug treatment. Currently used BD drugs, such as lithium (Malhi et al., 2013; Alda, 2015), and potential BD drugs, such as calcium channel antagonists, are linked to calcium in their pharmacological mechanism as a BD therapeutic (Cipriani et al., 2016). In fact, calcium channel antagonists were tested as potential BD treatment drugs because of the role calcium plays in BD.

Given the important role of calcium in BD cellular pathophysiology, and the calcium-linked pharmacological mechanisms of action of drugs such as lithium and calcium channel antagonists, future studies should take into account CACNA1C rs1006737 genotype when conducting clinical research trials. As the rs1006737 risk allele is associated with increased intracellular calcium concentrations, in a dose-dependent fashion (Bigos et al., 2010), it seems possible that rs1006737 genotype may influence BD treatment response to these drugs. Elucidating the relationship between rs1006737 and patient response to calcium-linked BD treatment drugs can potentially provide evidence for future genotype-specific drug therapies.

5.7 Conclusion

In conclusion, this study is the first of its kinds to find evidence of a relationship between cortical brain structure and CACNA1C rs1006737 in adolescents with BD and
HCs. Specifically, relative to non-carriers, A-carriers were found to have significantly greater SA in the ACC and occipital regions of the brain. Diagnosis-by-rs1006737 interactions were also found, such that, relative to BD non-carriers and HC A-carriers, BD A-carriers were found to have significantly greater SA in the bilateral ACC, prefrontal brain regions, and occipital brain regions. These findings demonstrate that rs1006737 interacts with BD diagnosis to have an effect on BD intermediate cortical neurostructural phenotypes in adolescents. In addition, these findings highlight the important role cortical SA plays in the developmental of BD neuropathology.

Future studies should build on findings from this study by widening the scope of investigations into the relationship between rs1006737 and the adolescent brain to include multi-modal imaging phenotypes, such as WM structure, neurofunction, in addition to subcortical and cortical structure. Future studies should also examine peripheral biomarkers and neuropsychological outcomes in order to gain a more holistic understanding regarding the role rs1006737 plays in adolescent BD pathology. Findings from this study and future studies may provide evidence for future BD therapeutic interventions, such as rs1006737 genotype-influenced calcium channel antagonist drug therapy.
6.0 References


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7.0 Appendix

Appendix 1

To:  

From:  

Date: December 21, 2011  

Subject: Assessing Changes in Cerebral Perfusion and Neuropsychological Function in Response to Aerobic Exercise among Adolescents with versus without Bipolar Disorder

Project Identification Number: 408-2011  
Approval Date: December 21, 2011  
Expiry Date: December 21, 2012

The Research Ethics Board of Sunnybrook Health Sciences Centre has conducted a Delegated Board review of the research protocol referenced above and approved the involvement of human subjects on the above captioned date. The quorum for approval did not involve any member associated with this project.

The approval of this study includes the following documents:

- Protocol dated December 19, 2011
- Informed Consent Form for Adolescents 13-19 Years of Age Version 1 dated December 19, 2011
- Informed Consent Form for Parents of Adolescents 13-19 Years of Age Version 1 dated December 19, 2011
- Recruitment Poster (Must submit to Sunnybrook Communications & Stakeholder Relations for approval prior to posting.)
- Study tools (received November 15, 2011):  
  - General Information Sheet
  - Child and Adolescent Health Screening Report
  - Family History Score Sheet – First Degree Relatives
  - Family History Score Sheet – Second Degree Relatives
  - Family Medical History
  - K-SADS Mania Rating Scale
  - K-SADS-P Depression Section
  - Diagnostic Interview K-SADS-PL
  - K-SADS-PL Screen Interview
  - Exercise-Induced Feeling Inventory
  - PRETIE-Q

The Research Ethics Board of Sunnybrook Health Sciences Centre operates in compliance with the Tri-Council Policy Statement 2nd edition, ICH GCP Guidelines, Part C Division 5 of the Food and Drug Regulations, Part 4 of the Natural Health Products Regulations, and Part 3 of the Medical Devices Regulations. All Health Canada regulated trials at Sunnybrook are conducted by a Qualified Investigator.

Fully affiliated with the University of Toronto
- PAR-Q
- BORG'S Rating of Perceived Exertion The 10-Point Scale
- DUSI
- Wong-Baker Faces Pain Rating Scale
- WAVE Adults/Adolescents
- Menstrual History Interview
- Tobacco Use – Lifetime
- Sleep Quality Questionnaire
- Petersen Pubertal Development Scale
- Stressful Life Events Schedule (Adolescent Self-Report)
- Stressful Life Events Schedule (Parent about Child)
- EndoPAT Booklet
- Wechsler Abbreviated Scale of Intelligence

All correspondence with the REB must include the assigned Project Identification Number. The REB requires immediate notification of all internal serious adverse events and significant deviations. Study continuation beyond one year requires submission of a renewal form prior to the expiry date or a study completion report must be received to close the file with the REB.

