PRELIMINARY STUDY INTEGRATING CACNA1C rs1006737, HIPPOCAMPAL AND AMYGDALA VOLUMES, AND PERIPHERAL ENDOTHelial FUNCTION IN YOUTH WITH BIPOLAR DISORDER

BY

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GRADUATE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY
UNIVERSITY OF TORONTO

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ABSTRACT (150/150 WORDS):

Multiple genome wide association studies have identified a bipolar disorder (BD) - risk allele polymorphism (CACNA1C rs1006737). rs1006737 is implicated in neurostructural differences in non-BD carriers. As CACNA1C codes for a protein subunit responsible for endothelial function, we predicted it might serve as a genetic underpinning for both the cardiovascular and neurological phenotypes seen in BD. Fifty adolescents (25 healthy controls, 25 BD) completed genotyping, structural scans, and an endothelial function test [reactive hyperemia index (RHI)]. No gene effects were observed in the amygdala or hippocampus, however healthy carriers of the risk allele showed trending reduced volume in the bilateral hippocampus and amygdala when compared to healthy non-carriers. Furthermore, RHI was non-significantly negatively correlated with left hippocampus volume in HC non-carriers, and a significant gene-RHI interaction was observed in the hippocampus of HC. These findings suggest CACNA1C rs1006737 warrants further study as a potential bridge between BD and vascular dysfunction.
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<th>Term</th>
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<tr>
<td>BD</td>
<td>Bipolar Disorder</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>C-GAS</td>
<td>Children’s Global Assessment Scale</td>
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<tr>
<td>CACNA1C</td>
<td>Calcium Channel, Voltage-Dependent, L type, Alpha 1C Subunit</td>
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<tr>
<td>CANTAB</td>
<td>Cambridge Neuropsychological Test Automated Battery</td>
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<td>CBF</td>
<td>Cerebral Blood Flow</td>
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<tr>
<td>CCB</td>
<td>Calcium Channel Blocker</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
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<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, 4th Edition</td>
</tr>
<tr>
<td>ED</td>
<td>Endothelial Dysfunction</td>
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<tr>
<td>FDR</td>
<td>False Discovery Rate</td>
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<tr>
<td>FMD</td>
<td>Flow-Mediated Dilation</td>
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<tr>
<td>GLM</td>
<td>General Linear Model</td>
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<tr>
<td>GWAS</td>
<td>Genome Wide Association Study</td>
</tr>
<tr>
<td>HC</td>
<td>Healthy Control</td>
</tr>
<tr>
<td>ICV</td>
<td>Intracranial Volume</td>
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<tr>
<td>MAP</td>
<td>Mean Arterial Pressure</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>RH-PAT</td>
<td>Reactive Hyperemia- Pulse Amplitude Tonometry</td>
</tr>
<tr>
<td>RHI</td>
<td>Reactive Hyperemia Index</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
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<tr>
<td>WMH</td>
<td>White Matter Hyperintensity</td>
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1.0 INTRODUCTION:

1.1 STATEMENT OF PROBLEM:

Bipolar Disorder (BD) is a highly heritable disorder, with a 60% concordance rate in monozygotic twins [1]. Individuals with BD cycle through periods of depression (prolonged periods of sadness, extreme apathy, low energy), mania (increased energy, prolonged euphoric mood, change in sleep and thought patterns), and euthymia (periods of mood stability) [2]. Although severity and duration of each episode vary, patients with BD experience a significantly decreased quality of life and overall functional impairments [3, 4]. In addition to mood dysfunction, patients with BD have excessive cardiovascular burden, demonstrating a cardiovascular mortality ratio of 1.5-2.5 when compared to healthy controls (HC) [5, 6]. In recent years, multiple genome-wide association studies (GWAS) in BD point to a single nucleotide polymorphism (G to A mutation) in the calcium channel, voltage-dependent, L type, alpha 1C subunit (CACNA1C rs1006737) gene as a potential susceptibility gene in BD [7, 8]. As CACNA1C codes for an subunit on a calcium channel (Ca,1.2) responsible for vascular health [9, 10], the risk allele may be interconnected to both cardiovascular and neurological differences in BD by influencing endothelial function [11].

Indeed, some studies have been linked CACNA1C rs1006737 with differences in brain structures that are associated with BD [12, 13]. More specifically, the risk allele has been associated with differences in grey matter volume in the frontal cortex and brain stem, as well as differences in grey matter density in both the hippocampus and amygdala [14-16]. The amygdala is a limbic structure responsible for processing and storage of fearful emotions for appropriate sensory output [17]. Thorough investigation has revealed significant increase in amygdala volume in adults, but diminished volumes in youth BD patients [18-20]. Similarly, the hippocampus is a subcortical region that plays a primary role in memory and mood regulation, such as inhibition to stress response, regulation of emotional behavior and encoding declarative
memory [21-25]. Studies have also demonstrated smaller hippocampal volumes in adults and adolescents with BD compared to controls, which may be reversed by mood stabilizers such as lithium [18, 26-28]. As CACNA1C rs1006737 has been highly associated with BD, further investigation into the role of the polymorphism on the subcortical differences observed in this disorder may shed light on the genetic mechanisms of BD.

In addition, investigation into CACNA1C rs1006737 provides an opportunity to study the influence of endothelial dysfunction (ED) on the structural differences outlined above. ED is defined as a decreased bioavailability of vasodilators and contributes to a myriad of CVD’s [29]. Although no studies have looked at the influence of the risk allele on endothelial function, CaV1.2 is associated with endothelial function through control of mean arterial pressure and cerebral artery diameter [10, 30]. Furthermore, there is also published evidence that changes in endothelial function have consequences on white matter integrity and differences in brain volumes during regional analyses [31]. Therefore, the BD-risk allele may potentially bridge the increased CVD risk and structural differences observed in BD through its contribution to ED.

The emergence of CACNA1C rs1006737 in this field presents clinicians with a potential use for already established calcium channel blockers (CCB) in a subset of individuals whom may benefit. Conventional therapies fail to provide optimal outcome in a large percentage of youth patients. In a four-year longitudinal study, 40% of the adolescents with BD spent 75% of the time symptomatic despite clinical intervention [32]. Knowledge regarding the clinical relationship between CACNA1C rs1006737 and BD however, is still limited. Therefore, further investigation into the role of CACNA1C rs1006737 is warranted to encourage the field towards novel or reworked interventions that may fill the gap left by current therapeutics.

1.2 Purpose of Study:

Recent GWAS have identified a BD susceptibility polymorphism (CACNA1C rs1006737) in a gene closely associated with endothelial function [8, 33]. Soon after, CACNA1C
rs1006737 was associated with structural brain differences in healthy adults, including altered whole brain grey matter volume and grey matter density in the right amygdala and hippocampus [15, 34]. We set out to explore two questions. Firstly, patients with BD have repeatedly shown differences in hippocampal and amygdala volumes. In addition, CACNA1C rs1006737 risk allele carriers have also shown differences in hippocampus and amygdala grey matter density [15]. Therefore, we looked at amygdala and hippocampal volumes in adolescent CACNA1C rs1006737 carriers with and without BD [35].

Secondly, BD is highly associated with cardiovascular burden. Although there are no studies looking directly at the relationship between endothelial function and subcortical brain volume, studies predict that endothelial function may play a role in both the increased cardiovascular burden and mood dysfunction seen in mood disorders [36-38]. Some studies have successfully linked endothelial function with differences in brain morphology. One reported endothelial function inversely associated with white matter hyperintensity volume [31]. Others have also reported indirect relationships between endothelial function and structural brain correlates [39, 40]. As CACNA1C codes for a protein that is associated with endothelial function [9], we explored the relationship between endothelial function and the differences in hippocampal and amygdala volume observed in youth risk allele carriers with and without BD.

To date, this is the first study to look at effects of CACNA1C in youth. Regardless of developmental differences in adult and youth BD, results from this study will supplement current knowledge on effects of CACNA1C rs1006737 in BD and may further renew interest in, and refine approaches toward, calcium channel blockers as an alternative intervention in mental illness that has already been safely established in clinical settings [41]. Furthermore, the study may aid in identifying endophenotypes (measureable phenotypes that are closely tied to the biological processes which give rise to psychiatric illness), which can be used to assess the genetic contribution of CACNA1C rs1006737 in BD [42].
1.3 **Hypotheses and Rationale:**

### 1.3.1 Primary Hypothesis:

- Within the total sample (diagnosis independent), risk allele carriers (CACNA1C rs1006737; A/A+A/G) will demonstrate lower bilateral hippocampal and amygdala volumes when compared to non-carriers.
- Within each diagnosis group (BD or HC), risk allele carriers will demonstrate lower hippocampal and amygdala volumes when compared to non-carriers. This association will be more evident in BD than HC.

**Rationale:** Currently, there is very limited literature looking at CACNA1C rs1006737 and differences in subcortical volumes. A total of 4 studies have reported subcortical structural findings in both BD and HC risk allele carriers. Three studies, however, did not find any significant differences [13, 14, 43]. One study reported an increased amygdala and hippocampus grey matter density in healthy risk allele carriers [14, 15].

Relatedly, youth with BD have consistently exhibited smaller amygdala and hippocampal volumes when compared to their healthy counterparts [44, 45]. Furthermore, presentation of emotional dysregulation and irregular responses on emotional face tasks have pointed to possible functional deficits in the amygdala, while poor performance in verbal learning and declarative and procedural memory tasks are highly associated with hippocampal dysfunction [46-48]. Therefore, despite limited association between subcortical volumes and the risk allele, examining these regions in youth carriers may shed light on how this candidate gene relates to brain structure in regions that are relevant to youth BD.

Focusing on endophenotypes may yield a better understanding of the biology underlying BD. Endophenotypes measure components that reside between the visible disease and the underlying genetic influences [42]. Gottesman et al suggests the use of endophenotypes can help
identify downstream clinical phenotypes as well as additional upstream genetic influences, which are often masked by the complexity of studying the entire illness as a whole entity [42]. In this case, the study may pinpoint changes in subcortical volume as a potential endophenotype of CACNA1C rs1006737 in adolescent carriers with BD.

1.3.2 **Secondary Hypothesis:**

- Within the total sample (diagnosis independent), better endothelial function, as measured by reactive hyperemia pulse amplitude tonometry (RH-PAT) will be associated with greater hippocampal and amygdala volumes in non-carriers when compared to carriers.
- Within each cohort (BD/HC) better endothelial function will be associated with greater hippocampal and amygdala volume in adolescent CACNA1C rs1006737 non-carriers when compared to carriers. This association will be more evident in BD than HC.

**Rationale:** Given the role of L-type calcium channels (more specifically the CaV1.2 alpha subunit coded by CACNA1C) in both peripheral and cerebral vascular function, it is reasonable to predict that the polymorphism may play a role in endothelial function of risk allele carriers [9, 49]. Although no neurostructural studies in CACNA1C risk allele carriers have looked specifically at peripheral ED, there is evidence that an increase in endothelial function biomarker is linked to increased basal ganglia volumes in schizophrenic participants [40]. In addition, endothelial dysfunction in the elderly has been repeatedly linked to increased white matter hyperintensity volumes [31]. As a proxy of CVD, ED is also related to diminished scores in BD associated cognitive tasks (ie: executive functioning and memory tasks) [50-53]. Taken together, this suggests ED may play a role in the structural changes associated with BD. Therefore additional exploration could shed light on the contribution of CACNA1C rs1006737 and ED to the morphological changes seen in BD.
1.4 REVIEW OF LITERATURE:

1.4.1 BD IS A HEREDITARY ILLNESS HIGHLY PREVALENT AMONGST ADULTS AND YOUTH, AND IS ASSOCIATED WITH PRONOUNCED SYMPTOMATIC BURDEN AND FUNCTIONAL IMPAIRMENT:

BD is an impairing mood disorder characterized by episodes of depression and mania as described by Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV). It affects 2-5% of adolescents and adults and is listed as the fourth leading cause of non-lethal disease burden in the world, significantly decreasing quality of life of patients [3, 4, 54-56]. In addition to the direct burden of mood symptoms, BD is associated with multiple other comorbidities including high rates of substance abuse, anxiety, greater medical burden and cardiovascular disease (CVD) [6, 57, 58]. Furthermore, Cognitive impairment across multiple domains (e.g. working memory, attention, processing speed) is evident within episodic and euthymic (non-symptomatic) adult patients, with similar findings in adolescents with BD [59, 60].

Though similar in many ways to adult BD, adolescents with BD have increased risk for comorbidities, spend significantly greater time suffering from mood symptoms and have worse outcome compared to later onset BD [59, 61-63]. Adolescents with BD disorder also have significantly greater risks of suicide when compared to control, and suicidal ideation and suicide attempts are common [64-66]. There is no known cause of BD. However there is substantial evidence that BD is associated with structural and functional neuroanatomic features, as well as peripheral markers of inflammation, oxidative stress, and neurotrophic factors [67, 68].

