Differential DNA methylation as a predictor biomarker of antidepressant response in patients with major depressive disorder

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A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Masters in Science

August 2018

“All I know is that I know nothing”

– Socrates

This work is dedicated to everyone who has inspired me to be proactive in mental health research and raising awareness. Thank you for sharing your stories, and being vulnerable with your perspectives. I am humbled to have been trusted with your experiences, and given the opportunity to listen.

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Acknowledgements

The contents in the following pages would simply not have been possible without the support of many. Firstly, a big thanks to my supervisor, Dr. Gustavo Turecki, for mentoring me as a student and for fostering such a warm, collaborative research environment at the Douglas Hospital. Thank you for trusting me to work independently on unique, challenging opportunities, which have helped me grow as scientist and as an individual. It has been an incredibly memorable experience for the past two years.

I would like to thank the bioinformaticians, JF, Alex, and Zahia, for their guidance throughout my endeavours. JF, you "R" so wonderful. Thank you for answering my numerous troubleshooting questions, blessing me with your coding wizardry, and for being patient with me on several occasions.

Much appreciation and thanks to Laura, Kat, Corina, Daniel (2), Jeff, and Gary for guiding me along parts of my project. Along with Rixing, Marcus, Daniel (1), Saumeh, Malosree, Marina, John, Amanda, and Liam: Thank you for all the fun memories, enlightening lunch time debates, office banter, lab outings, and Scattergories breaks. It has been an incredible pleasure to surround myself with such accomplished and motivated individuals. Additionally, a warm thanks to all members of the MGSS.

Outside the lab, I'd like to thank all my friends who provide me with the best social support I could ever ask for. Finally, my wonderful parents, thank you so much. I know you don't have a clear idea of what exactly I've been researching in the past 2 years, but I always have appreciated the unconditional encouragement. Thank you so much for your warmth and support, and giving me the opportunity to pursue my passions in life wholeheartedly.

Abstract

Major depressive disorder (MDD) is a severe and debilitating disease that is primarily treated with antidepressants. However, many patients fail to respond to medication, even after multiple attempts. Given the lack of objective clinical diagnostic and treatment guidelines, a predictor biomarker for antidepressant response (ADR) would largely improve clinical practice for treating MDD, and decrease the time required to effectively treat patients.

In addition to genetic factors, environmental factors are also associated with MDD etiology and treatment efficacy. Epigenetic mechanisms better reflect the interaction between genetic and environmental induced effects through chromatin structure modifications, without affecting the DNA sequence directly. An epigenetic biomarker would thus be more sensitive and functional in accounting for the multifactorial basis of treatment response variation in patients. DNA methylation is the best-known type of epigenetic modification, and has been studied in the context of treatment response. However, current studies are based on hypothesis driven approaches, and no genome wide investigations have been made.

This thesis aims to identify predictive, functional biomarkers for ADR using a novel genome wide method from peripheral blood samples. Firstly, we observed multiple significantly differentially methylated positions (DMPs) from microarray-based data. When selecting DMPs for validation and replication, we selected DMPs located in differentially expressed genes identified from our genome wide expression analysis. This revealed three DMPs of interest that were validated and partially replicated. We also performed functional annotation analysis which provided further functional perspectives as well. Collectively, this thesis discusses our exploratory findings of new candidates for predicting ADR, along with an overview of possible molecular mechanisms that characterize ADR.

Résumé

La dépression majeure est un trouble mental sévèrement incapacitant, traité principalement pharmacologiquement par les antidépresseurs. Leur efficacité est cependant relative, puisque près d'un tiers des patients sous traitement n'atteignent pas la rémission et cela même après plusieurs traitements. Compte tenu de l'absence de critères objectifs et systématiques pour établir un diagnostic et pour définir une stratégie thérapeutique adaptée, la découverte de biomarqueurs prédictifs de la réponse aux antidépresseurs faciliterait considérablement la prise en charge clinique des patients vers la rémission.

Au-delà des facteurs génétiques, les facteurs environnementaux contribuent aussi grandement à l'étiologie de la dépression et la variabilité dans la réponse aux antidépresseurs. Dans ce contexte, les mécanismes épigénétiques sont des indicateurs pertinents de l'effet de l'environnement sur nos gènes. Ces mécanisme modifient l'architecture de la chromatine et donc l'expression de nos gènes sans pour autant affecter leur séquence ADN.
Pour cette raison, un biomarqueur épigénétiques sera plus à même de prendre en compte le caractère multidimensionnel des facteurs influençant la réponse individuelle aux antidépresseurs. La méthylation de l'ADN est le type de modification épigénétique le plus largement décrit et étudié dans le contexte de la réponse au traitement antidépresseur. Ces études sont cependant limitées à des approches basées sur des hypothèses précises se focalisant sur des gènes candidats. En effet aucune à ce jour n'a tenté de déterminer la pertinence des modifications de la méthylation de l'ADN en tant que biomarqueur de la réponse aux antidépresseurs dans l'ensemble du génome.

Ce travail de thèse vise à identifier des biomarqueurs prédictifs et fonctionnels de la réponse aux antidépresseurs. Il utilise une nouvelle méthode pangénomique à partir d'échantillons de sang périphérique. Premièrement, nous avons observé des changements significatifs dans plusieurs positions différentiellement méthylées (PDM) à partir de données basées sur des puces à ADN. Lors de la sélection des PDM pour la validation et la réplication des résultats, nous avons sélectionné des PDM situées dans des gènes différentiellement exprimés, sur la base de nos données préalablement obtenues d'expression transcriptomique. Cela a révélé

trois PDM d'intérêt dont la méthylation différentielle a été validée et partiellement reproduite. Nous avons également effectué une analyse d'annotation qui a fourni d'autres perspectives fonctionnelles. Collectivement, cette thèse propose de nouveaux candidats pour prédire la réponse individuelle aux antidépresseurs, avec un aperçu des mécanismes moléculaires possibles qui caractérisent ces réponses.