All REB approved studies may be subject to review by the Sunnybrook Quality Assurance and Education Program and, as Principal Investigator, you are responsible for the ethical conduct of this study. Approval by the Sunnybrook REB entails compliance with current legislation outlined in the Ontario Personal Health Information Protection Act (PHIPA) and all policies and guidelines established by Sunnybrook. All applicable contracts and agreements must be submitted to Sunnybrook Legal Services before this research may be initiated.
Appendix 2

CONSENT TO PARTICIPATE IN A RESEARCH STUDY
For Adolescents 13-20 years of age

TITLE OF PROJECT:
Assessing Changes in Cerebral Perfusion and Neuropsychological Function in Response to Aerobic Exercise among Adolescents with versus without Bipolar Disorder

PRINCIPAL INVESTIGATOR:

SPONSOR:
Ontario Mental Health Foundation

INFORMED CONSENT
You are being asked to consider taking part in a research study. It is important that you read and understand this document. It describes the purpose, procedures, benefits, risks, discomforts and precautions of the study. It also describes other options that are available to you and your right to withdraw from the study at any time. If this form contains anything you do not understand or would like to know more about, please ask the study doctor or study staff to explain it to you. Upon request, someone may verbally translate this form in your preferred language. You may take as much time as you need to decide whether or not to participate. Feel free to discuss it with your friends and family, or your family doctor. You must make sure that all of your questions are answered to your satisfaction before deciding whether or not you will participate in this study.

INTRODUCTION

You are being asked to participate in this research study because you are either being treated for bipolar disorder through the Youth Psychiatry Division of Sunnybrook or because you responded to an advertisement to participate in the study as a psychiatrically healthy participant.

WHAT IS THE USUAL TREATMENT?

Usually, bipolar disorder is treated by assessing symptom frequency and severity, safety and efficacy of medication therapy, and in some cases, psychosocial treatment. Height, weight, blood pressure, and, in some cases, waist circumference is collected. Non-invasive MRI scans of the brain can also be routine practice for some patients.

WHY IS THE STUDY BEING DONE?

This study aims to measure changes in brain activity and blood flow after aerobic exercise among adolescents with and without bipolar disorder, and to find out whether these changes are associated with performance on neurocognitive tests. Furthermore, this study aims to examine how these factors relate to blood vessel functioning, biomarkers, and certain genetic markers. By including these factors in the same study, we hope to learn about the mechanism behind these cognitive benefits of exercise, and how they relate to one another in adolescents with bipolar disorder and in healthy adolescents.

WHAT WILL HAPPEN DURING THIS STUDY?

Study Visit 1

Visit 1 involves taking part in a screening interview to see if you are eligible to participate in this study. The interview will consist of questions about you regarding specific medical
illnesses and medications that might interfere with the assessment of the factors listed above, and it will take about 10-15 minutes. If you do not have these illnesses or take these medications, you will be asked to complete a psychiatric interview and to answer questions regarding your medical history, eating habits, physical activity, life events including family conflict, and use of nicotine, alcohol and street drugs. In addition, an intelligence test will be completed with the interviewer. The interview will take about 3 hours to complete.

**Study Visit 2**

If you meet the study criteria for being a participant with bipolar disorder or a control participant, you will be asked to return to Sunnybrook for a second visit to complete the following tasks:

**IMPORTANT:** Before arriving for Visit 2, you will be asked to abstain from all food and drink (no caffeine and alcohol, water is permitted) for at least 8 hours prior. You must also not drink water, smoke or chew gum 30 minutes prior.

*Saliva Collection:* We will first ask you to provide us with a 4mL sample of your saliva (about 1 teaspoonful) by spitting into a special tube. This will take approximately 10-15 minutes. Additionally, we will ask you to provide us with a sample of your saliva at 5 time points during the course of the study visit by asking you to place a cotton swab in your mouth for 60 seconds. Altogether, this additional saliva collection may take up to 10 minutes.

*Blood Vessel Functioning:* Next, we will measure your blood vessel functioning using a device called the EndoPAT. This will involve gently placing non-invasive probes on the index fingers of your hands while you are lying on your back. The EndoPAT will gather information for 10 minutes while you are resting. Then a blood pressure cuff will be tightly inflated on your arm for 5 minutes to prevent blood flow. The ultrasound will again gather information for 10 minutes after the blood pressure cuff is released. This will take up to about 60 minutes to complete.

*Break:* After the completion of these two tasks, you will be given a 30 minute break. Food and drink (non-caffeinated) will be provided.

*Questionnaires:* After returning from your break, you will be asked to complete questionnaires regarding your medical history, eating habits, physical activity, life events including family conflict, and use of nicotine, alcohol and street drugs. This should take about 30 minutes.