In addition, high degree of heritability of BD has been recognized for decades although the exact method of heritability is unknown [68]. One study looked at 67 twin pairs and estimated a heritability of BD by about 85% with no environmental influences detected. Other twin studies have also reported heritability in BD however lack in sample numbers making data
interpretation difficult [69, 70]. Nevertheless, there is clear indication that BD is in part subject to genetic influences that may become more apparent as further evidence emerges.

**1.4.2 Genome-wide Association Studies Point to a Calcium Channel Polymorphism That is Highly Associated with BD:**

Recently, two GWAS reported a significant association between a single nucleotide polymorphism and BD on the voltage dependent, L type, alpha 1C subunit gene, rs1006737 [8, 71]. The gene codes for an alpha-subunit on the Ca\(_{\text{1.2}}\) calcium channel gene, and is biologically associated with endothelial function [72]. Since then, multiple studies have replicated findings between CACNA1C rs1006737 and BD [7, 73-75]. Together, these studies provide the strongest current evidence for existence of a BD-associated genotype. However, as the magnitude of the association between rs1006737 and BD is not substantial (odds ratio approximately 1.2), and given similar findings in other mental illnesses such as major depressive disorder and schizophrenia [7, 74, 76-79], the polymorphism is likely linked with a cluster of phenotypes (endophenotypes), instead of BD as a monolithic construct.

**1.4.3 BD and CACNA1C rs1006737 are Both Associated with Differences in Subcortical Brain Structure:**

**1.4.3A Volumetric Amygdala and Hippocampal Differences are Associated with BD:**

Structural studies have reported differences in symptomatic adolescents with BD [80-82]. More specifically, youth with BD have demonstrated diminished amygdala volumes when compared to control [18, 44, 83, 84]. One study compared 14 BD youth with 23 controls and found significantly lower amygdala volume in the BD cohort [18]. Similarly, another study compared 23 BD adolescents with 20 controls and found that adolescents with BD exhibited smaller amygdala volumes when compared to HC [83]. Others have replicated these results [85, 86]. In addition, children of patients with BD have amygdala surface deformations but no significant decrease in volume when compared to controls, which suggests subcortical
differences may be due to prolonged exposure to the disorder but do contain a hereditary component [27, 80, 87, 88].

Similar structural findings are also reported in the hippocampus [18, 84, 85, 89, 90]. A study looking at 18 adolescents with BD and 18 matched controls reported decreased left hippocampal volumes in the BD cohort [90]. Volumes have been reported to be as much as 9.2% lower when compared to control [89]. Two other studies also reported diminished hippocampal volumes in adolescents with BD when compared to control [45, 91]. Hippocampal atrophy may be reversed by medication, as many studies have reported increased hippocampal volume and improved mood in patients being treated with lithium [28, 92-94]. As lithium is a prescribed mood stabilizer, this suggests subcortical atrophy may in part contribute to mood symptoms observed in BD.

**1.4.3B AMYGDALA AND HIPPOCAMPAL DIFFERENCES ARE ALSO ASSOCIATED WITH CACNA1C**

**RS1006737 RISK ALLELE CARRIERS:**

Currently, there are 6 structural studies looking at healthy risk allele carriers. 4 studies have examined the effects of CACNA1C rs1006737 on amygdala and hippocampal volume. 3 studies found no association between rs1006737 and amygdala/hippocampal GM volume [13, 14, 43]. In one study however, risk allele carriers (A/A, A/G) demonstrated significantly increased GM density in both the left amygdala and hippocampus [15].

Although preliminary findings are not thoroughly convincing, the abundant evidence of BD-related amygdala and hippocampal volumetric differences (see Section 1.4.3A) pinpoint both regions as highly associated with this disorder. Therefore, it is important to further explore the association between rs1006737 and subcortical volumes before dismissing the role of this polymorphism in regional differences observed in BD.
1.4.4 **BD AND CACNA1C rs1006737 ARE BOTH RELATED TO CARDIOVASCULAR FACTORS:**

CVD is the leading cause of premature mortality in BD, largely owing to the early onset CVD in this population [95]. Adults with BD are 5 times more likely to develop CVD, which manifests 14 years sooner than healthy individuals [6]. In addition, BD is highly associated with comorbidities closely linked to CVD. In one study, 35% of patients with BD-I fit criteria for obesity, which further predicted their quality of life and time before episodic recurrence [96]. In addition, patients with BD are significantly more likely to develop diabetes, hypertension and metabolic syndrome [6, 97].

1.4.4A **ED IS HIGHLY ASSOCIATED WITH CVD:**

Although many risk factors contribute to cardiovascular risk, ED is one of particular interest to CACNA1C rs1006737. ED is characterized by reduced bioavailability of vasodilators (such as nitric oxide), which eventually leads to atherogenesis [98]. ED predicts CVD mortality and undermines the primary mechanism in which most risks of CVD facilitate their effects [29, 99]. In adults, ED is highly associated with diabetes, obesity and hypertension [100-102]. In the presence of already existing coronary artery disease, ED also increases the risks of additional CVD such as acute myocardial infarction and ischemic stroke [103].

Like in adults, studies have reported ED as a marker of CVD risk in children. One study reported the presence of ED in children with hypercholesterolemia before anatomical signs of plaque [104]. Another was able to pinpoint ED as an early predictor of hypertension in later adulthood [105]. Furthermore, youth with ongoing CVD do repeatedly show signs of ED. One study reported significant ED in children with Type 1 Diabetes when compared to HC [106-110]. Another study compared obese children with those of normal weight, and found a significant correlation between obesity and microvascular endothelial dysfunction in this sample [111].
1.4.4B ENDOTHELIAL FUNCTION, A RISK FACTOR OF CVD, IS ALSO ASSOCIATED WITH NEUROSTRUCTURAL DIFFERENCES AND COGNITION:

Currently, limited studies have looked at ED and neurostructural differences. One study with 25 elderly adults reported increased white matter hyperintensities in individuals with impaired endothelial-dependent vasodilatation [31]. Likewise, another study reported greater white matter hyperintensities in elderly with decreased cerebral perfusion [112]. Studies have also looked at markers of vascular dysfunction in relation to structural differences. Dieset et al. reported increased plasma levels of von Willebrand factor (a marker of endothelial cell activation) with increased total volumes of the basal ganglia of patients with schizophrenia [40]. Another study positively correlated markers of vascular dysfunction with decreased white matter integrity [39].

Some studies have also looked at functional MRI and ED. One adult study reported a positive association between ED [as measured by brachial flow-mediated dilation (FMD)] and decreased working-memory associated activation in the right superior parietal lobule [113]. Another found that increased intima-media thickness was related to improved amygdala reactivity and functional connectivity between the amygdala and perigenual anterior cingulate cortex [114].

The majority of studies in this area have examined ED and cognitive deficits. More specifically, cerebral ED is positively associated with geriatric cognitive and mood dysfunctions such as major depression and sub-vascular dementia [115-117]. For example, one study looking at an elderly sample reported a positive correlation between memory deficits and endothelial dysfunction [118]. Most other studies in adults report executive and attention deficits in patients with ED [119-121]. One study looking at elderly woman found that individuals with vascular dementia had significantly greater arterial stiffness as well as higher rates of memory impairment [122]. A study in a younger cohort of obese adults (age ≥ 35) found that individuals with better
vascular health exhibited better executive function [121]. In addition, some studies have linked ED with depressive states in adults [123, 124].

Studies have also looked at ED and mood in children. One study looking at pediatric sleep apnea found participants with ED more highly associated with ED had greater neurocognitive deficits (as measured by the Differential Ability Scales and NeuroPSychological Assessment Battery) [52]. Another study reported increased feelings of hopelessness in adolescent girls with increased endothelial dysfunction, but independent of any underlying psychiatric disorder [125]. Similarly, studies looking at healthy adolescent woman report a significantly negative correlation between endothelial function and depression [124, 126]. This suggested that individuals with poor endothelial function and greater feelings of depression.

To date, only two studies have looked directly at ED in participants with BD. One study reported impaired endothelial function in both unipolar and bipolar participants during episodes of depression and after remission [36]. Another looked at a younger cohort, and found no evidence of ED in this sample despite the presence of other signs of CVD [127]. Therefore, further studies are warranted before any conclusive associations between ED and BD can be made.

1.4.4C The role of CACNA1C in BD may also be mediated by endothelial function:

Some studies report anomalous cerebral blood flow (CBF) in BD, although none have examined this affect in relation to CACNA1C rs1006737 [128-131]. For example, one recent study reported better inhibitory response in BD with improved resting state CBF to certain regions of the brain (anterior cingulate cortex, inferior parietal lobe, and dorsal lateral prefrontal cortex) [132]. It is therefore possible that CACNA1C rs1006737 may subserve, in part, the association between BD and CVD by affecting CBF through arterial endothelial function. Indeed, some animal studies have looked at the effects of CCB on CBF.
Animal studies have reported reduced CBF after administration of nimodipine (a dihydropyridine CCB) and other dihydropyridine CCBs [133, 134]. *In vitro* animal studies point to similar relationships during cerebral vasoconstriction [10, 135]. Studies have also looked at the influence of CCB’s on CBF. In one study, rats that were given nimodipine showed diminished cerebral autoregulation when compared to a control group [136]. A more recent study reported an increased CBF in the hippocampus of rats administered nimodipine [137].

In addition, rs1007636 may affect vasoconstriction, including controlling mean arterial pressure (MAP), and moderating response to anti-hypertensive drugs [30, 72]. In one study, mibefradil (a T-type calcium channel inhibitor) reduced peripheral conduit vessel resistance in hind limbs of wild-type mice, but had no effect on Ca<sub>v</sub>1.2 knockout mice [30]. Similar effects were observed in the microcirculatory system, suggesting that Ca<sub>v</sub>1.2 channels are important in regulating MAP and smooth muscle vasoconstriction. Taken together, these studies may point to Ca<sub>v</sub>1.2s involvement in vasodilation of cerebral arteries and subsequently cerebral perfusion [9]. Little has been published regarding the implications of Ca<sub>v</sub>1.2 on CBF; however, a more recent *in vitro* study identified the expression of Ca<sub>v</sub>1.2 in rat cerebral arteries and demonstrated their contribution to maintaining myogenic tone [138].

In addition to animal studies, investigations in human participants were also conducted. Earlier studies looking at healthy participants did not report a significant change in cerebral autoregulation after nimodipine treatment (n=12); however, in a more recent study (n=8), nimodipine increased blood volume and decreased arterial blood pressure, suggesting that nimodipine induced vasodilation and increased CBF [139, 140]. In patients with subarachnoid hemorrhage, nimodipine has also been shown to decrease both MAP and CBF [141]. Taken together, these studies suggest that CBF may be one potential mechanism through which rs1006737 asserts its effects on brain structure and function. No studies to date have directly examined the neurovascular effects of CCBs among adolescents with BD specifically [77].
Some studies have, however, reported cognitive benefits of CCB in the elderly population [142]. In mood disorders, limited studies have properly examined the extended effects of CCB in mood regulation. One early double-blind placebo trial found that verapamil had no significant difference on symptoms of major depressive disorder when compared to placebo, while another study (which did not run to completion) did find a potential benefit of CCB’s in depression [143, 144]. Most studies looking specifically at BD have study limitations that restrict the usefulness of data. Some controlled studies looking at nimodopine did find that BD patients showed significant improved clinical symptoms [37, 145]. Compared to nimodopine, more controlled studies looking at verapamil as a potential therapeutic have been done. Some studies do report improved mood scores in patients on verapamil, however many also find verapamil to be equally effective as lithium [38, 143, 146-149]. Therefore, existing studies have highlighted an interesting therapeutic direction that needs to be further explored in an area of medicine that is constantly thirsty for new interventions. However, current literature, although pointing to promising findings, require further investigation before any concrete evidence can be made.

1.4.5 RHI AS A MEASURE OF ED:

Measurement of ultrasound-based flow-mediated dilation (FMD) is the conventional non-invasive technique of measuring endothelial dysfunction that has proven reproducibility and significantly correlation with certain CVD in patient populations [150, 151]. FMD is an ultrasound based technique that measures changes in conduit artery diameter in response to shear-stress induced vasodilator mediators. Although highly correlated with established invasive measures of ED, FMD has several disadvantages that complicates its use in research [152]. The method is technically demanding and operator-dependent, and as such is subject to measurement error and variability, such as viral illness and other physiological events that alter results [153].

In recent years, pulse amplitude tonometry is a candidate for digital non-invasive measures of ED. In particular, peripheral measures of endothelial vasomotor response to reactive
hyperemia by pulse amplitude tonometry has peaked inquiry in this field [154]. Digital RH-PAT measures resting arterial pulsatile volume during arterial occlusion that induces acute nitric oxide-mediated reactive hyperemia [155]. The technique utilizes a peripheral probe placed on the index finger of the dominant hand, with any systematic variability controlled using an identical probe on the contralateral index fingertip. By comparing results pre- and post- occlusion, a reactive hyperemia index (RHI) is calculated.