Author Contributions

C.J. conducted and coordinated the research project, planning experiments, pre-processing and analyzing microarray data, validation of results, data analysis, interpretation, and preparing the manuscript. L.M.F. also planned experiments for genome wide methylation (for the discovery samples) and expression analysis, extracted RNA and DNA from peripheral blood samples, and reviewed the manuscript. Q.S.L planned and designed the genome wide methylation experiment for the replication samples. J.F.T. conducted pre-processing of the preliminary expression microarray data. G.G.C. assisted in targeted sequencing and loaded samples onto the MiSeq. Z.A. processed raw sequencing data. Bioinformatic and statistical analyses were performed by C.J. and R.B. G.T. and S.H.K. conceived, supported and designed this study. G.T. oversaw all experiments, including design, data interpretation, and manuscript preparation.

Abbreviations

5-HT: 5-hydroxytryptamine; serotonin
ACTH: Adrenocorticotropic hormone
ADR: Antidepressant response
AVP: Arginine vasopressin
BBB: Blood Brain Barrier
BDNF: Brain derived neurotrophic factor
BMIQ: Beta mixture quantile dilation
CAN-BIND: Canadian Biomarker Integration Network in Depression
CBT: Cognitive behavioural therapy
CGI: CpG island
CNS: Central nervous system
CpG: Cytosine-phosphate-guanine dinucleotides
CRH: Corticotropin releasing hormone
CRP: C-reactive protein
DAVID: Database for Annotation, Visualization, and Integrated Discovery
DEG: Differentially expressed gene
DMP: Differentially methylated position
DNMT: DNA methyltransferase
DSM: Diagnostic and statistical manual of mental disorders
ECT: Electroconvulsive therapy
GABA: γ-aminobutyric acid
GENDEP: Genome-based Therapeutic Drugs for Depression project
GPCR: G-protein coupled receptor
GQ: McGill University and Génome Québec innovation center; Génome Québec
GR: Glucocorticoid receptor
HAM-D: Hamilton Depression Rating Scale
HPA: Hypothalamic-pituitary-adrenal
IL-11: Interleukin 11
MADRS: Montgomery-Åsberg Depression Rating Scale
MAOi: Monoamine oxidase inhibitor
MDD: Major depressive disorder, depression
NF-kβ: Neuronal factor kappa beta
QC: Quality control
RBC: Red blood cell
RIN: RNA integrity number
RRBS: Reduced representation of bisulfite sequencing
SAM: S-adenosylmethionine
SLC6A4: Serotonin transporter
sncRNA: Small non-coding RNA
SNRI: Serotonin and norepinephrine reuptake inhibitor
SSRI: Selective serotonin reuptake inhibitors
TAD: Topologically assisting domains
TCA: Tricyclic antidepressant
TLR: Toll-like receptor
WBC: White blood cell
WHO: World Health organization
WGBS: Whole genome bisulfite sequencing

CHAPTER 1: Introduction

Major Depressive Disorder (MDD)

MDD is a severe, affective disorder that affects millions of individuals and their families worldwide. It was recently deemed by the World Health Organization (WHO) in 2017 to be the leading cause of global disability (WHO 2017). In Canada, 11 per cent of Canadians aged 15 to 24 have experienced depression and an estimated 14% have had suicidal thoughts at some point throughout their life (CBC 2017).

Common symptoms of MDD can include depressed mood, anhedonia (inability to experience pleasure throughs previously rewarding activities), significant weight loss or gain, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, excessive guilt or feelings of worthlessness, and decreased concentration (Paris 2014). As outlined by the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV), MDD is diagnosed in patients who present with at least five of these symptoms for at least a two week period, where one of the symptoms must be either depressed mood or anhedonia (Bentley, Pagalilauan et al. 2014). In severely depressed patients, suicide is the worst possible outcome. It is estimated that around 50% of worldwide suicides occur within individuals experiencing a depressive episode, and patients with MDD are twenty times more likely to die by suicide compared to the general population (Chesney, Goodwin et al. 2014). Aside from suicide-related mortality, chronic diseases can be exacerbated by the onset of MDD, and patients are also more susceptible to developing chronic diseases such as coronary vascular diseases, diabetes, and increased risk of stroke (Krishnan and Nestler 2008). Overall, MDD is associated with personal,

social and economic morbidity, loss of productivity, and increases demands of health service provision (Steger and Kashdan 2009).

Although MDD is highly prevalent and its considerable impact on mental, physical and social health are well documented, our current understanding of its pathophysiology is primitive compared to other diseases due to several reasons. Firstly, observing brain pathology is much more complex compared to other organs, and increases or decreases in brain region size or activity does not sufficiently account for the spectrum of symptoms exhibited by MDD patients. Secondly, depression occurs spontaneously, and its etiology varies across patients, making it difficult to define a consistent model of MDD. To this day, there are no robust explanations for disease pathophysiology, and this mainly accounts for why clinical diagnosis and treatment paradigms still rely upon subjective evaluation. Much research suggests that MDD etiology results from a genetic predisposition coupled with abnormal regulation of multiple neurotransmitter pathways and metabolic processes due to a plethora of biological and psychosocial factors.

Neurobiological bases of MDD

No established neurobiological mechanism exists to explains all components of MDD pathophysiology despite several advances made to achieve our current understanding of the disease. Overall, MDD pathophysiology is likely attributable to multiple dysregulated neurobiological networks with interconnecting pathways and mechanisms. There are also implications in functional and structural brain related to the dysregulation of various neurobiological systems associated with MDD (Boku, Nakagawa et al. 2017). Specifically, MDD has been extensively linked with neurotransmitter imbalance, along with alterations in

the neurobiological systems that mediate stress response, particularly components of the hypothalamic-pituitary-adrenal (HPA) axis (Varghese and Brown 2001) and immune response systems (Slavich and Irwin 2014).