*Aerobic Exercise and MRI Scans:* Finally, you will be asked to complete a task that assesses brain changes while you perform a cognitive test. This will include a practice of the test, a pre-exercise assessment, a bout of aerobic exercise, and a post-exercise assessment. You will practice the cognitive test for 10 minutes so that you are familiar with it, and complete it two more times both before and after the exercise session. The test gathers information on cognitive function (thinking and memory) by using a reaction test, and may require you to press an appropriate button quickly after a stimulus appears.

After the practice, your brain will be imaged using non-invasive magnetic resonance imaging (MRI) at rest and while you complete the cognitive test. This will take
approximately 1 hour. This scan assesses changes in activity and blood flow in the brain, and involves lying stationary on a bed that moves into the centre of the main magnetic field. MRI technologists will perform all MRI scans and are trained to address participant needs and maximize comfort. You will have constant communication with the MRI technologists and study staff while undergoing the MRI and you are free to withdraw at any time.

During one of the MRI scans, there will also be a breath hold task that will require your active participation. This task measures how breath holding may affect blood flow to your brain. You will be asked to hold your breath six separate times for 15 seconds each. You will see instructions on the screen that will switch from “rest” for 30 seconds to “breathe out” for 5 seconds followed by “hold breath” for 15 seconds.

After the MRI, you will be asked to ride a stationary bike for 25 minutes just outside of the MRI scanning room. This will include a five minute warm-up period and 20 minutes of exercise that will increase your breathing and heart rate. The goal is to maintain a constant rate and workload such that your heart rate stays between 60-80% of your age calculated maximum (208-0.7*AGE). You will be monitored for safety and are free to stop exercising at any time. After the exercise, your brain will be imaged again while at rest and during the cognitive test. This will take approximately 30 minutes. In total, this study phase will take about 2.5 hours to complete.

Your parent can accompany you to the MRI scan and wait just outside the testing room. Since the procedures must be the same for all participants, parents may not be inside the testing room.

<table>
<thead>
<tr>
<th>Visit 1</th>
<th>Visit 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOTAL TIME:</strong></td>
<td><strong>Approximately 4.5 hours</strong></td>
</tr>
<tr>
<td>1 – 4 hours</td>
<td></td>
</tr>
<tr>
<td>Informed Consent = 45 minutes</td>
<td>Saliva Collection = 10 minutes</td>
</tr>
<tr>
<td>Screening = 10 – 15 minutes</td>
<td>Blood Vessel Assessment = 60 minutes</td>
</tr>
</tbody>
</table>
Psychiatric Interview / Complete self–report forms = 3 hours | Break = 30 minutes
---|---
Questionnaires = 30 minutes
Cognitive Practice Test = 10 minutes
Aerobic Exercise = 25 minutes
MRI Scans = 1.5 hours

**HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?**
It is expected that about 120 adolescents and their parents will take part in this study at Sunnybrook. The length of this study for participants include 2 separate sessions lasting approximately 8.5 hours total. The entire study is expected to take about 4 years to complete and the results should be known in 1 year following the completion of study procedures.

**WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?**
Although participation in this study is entirely voluntary, you are responsible for completing the full procedure for each visit, as outlined above. If you choose not to complete any of the requirements, you will not be able to participate in the study.

Please note the following information regarding the use and storage of the saliva sample you will provide at visit 2:

*Duration of Storage of Information*
All saliva samples will be stored at Sunnybrook Health Sciences Centre. Your individual results of genetic markers and other results pertaining to cognitive test performance will not be reported to you because, at this point in time, these are research measurements, and they do not currently have any clear relevance to your medical health.

Any samples obtained from you will be destroyed once analysis is complete. If the research study is extended beyond this time, you will be asked once again to give consent to extend the storage period for a specified amount of time. If you cannot be reached, your samples will be destroyed at that time.

*Limits to Sharing Information with Collaborators and Laboratories*
The saliva samples obtained from you will not be used for any other investigations outside of this study (i.e. for the purpose of investigating bipolar disorder). The information may be sent for specific testing to the laboratories of collaborators with Dr. Goldstein’s team; however
information will not be shared with any individuals who are not involved in this study.

**WHAT ARE THE POTENTIAL RISKS AND/OR DISCOMFORTS OF PARTICIPATING IN THIS STUDY?**

You may experience side effects from participating in this study. Some side effects are known and are listed below, but there may be other side effects that are not expected. If you decide to take part in this study, you should contact the study doctor (Dr. Benjamin Goldstein) or study staff during business hours with questions or concerns regarding any side effects or study-related injuries that you experience. The telephone number for this purpose is: [416-480-5328].