Studies using RH-PAT have demonstrated reproducible results consistent with FMD, which points to RH-PAT as an alternative, but less operator dependent, non-invasive measure of ED [156, 157]. Studies have also linked RHI with multiple CVD risk factors, including smoking, overweight and low level of high-density lipoprotein cholesterol [158]. In children, higher RHI (associated with ED) has been linked to adolescents with blood pressure hyperactivity and youth at risk for type 1 diabetes mellitus [159, 160]. Inconsistencies do exist, as one study found that RHI could not predict coronary artery disease but may be useful for predicting the risk of future CVD onset [161]. Thus additional studies remains to be done to further assess the utility of RH-PAT as a clinical detector of CVD.

1.4.6 SUMMARY OF LITERATURE:

BD is a debilitating disorder seen in 2-5% of adolescents and adults that presents with both manic and depressive episodes [54, 55]. Recently, several GWAS have identified a polymorphism in the CACNA1C (rs1006737) gene to be associated with BD. Following this, multiple studies have also examined the effects of the risk allele on morphological differences in regions of the brain often closely associated with BD. To date, no studies have specifically examined the effects of CACNA1C rs1006737 on subcortical volumes in youth with BD. Similarly, despite the compelling evidence of vascular dysfunction in mental illness, no prior studies in BD have examined for neurostructural correlates of vascular measures such as RH-
PAT. There is reason to expect that rs1006737 and endothelial function are independently, and perhaps interactively, relevant to brain structure in BD (Figure 1). Integration of these two variables may inform treatment approaches such as the use of CCBs, as well as incorporating therapeutic strategies that optimize endothelial function, in the management of adolescents with BD.

**Figure 1: Summary of Background and Rationale:** Recently, a polymorphism in CACNA1C (rs1006737) has been associated with both BD and BD-related cognitive deficits and structural differences. As the gene codes for a calcium channel that is biologically associated with endothelial function (which is subsequently associated with cardiovascular risk), we predict the polymorphism may be linked to both neurological and cardiovascular differences observed in BD.

### 2.0 Methods and Materials:

#### 2.1 Study Design:

This study is a cross-sectional observational study of adolescents with BD and comparison healthy adolescents. All consenting participants completed semi-structured diagnostic interviews and other psychiatric assessments, anthropomorphic measurement,
peripheral arterial tonometry to test endothelial function, and structural brain MRI T1 scans for neuroimaging analysis.

2.2 **Participant Recruitment:**

The study aims to recruit 60 adolescent participants with BD (type I, II or not otherwise specified) and 60 HC. Currently, 25 BD and 25 HC adolescents have been recruited. The greater number of participants with BD is required for anticipated sensitivity analyses examining the impact of comorbidities, current mood state, medication and other covariates. Participants with BD were recruited from the Youth Psychiatry Division of Sunnybrook and from the community via advertisements. Controls were recruited from the community via advertisements. The research ethics board at Sunnybrook Health Science Centre approved this study (**Appendix 1**) and all participants as well as one parent provided written informed consent before study initiation (**Appendix 2** and **Appendix 3** respectively).

2.2.1 **Inclusion Criteria:**

English speaking individuals between the ages of 13-20 years of age were recruited as BD or HC respectively, if they fell into either criteria: 1) meet the DSM-IV diagnostic criteria for BD or 2) no history of major psychiatric disorders (no lifetime mood or psychotic disorders, no alcohol or drug dependence in the past 3 months and no anxiety disorders in the past 3 months) and has no family history of BD or psychosis (first or second degree relatives).

2.2.2 **Exclusion Criteria:**

Possible participants were excluded if they were one of the following: 1) unable to give informed consent (ie: severe mania or psychosis or unable to speak English), 2) had a pre-existing cardiac condition, auto-immune illness, or inflammatory illness, 3) taking any anti-inflammatories, anti-platelet, anti-lipidemic, anti-hypertensive or hypoglycemic agents including insulin and metformin, 4) had an infectious disease within the past 14 days, 5) had contradictions
with MRI (ie: cardiac pacemaker) 6) had a health condition or physiological impairment that prohibits intense exercise, 7) had a neurological or severe cognitive impairment (ie: autism)

2.2.3 DEMOGRAPHICS AND MEDICAL HISTORY:

Patient characteristics such as age, sex, diagnosis and concomitant medication were obtained from Kiddie-Schedule for Affective Disorders and Schizophrenia interview during research assessment at the clinic for Youth Bipolar Disorder [162]. Other demographics [body mass index (BMI), waist circumference, weight, current mood status (including mania and depression scores), current and past medication] were collected using standardized inventories during the parent child interview and post-interview self-questionnaires [163-166]. Additional information (ie: family medical history, CARDIO medical history and family medical history) was also collected for completeness but was not relevant to the current study.

2.3 STUDY CHRONOLOGY:

New and existing adolescent patients were initially identified by a clinician or from advertisements within the Centre for Youth Bipolar Disorder at Sunnybrook Health Sciences Centre. Adolescent controls were primarily recruited from community advertisements. All eligible participants were contacted via phone. An overview of the study was presented to both parent and adolescent participants, who then provided written informed consent. Study intake proceeded in two visits. During the first visit, the participant and accompanying parent provided consent and a semi-structured diagnostic interview (see Section 2.3.1). During the second visit, eligible participants provided saliva samples and underwent an endothelial function assessment, a clinical interview and a T1 magnetic resonance imaging (MRI) scan.

2.3.1 PRIMARY INTERVIEW INSTRUMENTS:

Demographics and current mood were assessed during a clinical interview using the Affective Disorder and Schizophrenia for School Aged Children, Present and Life Version – mania and depression scales (K-SADS-PL/ MRS and DEP-P respectively), Cambridge
Neuropsychological Test Automated Battery (CANTAB) medication listing, Children’s Global Assessment Scale (C-GAS), family medical history screen, CARDIA medical and family medical history, and tobacco use form [162-164, 166]. The K-SADS-PL is a semi-structured interview that asks about present and lifetime history of mania and depression (based on DSM-IV criteria). The CANTAB medication listing asks the participant about medication recently taken the day of the study and the day before. C-GAS is an adaptation of the Global Assessment Scale that assesses the overall level of functioning of a child or adolescent during a specific period of time. The family history screen records psychiatric history in all first degree and second-degree relatives and CARDIA medical/ family medical history screens for present and past CVD in participants and first and second-degree relatives respectively.

2.4 GENOTYPING:

2.4.1 SALIVA COLLECTION:

For saliva collection, participants were asked to abstain from drinking, eating, or smoking and chewing gum 30 minutes prior to collection. Saliva was collected in Oragene DNA Self-Collection Kits (OG-500) for collection of human DNA. Participants were asked to salivate into the collection tube up to the indicated line and samples were stored in room temperature before genetic DNA extraction and genotyping was completed at Dr. James L. Kennedy’s lab at the Centre for Mental Health and Addiction.

2.4.2 GENOTYPING:

DNA was extracted in the Neurogenetics Laboratory at the Centre for Addiction and Mental Health (Toronto, Canada) from collected saliva samples (~2mL) on a chemagen MSM I DNA extractor (Perkin-Elmer, Waltham, MA) as per manufacturer’s instructions. The extracted DNA was quantified on a Nanodrop 8000 spectrophotometre (ThermoFisher Scientific, Waltham, MA) and an aliquot diluted to 20 ng/µL for use in downstream genotyping applications.
CACNA1C rs1006737 was genotyped using the TaqMan® OpenArray® Format32 method (ThermoFisher Scientific; Waltham, MA) as per manufacturer’s directions on the QuantStudio™ 12K Flex Real-Time PCR System (ThermoFisher Scientific; Waltham, MA). Within this list, a custom assay for the Amelogenin region was included for quality control purposes. Briefly, 2 μL of DNA at a concentration of 20 ng/μL and 2 μL of 2X TaqMan® OpenArray® Master Mix were combined manually in 384-well plates and loaded onto the OpenArray® genotyping plates using the AccuFill System (ThermoFisher Scientific; Waltham, MA). Prepared arrays were amplified, visualized and analyzed on the QuantStudio™ system. Genotyping of 10% of samples from each run were replicated for quality control purposes for each marker. Genotypes were finally imported into the TaqMan® Genotyper software v1.3 and confirmed manually by two independent lab personnel.

2.4.3 **HARDY-WEINBERG CALCULATIONS:**

In order to assess the similarly of allele frequency between the current sample and the sample population, deviations from Hardy-Weinberg equilibrium was tested to using a Pearson’s chi-squared (χ²) test (alpha: 0.05, Degrees of Freedom: 2) with an overall population minor allele (A) frequency of 0.356 for BD and 0.324 for HC, as reported in a recent GWAS [8]. Deviations from Hardy-Weinberg equilibrium may indicate sampling bias, population stratification, or genotyping errors.

2.5 **MAGNETIC RESONANCE IMAGING AND ANALYSIS:**

2.5.1 **PATIENT PREPARATION:**

All participants were asked to avoid wearing jewelry (including body piercings, mascara and glasses) and screened for MRI safety (Appendix 4 and Appendix 5 respectively) by a licensed MRI technologist before entering the MRI room.


2.5.2 **IMAGE ACQUISITION:**

All MRI scans were done at Sunnybrook Health Science Centre, Toronto, Ontario, Canada, in a research dedicated 3 Tesla Philips Achieva system (Amsterdam, Netherlands) scanner using a high-resolution 8-channel head coil.

2.5.3 **T1 WEIGHTED IMAGING:**

3D T1-weighted Fast Field Echo scans quantified grey and white matter using a single slab Fast Field Echo sequence with 140 slices, an echo time of 2.3 milliseconds (ms), repetition time of 9.5ms, and flip angle of 8 degrees. Field view was 240mm x 191mm. Time of acquisition was 8 minutes and 56 seconds.

![Figure 2: Example of T1 Weighted Images from Varying Participants](image)

**Figure 2:** Example of T1 Weighted Images from Varying Participants: A and B show scans of high resolution and minimal artifacts. Figures C and D show ring artifacts introduced by subtle head movements.
2.5.4 **IMAGE PROCESSING:**

Digital Imaging and Communications in Medicine images obtained from the MRI were transformed into 3D Neuroimaging Informatics Technology Initiative files using MRIcron (MRIcron; version 4th Aug, 2014; http://www.mccauslandcenter.sc.edu/mricro/mricron/). Images were inspected visually for quality and presentation of artifacts, then co-registered individually to a publically available standard space reference brain (Talairach atlas) [167]. Subcortical volumes (bilateral hippocampus and amygdala) were extracted in regions of interest (ROI) using a free Linux based automatic labeling tool called FreeSurfer (FreeSurfer; version 5.3.0; freesurfer.net). FreeSurfer runs a probabilistic volume-based analysis to map subcortical regions, which consists of five stages. The first stage involves an affine transformation with the Talairach space followed by an initial volumetric labeling. Variations in intensity due to B1 bias field were corrected algorithmically before a final nonlinear volumetric alignment with the Talairach atlas. Finally, the program labels the volumes.

**FIGURE 3: SEGMENTATION OF SUBCORTICAL VOLUMES:** Examples of segmentation performed using automatic surface parcellation and volumetric segmentation based on Talairach Atlas.
2.6 **Endothelial Function Assessment:**

Participants were asked to fast for 8 hours prior to endothelial function assessment. Endothelial function was assessed by RHI using a RH-PAT (EndoPAT™, Itamar Medical, Isreal). RH-PAT occurred during 9-11 am in a thermally controlled (21-24 Celsius) environment after participant has acclimatized from extreme outdoor surroundings. Participants were asked to lie in a dimmed room with reduced noise and the device was placed 1-2 feet away from examination bed after a 20 min initial warm up. Before starting the assessment, BP, height, weight and waist circumference were measured by a trained research assistant. A BP cuff for vein occlusion was applied to the non-dominant arm 2-3 inches above the elbow. The experimental timeline was explained and patients were asked to lie very still, avoiding any finger movement. They were asked not to listen to music but were allowed to fall asleep.

Participants were asked to remove nail polish before arrival. Non-invasive pneumatic probes were inserted on the index finger (trimmed nails if necessary) of participants in supine position. The probes continuously record pulse wave amplitude in the fingertips. Patients were reminded before initiation to avoid touching probes during assessment. Forearms were placed on an arm-support palm-side down to allow probe to dangle freely. Assessment began if a good signal was observed for a minimum of 1 minute. After a 10-minute calibration period, the non-dominant arm (self-reported) was rapidly occluded using the pressure cuff (inflated to 200mmHg) for 5 minutes, followed by a release and a 5-minute post-occlusion period. The RHI was then calculated automatically as the ratio of average pulse wave amplitude one minute post-occlusion over the average amplitude during the 10-minute calibration period. Following RH-PAT, participants were asked to base their pain during the procedure using the Wong-Baker FACES Pain Rating Scale [168].
2.7 **Statistical Analysis:**

All continuous variables were presented as a mean and standard deviation while categorical variables were presented as a percentage of the total. SPSS was used for all statistical analysis (SPSS statistical software, version 22; IBM, Armonk NY). Continuous demographic variables were compared between HC and BD using a Student’s t-test and Cohen’s d for equal samples was used as an indicator of effect size. Cohen’s d for unequal samples was used as an indicator of effect size for demographic samples of unequal numbers (waist circumference, DEP-P Scores). Normality of each sample was tested using Shapiro-Wilk tests. Categorical demographic variables were compared between HC and BD using a Fischer’s Exact test and Cramer’s V values were used to indicate effect size. To accommodate for the low rate of A/A homozygotes, A/A and A/G participants were grouped as ‘risk allele carriers’ in all analyses. Results were corrected for multiple comparisons using the False Discovery Rate correction (FDR) [169]. The threshold for statistical significance was set to 0.05.