Differential brain function and structural modulations in MDD

Mood disorders in general have been characterized by elevated rates of neurodegeneration and decreased neurogenesis (Drevets, Price et al. 2008). Specifically, in depressed patients, neurogenesis occurs in the adult brain, most prominently in subventricular zone, and subgranular zones of the hippocampus. Decreased neurogenesis and hippocampal neuronal death has been noted to induce a shrinkage in hippocampal volume (Bremner JD 1998), that partially accounts for neurocognitive deficits commonly seen across MDD patients (Brown ES 2004). Structural and volumetric changes observed in unipolar depressed patients have been reported in regions of the hippocampus, amygdala, prefrontal cortex, anterior cingulate, and basal ganglia (Campbell and MacQueen 2006). Apart from the hippocampus, a decreased number of astrocytes and neurons in the prefrontal cortex and striatum has also been observed, (Sheline YI 1999), which have been also noted in studies conducted with postmortem brain samples (Stockmeier CA 2004). At the molecular level, significant decreases in neurotrophins such as brain-derived neurotrophic factor (BDNF) have been detected in depressed patients and stress-induced animal models of depression (Smith MA 1995 , Angelucci F 2005). Reduced amounts of hippocampal neurogenesis (Goshen I 2008) and BDNF in brain regions associated with depression (hippocampus, amygdala and prefrontal cortex) were observed in rodents induced to exhibit depressive symptoms (Schmidt HD 2007). Collectively, these findings suggest

that neurodegeneration and neurogenesis, particularly in the hippocampus, are two mechanisms importantly implicated in MDD.

The Monoamine Hypothesis

Monoamines such as serotonin, norepinephrine, and dopamine are heavily implicated in MDD pathophysiology. As suggested by their name, monoamines are a type of neurotransmitters characterized by an amino group attached to an aromatic ring. They exert their effects by binding to G-protein coupled receptors (GPCRs) in the postsynaptic cell membrane and modifying their response to glutamate and γ-aminobutyric acid (GABA). Two main observations led to the development of the monoamine hypothesis: the serendipitous discovery of first generational antidepressants, and the subsequent investigations made into their pharmacological mechanisms of action. In the 1950s, high doses of reserpine, used to treat hypertension were found to depressed affect in patients (Freis 1954), and it was later identified as a vesicular monoamine transporter inhibitor, which depletes brain monoamines. This provided the first evidence that monoamines had an important role in affective disorders (Shore Parkhurst, Pletscher et al. 1957). Iproniazid, a MAOi, incidentally produced an antidepressant effect in tuberculosis patients which were later confirmed to produce similar effects in non-tubular, depressed patients (Delgado 2000). Administering the serotonin (5-hydroxytryptamine; 5-HT) precursor tryptophan in the presence or absence of standard antidepressant treatments were also shown to be beneficial in treating or preventing MDD (Caspi, Sugden et al. 2003) (Praag and Haan 1981). Further investigation into the mechanism of action of monoamine oxidase inhibitors (MAOis), tricyclic antidepressants (TCAs), and serotonin specific reuptake inhibitors (SSRIs) demonstrated that they primarily increase synaptic monoamine concentrations (Hillhouse and Porter 2015).

Overall, these observations collectively formed the "monoamine hypothesis of depression": depression is caused by a depletion in monoamines (particularly serotonin and norepinephrine) and an increase in monoamines are shown to have antidepressant like effects. However, depleted concentrations of monoamines do not fully explain all facets of MDD pathophysiology, and restoring their balance does not guarantee a relief of depressive symptoms. For example, monoamine depletion in healthy subjects do not produce depressive phenotype (Salomon, Miller et al. 1997), nor does it promote depressive symptoms in MDD patients (Berman, Sanacora et al. 2002). Finally, not all patients respond to antidepressants, clearly showing the caveats of the monoamine hypothesis.

The HPA Axis

The hypothalamic-pituitary-adrenal (HPA) axis is a feedback loop comprised of the hypothalamus, pituitary and adrenal glands that is activated upon exposure to a physical or emotional stressor. Corticotropin-releasing hormone (CRH), the primary regulator of mammalian stress response, and arginine vasopressin (AVP) are released by the hypothalamus to stimulate adrenocorticotropin hormone (ACTH) release from the anterior pituitary. ACTH is released into the blood and interacts its receptors on the adrenal cortex to stimulate production and release of the glucocorticoid cortisol, the main stress hormone secreted in humans and primates. Neuroendocrine challenge tests such as the dexamethasone suppression test (DST) are used to assess level of HPA axis function, where a normal response after administering dexamethasone (synthetic corticosteroid) would be an inhibition of cortisol due to negative feedback processes.

Much evidence suggests that depressed patients exhibit hyperactivity of the HPA axis. Differential hormone levels at all levels of the HPA axis have been reported in depressed patients. At the hypothalamus, MDD patients were exclusively found to have elevated levels of CRH in cerebrospinal fluid samples, compared to schizophrenic (Banki CM 1987), demented, and manic patients (France, Urban et al. 1988). Increased CRH concentrations have also been reported in MDD patients from plasma samples (Claes 2004) and urine (Carroll, Curtis et al. 2009). Interestingly, MDD patients also exhibit a diminished ACTH response to CRH administration, (Owens MJ1 1993) which could be associated with canonical negative feedback of the HPA axis, and down-regulation of CRH receptors in the pituitary due to high CRH concentrations in a depressive episode. Given the imbalance seen at the level of the hypothalamus and the pituitary, it is unsurprising that excess cortisol levels and DST non-suppression are positively correlated with the number of depressive episodes (Yerevanian BI 1984) and a higher likelihood of MDD relapse (Greden 1983). MDD patients have also been found to have increased cortisol levels in saliva, plasma and urine samples, as well as hypertrophy of the pituitary and adrenal glands (Sachar EJ 1970).