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>Frequency</th>
<th>Severity</th>
<th>Long Term Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very Likely</td>
<td>Likely</td>
<td>Less Likely</td>
</tr>
<tr>
<td>Muscle Fatigue/Soreness</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Heart Trouble</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Heart or Attack</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light Headedness</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Eye Strain or Headache</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Emotional Discomfort</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Hunger Pains</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

There is a chance you may experience temporary muscle fatigue or soreness from the exercise. There are no known risks associated with magnetic resonance imaging other than discomfort while remaining still for the scanning period. You may experience temporary light headedness from the breath hold task. You may experience eye strain or headaches while concentrating on the computerized cognitive test. You may experience emotional discomfort when completing the psychiatric interview and questionnaires. You may experience hunger and/or hunger pains while fasting.
There is a minimal risk of heart trouble with exercise which could make you feel short of breath, pain or pressure in your chest, or pain down your arm. The risk includes the rare possibility of a heart attack. We will minimize the risk by monitoring your heart rate and having appropriate emergency services on hand.

You may discontinue any of the procedures at any time.

You will be told about any new information that might reasonably affect your willingness to continue to participate in this study as soon as the information becomes available to study staff.

WHAT ARE THE POTENTIAL BENEFITS OF PARTICIPATING IN THIS STUDY?
There are no direct benefits from participation in this study. However, this study relies on your participation in order to explore bipolar disorder among adolescents, which will broaden understandings of the illness and may eventually lead to novel assessment, prevention and treatment strategies. Findings from this study may therefore benefit future individuals or families affected with or at risk for bipolar disorder.

CAN PARTICIPATION IN THIS STUDY END EARLY?
The investigator(s) may decide to remove you from this study without your consent for any of the following reasons:

- You are unable or unwilling to follow the study procedures
- If you are disruptive to the study

If you are removed from this study, the investigator(s) will discuss the reasons with you.

You can also choose to end your participation at any time without having to provide a reason. If you choose to withdraw, your choice will not have any effect on your current or future medical treatment or health care. There will be no penalty or loss of benefits to which you are otherwise entitled. If you withdraw voluntarily from the study, you are encouraged to contact: Dr. Benjamin Goldstein at 416-480-5328; 2075 Bayview Avenue, Toronto, Ontario, M4N 3M5. If you withdraw consent to participate after beginning the study, the data collected up to that time point will be used.

WHAT ARE THE COSTS FOR PARTICIPATING IN THIS STUDY?
There is no cost for participation.
WHAT HAPPENS IF I HAVE A RESEARCH RELATED INJURY?

If you become sick or injured as a direct result of your participation in this study, your medical care will be provided. Financial compensation for such things as discomfort due to injury is not routinely available. By signing this consent form, you do not give up any of your legal rights.

ARE STUDY PARTICIPANTS PAID TO PARTICIPATE IN THIS STUDY?

Parents will be compensated $50 for travel expenses and parking. Adolescents will be compensated $20 for completing study screening procedures. Eligible participants will also receive $90 at the completion of Visit 2.

HOW WILL MY INFORMATION BE KEPT CONFIDENTIAL?

You have the right to have any information about you and your health that is collected, used or disclosed for this study to be handled in a confidential manner.

If you decide to participate in this study, the investigator and study staff will look at your personal health information and collect only the information they need for this study. Personal health information refers to health information about you that could identify you because it includes information such as your:

- Name,
- Address,
- Telephone number,
- Date of birth,
- New and existing medical records, or
- The types, dates and results of various tests and procedures.

You have the right to access, review and request changes to your personal health information.

The following people may come to the hospital to look at your personal health information to check that the information collected for the study is correct and to make sure the study followed the required laws and guidelines:

- Representatives of the Sunnybrook Research Ethics Board, a group of people who oversee the ethical conduct of research studies at Sunnybrook
Access to your personal information will take place under the supervision of the Principal Investigator.

“Study data" is information about you that is collected for the study, but that does not directly identify you. Any study data that is sent outside of the hospital will have a study code and will not contain your name or address or any information that directly identifies you. Study data that is sent outside of the hospital will be used for the research purposes explained in this consent form.

The investigator(s), study staff and the other people listed above will keep the information they see or receive about you confidential, to the extent permitted by applicable laws. Even though the risk of identifying you from the study data is very small, it can never be completely eliminated. All study data will be stored in a secure and confidential location for a period of at least 5 years. All reasonable measures to protect the confidentiality of participants’ study records and their identity will be taken to the extent permitted by the applicable laws and/or regulations, and will not be made publicly available. The results of this study may be presented at meetings or in publications; however, participant’s identity will not be disclosed.

When the results of this study are published, your identity will not be disclosed.

You have the right to be informed of the results of this study once the entire study is complete. If you would like to be informed of the results of this study, please contact the study doctor: Dr. Benjamin Goldstein, 416-480-5328.