We chose to perform statistics on a priori selected ROI’s to reduce the severity of multiple corrections, which is present in whole-brain analysis [170]. Therefore, multiple correction adjustments using FDR were applied individually to each set of analyses within each ROI (bilateral hippocampus and amygdala). Finally, graphical representations depicting the ratio of ICV and ROI volumes were created to visually assess associations only. All statistical tests were done using general linear models (GLM’s).

Intracranial volume (ICV) was included as a covariate to correct for fluctuating cranial sizes in all hypotheses [171]. Age is positively correlated with brain volume, and in the current sample this variable was also significantly correlated with ICV (Table 1). Therefore, age was excluded in the GLM’s to avoid the problem of multicollinearity. No other covariates were included due to sample size limitations, and standardized β values were calculated to gauge the effect size of every comparison.
<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>F</th>
<th>Sig.</th>
<th>Standardized Beta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>5.61</td>
<td>0.022</td>
<td>-0.32</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*R = 0.32; R squared = 0.11*

**TABLE 1: SUMMARY OF LINEAR REGRESSION EXAMINING CORRELATION BETWEEN ICV AND AGE.** As ICV was significantly correlated with age (p≤0.05), age was removed from covariates during subcortical analysis.

### 2.7.1 PRIMARY HYPOTHESIS: ANALYSIS OF SUBCORTICAL VOLUMES AND CORTICAL THICKNESS BETWEEN GENOTYPE COHORTS:

In the primary hypothesis (Section 3.5), total hippocampal and amygdala volumes were compared between carriers and non-carriers using multiple general linear univariate models for one dependent variable and multiple independent variables [44]. An interaction term (Genotype*Diagnosis) was added to the GLM to determine whether volumetric effects of CACN1AC rs1006737 were more apparent in BD or HC. Each region of interest was entered as a dependent variable and diagnosis, genotype, ICV were entered as independent variables. This GLM was first run between carriers and non-carriers in the entire sample. The sample was then split into two cohorts: carriers and non-carriers. The GLM was then run once more between carriers and non-carriers in each individual cohort (Figure 4).

In addition, the GLM was run in bilateral amygdala and hippocampal volumes to detect any possible differences between the left and right regions. This analysis was also performed in each individual cohort (as depicted by Figure 4).
Figure 4: Statistical Analysis Cohorts: Three cohorts were used in each analysis. The first one consists of carriers and non-carriers in the entire sample. This combined both BD and HC participants. Two cohorts were then split from the original sample: BD and HC. Differences between carriers and non-carriers were then additionally analyzed in these individual groups. The figure shows the definition of a ‘diagnostic independent’ and ‘diagnosis dependent’ analysis as used in this study.

2.7.2 Secondary Hypothesis: Analysis of RHI and Subcortical Volumes

A GLM was used to model a potential correlation between RHI and total amygdala and hippocampal volumes using RHI and ICV as covariates (Section 3.6). In addition, an interaction term (RHI*Diagnosis) was added to the GLM to determine whether volumetric effects of RHI were more apparent in BD or HC. In order to observe the specific interactions between RHI and subcortical volumes, the sample was split two ways (Figure 5). Firstly, GLM was run in the entire sample for the both carriers and non-carriers (diagnosis independent). Secondly, the identical GLM was then re-run in both carriers and non-carriers once the dataset was split into BD and HC (Figure 5). GLM’s were run in total hippocampus and amygdala volumes, as well as in bilateral amygdala and hippocampal volumes.
**FIGURE 5: SAMPLE SPLITTING DONE IN SECONDARY HYPOTHESIS:** GLM’s were run initially in the total sample to assess the association between RHI and subcortical volumes in both carriers and non-carriers. The GLM’s were then run after the total sample was split into HC and BD. In summary, GLM’s between RHI and subcortical volumes were run in each individual cohort labeled as diamonds.

**2.7.3 POST-HOC ANALYSIS:**

3 post hoc analyses were run after completing the primary, secondary and exploratory hypothesis. Firstly, bilateral hippocampal and amygdala volumes were compared between BD and HC using a GLM with ICV as a covariate. Secondly, differences in ICV were checked between carriers and non-carriers in the total dataset using a Student’s t-test. This test was done once the entire dataset, and was then repeated in BD and HC (Figure 4). Finally, an interaction between RHI and genotype was assessed by adding an interaction term (RHI * genotype) into the main-effects GLM used in section 2.7.2.

**2.7.4 ICV AS A COVARIATE:**

Total brain volume (or ICV) has a great influence on interpretation of morphological findings. By accounting for differences in ICV, results will reflect morphological fluctuations in regional areas that are due primarily to the variable of interest (in this case genotype or
diagnosis) rather than differences in physiology. Use of ICV as a proper correction for brain size variability has widely dominated more traditional measures such as head circumference [172]. It is considered a more accurate measure of total brain volume in youth and is an appropriate adjustment factor in studies looking at grey matter volume [173-175]. Therefore, ICV was included as a covariate in the present study.

2.7.5 **POWER CALCULATION:**

A *post hoc* power calculation was performed for linear multiple regression using G*power 3.1 (G*Power: Statistical Power Analysis for Windows and Mac, Version 3.1.9.2, http://www.gpower.hhu.de/). The post hoc analysis was performed on the entire dataset (n=50), which consisted of 23 carriers and 27 non-carriers (Figure 4). A power calculation was also performed for each individual diagnosis cohort (BD and HC), with BD containing 7 risk allele carriers and 18 non-carriers, while HC had 16 risk allele carriers and 9 non-carriers (Figure 4). Assuming a type I error rate of 0.05, the study is currently underpowered to detect small effect sizes of $f^2 = 0.02$ (entire dataset power = 0.165, BD/HC cohort power = 0.104), medium effect sizes of $f^2 =0.15$ (entire dataset = 0.765, HC/BD cohort power =0.46). The study however, is powered to detect a large effect size of $f^2 = 0.35$ (entire dataset power = 0.984, HC cohort power = 0.452, BD cohort power = 0.806).

3.0 **RESULTS:**

3.1 **DEMOGRAPHICS:**

Demographics of 50 adolescents (BD=25, HC=25) are shown in table 2. A similar comparison (but between carriers and non-carriers) is shown in table 3. Finally, table 4 and table 5 show demographics between carriers and non-carriers in HC and BD respectively. Some individuals did not provide certain demographics. 2 HC were missing blood pressure measurements (BP) and 6 HC + 10 BD did not provide waist circumference. One participant failed to provide DEP-P scores. As none of these demographics were used directly in analysis, these participants were not
excluded from the data. 2 BD and 2 HC did not provide satisfactory RHI. These individuals were excluded in the secondary hypothesis. Not all individuals who participated were screened for CACNA1C due to time constraints. The BD cohort had significantly greater age, BMI, weight, weight circumference, mania and depression scores, and antipsychotic use when compared to control. In total, 23 out of 25 BD were medicated, while 3 out of 25 HC were medicated.
<table>
<thead>
<tr>
<th>SOCIODEMOGRAPHICS OF ENTIRE SAMPLE (N=50)</th>
<th>HC (n=25)</th>
<th>BD (n=25)</th>
<th>p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.64 ± 1.78</td>
<td>16.90 ± 1.35</td>
<td>0.0061</td>
<td>0.83</td>
</tr>
<tr>
<td>Gender [ N (%) Male]</td>
<td>13 (52%)</td>
<td>10 (40%)</td>
<td>0.57</td>
<td>0.12</td>
</tr>
<tr>
<td>Ethnicity [N (%) Caucasian]</td>
<td>21 (84%)</td>
<td>24 (96%)</td>
<td>0.35</td>
<td>0.20</td>
</tr>
<tr>
<td>GENETIC PARAMETERS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CACNA1C Genotype [A/A + A/G (%)/ G/G (%)]</td>
<td>16 (64%)/9 (36%)</td>
<td>7 (28%)/18 (72%)</td>
<td>0.022</td>
<td>0.36</td>
</tr>
<tr>
<td>RECENT PSYCHIATRIC STATUS (MEAN ± ST DEV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-SADS Mania Score</td>
<td>0</td>
<td>11.84 ± 9.14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Depression Score</td>
<td>1.04 ± 2.40</td>
<td>12.88 ± 10.69</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CARDIAC PARAMETERS (MEAN ± ST DEV)</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Adjusted BMI</td>
<td>20.63 ± 2.75</td>
<td>24.4 ± 3.14</td>
<td>p&lt;0.001</td>
<td>1.33</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>73.88 ± 6.72</td>
<td>83.20 ± 9.03</td>
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<tr>
<td>RHI</td>
<td>1.63 ± 0.52</td>
<td>1.86 ± 0.57</td>
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<td>0.42</td>
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<td>CONCOMITANT MEDICATION [N (%) yes]</td>
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<tr>
<td>Anticonvulsant</td>
<td>0</td>
<td>7 (28%)</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Antipsychotic</td>
<td>0</td>
<td>19 (76%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>0</td>
<td>5 (20%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stimulant</td>
<td>3 (12%)</td>
<td>1 (4%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lithium</td>
<td>0</td>
<td>4 (16%)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**TABLE 2: COMPLETE DEMOGRAPHIC CHARACTERISTICS (COMPAARED BY DIAGNOSIS):** Continuous variables (age, cardiac parameters, recent psychiatric status) were compared between HC and BD using a Student’s t-test. Cohen’s d’s are reported as effect size. Categorical variables (gender, ethnicity, allele frequency) were compared between HC and BD using a Fisher’s Exact test. Cramer’s V values are reported as effect size.
### Sociodemographics of Entire Sample (N=50)

<table>
<thead>
<tr>
<th></th>
<th>A/A+A/G (n = 23)</th>
<th>G/G (n=27)</th>
<th>p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>16.35 ± 1.77</td>
<td>16.22 ± 1.65</td>
<td>0.80</td>
<td>0.075</td>
</tr>
<tr>
<td>Gender [ N (%) Male]</td>
<td>9 (39.13%)</td>
<td>14 (51.85%)</td>
<td>0.41</td>
<td>0.13</td>
</tr>
<tr>
<td>Ethnicity [N (%) Caucasian]</td>
<td>22 (95.65%)</td>
<td>23 (85.18%)</td>
<td>0.36</td>
<td>0.17</td>
</tr>
</tbody>
</table>

### Recent Psychiatric Status (Mean ± ST Dev)

<table>
<thead>
<tr>
<th></th>
<th>A/A+A/G (n = 23)</th>
<th>G/G (n=27)</th>
<th>p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-SADS Mania Score</td>
<td>4.00± 8.12</td>
<td>7.56 ± 9.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Depression Score</td>
<td>6.13 ± 10.60</td>
<td>7.46 ± 8.91</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Cardiac Parameters (Mean ± ST Dev)

<table>
<thead>
<tr>
<th></th>
<th>A/A+A/G (n = 23)</th>
<th>G/G (n=27)</th>
<th>p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted BMI</td>
<td>22.33 ± 3.55</td>
<td>22.68 ± 3.53</td>
<td>0.73</td>
<td>0.10</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>78.00 ± 8.78</td>
<td>77.98 ± 9.47</td>
<td>0.99</td>
<td>0.0020</td>
</tr>
<tr>
<td>RHI</td>
<td>1.79 ± 0.46</td>
<td>1.711 ± 0.62</td>
<td>0.62</td>
<td>0.15</td>
</tr>
</tbody>
</table>

### Concomitant Medication [N (%) Yes]

<table>
<thead>
<tr>
<th></th>
<th>A/A+A/G (n = 23)</th>
<th>G/G (n=27)</th>
<th>p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticonvulsant</td>
<td>2 (8.70%)</td>
<td>5 (19%)</td>
<td>0.43</td>
<td>0.14</td>
</tr>
<tr>
<td>Antipsychotic</td>
<td>6 (26.01%)</td>
<td>13 (48.14%)</td>
<td>0.15</td>
<td>0.23</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>2 (8.70%)</td>
<td>3 (11.11%)</td>
<td>1.00</td>
<td>0.040</td>
</tr>
<tr>
<td>Stimulant</td>
<td>2 (8.70%)</td>
<td>2 (7.40%)</td>
<td>1.00</td>
<td>0.024</td>
</tr>
<tr>
<td>Lithium</td>
<td>3 (13.04%)</td>
<td>1 (3.70%)</td>
<td>0.322</td>
<td>0.17</td>
</tr>
</tbody>
</table>