Cortisol regulates neuronal survival, neuronal excitability, neurogenesis, and memory acquisition, and high levels can lead to depressive symptoms by impairing these critical brain functions. Impairments in the glucocorticoid receptor (GR) can also contribute to structural changes seen in a depressed brain, and suggested for HPA axis hyperactivity in depression. GR regulates neurotrophic factor (i.e. Brain-derived neurotrophic factor, BDNF) expression, induces neuronal death and alters adult hippocampal neurogenesis. Broadly, studies have shown that modified expression, nuclear translocation, co-factor binding and GR-mediated gene

transcription in the peripheral and central nervous systems have been shown to contribute to HPA axis hyperactivity (Lowy MT 1984, Wodarz N 1991, Pariante CM 2001).

Inflammation and immune response dysfunction

Much evidence supports the role of inflammation in depression. Overall, most depressed patients exhibit increased expression of pro-inflammatory cytokines and their canonical receptors, along with increased levels of acute-phase reactants and chemokines in peripheral blood and CSF (Miller, Maletic et al. 2009). Specifically, in their peripheral blood, gene expression profiles are commonly found to have an over-representation of components in IL-6, IL-8, and IFN-induced signalling pathways (Mostafavi, Battle et al. 2014). Blocking production of cytokines or various components of inflammatory cytokine signalling pathways have been shown to provide symptomatic relief in MDD patients. (Kohler O 2014). Interestingly, when healthy individuals were injected with cytokines or their inducers (i.e. vaccines or endotoxins) they began showing depressive symptoms (Bonaccorso S 2002). Overall, IL-1β, IL-6, TNF and C-reactive protein (CRP) were revealed through a meta-analysis to be the most reliable inflammatory biomarkers for depression (Miller, Maletic et al. 2009). Though these studies all provide robust insight on the role of inflammation in MDD, some inflammatory markers are not specific to depression. Patients with other psychiatric disorders such as anxiety and schizophrenia have demonstrated elevated CRP levels in peripheral blood (Michopoulos, Rothbaum et al. 2015) (Fernandes BS 2015). In conclusion, peripheral blood inflammatory markers are indeed associated with MDD, but it may be more accurate to broadly associate them rather with specific affective symptoms (Miller and Raison 2016)

The brain was initially considered to be protected from the peripheral immune system by the blood-brain-barrier (BBB). However, it is now understood that the CNS is directly influenced by peripheral cytokines, chemokines, glucocorticoids and other various immune cells, which is linked to neural system malfunctioning seen across depressed patients (Leonard 2010). In post-mortem brain samples of depressed suicide victims, some studies have demonstrated increased expression of innate immune genes including IL-1β, IL-6, TNF, Toll-like receptor 3 (TLR3) and TLR4 (Miller, Maletic et al. 2009) (Drago, Crisafulli et al. 2015) (Maes 1995). Much like peripheral inflammatory responses, pro-inflammatory cytokines such as interleukin 1 (IL-1) and tumour necrosis factor α (TNFα) activates neuronal and non-neuronal cells through the nuclear factor-kappa-beta (NF-kβ) pathway in the brain (Anisman H 2008). Chronic inflammation in the brain also modulates monoamine neurotransmitter function; for example, pro-inflammatory cytokines lower the amount of tryptophan converted to serotonin by redirecting the pathway towards kynurenine synthesis, and decrease dopamine production in the brain (Moron JA 2003). Additionally, previously described brain structural changes that are associated with MDD (hippocampus, amygdala, and the prefrontal cortex) can also be linked to chronic low-grade inflammation, where pro-inflammatory cytokines, nitric oxide, prostaglandin E2 and other inflammatory mediators contribute to inflammatory insult (Leonard 2010).

Cytokines also increase re-uptake of monoamine neurotransmitters; for example, IL-1 and TNF activate the neuronal 5-HT transporter by stimulating the p38 mitogen-activated protein kinase pathway (Zhu CB 2006). Monoamine neurotransmitter imbalance can also activate the HPA axis via increased CRH release and decreased GR sensitivity, which confers GR resistance over periods of chronic inflammation (Pariante CM 2001).

Psychosocial and environmental factors of depression

In addition to molecular and genetic bases of depression, multiple psychosocial and environmental factors are related to MDD pathophysiology and etiology. Stressful life events, particularly in childhood, are a large predisposing factor for an increased risk of MDD and will be discussed in detail in the following section. In general, depression-related outcomes of stressful life circumstances are influenced by appraisal and coping responses developed in childhood years (Billings and Moos 1982) and it is thought that childhood trauma can predispose an individual to psychosocial vulnerability. Besides stressful life events and childhood adversity, a family history of mental illness, chronic physical ailments, lack of social relationships, low socioeconomic status, insomnia, and are other examples of social and environmental components that can contribute to a higher risk of MDD (Brown 1996, Bosworth HB. 2003).

Early life adversity and childhood trauma

Early life adversity is defined as exposure to stressful events such as physical, emotional or sexual abuse, caregiver neglect, and bullying in childhood which negatively impacts future outcomes of physical and emotional health (Goff and Tottenham 2015). It has been linked to many negative outcomes in adult life, including an increased risk of cardiovascular diseases, diabetes, other chronic diseases, cancer and inflammation which all decrease mortality (Friedman 2015). Childhood trauma is also correlated with an increased vulnerability for developing psychiatric disorders such as MDD, anxiety and substance abuse (Hill 2003). In the context of depression, early life adversity is consistently associated with onset of depression at an earlier age (Gladstone GL 2004), greater number of depressive episodes, increased risk of suicidality (Bahk, Jang et al. 2017), and more chronic cases of depression (Zlotnick C 2001).