**DOES (DO) THE INVESTIGATOR(S) HAVE ANY CONFLICTS OF INTEREST?**

The study doctors do not have any conflicts of interest regarding this study.

**WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?**

You have the right to receive all significant information that could help you make a decision about participating in this study. You also have the right to ask questions about this study and your rights as a research participant, and to have them answered to your satisfaction, before you make any decision. You also have the right to ask questions and to receive answers throughout this study.
If you have any questions about this study, you are encouraged to contact the study doctor: Dr. [redacted].

The Sunnybrook Research Ethics Board has reviewed this study. If you have questions about your rights as a research participant or any ethical issues related to this study that you wish to discuss with someone not directly involved with the study, you may call Chair of the Sunnybrook Research Ethics [redacted].

Assessing Changes in Cerebral Perfusion and Neuropsychological Function in Response to Aerobic Exercise among Adolescents with versus without Bipolar Disorder

Name of Participant: _____________________________________________

Participant:

By signing this form, I confirm that:

- This research has been fully explained to me and all of my questions answered to my satisfaction
- I understand the requirements of participating in this research study
- I have been informed of the risks and benefits, if any, of participating in this research study
- I have been informed of any alternatives to participating in this research study
- I have been informed of the rights of research participants
- I have read each page of this form
- I authorize access to my personal health information, medical record and research study data as explained in this form
- I have agreed to participate in this research study, or agree to allow the person I am responsible for, to participate in this research study
- I understand that my family doctor may be informed of my participation in this research study
- This informed consent document may be placed in my medical records

___________________________      _________________________________     ___________
Name of Adolescent (print)                Signature                                                          Date

Assistance Declaration

Was the participant assisted during the consent process?  ☐ Yes   ☐ No
☐ The consent form was read to the participant/substitute decision-maker, and the person signing below attests that the study was accurately explained to, and apparently understood by, the participant/substitute decision-maker.
☐ The person signing below acted as a translator for the participant/substitute decision-maker during the consent process. He/she attests that they have accurately translated the information for the participant/substitute decision-maker, and believe that that participant/substitute decision-maker has understood the information translated.

___________________________      _________________________________     ___________
Name of Person Assisting (print)       Signature                                                        Date
By signing this form, I confirm that:

- This study and its purpose has been explained to the participant named above
- All questions asked by the participant have been answered
- I will give a copy of this signed and dated document to the participant

___________________________      _________________________________     ___________
Name of Person Obtaining          Signature                                      Date
Consent (print)

CONSENT TO PARTICIPATE IN A RESEARCH STUDY
For Parents of Adolescents 13-20 years of age

TITLE OF PROJECT:
Assessing Changes in Cerebral Perfusion and Neuropsychological Function in Response to Aerobic Exercise among Adolescents with versus without Bipolar Disorder
INFORMED CONSENT

Your adolescent is being asked to consider taking part in a research study. As part of the study, you will be asked to answer questions and fill out questionnaires about your adolescent. It is important that you read and understand this document. It describes the purpose, procedures, benefits, risks, discomforts and precautions of the study. It also describes other options that are available to your adolescent and his/her right to withdraw from the study at any time. If this form contains anything you do not understand or would like to know more about, please ask the study doctor or study staff to explain it to you. Upon request, someone may verbally translate this form in your preferred language. You may take as much time as you need to decide whether or not to participate. Feel free to discuss it with your friends and family, or your family doctor. You must make sure that all of your questions are answered to your satisfaction before deciding whether or not you will participate in this study.
INTRODUCTION

Your adolescent is being asked to participate in this research study because he/she is either being treated for bipolar disorder through the Youth Psychiatry Division of Sunnybrook or because he/she responded to an advertisement to participate in the study as a psychiatrically healthy participant.

WHAT IS THE USUAL TREATMENT?
Usually, bipolar disorder is treated by assessing symptom frequency and severity, safety and efficacy of medication therapy, and in some cases, psychosocial treatment. Height, weight, blood pressure, and, in some cases, waist circumference is collected. Non-invasive MRI scans of the brain can also be routine practice for some patients.

WHY IS THE STUDY BEING DONE?
This study aims to measure changes in brain activity and blood flow after aerobic exercise among adolescents with and without bipolar disorder, and to find out whether these changes are associated with performance on neurocognitive tests. Furthermore, this study aims to examine how these factors relate to blood vessel functioning, and certain genetic markers. By including these factors in the same study, we hope to learn about the mechanism behind these cognitive benefits of exercise, and how they relate to one another in adolescents with bipolar disorder and in healthy adolescents.

WHAT WILL HAPPEN DURING THIS STUDY?