**Table 3: Compared Demographic Characteristics (Compared by Genotype):** Continuous variables (age, cardiac parameters, recent psychiatric status) were compared between carriers and non-carriers using a Student’s t-test. Cohen’s d’s are reported as effect size. Categorical variables (gender, ethnicity, allele frequency) were compared between carriers and non-carriers using a Fisher’s Exact test. Cramer’s V values are reported as effect size.
<table>
<thead>
<tr>
<th><strong>SOCIODEMOGRAPHICS OF HC (N=25)</strong></th>
<th><strong>A/A+A/G</strong></th>
<th><strong>G/G</strong></th>
<th><strong>p-value</strong></th>
<th><strong>Effect Size</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.75 ± 1.69</td>
<td>15.44 ± 2.0</td>
<td>0.69</td>
<td>0.17</td>
</tr>
<tr>
<td>Gender [ N (%) Male]</td>
<td>8 (50%)</td>
<td>5 (55.55%)</td>
<td>1.00</td>
<td>0.053</td>
</tr>
<tr>
<td>Ethnicity [N (%) Caucasian]</td>
<td>15 (93.75%)</td>
<td>6 (66.66%)</td>
<td>0.12</td>
<td>0.35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>RECENT PSYCHIATRIC STATUS (MEAN ± ST DEV)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>K-SADS Mania Score</td>
</tr>
<tr>
<td>Depression Score</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>CARDIAC PARAMETERS (MEAN ± ST DEV)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted BMI</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
</tr>
<tr>
<td>RHI</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>CONCOMITANT MEDICATION [N (%) YES]</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulant</td>
</tr>
</tbody>
</table>

**TABLE 4: DEMOGRAPHIC CHARACTERISTICS OF CARRIERS AND NON-CARRIERS IN HC:** Continuous variables (age, cardiac parameters, recent psychiatric status) were compared between carriers and non-carriers using a Student’s t-test. Cohen’s d’s are reported as effect size. Categorical variables (gender, ethnicity, allele frequency) were compared between carriers and non-carriers using a Fischer’s Exact test. Cramer’s V values are reported as effect size.
<table>
<thead>
<tr>
<th><strong>SOCIODEMOGRAPHICS OF BD (N=25)</strong></th>
<th><strong>A/A+A/G (n = 7)</strong></th>
<th><strong>G/G (n=18)</strong></th>
<th><strong>p-value</strong></th>
<th><strong>Effect Size</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>17.71 ± 1.11</td>
<td>16.61 ± 1.33</td>
<td>0.065</td>
<td>0.81</td>
</tr>
<tr>
<td>Gender [ N (%) Male]</td>
<td>1 (14.29%)</td>
<td>9 (50%)</td>
<td>0.18</td>
<td>0.33</td>
</tr>
<tr>
<td>Ethnicity [N (%)] Caucasian</td>
<td>7 (100%)</td>
<td>17 (94.44%)</td>
<td>1.00</td>
<td>0.13</td>
</tr>
</tbody>
</table>

**RECENT PSYCHIATRIC STATUS (MEAN ± ST DEV)**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>K-SADS Mania Score</td>
<td>13.14 ± 10.09</td>
<td>11.33 ± 9.00</td>
<td>0.67</td>
<td>0.18</td>
</tr>
<tr>
<td>Depression Score</td>
<td>18.14 ± 12.36</td>
<td>10.70 ± 9.47</td>
<td>0.12</td>
<td>0.68</td>
</tr>
</tbody>
</table>

**CARDIAC PARAMETERS (MEAN ± ST DEV)**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted BMI</td>
<td>25.07 ± 2.91</td>
<td>24.19 ± 3.28</td>
<td>0.54</td>
<td>0.26</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>83.50 ± 8.27</td>
<td>83.05 ± 9.81</td>
<td>0.93</td>
<td>0.05</td>
</tr>
<tr>
<td>RHI</td>
<td>1.96 ± 0.46</td>
<td>1.81 ± 0.62</td>
<td>0.53</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**CONCOMITANT MEDICATION [N (%) YES]**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticonvulsant</td>
<td>2 (28.57%)</td>
<td>5 (28%)</td>
<td>1</td>
<td>0.0079</td>
</tr>
<tr>
<td>Antipsychotic</td>
<td>6 (85.71%)</td>
<td>13 (72%)</td>
<td>0.64</td>
<td>0.14</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>2 (28.57%)</td>
<td>3 (16.67%)</td>
<td>0.60</td>
<td>0.13</td>
</tr>
<tr>
<td>Stimulant</td>
<td>1 (14.28%)</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lithium</td>
<td>3 (42.86%)</td>
<td>1 (5.6%)</td>
<td>0.053</td>
<td>0.46</td>
</tr>
</tbody>
</table>

**Table 5: Demographic Characteristics of Carriers and Non-Carriers in BD**: Continuous variables (age, cardiac parameters, recent psychiatric status) were compared between carriers and non-carriers using a Student’s t-test. Cohen’s d’s are reported as effect size. Categorical variables (gender, ethnicity, allele frequency) were compared between carriers and non-carriers using a Fisher’s Exact test. Cramer’s V values are reported as effect size.

### 3.2 Hardy-Weinberg Calculation:

Hardy-Weinberg equilibrium was tested using a Pearson’s $\chi^2$ test. In total, the HC group had 16 carriers (3A/A+13A/G) and 9 non-carriers (G/G), while the BD group had 7 carriers (2A/A+ 5A/G) and 18 non-carriers. The HC cohort was in Hardy-Weinberg equilibrium ($\chi^2$=...
The BD cohort had significantly different frequencies than expected ($\chi^2 = 9.692; p = 0.008$).

### 3.3 Neuroimaging Outcomes

Mean ROI volumes are listed in Tables 6. In total, 11 major areas were linked to the FC.

<table>
<thead>
<tr>
<th>ROI</th>
<th>HC Volume (mm$^3$) ± st. dev.</th>
<th>BD Volume (mm$^3$) ± st. dev.</th>
<th>P value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>4579.70 ± 398.55</td>
<td>4434.21 ± 390.49</td>
<td>0.19</td>
<td>0.38</td>
</tr>
<tr>
<td>Amygdala</td>
<td>1638.08 ± 159.48</td>
<td>1592.46 ± 176.026</td>
<td>0.34</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Right</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>4611.92 ± 373.22</td>
<td>4326.28 ± 357.05</td>
<td>0.0010</td>
<td>0.80</td>
</tr>
<tr>
<td>Amygdala</td>
<td>1710.23 ± 174.32</td>
<td>1689.22 ± 189.80</td>
<td>0.69</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>9191.62 ± 747.81</td>
<td>8760.49 ± 709.54</td>
<td>0.041</td>
<td>0.60</td>
</tr>
<tr>
<td>Amygdala</td>
<td>3348.32 ± 315.66</td>
<td>3281.69 ± 189.80</td>
<td>0.48</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Estimated Intracranial Volume (eICV)</strong></td>
<td>1.54E+06 ± 1.92E+05</td>
<td>1.44E+06 ± 1.74E+05</td>
<td>0.058</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>Total Grey Matter (GM)</strong></td>
<td>7.12E+05 ± 7.71E+04</td>
<td>6.72E+05 ± 6.13E+04</td>
<td>0.048</td>
<td>0.59</td>
</tr>
</tbody>
</table>

**TABLE 6: MEAN ROI VOLUMES.** Regions were identified using the Talairach atlas in FreeSurfer. Areas were compared using a Student’s t-test, and covariate correction was not performed. Cohen’s d’s are reported as effect size.

### 3.4 Primary Hypothesis:

As differences in amygdala and hippocampal morphology are a well-replicated finding in both youth and adult BD, we investigated the possible contribution of the BD associated susceptibility polymorphism (CACNA1C rs1006737) to these specific morphological differences. We summated bilateral hippocampal and amygdala volumes as conventional in literature [176]. The genotype*diagnosis interaction term was not significant after correcting for multiple comparisons, therefore was excluded from the final GLMs ($F=0.034$, $p=0.854$). In the total complete cohort, carriers did not demonstrate a significantly different total hippocampal volume when compared to non-carriers after correction for ICV ($F=0.367$, $p=0.547$). Similarly,
carriers did not demonstrate significantly different total amygdala volumes when compared to carriers after ICV correction (F = 0.074, p = 0.79)

The group was then split into two separate groups: BD and HC. Within the BD cohort, carriers did not show significantly different amygdala (F = 0.111, p = 0.742) or hippocampal (F = 1.2, p = 0.269) volumes after ICV correction. Similarly in the HC cohort, carriers also did not show significantly different amygdala (F = 0.7, p = 0.412) or hippocampal (F = 2.62, p = 0.12) volumes after ICV correction.

Some studies have also reported differences between left and right ROI’s, therefore we looked at interactions in the left and right hippocampus and amygdala independently [177, 178]. In the entire dataset, carriers did not have significantly different bilateral hippocampal (F_{left} = 0.263, p_{left} = 0.61; F_{right} = 0.38, p_{right} = 0.541) and amygdala volumes (F_{left} = 0.342, p_{left} = 0.56; F_{right} = 0.001, p_{right} = 0.978) when compared to non-carriers. This was after correction for ICV.

When the complete dataset was split into HC and BD, HC carriers were significantly associated with greater bilateral hippocampal volume when compared to HC non-carriers (t_{left}=2.78, p_{left}=0.01; t_{right}=2.167, p_{right}=0.041), however these associations did not survive correction for intracranial volume as a covariate (F_{left} = 3.79, p_{left} =0.06; F_{right}=1.2, p_{right}=0.285).

No significant differences in bilateral hippocampal volumes associates were observed between BD carriers and non-carriers after correction for ICV (Appendix 6: Table 7). No significant findings were observed in the bilateral amygdala (Appendix 6: Table 7). Figure 7 and 8 represent volumes graphically as a ratio of ICV.
FIGURE 6: COMPARISON OF AVERAGE BILATERAL HIPPOCAMPAL AND AMYGDALA VOLUMES IN HEALTHY CONTROL (CORRECTED FOR ICV). Groups were compared using a GLM. No significant differences were observed. Volume corrected = ROI volume / ICV.

FIGURE 7: COMPARISON OF AVERAGE BILATERAL HIPPOCAMPAL AND AMYGDALA VOLUMES IN ADOLESCENTS WITH BD (CORRECTED FOR ICV). Groups were compared using a GLM. No significant differences were observed. Volume corrected = ROI volume / ICV.
3.5 **SECONDARY HYPOTHESIS:**

As CACNA1C rs1006737 codes for a subunit of an L-type calcium channel integrated in endothelial health, we investigated the significance of this risk allele in predicting the relationship between peripheral endothelial function (as measured by RHI) and subcortical volumes in youth with and without BD. The RHI*diagnosis interaction term was not significant after correcting for multiple comparisons, therefore was excluded from the final GLMs. In the total sample, no significant associations between RHI and total hippocampal volume (corrected for ICV) were observed in carriers (F = 2.25E-4, p = 0.988) or non-carriers (F = 3.683, p = 0.068). Similarly, no significant associations between RHI and total amygdala volume (corrected for ICV) were observed in carriers (F = 0.118, p = 0.736) or non-carriers (F = 1.898, p = 0.182). The dataset was then split into HC and BD. No significant associations between RHI and total subcortical volumes were observed in either BD or HC cohorts (Appendix 6: Table 8 and 9).

As with the primary hypothesis, the ROI’s where then split into the bilateral hippocampus and amygdala. GLM’s were then run in a similar fashion as above. Firstly, carriers and non-carriers where compared within the entire dataset. No significant associations between RHI and subcortical volumes (bilateral amygdala and hippocampus) were observed.