Specifically for the latter, the greater number of early life traumatic experiences, the more chronic the course of MDD (Negele, Kaufhold et al. 2015). In the developing brain, early life adversity can induce abnormal development of stress response pathways (Goff and Tottenham 2015). As a result, increased levels of autonomic responses to stress, elevated adrenal response to adversity, increased CRF levels in the CNS, increased baseline levels of cortisol and decreased hippocampal volume are observed in victims of childhood abuse and in patients with MDD (Arborelius L 1999, Cicchetti D1 2001, Heim 2008). One particular theory suggests that the early trauma induced vulnerability towards developing MDD is mediated by stress-induced immune activation of transcription factor NF- $\varkappa$B, and the sequential rise of circulating pro-inflammatory cytokines (i.e. TNF-α, IL-1, and Il-6) (Raison 2003). In the peripheral blood of depressed patients with a history of childhood trauma, a psychosocial stressor results in abnormally elevated CRF stimulation, which in turn causes associated increases in plasma IL-6 and activation of the NF- $\varkappa$B pathway (Heim 2008). However, not all those who suffered early life adversity develop adulthood affective disorders (Maples, Park et al. 2014, Beutel, Tibubos et al. 2017). Although there are clear correlations between early life adversity and depression that are likely mediated by HPA-axis stress response and/or immune response dysregulation, the exact mechanisms behind this important association remains to be elucidated.

Antidepressant Treatment and Response

Antidepressant medications are used primarily to treat clinical depression. Other methods such as cognitive behavioural therapy (CBT), electroconvulsive therapy (ECT), exercise, mindfulness and sleep deprivation are also shown to have antidepressant effects (Blumenthal, Smith et al. 2012, Dallaspezia and Benedetti 2015, Kuyken, Hayes et al. 2015), but often are

used as secondary and/or adjunct therapies to medication. The most commonly prescribed group of antidepressants are the selective serotonin reuptake inhibitors (SSRIs), followed closely by serotonin and norepinephrine reuptake inhibitors (SNRIs). Others such as tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOis) and are used in cases of treatment-resistant depression (Moret 2005). All three types of antidepressants mediate their effects by increasing synaptic monoamine levels, especially 5-HT levels, either by disrupting presynaptic reuptake processes or disrupting degradation of these molecules (Taylor, Fricker et al. 2005).

History of Antidepressant Development

Antidepressants were accidentally discovered around 60 years ago when tuberculosis patients being treated with the MAOi iproniazid noted incidental improvements in their mood, regardless of the progression in their physical disease. However, soon after being established as a depression treatment, the high risk of hypertensive crises and overdose in patients were concerning factors when prescribing MAOis (Fiedorowicz and Swartz 2004). TCAs were also incidentally discovered as antidepressants while being investigated as a treatment for schizophrenia and they were found to be more effective than MAOis in treating depression. However, patients still exhibited the same negative side effects, along with an increased risk of seizures (Ramachandraih, Subramanyam et al. 2011). The large range and severity of MAOi and TCA side effects observed in MDD patients are attributed to their interactions with multiple neurotransmitter receptors. This inspired further development of antidepressants with more specific binding activity. At that time, there was increasing evidence pointing towards serotonin as the main neurotransmitter implicated in MDD pathophysiology; for example, decreased levels

of serotonin were detected in post mortem brain samples of depressed, suicidal patients (Shaw DM 1967). In 1974, fluoxetine was developed as the first SSRI antidepressant. SSRIs are effective for treating symptoms and avoiding extreme side effects, but other side effects such as sexual dysfunction, nausea, weight gain, and insomnia are still commonly reported by patients being treated with SSRIs, particularly in long term users (Nutt 2008). Buproprion was developed shortly afterwards as an atypical antidepressant, with selective reuptake inhibition of dopamine and norepinephrine. Buproprion's mechanism of action highlighted the importance of other monoamines in MDD pathophysiology (Stahl, Pradko et al. 2004). Coupled with the increasing body of literature that proposed norepinephrine to be another important neurotransmitter in MDD pathophysiology, serotonin-norepinephrine reuptake inhibitors (SNRIs) were also developed later on for treatment purposes, with similar side effects and efficacy to SSRI use.

SSRIs: mechanism of action

SSRIs inhibit serotonin 20-1500 fold more than norepinephrine at respective transporter proteins, and have very little to no binding affinity for other adrenergic (α_1, α_2, and β), histamine H_1, muscarinic, and dopamine D_2 receptors (Owens, Morgan et al. 1997). They increase postsynaptic serotonin receptor activity primarily by inhibiting presynaptic reuptake and increasing serotonin concentrations in the synaptic cleft. They do not stimulate presynaptic serotonin or norepinephrine release, and have little to no interactions with postsynaptic serotonin receptors (specifically, 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2c}) (Owens, Morgan et al. 1997, Sánchez C 1999). Various SSRIs currently on the market include fluoxetine, sertraline, citalopram, paroxetine, and escitalopram.

Current perspectives: Antidepressant Treatment Response Efficacy

Antidepressants are the first line pharmacological treatment prescribed for MDD, and multiple types and combinations of pharmacological therapy are available. Yet, selection is clinically subjective and based largely on trial and error which elongates the assessment period for response and remission (Madhukar H. Trivedi, A. John Rush et al. 2006). After initializing treatment, improvement of symptoms is seen only after two to four weeks, and it is difficult to distinguish actual improvement from a placebo effect (Mitchell 2018). During this period, patients can experience exacerbated symptoms, and become noncompliant with their medication (Masand 2003). The largest concern with currently available antidepressant medications is that roughly 60% of patients fail to respond to their initial course of antidepressants, and 20-30% do not respond after multiple interventions (Labermaier, Masana et al. 2013). In the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study, one of the largest ADR studies to date, more than 40% of MDD patients did not report remission of symptoms despite being given two different trials of antidepressant treatment (Nierenberg AA1 2006). Similar to MDD symptoms, ADR is quite heterogeneous across patients, and this could be attributed to a variety of physiological and social factors.