Study Visit 1

Visit 1 involves taking part in a screening interview to see if you and your adolescent are eligible to participate in this study. The interview will consist of questions about your adolescent regarding specific medical illnesses and medications that might interfere with the assessment of the factors listed above, and it will take about 10-15 minutes. If your adolescent does not have these illnesses or take these medications, you will be asked to complete a psychiatric interview regarding your adolescent and to answer questions regarding his/her medical history, eating habits, physical activity, life events including family conflict, and use of nicotine, alcohol and street drugs. In addition, your adolescent will complete an intelligence test with the interviewer. The interview will take about 3 hours to complete.

Study Visit 2
If your adolescent meets the study criteria for being a participant with bipolar disorder or a control participant, you will both be asked to return to Sunnybrook for a second visit to complete the following tasks:

IMPORTANT: Before arriving for Visit 2, your adolescent will be asked to abstain from all food and drink (no caffeine and alcohol, water is permitted) for at least 8 hours prior. Your adolescent must also not drink water, smoke or chew gum 30 minutes prior.

Saliva Collection: We will first ask your adolescent to provide us with a 4mL sample of his/her saliva (about 1 teaspoonful) by spitting into a special tube. This will take approximately 10-15 minutes. Additionally, we will ask your adolescent to provide us with a sample of his/her saliva at 5 time points during the course of the study visit by asking them to place a cotton swab in their mouth for 60 seconds. Altogether, this additional saliva collection may take up to 10 minutes.

Blood Vessel Functioning: Next, we will measure your adolescent’s blood vessel functioning using a device called the EndoPAT. This will involve gently placing non-invasive probes on the index fingers of your adolescent’s hands while he/she is lying on his/her back. The EndoPAT will gather information for 10 minutes while your adolescent is resting. Then a blood pressure cuff will be tightly inflated on your adolescent’s arm for 5 minutes to prevent blood flow. The ultrasound will again gather information for 10 minutes after the blood pressure cuff is released. This will take up to about 60 minutes to complete.

Break: After the completion of these two tasks, you will be given a 30 minute break. Food and drink (non-caffeinated) will be provided.

Questionnaires: After returning from your break, your adolescent will be asked to complete questionnaires regarding his/her medical history, eating habits, physical activity, life events including family conflict, and use of nicotine, alcohol and street drugs. This should take about 30 minutes.

Aerobic Exercise and MRI Scans: Finally, your adolescent will be asked to complete a task that assesses brain changes while he/she performs a cognitive test. This will include a practice of the test, a pre-exercise assessment, a bout of aerobic exercise, and a post-exercise assessment. Your adolescent will practice the cognitive test for 10 minutes so that he/she is familiar with it, and complete it two more times both before and after the exercise session. The test gathers information on cognitive function (thinking and memory) by using a reaction test, and may require your adolescent to press an appropriate button quickly after a stimulus appears.

After the practice, your adolescent’s brain will be imaged using non-invasive magnetic resonance imaging (MRI) at rest and while he/she completes the cognitive test. This will take approximately 1 hour. This scan assesses changes in activity and blood flow in the brain, and involves your adolescent lying stationary on a bed that moves into the centre of the main magnetic field. MRI technologists will perform all MRI scans and are trained to address participant needs and maximize comfort. Your adolescent will have constant communication with the MRI technologists and study staff while undergoing the MRI and he/she is free to withdraw at any time.
During one of the MRI scans, there will also be a breath hold task that will require the active participation of your adolescent. This task measures how breath holding may affect blood flow to his or her brain. Your adolescent will be asked to hold his or her breath six separate times for 15 seconds each. They will see instructions on the screen that will switch from “rest” for 30 seconds to “breathe out” for 5 seconds followed by “hold breath” for 15 seconds.

After the MRI, your adolescent will be asked to ride a stationary bike for 25 minutes just outside of the MRI scanning room. This will include a five minute warm-up period and 20 minutes of exercise that will increase your adolescent’s breathing and heart rate. The goal is to maintain a constant rate and workload such that your adolescent’s heart rate stays between 60-80% of his/her age calculated maximum (208-0.7*AGE). Your adolescent will be monitored for safety and he/she is free to stop exercising at any time. After the exercise, your adolescent’s brain will be imaged again while at rest and during the cognitive test. This will take approximately 30 minutes. In total, this study phase will take about 2.5 hours to complete.

You can accompany your adolescent to the MRI scan and wait just outside the testing room. Since the procedures must be the same for all participants, parents may not be inside the testing room.

<table>
<thead>
<tr>
<th>Visit 1</th>
<th>Visit 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL TIME: 1 – 4 hours</td>
<td>Approximately 4.5 hours</td>
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<tr>
<td>Informed Consent = 45 minutes</td>
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<td>Psychiatric Interview / Complete self – report forms = 3 hours</td>
<td>Break = 30 minutes</td>
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<td></td>
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<tr>
<td></td>
<td>Aerobic Exercise = 25 minutes</td>
</tr>
</tbody>
</table>
HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?
It is expected that about 120 adolescents and their parents will take part in this study at Sunnybrook. The length of this study for participants include 2 separate sessions lasting approximately 8.5 hours total. The entire study is expected to take about 4 years to complete and the results should be known in 1 year following the completion of study procedures.

WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?
Although participation in this study is entirely voluntary, you and your adolescent are responsible for completing the full procedure for each visit, as outlined above. If you or your adolescent chooses not to complete any of the requirements, you will both not be able to participate in the study.

Please note the following information regarding the use and storage of the saliva sample your adolescent will provide at visit 2:

Duration of Storage of Information
All saliva samples will be stored at Sunnybrook Health Sciences Centre. Your adolescent’s individual results of genetic markers and other results pertaining to his/her cognitive test performance will not be reported to you or your adolescent because, at this point in time, these are research measurements, and they do not currently have any clear relevance to your adolescent’s medical health.

Any samples obtained from your adolescent will be destroyed once analysis is complete. If the research study is extended beyond this time, your adolescent will be asked once again to give consent to extend the storage period for a specified amount of time. If your adolescent cannot be reached, his/her samples will be destroyed at that time.

Limits to Sharing Information with Collaborators and Laboratories
The saliva samples obtained from your adolescent will not be used for any other investigations outside of this study (i.e. for the purpose of investigating bipolar disorder). The information may be sent for specific testing to the laboratories of collaborators with Dr. Goldstein’s team; however information will not be shared with any individuals who are not involved in this study.

WHAT ARE THE POTENTIAL RISKS AND/OR DISCOMFORTS OF PARTICIPATING IN THIS STUDY?
Your adolescent may experience side effects from participating in this study. Some side effects are known and are listed below, but there may be other side effects that are not expected. If your adolescent decides to take part in this study, he/she should contact the study doctor (Dr.
Benjamin Goldstein) or study staff during business hours with questions or concerns regarding any side effects or study-related injuries that he/she experiences. The telephone number for this purpose is: 416-480-5328.

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>Frequency</th>
<th>Severity</th>
<th>Long Term Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very Likely</td>
<td>Likely</td>
<td>Less Likely</td>
</tr>
<tr>
<td>Muscle Fatigue/Soreness</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Heart Trouble or Heart Attack</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Light Headedness</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Eye Strain or Headache</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Emotional Discomfort</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Hunger pains</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

There is a chance your adolescent may experience temporary muscle fatigue or soreness from the exercise. There are no known risks associated with magnetic resonance imaging other than discomfort while remaining still for the scanning period. Your adolescent may experience temporary light headedness from the breath hold task. Your adolescent may experience eye strain or headaches while concentrating on the computerized cognitive test. Your adolescent may experience emotional discomfort when completing the psychiatric interview and questionnaires. Your adolescent may experience hunger or hunger pains while fasting.

There is a minimal risk of heart trouble with exercise which could make your adolescent feel short of breath, pain or pressure in his/her chest, or pain down his/her arm. The risk includes the rare possibility of a heart attack. We will minimize the risk by monitoring your adolescent’s heart rate and having appropriate emergency services on hand.

Your adolescent may discontinue any of the procedures at any time.
You and your adolescent will be told about any new information that might reasonably affect your willingness to continue to participate in this study as soon as the information becomes available to study staff.

**WHAT ARE THE POTENTIAL BENEFITS OF PARTICIPATING IN THIS STUDY?**

There are no direct benefits from participation in this study. However, this study relies on you and your adolescent’s participation in order to explore bipolar disorder among adolescents, which will broaden understandings of the illness and may eventually lead to novel assessment, prevention and treatment strategies. Findings from this study may therefore benefit future individuals or families affected with or at risk for bipolar disorder.

**CAN PARTICIPATION IN THIS STUDY END EARLY?**

The investigator(s) may decide to remove your adolescent from this study without his/her consent for any of the following reasons:

- He/she is unable or unwilling to follow the study procedures
- He/she is disruptive to the study

If your adolescent is removed from this study, the investigator(s) will discuss the reasons with him/her.

You and your adolescent can also choose to end participation at any time without having to provide a reason. If your adolescent chooses to withdraw, his/her choice will not have any effect on his/her current or future medical treatment or health care. There will be no penalty or loss of benefits to which you are otherwise entitled. If you withdraw voluntarily from the study, you are encouraged to contact: Dr. Benjamin Goldstein at 416-480-5328; 2075 Bayview Avenue, Toronto, Ontario, M4N 3M5. If you withdraw consent to participate after beginning the study, the data collected up to that time point will be used.

**WHAT ARE THE COSTS FOR PARTICIPATING IN THIS STUDY?**

There is no cost for participation.

**WHAT HAPPENS IF I HAVE A RESEARCH RELATED INJURY?**

If your adolescent becomes sick or injured as a direct result of his/her participation in this study, his/her medical care will be provided. Financial compensation for such things as discomfort due to injury is not routinely available. By signing this consent form, you or your adolescent do not give up any of your legal rights.
ARE STUDY PARTICIPANTS PAID TO PARTICIPATE IN THIS STUDY?