Afterwards, the dataset was split into HC and BD. In HC, RHI was significantly associated with left hippocampal volume in HC non-carriers (F=6.781, p=0.04) (Table 10). However, this significant association did not survive FDR multiple comparisons. In the HC cohort, no significant trends were observed in the right hippocampus (Appendix 6: Table 11). No significant associations in the bilateral hippocampus were observed in the BD cohort (Appendix 6: Table 12 and 13). No significant associations were observed in the bilateral amygdala.
A negative trend seen in HC non-carriers can be visually observed when left hippocampal volume is plotted as a ratio of ICV (Figure 9). In contrast, HC carriers seem to demonstrate a non-significant positive trend in the left hippocampal (Figure 10).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Genotype</th>
<th>Source</th>
<th>df</th>
<th>F</th>
<th>p value</th>
<th>Standardized Beta</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>Carrier</td>
<td>Corrected Model</td>
<td>2</td>
<td>1.61</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intercept</td>
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<td>12.55</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ICV</td>
<td>1</td>
<td>0.329</td>
<td>0.58</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RHI</td>
<td>1</td>
<td>2.50</td>
<td>0.142</td>
<td>0.48</td>
</tr>
<tr>
<td>Non-Carrier</td>
<td></td>
<td>Corrected Model</td>
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<td>5.45</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intercept</td>
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<td>24.34</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ICV</td>
<td>1</td>
<td>4.67</td>
<td>0.074</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RHI</td>
<td>1</td>
<td>6.81</td>
<td>0.040</td>
<td>-0.46</td>
</tr>
<tr>
<td>BD</td>
<td>Carrier</td>
<td>Corrected Model</td>
<td>2</td>
<td>1.66</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intercept</td>
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<td>20.71</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ICV</td>
<td>1</td>
<td>0.14</td>
<td>0.73</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RHI</td>
<td>1</td>
<td>3.003</td>
<td>0.16</td>
<td>-0.46</td>
</tr>
<tr>
<td>Non-Carrier</td>
<td></td>
<td>Corrected Model</td>
<td>2</td>
<td>10.67</td>
<td>0.0020</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intercept</td>
<td>1</td>
<td>13.70</td>
<td>0.0030</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ICV</td>
<td>1</td>
<td>21.23</td>
<td>p&lt;0.001</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RHI</td>
<td>1</td>
<td>1.093</td>
<td>0.32</td>
<td>-0.17</td>
</tr>
</tbody>
</table>

a. $R^2 = .226$ (Adjusted $R^2 = .085$)  
b. $R^2 = .645$ (Adjusted $R^2 = .526$)  
c. $R^2 = .454$ (Adjusted $R^2 = .181$)  
d. $R^2 = .621$ (Adjusted $R^2 = .563$)

**Table 10: Summary of GLMs examining association between RHI and left hippocampal volumes.** The total dataset was split into BD and HC, where the association between RHI and bilateral subcortical volumes could be examined independently in carriers and non-carriers. Covariates entered into the model include ICV. A significant association between RHI and left hippocampal volume was observed in HC non-carriers. This association did not survive correction for multiple comparisons (not shown).
Figure 8: Comparison of Left Hippocampal Volumes in HC (Corrected for ICV). The significant association in non-carriers did not survive FDR correction. A possible interaction is observed between groups (see Section 3.7.3). ROI corrected = ROI volume / ICV.

Figure 9: Comparison of Left Hippocampal Volumes in BD (Corrected for ICV). Both genotype cohorts demonstrated similar trends. No significant associations were found. ROI corrected = ROI volume / ICV.
3.6 POST HOC ANALYSES

3.6.1 COMPARISON OF ROI’S IN BD VS. HC:

Previous studies strongly support morphological differences between BD and HC in amygdala and hippocampal volumes. In particular, adolescents with BD have smaller amygdala and hippocampal volumes [44, 90, 179, 180]. In the interest of replicating previous findings with the current sample, comparisons between aforementioned areas were completed. Adolescents with BD had significantly lower right hippocampal volumes when compared to HC (F=7.086, p=0.01), which did not survive correction for ICV (F=3.377, p=0.071) (Table 6 and Table 14 respectively).

<table>
<thead>
<tr>
<th>Model</th>
<th>ROI (Volume)</th>
<th>df</th>
<th>F</th>
<th>p Value</th>
<th>Standardized Beta</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICV corrected</td>
<td>Left Hippocampus</td>
<td>1</td>
<td>0.03</td>
<td>0.86</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>Right Hippocampus</td>
<td>1</td>
<td>3.59</td>
<td>0.064</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Left Amygdala</td>
<td>1</td>
<td>0.0016</td>
<td>0.97</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Right Amygdala</td>
<td>1</td>
<td>0.10</td>
<td>0.75</td>
<td>-0.09</td>
</tr>
</tbody>
</table>

**Table 14: Summary of GLMs Examining Mean Hippocampal and Amygdala Volumes Between BD and HC.** ICV was entered as a covariate during the “ICV corrected” Model. Values in the table have not been corrected for multiple comparisons.

3.6.2 INTRACRANIAL VOLUME INCONSISTENCIES:

Graphical representations of amygdala and hippocampal volumes (with vs. without ICV correction) show contrasting trends (Figure 7 vs. 11, and Figure 8 vs. 12). When corrected for ICV, non-carriers demonstrated non-significantly greater bilateral hippocampal and amygdala volumes when compared to non-carriers (Figures 7 and 8). Without correcting for ICV however, the opposite was observed (Figures 11 and 12). To further investigate this contrast in trends, mean ICV was compared between HC and BD in the entire sample, as well as between carriers and non-carriers once the dataset was split into HC and BD cohorts. On average, BD had smaller ICV when compared to HC (t=1.943, p=0.058). In HC, carriers had trending greater
average ICV when compared to non-carriers (t=2.031, p=0.054). This was not seen within the BD carriers when compared to BD non-carriers (t = -1.603, p = 0.123).

**Figure 11:** Comparison of average bilateral hippocampal and amygdala volumes in HC (uncorrected for ICV). Groups were compared using a Student’s t-test. A significant difference within group was observed in the hippocampus. p≤0.05.

**Figure 12:** Comparison of average bilateral hippocampal and amygdala volumes in BD (uncorrected for ICV). Groups were compared using a Student’s t-test. No significant differences were observed.
3.6.3 **Possible RHI vs Genotype Interaction within HC:**

Figures 9 presents a possible interaction between RHI and genotype in the left hippocampus within the HC group. To assess this possibility, a RHI*genotype interaction term was added to the main effects model (which consists of RHI and ICV as covariates, and ROI as a dependent variable). Within HC, a significant interaction was indeed observed in HC bilateral hippocampus between genotype and RHI (Table 15 and 16; \( F_{\text{left}} = 6.411, p_{\text{left}} = 0.021; F_{\text{right}} = 4.755, p_{\text{right}} = 0.043 \)). Both interactions survived correction for multiple comparisons (\( q = 0.04 \)). This was not seen in the BD group. No significant interactions were observed in the bilateral amygdala.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Source</th>
<th>df</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC(^a)</td>
<td>Corrected Model</td>
<td>4</td>
<td>5.00</td>
<td>0.0070</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>1</td>
<td>30.21</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ICV</td>
<td>1</td>
<td>2.26</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>RHI</td>
<td>1</td>
<td>p&lt;0.001</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Genotype * RHI</td>
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<td>6.41</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Genotype</td>
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<td>2.84</td>
<td>0.11</td>
</tr>
<tr>
<td>BD(^b)</td>
<td>Corrected Model</td>
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<td>6.04</td>
<td>0.0030</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
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<td>21.58</td>
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<tr>
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<td>RHI</td>
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<td>0.168</td>
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<tr>
<td></td>
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<td>0.50</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Genotype</td>
<td>1</td>
<td>0.34</td>
<td>0.57</td>
</tr>
</tbody>
</table>

* a. \( R \text{ Squared} = .526 \) (Adjusted \( R \text{ Squared} = .421 \))
* b. \( R \text{ Squared} = .573 \) (Adjusted \( R \text{ Squared} = .478 \))

**Table 15: Summary of GLMs Examining a Possible Interaction between RHI and Genotype in the Left Hippocampus of BD and HC.** The data was split into HC and BD and analyzed in a diagnosis dependent way. There was a significant interaction term in the HC. ICV and RHI were entered as a covariate.
TABLE 16: SUMMARY OF GLMS EXAMINING A POSSIBLE INTERACTION BETWEEN RHI AND GENOTYPE IN THE RIGHT HIPPOCAMPUS OF BD AND HC. The data was split into HC and BD and analyzed in a diagnosis dependent way. There was a significant interaction term in the HC. ICV was entered as a covariate.

4.0 DISCUSSION:

In the current study, adolescent carriers of the BD risk allele gene were compared within the entire dataset (n=50; carriers vs. non-carriers) as well as split between individual diagnosis groups (Figure 4). In both HC and BD, carriers did not demonstrate significantly different amygdala or hippocampal volumes when compared to non-carriers (corrected for ICV).

No associations between RHI and total amygdala and hippocampal volumes were found (in both diagnostic independent and diagnostic dependent analyses). When examining bilateral amygdala and hippocampal volumes, no significant associations were observed in carriers or non-carriers (diagnosis independent). However, when split by genotype, RHI was associated to left hippocampal volume of HC non-carriers (although this association was no longer significant after FDR correction).
In post hoc analyses, BD did not have diminished bilateral amygdala and hippocampal volumes when compared to HC (genotype independent). In addition, BD did display smaller ICV when compared to HC (genotype independent), although this was not statistically significant. Within HC, carriers subsequently demonstrated trending smaller ICV when compared to non-carriers. Finally, there was a significant interaction between RHI and genotype in the hippocampus of HC.

4.1 Subcortical Volumes and CACNA1C rs1006737:

In the present study, bilateral hippocampus and amygdala atrophy in BD were not replicated. In addition, CACNA1C rs1006737 did not significantly affect subcortical volumes. Current lack of literature makes meaningful interpretations difficult, however a trend in HC, where carriers had non-significantly lower volumes across the bilateral amygdala and hippocampus after correcting for ICV, suggests CACNA1C rs1006737 may in part contribute to subcortical atrophy in risk allele carriers (Figure 8). In addition, as the diagnosis*genotype interaction term was non-significant, the association in HC was not significantly stronger than what was observed in BD. Nevertheless, the trend observed in HC might hint at a genetic contribution that is currently more apparent in the HC cohort and may be revealed in the BD cohort as the sample size grows (please see limitations in Section 4.5).

Indeed, previous studies have suggested that subcortical size in humans may be genetically driven [181, 182]. Findings specific to CACNA1C rs1006737 however, are still limited. Thus far, three studies have reported inconclusive subcortical findings in adult carriers. One study examined 41 euthymic adults with BD-I and 50 HC [15]. In the combined sample, independent of diagnosis, risk allele carriers had increased grey matter density compared to GG homozygotes in the right amygdala and hippocampus. Another study scanned and genotyped 585 healthy participants of European Caucasian decent and found no association with white matter
volume, hippocampal volume or subcortical brain structures [14]. Soeiro-de-Souza et al. scanned 39 BD-I patients and 40 healthy individuals who also completed an out-of-scanner facial emotion recognition test (Ekman 60 Faces Test) and did not find any significant association between rs1006737 and limbic structural volumes [13]. Similar results were seen in 298 psychiatric cases (including 121 BD, 116 SCZ, 61 other psychosis cases) when compared to 219 HC [43]. The authors did not find any significant difference in brain volume between risk allele carriers and controls after correcting for multiple comparisons. No studies have reported findings in adolescents. Additional studies in CACNA1C rs1006737 carriers are required before any concrete relationships between the polymorphism and subcortical volume can be established.

### 4.1.1 CACNA1C rs1006737 Functionality and Role in the Brain and Cardiovascular System:

On a molecular level, CACNA1C rs1006737 falls under an intron with no known purpose. Some studies however, have reported altered mRNA expression in carriers, which may contribute to decreased cerebral protein expression [183, 184]. Furthermore, mutations in CACNA1C may change expression in the substrate selective α1-subunit that ultimately could affect Ca$_{v}$1.2 functionality in neuronal signalling, as the α1-subunit plays a significant role in membrane conductance and substrate selection [185, 186]. In addition, the α–subunits are highly regulated by pharmacological agents [186]. Therefore, the polymorphism may identify a cohort of BD patients that can benefit from calcium channel blockers.

The CACNA1C gene influences both the cardiovascular system as well as the neurological system. More severe CACNA1C mutations seen in Timothy Syndrome show longer action potentials in the heart and increased Ca$^{2+}$ permeability into cells [187]. Mutations in Timothy syndrome have also been shown to affected dendritic processes in cortical areas as well as synaptic plasticity associated with long-term potentiation and fear processing [188, 189]. Fear
processing, in return, seems to up regulate expression of Ca,1.2 in the amygdala [190]. In BD, CACNA1C rs1006737 has recently been associated with greater B lymphoblast intracellular Ca\(^{2+}\) concentrations in carriers [191]. Given this evidence, it is possible CACNA1C rs1006737 may play a role in subcortical development and morphological differences observed in BD. Although this is not apparent in the current BD cohort, it is possible such an association will be unmasked as our sample size grows.

4.1.2 **Explanation for negative results:**

There are a number of factors that may explain non-significant differences in subcortical volumes. One possibility may lie with uncontrolled variables given the current sample size, such as medication and BMI (others are discussed in study limitations in Section 4.5). Indeed, many studies have reported neurostructural normalization in lithium responders. In other words, individuals who responded positively with lithium did not have significantly different hippocampus and amygdala volumes when compared to HC [28, 192]. As sample size increases, controlling for lithium positive participants will have a minimal effect on power, and may account for associations masked by lithium positive participants.