Higher levels of peripheral inflammation in MDD patients has been repeatedly associated with treatment non-response (Miller, Maletic et al. 2009, Michopoulos, Rothbaum et al. 2015). For example, in one particular study, high inflammation levels reflected by CRP levels greater than 3mg/L were detected in 45% of patients who did not respond to conventional antidepressants (Raison, Rutherford et al. 2013). Additionally, non-steroidal anti-inflammatory drugs are shown to be possible adjunctive treatments in unipolar depression (Akhondzadeh 2009), while infliximab, a TNFα antagonist, has been shown to assist treatment resistant MDD

patients with high levels of peripheral inflammation (Raison, Rutherford et al. 2013). Furthermore, decreasing TNFα levels over a course of antidepressants were noted in responders, but not in treatment-resistant patients (Strawbridge 2015). MDD patients with a history of partial or lack of ADR are noted to have elevated levels of IL-6 and acute immune phase proteins that persist, despite antidepressant treatment (Sluzewska, Rybakowski et al. 1996).

Differential levels of baseline serotonin and norepinephrine are also associated with unique effects on the immune system that are possibly associated with differential ADR outcomes. Norepinephrine does not directly affect cytokine production from Th2 cells, given their lack of β2 adrenergic receptors (Sanders, Baker et al. 1997). However, these receptors are expressed on Th1 cells, and when activated by norepinephrine, IL-12 production is suppressed, decreasing release of pro-inflammatory cytokines such as IFN-γ and IL-4. This shifts the Th1/Th2 balance towards Th2-mediated up-regulation of other cytokines such as IL-6, and IL-10 (Elenkov 2008). Conversely, 5-HT mediates a Th1 shift, and induces the secretion of the IL-1β, IFN-γ and TNF-α (Durk 2005). Several studies have shown that SSRIs alter lymphocyte production (Edgar VA 1999), (F. Fazzino 2008) (M. Taler 2007) and one possibility to explain differential treatment outcomes (i.e. SNRIs vs. SSRIs) or lack of ADR overall could be the differential levels of cytokines induced by varying Th1/Th2 balances in MDD patients.

Social factors such as childhood abuse have also been linked to differential ADR outcomes. For example, a meta-analysis revealed that history of early-life stress predicts poorer response to pharmacotherapy, psychotherapy, and combinational pharmacotherapy with CBT, and that it can also act as a moderator biomarker for differential response to these treatments (Nanni, Uher et al. 2012). The international Study to Predict Optimized Treatment for Depression (iSPOT-D) compared 1008 MDD patients randomly assigned to eight weeks of

treatment with escitalopram, sertraline or venlafaxine, and 336 healthy controls. Abuse, especially before the age of seven, predicted poorer ADR outcomes after eight weeks across all three treatment groups. Abuse between the ages four and seven differentially predicted the poorest outcome following the treatment with sertraline (Williams, Debattista et al. 2016). Another study conducted with 681 MDD patients demonstrated that those without a history of childhood trauma had an equal response to either CBT, or nefazodone (an atypical antidepressant), but those with a history of abuse or neglect were less likely to respond to antidepressant monotherapy (Nemeroff, Heim et al. 2003) From the Treatment of Resistant Depression in Adolescents Study, adolescents that failed to respond to an SSRI medication were either given SSRI or SNRI monotherapy, or combinational therapy with CBT. Non-abused individuals responded better to combinational therapy compared to antidepressant monotherapy (Shamseddeen, Asarnow et al. 2011). These results collectively show that childhood abuse and/or trauma can act as predictors or moderators of antidepressant response, albeit with varying results (Nanni, Uher et al. 2012). Despite these findings, antidepressant treatment is still subjective. Discovery of clinical and biological biomarkers for ADR would better inform clinical decision making and cater towards an individualized approach to treating MDD.

Biomarkers

A biomarker can be broadly defined as a "characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathologic processes or biological responses to a therapeutic intervention" (Atkinson 2001). This could for example involve a gene, a set of genes, proteins, epigenetic marks, or anatomical indices. In the case of MDD and ADR, measurement of these components requires biological sampling from tissues such as peripheral

blood or cerebrospinal fluid (CSF), or the use of brain imaging to detect changes in composition or function of nervous system components (Mayeux 2004).

Biomarkers aid in understanding etiology, cause, diagnosis, progression, regression, and treatment outcomes of a disease. Many biomarkers are well established, and widely used in diagnosis or prognosis of medical ailments such as cardiovascular disease, infections, immune and genetic disorders, and cancer (Hulka 1990). No robust biomarkers are implemented for clinical decision making with psychiatric disorders, and none exist for diagnosing MDD or treatment response. This is partially due to the lack of a consistent, standardized classification of MDD, and the lack of in-vitro animal models that can effectively portray MDD pathophysiology and treatment response. Methodological limitations such as small sample sizes and lack of replication in other patient cohorts also decrease confidence of results from existing studies.

Many single biomarkers with small effect sizes have been proposed for MDD and/or ADR, suggesting that a single biomarker is unlikely to largely impact diagnosis and treatment (Venkatasubramanian and Keshavan 2016). To address the concerns of single biomarker impracticality, some groups have started looking at groups of candidate genes, or "biomarker signatures" (Breitfeld, Scholl, Steffens, Laje, & Stingl, 2017). Others aim to incorporate clinical, socio-environmental, molecular, neuroimaging and neurobiological findings (Breitfeld, Scholl et al. 2017)in hopes of detecting a biomarker signature (Lam, Milev et al. 2016) (Pangalos, Schechter et al. 2007) . Eventually, the use of a panel of biomarkers would be much more comprehensive, reflecting the complexities associated with symptom heterogeneity in MDD and ADR.