Parents will be compensated $50 for travel expenses and parking. Adolescents will be compensated $20 for completing study screening procedures. Eligible participants will also receive $90 at the completion of Visit 2.

HOW WILL MY INFORMATION BE KEPT CONFIDENTIAL?

Your adolescent has the right to have any information about him/her and his/her health that is collected, used or disclosed for this study to be handled in a confidential manner.

If your adolescent decides to participate in this study, the investigator and study staff will look at his/her personal health information and collect only the information they need for this study. Personal health information refers to health information about your adolescent that could identify him/her because it includes information such as your adolescent’s:

- Name,
- Address,
- Telephone number,
- Date of birth,
- New and existing medical records, or
- The types, dates and results of various tests and procedures.

Your adolescent has the right to access, review and request changes to his/her personal health information.

The following people may come to the hospital to look at your adolescent’s personal health information to check that the information collected for the study is correct and to make sure the study followed the required laws and guidelines:

- Representatives of the Sunnybrook Research Ethics Board, a group of people who oversee the ethical conduct of research studies at Sunnybrook

Access to your adolescent’s personal information will take place under the supervision of the Principal Investigator.
“Study data" is information about your adolescent that is collected for the study, but that does not directly identify your adolescent. Any study data that is sent outside of the hospital will have a study code and will not contain your adolescent’s name or address or any information that directly identifies him/her. Study data that is sent outside of the hospital will be used for the research purposes explained in this consent form.

The investigator(s), study staff and the other people listed above will keep the information they see or receive about you confidential, to the extent permitted by applicable laws. Even though the risk of identifying your adolescent from the study data is very small, it can never be completely eliminated.

All study data will be stored in a secure and confidential location for a period of at least 5 years. All reasonable measures to protect the confidentiality of participants’ study records and their identity will be taken to the extent permitted by the applicable laws and/or regulations, and will not be made publicly available. The results of this study may be presented at meetings or in publications; however, participant’s identity will not be disclosed.

When the results of this study are published, your adolescent’s identity will not be disclosed.

You and your adolescent have the right to be informed of the results of this study once the entire study is complete. If either of you would like to be informed of the results of this study, please contact the study doctor: Dr. Benjamin Goldstein, 416-480-5328.

**DOES (DO) THE INVESTIGATOR(S) HAVE ANY CONFLICTS OF INTEREST?**

The study doctors do not have any conflicts of interest regarding this study.

**WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?**

You have the right to receive all significant information that could help you make a decision about participating in this study. You also have the right to ask questions about this study and your rights as a research participant, and to have them answered to your satisfaction, before you make any decision. You also have the right to ask questions and to receive answers throughout this study.

If you have any questions about this study, you are encouraged to contact the study doctor: Dr. Benjamin Goldstein, 416-480-5328.

The Sunnybrook Research Ethics Board has reviewed this study. If you have questions about your rights as a research participant or any ethical issues related to this study that you wish to discuss with
someone not directly involved with the study, you may call Chair of the Sunnybrook Research Ethics
Board at 416-480-6100 ext. 88144.

Assessing Changes in Cerebral Perfusion and Neuropsychological Function in Response to Aerobic Exercise among Adolescents with versus without Bipolar Disorder

Name of Participant: _____________________________________________

Parent:

By signing this form, I confirm that:

- This research has been fully explained to me and all of my questions answered to my satisfaction
- I understand the requirements of participating in this research study
- I have been informed of the risks and benefits, if any, of participating in this research study
- I have been informed of any alternatives to participating in this research study
- I have been informed of the rights of research participants
- I have read each page of this form
- I have agreed to participate in this research study, or agree to allow the person I am responsible for, to participate in this research study

___________________________      _______  ____________________________     ___________
Name of Parent (print)                        Signature                                                          Date
Assistance Declaration

Was the participant assisted during the consent process?  □ Yes  □ No

☐ The consent form was read to the participant/substitute decision-maker, and the person signing below attests that the study was accurately explained to, and apparently understood by, the participant/substitute decision-maker.

☐ The person signing below acted as a translator for the participant/substitute decision-maker during the consent process. He/she attests that they have accurately translated the information for the participant/substitute decision-maker, and believe that that participant/substitute decision-maker has understood the information translated.

_________________________________  ___________________________  ___________
Name of Person Assisting (print)  Signature  Date

Person Obtaining Consent

By signing this form, I confirm that:

• This study and its purpose has been explained to the participant named above
• All questions asked by the participant have been answered
• I will give a copy of this signed and dated document to the participant

_________________________________  _________________________________  ___________
Name of Person Obtaining Consent (print)  Signature  Date