Studies have also suggested a negative correlation between subcortical brain volume and illness duration. One study looking at elderly adults with BD (mean age 64.4) reported lower hippocampal and amygdala volumes associated with longer duration of disease [193]. Another looking at 24 adults with BD also found a negative correlation between hippocampal volume and illness duration, suggesting that hippocampal volume may be comparable to HC in early stages of the disease, but decrease over time [177]. Finally, studies have also demonstrated a negative correlation between subcortical volume and number of affective episodes in patients [177]. Along with study limitations, this suggests the current sample population may have differences that are currently undetectable and may resolve as our sample size grows, or may become more
apparent as the sample ages.

4.2 **CACNA1C rs1006737 and RHI:**

Within groups, HC non-carriers also demonstrated a negative correlation between RHI and corrected left hippocampal volume (which did not survive multiple corrections). No other significant associations were observed. Parallel trends in both carriers and non-carriers suggest a CACNA1C-independent relationship in the BD cohort where individuals with poor endothelial function may demonstrate greater subcortical volumes, and lack of significance may be in part due to limited detection power restricted by sample size (please see Section 4.5).

Currently there are no studies looking specifically at the effects of CACNA1C rs1006737 and peripheral endothelial dysfunction, as measured by RHI, on subcortical volumes. Therefore our results require future exploration before confident interpretations can be made. Nevertheless, we predicted the observed negative association between subcortical volumes and RHI based on previous literature in peripheral endothelial dysfunction and its role in other mental illnesses. For example, one recent study reported a correlation between endothelial cell activation and changes in brain structure in SCZ [40]. Another study reported diminished subcortical volumes in CVD patients whom experienced longitudinal declining blood pressure, suggesting a possible association between changes in peripheral perfusion and subcortical health [194]. Therefore despite current negative findings, there is growing evidence supporting a role of endothelial function in brain morphology, which may ultimately present clinically as cognitive symptoms seen in mental illness.

4.3 **Post Hoc Analysis:**

4.3.1 **Comparison of Subcortical Volumes in BD vs. HC:**

As the primary and secondary hypotheses did not specifically address comparisons
between groups (ie. BD vs HC), the question was tested as a post hoc analysis. In the current study, decreases in hippocampal and amygdala volume, as established by past literature, were not replicated. Studies in youth with BD report a significantly diminished amygdala in patients, which are contrary to hypertrophy observed in adults [81, 83, 195]. Similarly, diminished hippocampal volumes are also observed in youth and adults [18, 195].

Non-significant findings can be explained by two possibilities. Firstly, it may be the current sample of youth with BD does not properly represent what is observed in the population and a larger sample size is required before any conclusive interpretations could be made. This is additionally evident in the BD gene frequency, which is not in Hardy-Weinberg equilibrium (see Section 4.5). Secondly, it is again possible the current cohort reflects a consequence of early disease onset, where alterations to brain morphology are not yet obvious. Indeed one meta-analysis does suggest that, despite replicated findings in youth, these differences may in part reflect exposure to medication and comorbidities which accumulate over illness duration [178]. Finally, it is again possible any differences may currently be masked by lithium response in the BD cohort. The smaller effect size in youth, coupled with small sample size, suggests our study may not be completely powered to detect differences in subcortical volumes.

4.3.2 DIFERENCES IN ICV:

Trends in subcortical volume were largely affected by correction for ICV. Curiously, the BD cohort had smaller mean ICV’s when compared to HC and a difference between carriers and non-carriers were observed in the HC only. The discrepancy is possibly due to confined sample selection will even out as the sample size grows (see Section 4.5). Alternatively this may reflect a clinical presentation of early onset of mental illness. ICV in BD have not been extensively studied. To our knowledge, only one study has reported ICV findings in youth with BD. Like in
the current cohorts, authors found significantly smaller ICV in a modest sample of BD and SCZ adolescents that may be a subtle reflection of defective brain development, as seen in clinical microcephaly [196].

In adults, one meta-analysis looked at 45 studies, demonstrating that patients with BD had a significantly diminished ICV when compared to HC [197]. Harvey et al looked at 26 BD and 34 HC, but did not find any differences in ICV [198]. Similarly, Reite et al also reported no significant difference between 51 BD and 89 HC [199]. No studies have reported findings in ICV between CACNA1C rs1006737 carriers and non-carriers. Therefore, at the current time it is difficult to credit differences seen between and within cohorts as clinical presentation or selection bias.

4.3.3  **RHI – Genotype Interaction:**

In HC, a significant interaction was observed between RHI and genotype in the bilateral hippocampus of HC. Individuals with the risk allele showed a positive association between RHI and left corrected hippocampal volume. On the other hand, individuals without the risk allele showed a negative trend. This suggests the effect of peripheral vascular function on subcortical brain volume may be driven partially by genotype.

Genetic interactions are not an uncommonly explored area in mental illness. The gene-environment interaction dictates that certain environmental factors contribute to mental illness, and the susceptibility of individuals to these factors is determined by their genetics [200]. Studies in dementia have demonstrated this event in the elderly population. For example, one study followed 480 participants (≥65 years) over a span of 5.4 years, and reported in this population, that exercise diminished the risk of dementia over time, but only in apolipoprotein E genotype ε4 non-carriers [201].
It is possible a similar interaction exists with CACNA1C rs1006737 and factors for cardiovascular risk in BD, possibly through a behavioral mechanism. Comorbidity in mental illness can often time result from associated behavioral changes. For example, individuals with BD are more likely to suffer from substance abuse, smoking, and other factors increasing cardiovascular risk [202]. In addition, individuals suffering from depressive episodes may suffer from non-compliance to a healthy lifestyle due to mood instability, all of which contributes to the increased risk of CVD in BD [202, 203]. Therefore, factors that affect behavior may in turn affect associated comorbidities. Indeed CACNA1C rs1006737 has been previously linked to changes in brain morphology of healthy carriers (see Section 1.4). In addition, CACNA1C rs1006737 is associated with changes in neurocognition, in particular emotional processing, memory, and executive control [204-206]. These neurocognitive changes may result in additional behavioral changes. Alternatively, a molecular mechanism may dictate the relationship between RHI and subcortical volumes. More studies would be required to confirm the significance of the interaction, as well as explore possible underlying mechanisms.

4.4 LIMITATIONS:

There are several limitations that may have affected the results of this study. Firstly, our study currently has a small sample size, which may have contributed to non-significant associations after correction for multiple comparisons. Indeed the power calculation in Section 2.7.5 suggests the current study is underpowered to detect medium and small effect sizes. An increase in sample size could improve the power to detect differences in cohort analysis and significant trends within groups may become apparent as more participants are recruited. An increase in sample size also increase the power to include additional covariates. For example, an increased power will allow the study to assess the effects of medication or comorbidities on the primary dependent variables.
Secondly, selection bias may explain why the BD cohort was not in Hardy-Weinberg equilibrium, again suggesting that cohorts may not be correctly representing the sample population. However, three factors may undermine the importance of this limitation. Firstly, populations in mental illness may partially violate assumptions in Hardy-Weinberg equilibrium, more specifically random mating, as behavioral symptoms of mental illness often negatively impact social relationships and interactions. Secondly, the population allele frequency used in the study was concluded from an adult sample, as no other known study has reported similar findings in youth [8]. Thirdly, the study design may represent a biased selection of BD participants, as the risk-allele may also be associated with behavioral differences that interfere with participant recruitment (ie: increased anxiety) (please see Section 4.3.3). Therefore, it is also difficult to gauge the full consequences of working with a youth sample that do not reflect expected allele frequencies in adults.

Finally, this study is restricted by all limitations of a cross-sectional design study. For example, the study design does not reveal any temporal information and makes casual inferences difficult [207].

4.5 CONCLUSION:

In conclusion, the current study is the first to examine subcortical and cardiovascular associations in adolescents with CACNA1C rs100737. However, no significant differences were observed in the bilateral hippocampus or bilateral amygdala when comparing carriers to non-carriers. Furthermore, RHI was not associated with ROI volumes, and a significant gene-RHI interaction was observed in the hippocampus of HC. Changes in hippocampal and amygdala function and morphology have been a particular interest in BD as they are often linked to onset of psychosis and other signs of mood instability [81, 93]. In addition, CACNA1C rs1006737 has also been linked to cognitive dysfunction and subcortical changes in BD, although the exact
effects of CACNA1C rs1006737 on BD are still inconclusive [13, 15]. The current results suggest further investigation is required to understand the relationship between CACNA1C rs1006737, ED and subcortical volumes, and more significant associations may be observed as the sample size grows.

This study is important as it outlines a putative mechanism in which CACNA1C rs1006737 influences both cardiovascular and cognitive deficits seen in BD patients. Although CACNA1C rs1006737 confers a limited risk to individual carriers, this polymorphism is robustly and consistently associated with BD. Identifying measurable endophenotypes associated directly with the polymorphism can help further predict other behavioral symptoms also associated with the gene, therefore paving the way to improved personalized medicine. In a disorder with large heterogeneity in treatment response, progress towards personalized medicine is always welcomed. Indeed one study looking at a wide range of randomized trails looking at drugs used during the maintenance phase of BD showed wide variability between compliance, tolerance and efficacy of current therapeutics [208]. As L-type calcium channel blockers, such as nimodipine, are readily available, studies in CACNA1C rs1006737 revives an additional avenue for combined therapy in a special cohort of BD, which faded from interest following initial inconclusive results [209].

4.6 FUTURE DIRECTIONS:

The current study highlights two important questions. Firstly, as this is the first study to look at CACNA1C rs1006737 in youth with BD, investigating the effects of CACNA1C rs1006737 on cognition in this population may supplement the current study. In adults, CACNA1C rs1006737 may contribute to cognitive dysfunction in both healthy adults and adults with BD. Although not unanimous, current studies have reported a decreased hippocampal activity during frontal-executive tasks and increased amygdala activation during emotional tasks.
Furthermore, studies also report decreasing prefrontal cortex (PFC) activation in healthy carriers during fear-face recognition and working memory tasks [210, 213]. Healthy carriers have also demonstrated diminished connectivity between PFC regions and subcortical regions [210]. In adults with BD, carriers seemed to demonstrate an increased amygdala activity during facial tasks, which increased in severity with increasing risk alleles (G/G<A/G<A/A) [43]. BD carriers also demonstrated decreased PFC activity as well as increased PFC connectivity [210, 214]. Therefore, it is possible such cognitive deficits are also observed in adolescent risk-allele carriers.

In addition, the investigation can be further expanded to examine the effects of peripheral endothelial dysfunction on cognition in CACNA1C rs1006737. Peripheral endothelial dysfunction has been identified as a possible indicator of cerebral small vessel disease and mood, though results are still unclear [215, 216]. One fMRI study in the middle-aged population correlated poor peripheral endothelial dysfunction (as measured by flow mediated dilation) to reduced working memory-related activation [113]. Another study measured the flow mediated dilation (FMD) in elderly patients and found endothelial dysfunction (low FMD values) was correlated with Alzheimer’s disease independent of age [217]. Similarly, another study linked poor forearm reactive hyperemia with more severe depression scores in adults [123]. To date no study has looked specifically at peripheral endothelial dysfunction and cognition in CACNA1C rs1006737 carriers, and such a study could shed light on the impact of this polymorphism in clinical cognitive dysfunction, as well as increased risk for CVD seen in BD.

Finally, investigation into the relationship between white matter hyperintensities and the risk allele will further supplement this imaging study. Presence of excessive white matter hyperintensities (WMH) in adults with BD is a very well replicated finding [218, 219]. Youth with BD also demonstrate increased WMH when compared to HC, with an odds ratio of 5.7
In one study looking at youth with BD, 10 out of 15 patients demonstrated WMH [220]. Another study reported greater prevalence of severe WMH in youth with BD when compared to controls (17.9% vs. 1.2%) [221]. Furthermore, WMH are also associated with both changes in cerebral blood flow and decrease in subcortical grey matter volumes in elderly adults [222]. In elderly patients with Alzheimer’s disease, increased WMH was positively associated with decreased CBF and lower total brain volume [223]. Therefore, the evidence suggests WMHs are associated with both neurostructural and neurovascular differences. As the risk allele polymorphism may also play a similar dual role in the brain, an association between WMH and CACNA1C rs1006737 might surface with additional investigation. Currently, 2 studies have reported non-significant differences in white matter volume between healthy carriers when compared to non-carriers. No studies in risk allele carriers have looked directly at WMH, and further studies are warranted to assess the relationship between CACNA1C rs1006737 and WMH in youth with BD.

5.0 Bibliography:


APPENDIX 1  REB APPROVAL:

To: Dr. Benjamin Goldstein  
Psychiatry  
Room FG 53

From: Dr. Philip Hébert

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.

Philip C. Hebert, MD PhD FCFPC
Chair, Research Ethics Board

OR

Miriam Shuchman, MD
Vice-Chair, Research Ethics Board
APPENDIX 2  ADOLESCENT CONSENT:

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:
Benjamin I. Goldstein, MD, PhD, FRCPC
Sunnybrook Health Sciences Centre
2075 Bayview Avenue
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HSF Centre for Stroke Recovery
Sunnybrook Research Institute
2075 Bayview Avenue
Toronto, Ontario M4N 3M5

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, 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.