Biological sampling for biomarker investigations

Clinical diagnostic tests aim to be as non-invasive as possible in order to minimize the number of risks associated with invasive sampling methods. Peripheral biological tissues are thus the most standardized and common material to sample, as they are efficient, easy and quick to retrieve from multiple patients. Blood is the most commonly used peripheral material in biomarker studies since sampling methods are standardized in clinical settings (Perez-Gracia, Sanmamed et al. 2017). Additionally, whole blood has multiple components that can be analyzed holistically or separately. Whole blood consists of red blood cells (RBCs), white blood cells (WBCs), platelets, and plasma. WBCs are commonly isolated from whole blood for genomic assessments since the majority of genetic material from whole blood samples are provided by nucleated WBCs. By isolating WBCs, this avoids the effect of globin mRNA on sample integrity, and eliminates the diluting effect of other blood cell types on signal detection (Liu, Walter et al. 2006). Plasma or serum are less common methods of patient sampling in psychiatry, but they can be used to assess DNA and/or RNA molecules released from the brain and circulated around the body as microvesicles, exosomes, or bound to high-density lipoproteins (Arnold, Xie et al. 2012). The use of plasma or serum samples potentially allows for greater characterization of how communication between the brain and periphery differs between depressed patients and healthy controls, and how it is influenced by antidepressant treatment. Peripheral tissues can reflect holistic physiological changes, and since MDD is viewed as a systemic disorder that affects multiple organ systems, they are the most enticing biological material to utilize for biomarker studies.

Besides blood, other types of biological materials used include saliva, buccal and skin cells, and cerebrospinal fluid (Levenson 2010) . Saliva and buccal cells are easily retrieved from

patients, but has high levels of buccal cell heterogeneity which affect accurate signal detection. It can also contain varying levels of non-human DNA which lower the likelihood of performing accurate downstream genetic or epigenetic assays (Smith, Kilaru et al. 2015). Cerebrospinal fluid provides the most accurate surrogate assessment of brain tissue, but is very difficult to retrieve from patients (Spector, Robert Snodgrass et al. 2015).

Epigenetic mechanisms

Regulation of gene transcription is a biological process important for all organisms throughout their lifespan. Gene expression is activated or repressed through the interaction of transcription factors that bind to specific sequence motifs in regulatory gene regions (Yilmaz and Grotewold 2010). However, another large aspect of regulating gene expression is through epigenetic mechanisms. The term "epigenetic" is used to describe multiple different molecular processes that exhibit effects on gene expression without any alterations in DNA sequence. They consist of various pre-transcriptional to post-translational events such as DNA methylation (discussed in depth in the following section), non-coding RNAs (ncRNAs), histone modifications and chromatin conformation remodeling.

ncRNAs consist of short ncRNAs (i.e. microRNAs, short interfering RNAs, and piwi-interacting RNAs) less than 30 nucleotides in length, and long ncRNAs greater than 200 nucleotides in length. Generally, ncRNAs have been shown to play a role in heterochromatin formation, histone modifications, targeting DNA methylation, and gene silencing (Holoch and Moazed 2015). Histone modifications are broadly defined as a covalent, post-translational modification (i.e. methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation) on histone proteins to regulate chromatin density and/or to recruit further histone modifiers. Depending on the type of histone modification, this can either loosen or tighten the chromatin

structure to increase or decrease access to regulatory gene regions, respectively. Finally, extensive chromatin remodeling has been closely linked to enhancer activity, and also can have impacts on transcriptional regulation. Higher order organization of the genome keeps chromatin compact, but multiple levels of folding inevitably creates chromatin contact points around the genome, including regulatory regions positioned far away from promoter sites. Chromatin conformation capture techniques have shown that chromosomes are organized in topologically associating domains (TADs) which are discrete genomic regions that contain intra-TAD chromatin interactions such as enhancer-promoter loops. TADs can be rearranged depending on the specific genomic contact points, size of intra-TAD loops, access to regulatory regions, and interactions with other TADs (Hu and Tee 2017). Epigenetic mechanisms can reflect the effect of environmental factors such as food, drugs, toxin exposure and/or social adversity on modifying gene expression (Kubota, Miyake et al. 2012). Especially in the context of psychiatric diseases, understanding their influence can help us potentially explain etiology, severity, and differential ADR outcomes.

DNA methylation

DNA methylation is a prominently studied heritable epigenetic mark, associated with various cellular mechanisms such as X chromosome inactivation, genomic imprinting, and chromatin remodeling (Curradi, Izzo et al. 2002). It is defined as the covalent transfer of a methyl group, from the methyl donor S-adenosylmethionine (SAM), onto the 5^{th} position of cytosine rings, resulting in 5'-methylcytosine (5mC). These events are catalyzed by members of the DNA methyltransferase (DNMT) family. DNMT1, DNMT3a and DNMT3b all contribute to maintain the integrity of the methylome during strand replication, while DNMT3a and DNMT3b

are additionally involved with the addition of new methylation of double stranded DNA (Menke and Binder 2014), (Jones 2012). In mammals, DNA methylation events occur almost exclusively at cytosines immediately followed by a guanine (CpG). Non-CpG dinucleotide methylation isbe observed, but these events are uncommon in mammalian genomes, and their contributions to gene regulation are less understood (Lutz and Turecki 2014). The mammalian genome has an underrepresentation of CpG dinucleotides, attributed to the high conversion rate of methylcytosine-to-thymine (Laird 2003). However, large clusters of non-methylated CpGs are dispersed in clusters across the genomic landscape. These CpG "islands" (CGIs) are canonically unmethylated and most often found in the promoter regions of conserved house-keeping genes (Mamrut, Harony et al. 2013) (Maunakea, Nagarajan et al. 2010). On the other hand, promoters with less CpG content tend to be methylated, and concomitant with tissue specific genes (Vinson and Chatterjee 2012) (Weber, Hellmann et al. 2007).