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.

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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.
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**HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?**
It is expected that about 100 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 3 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.

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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.

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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. Benjamin Goldstein at 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: _____________________________________________

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

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
APPENDIX 3  PARENT CONSENT:

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

PRINCIPAL INVESTIGATOR:
Benjamin I. Goldstein, MD, PhD, FRCPC
Sunnybrook Health Sciences Centre
2075 Bayview Avenue
Toronto, Ontario M4N 3M5

CO-INVESTIGATORS:
Dr. Daphne Korczak, MD, FRCPC, FRCPC Dr. Bradley MacIntosh, PhD
Hospital for Sick Children HSF Centre for Stroke Recovery
555 University Avenue Sunnybrook Research Institute
Toronto, Ontario M5G 1X8 2075 Bayview Avenue
Toronto, Ontario M4N 3M5

SPONSOR:
Ontario Mental Health Foundation

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.

**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.

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**HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?**
It is expected that about 100 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 3 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.

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**Side Effect Summary**

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 headness 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.

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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.
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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.

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- 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 at 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                                                        D

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                  Signature                                  Date
Consent (print)
APPENDIX 4  MRI PRE-PROCEDURAL SCREENING FORM:

1.5T AND 3.0T MR
PRE-PROCEDURE SCREENING FORM

Date ____________________________ / /

Name ____________________________  First Name ____________________________  Height _______  Weight _______

Birthdate ____________________________

1. Have you ever worked as a machinist, metal worker, or in any profession or hobby grinding metal?  □ Yes  □ No

2. Have you ever had an injury to the eye involving a metallic object (e.g. metallic slivers, shavings, or foreign body)?  □ Yes  □ No

3. Are you pregnant, experiencing a late menstrual period, or having fertility treatments?  □ Yes  □ No

4. Are you currently taking or have recently taken any medication?  □ Yes  □ No  Please List: ____________________________

5. Do you have drug allergies or have you had an allergic reaction?  □ Yes  □ No  Please List: ____________________________

Some of the following items may be hazardous to your safety and some can interfere with the MRI examination. Please check the correct answer for each of the following:

☐ Yes  ☐ No  Cardiac pacemaker

☐ Yes  ☐ No  Implanted cardiac defibrillator

☐ Yes  ☐ No  Antisense or brain clip

☐ Yes  ☐ No  Carotid artery vascular clamp

☐ Yes  ☐ No  Neurostimulator

☐ Yes  ☐ No  Insulin or infusion pump

☐ Yes  ☐ No  Implanted drug infusion device

☐ Yes  ☐ No  Spinal fusion stimulator

☐ Yes  ☐ No  Cochlear, otologic, or ear implant

☐ Yes  ☐ No  Tissue expander (brest)

☐ Yes  ☐ No  Prostheses (eye/orbital, penile, etc.)

☐ Yes  ☐ No  Implant held in place by a magnet

☐ Yes  ☐ No  Heart valve prosthesis

☐ Yes  ☐ No  Artificial limb or joint

☐ Yes  ☐ No  Other implants in body or head

☐ Yes  ☐ No  Electrodes (on body, head, or brain)

☐ Yes  ☐ No  Intravascular stents, filters, or cells

☐ Yes  ☐ No  Shunt (spinal or intraventricular)

☐ Yes  ☐ No  Vascular access port or catheters

☐ Yes  ☐ No  Swan-Ganz catheter

☐ Yes  ☐ No  Medication patch (remove before scan)

☐ Yes  ☐ No  Shaped, buckshot, or bullets

☐ Yes  ☐ No  IUD or diaphragm

☐ Yes  ☐ No  Pessary or bladder ring

☐ Yes  ☐ No  Tattoos, permanent makeup

☐ Yes  ☐ No  Body piercing(s)

☐ Yes  ☐ No  Metal fragments (eye, head, ear, skin)

☐ Yes  ☐ No  Facelift or other cosmetic surgery on body

☐ Yes  ☐ No  Internal pacing wires

☐ Yes  ☐ No  Acrylic clips

☐ Yes  ☐ No  Venous umbrellas

☐ Yes  ☐ No  Metal or wire mesh implants

☐ Yes  ☐ No  Wire sutures or surgical staples

☐ Yes  ☐ No  Harrington rods (spine)

☐ Yes  ☐ No  Metal rods in bones, joint replacements

☐ Yes  ☐ No  Bone/joint pin, screw, nail, wire, plate

☐ Yes  ☐ No  Wig, toupee, or hair implants

☐ Yes  ☐ No  Hearing aid (remove before scan)

☐ Yes  ☐ No  Dentures (remove before scan)

☐ Yes  ☐ No  Asthma or breathing disorders

☐ Yes  ☐ No  Seizures or nervous disorders

☐ Yes  ☐ No  Claustrophobia

Please remove all metallic objects before MR examination including: keys, hair pins, barrettes, jewelry, watch, safety pins, paperclips, money clip, credit cards, coins, pens, belt, metal buttons, pocket knife, & clothing with metal in the material.

Earplugs are required during the MR examination.

I attest that the above information is correct to the best of my knowledge. I read and understand the contents of this form and had the opportunity to ask questions regarding the information on this form and regarding the MR procedure that I am about to undergo.

Signature of Person Completing Form ____________________________  Date ____________________________ / /

Form Completed By  □ Patient  □ Relative

Print Name ____________________________  Relationship to Patient ____________________________

Form Information Reviewed By ____________________________  Print Name ____________________________  Signature ____________________________

☐ MR Technologist  ____________________________  ☐ Other  ____________________________
Request for MRI Consultation
Sunnybrook Campus AG-256, 2075 Bayview Avenue, Toronto, Ontario M4N 3M5
Tel: 416.480.6177    Fax: 416.480.5727

Referring Physician’s OHIP:

Sunnybrook MRN:

Name (Last, First):

Health Card #:

DOB (dd/mm/yyyy):

Address:

City: Province:

Tel: Fax:

Other

19 YES

A

D

Patient

Other

20 Hypertension/Hypotension

Bloodwork Required (two hours early)

Fax:

Relative

DOB (dd/mm/yyyy):

I

Will you be coming via Ambulance or public transportation?

IUD, Diaphragm or Pessary

MRI USE ONLY

Outside Facility

11

13

14

NSAIDs (Including Coxibs)

MRI Technologist

removed by a physician

23

Are you claustrophobic?

Heart Disease

Vancomycin

22

A

4

Form information reviewed by:

Cochlear, Otologic, or other ear implant

Any type of prosthesis (eye, penile, etc.)

Orthopaedic Hardware

Have you ever had a metallic, orbital foreign body?

17

1

Inpatient

Current weight lbs or kgs (Max. 350lbs or 159 kgs)

MR

Unit:

Tattoo, Permanent Makeup or Body Piercing Jewelry

Prosthetic Heart Valve, Cardiac Closure or Occluder Device

18

3

Province:

Insulin or other infusion pump

NO

Multiple Myeloma

NO

12

Electronic or Magnetically Activated Implant or Device

Cardiac Pacemaker

Chemotherapy and/or immunosuppressants

Intracranial Aneurysm Clip(s)

YES

Over the age of 70

Tissue Expander (e.g. breast)

Will you require an interpreter?

Metallic Stent, Filter, or Coil

Evening

MRI USE ONLY

Form information reviewed by:

Implanted Cardioverter Defibrilator (ICD)

Transdermal Medication Patch or Silver Chloride Dressing

15

Programmable Shunt (spinal or intraventricular)

Are you pregnant or the possibility of being pregnant?

16

2

Do you have an allergy to Gadolinium Contrast Agents

Diabetes

On Dialysis

YES

History of Renal Disease

Single Kidney

Peripheral Vascular Disease

21

25

24

20

10

8

7

5

4

3

2

1

LEAVE VALUABLES AT HOME. DO NOT WEAR MAKE UP, WEAR CLOTHING FREE OF METAL ENTERING THE MR ENVIRONMENT.

THE MR SYSTEM HAS A STRONG MAGNETIC FIELD THAT MAY BE HAZARDOUS TO INDIVIDUALS ENTERING THE ENVIRONMENT. FOR PATIENT SAFETY THESE QUESTIONS MUST BE ANSWERED.

Do you have any of the following:

Inpatient

1 Intracranial Aneurysm Clip(s)

9 Cardiac Pacemaker

10 Implanted Cardioverter Defibrillator (ICD)

11 Electronic or Magnetically Activated Implant or Device

12 Neurostimulation System or any other Stimulator Device

13 Aortic Stent Graft (Zenith AAA)

14 Cochlear, Otologic, or other ear implant

15 Programmable Shunt (spinal or intraventricular)

16 Tissue Expander (e.g. breast)

17 Any type of prosthesis (eye, penile, etc.)

18 Metallic Stent, Filter, or Coil

19 Insulin or other infusion pump

20 Prosthetic Heart Valve, Cardiac Closure or Occluder Device

21 IUD, Diaphragm or Pessary

22 Orthopaedic Hardware

23 Any metallic fragment or foreign body (e.g. shrapnel, bullet, etc.)

24 Tattoo, Permanent Makeup or Body Piercing Jewelry

25 Transdermal Medication Patch or Silver Chloride Dressing

26 Any other implant or device(s):

Signature of person completing form:

Patient    Relative    Other

I attest that the above information is correct to the best of my knowledge.

I understand that the information provided must be accurate to ensure patient safety.

Signature:

Print name:

Form completed by:

Signature:

Print name:

NOT A CHART COPY - RETURN TO MRI DEPARTMENT AG-256

PR 26021 (10-2008)
### Appendix 6  Supplementary Tables:

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Table 7: Summary of GLMs examining mean subcortical volumes between carriers and non-carriers within both HC and BD. ICV was entered as a covariate. No significant differences were observed (p≤0.05). GLM was run in HC and BD groups separately once the entire dataset had been split by diagnosis.
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a. $R^2 = .308$ (Adjusted $R^2 = .182$)
b. $R^2 = .607$ (Adjusted $R^2 = .477$)
c. $R^2 = .507$ (Adjusted $R^2 = .261$)
d. $R^2 = .632$ (Adjusted $R^2 = .575$)

**Table 8: Summary of GLMs association between RHI and Total Hippocampal Volume in HC and BD:** Individual groups were split by diagnosis and genotype to observe the association between RHI and hippocampal volume in these subgroups individually. No significant associations were found. ICV was introduced as a covariate to correct for differences in total brain size.
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a. R Squared = .166 (Adjusted R Squared = .014)
b. R Squared = .127 (Adjusted R Squared = -.163)
c. R Squared = .342 (Adjusted R Squared = .013)
d. R Squared = .326 (Adjusted R Squared = .222)

**Table 9: Summary of GLMs association between RHI and Total Amygdala Volume in HC and BD:** Individual groups were split by diagnosis and genotype to observe the association between RHI and amygdala volume in these subgroups individually. No significant associations were found. ICV was introduced as a covariate to correct for differences in total brain size.
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*a. R Squared = .375 (Adjusted R Squared = .261)*
*b. R Squared = .535 (Adjusted R Squared = .380)*
*c. R Squared = .468 (Adjusted R Squared = .202)*
*d. R Squared = .515 (Adjusted R Squared = .440)*

**TABLE 11: SUMMARY OF GLMS EXAMINING ASSOCIATION BETWEEN RHI AND RIGHT HIPPOCAMPAL VOLUMES.** The total dataset was split into BD and HC, where the association between RHI and bilateral subcortical volumes could be examined independently in carriers and non-carriers. Covariates entered into the model include ICV. No significant associations were seen between RHI and right hippocampal volume.
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a. $R^2 = .090$ (Adjusted $R^2 = -.076$)
b. $R^2 = .415$ (Adjusted $R^2 = .220$)
c. $R^2 = .621$ (Adjusted $R^2 = .431$)
d. $R^2 = .317$ (Adjusted $R^2 = .212$)

**TABLE 12: SUMMARY OF GLMs EXAMINING ASSOCIATION BETWEEN RHI AND LEFT AMYGDALA VOLUMES.** The total dataset was split into BD and HC, where the association between RHI and bilateral subcortical volumes could be examined independently in carriers and non-carriers. Covariates entered into the model include ICV. No significant associations were seen between RHI and left amygdala volume.
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a. $R^2 = .268$ (Adjusted $R^2 = .135$)
b. $R^2 = .007$ (Adjusted $R^2 = -.324$)
c. $R^2 = .149$ (Adjusted $R^2 = -.277$)
d. $R^2 = .319$ (Adjusted $R^2 = .214$)

**Table 13: Summary of GLMs examining association between RHI and right amygdala volumes.** The total dataset was split into BD and HC, where the association between RHI and bilateral subcortical volumes could be examined independently in carriers and non-carriers. Covariates entered into the model include ICV. No significant associations were seen between RHI and right amygdala volume.