Indeed, methylation at CpG sites have significant roles in regulating transcription, particularly when at promoter regions, and generally in a repressive manner (Vinson and Chatterjee 2012). CpG methylation in promoter regions can directly affect gene expression by physically blocking transcription factors from binding and inducing their downstream activity (Pérez, Castellazzi et al. 2012). Indirectly, it can also disturb gene expression by recruiting proteins with methylated-DNA binding domains (MBDs) that bind to methylated sites and modify chromatin structure (Curradi, Izzo et al. 2002, Baylin and Jones 2011). The directional effects of DNA methylation on gene expression are mediated by what types of transcription factors or secondary proteins bind to the methylation site.

DNA methylation quantification methods

Multiple protocols exist for quantifying DNA methylation at the targeted or genome wide level. Most methods often require an initial treatment of sample DNA with sodium bisulfite, which converts unmethylated cytosines to uracil, while methylated cytosines are protected from this change by the methyl group, and remain as cytosine. Thus, this allows for the detection of differential methylation levels with various types of molecular assays (Levenson 2010). Early DNA methylation detection techniques combined standard DNA sequencing techniques with bisulfite treatment in order to target CpG sites in various genes of interest. Microarray based technologies and next-generation sequencing technologies were then developed for genome-wide methylation quantification at single nucleotide resolution, which were originally designed to specifically target CpG sites in promoter sites (Yong, Hsu et al. 2016). Whole genome bisulfite sequencing (WGBS) disregards the type of downstream nucleotide after cytosine, offering the most comprehensive DNA methylation assessment. However, the high cost of WGBS eventually led to the development of a reduced representation bisulphite sequencing (RRBS) technique. In RRBS, DNA is first pre-treated with the *Mspl* restriction enzyme to digest and eliminate non-informative sequences, particularly in repetitive elements (Menke and Binder 2014), reducing the number of sites assessed during sequencing. Third generation sequencing techniques are currently being established and optimized to allow for the assessment of novel functions associated with methylation marks. Two examples include real-time genome-wide methylation sequencing, and single cell methylome sequencing which address rate of methylation, and/or cell-specific effects on differential methylation levels, respectively (Yong, Hsu et al. 2016). Overall, multiple types of methylation detection assays are currently available to provide sufficiently fast and robust quantification of methylation levels (The 2016).

Suitability of DNA methylation as an epigenetic biomarker

DNA methylation-based biomarkers have already been successfully utilized in diagnosing multiple types of diseases, including multiple types of cancers (Laird 2003, deVos, Tetzner et al. 2009), neurodevelopmental disorders (Robertson and Wolffe 2000), and autoimmune diseases (Li 2002). They are suitable for *in vitro* applications for a variety of reasons. They have more molecular stability compared to volatile, RNA-based counterparts, and are faithfully retained in samples after an extended period of storage (The 2016). Furthermore, multiple techniques exist for quantifying DNA methylation, many of which are similar to gene expression assays. Thus, the infrastructure for analyzing DNA methylation is already standardized in most molecular laboratories.

Current evidence: differential DNA methylation as a predictor of treatment response

Presently, a select number of target-based studies have evaluated DNA methylation as an ADR biomarker. A majority of them are follow-up studies to investigate expression-based findings in genes encoding pro-inflammatory cytokines (i.e. *IL11*), neurotrophic factors (i.e. *BDNF*), and the serotonin transporter (*SLC6A4*), which have been key players proposed for MDD pathophysiology and differential ADR.

IL11 is one of the many pro-inflammatory cytokine implicated in immune dysregulation aspects of MDD and ADR. A GWAS performed using baseline peripheral blood samples of participants in the Genome-based Therapeutic Drugs for Depression project (GENDEP) cohort revealed that no differences in *IL11* expression are predictive of ADR, but differential methylation at a SNP (rs1126757) in *IL11* was capable of predicting ADR to escitalopram (Uher, Perroud et al. 2010). As a follow-up study in the same cohort, differential methylation analysis of

eleven CpG sites located near rs1126757 was conducted between non-responders and responders to escitalopram or nortriptyline. Methylation at select CpG sites within *IL11* were discovered to be predictive of ADR, and differential response between the two types of antidepressants. (Powell, Schalkwyk et al. 2013).

Lower levels of SLC6A4 protein expression are associated with an increased risk of developing MDD following an adverse experience, a delayed therapeutic response to antidepressants, and a larger amount of negative side effects during a course of treatment.(Luddington, Mandadapu et al. 2009). In an epigenetic context, hypermethylation of *SLC6A4* CpG sites were identified in MDD patients who responded to a six-week treatment with escitalopram (Domschke et al., 2014). Conversely, another similar study with a 12-week treatment period of escitalopram did not replicate these findings. SLC6A4 methylation did not differ between escitalopram-treated MDD patients and healthy controls, but they did correlate with increased MDD course severity, and the presence of childhood adversity (Kang, Kim et al. 2013). Collectively, these findings suggest that differential SLC6A4 methylation has potential as a predictor biomarker of ADR to escitalopram, but further replications of these results are required to increase confidence.

BDNF encodes for a neurotrophin necessary for neurodevelopment, cell differentiation, and synaptic plasticity (Binder and Scharfman 2004). The neurotrophin hypothesis of depression states that depression may result from stress-induced decreases in BDNF expression which can be reversed by antidepressant treatment (Molendijk, Bus et al. 2011). Indeed, MDD has been observed to be correlated with decreased central and peripheral BDNF levels (Sen S 2008), and antidepressants are shown to produce an increase in BDNF levels in depressed patients (Wolkowitz, Wolf et al. 2011). Follow-up studies were interested in whether differential

methylation of BDNF promoter regions could act as potential biomarkers for ADR as well. Analysis of 13 CpG sites in the BDNF promoter IV retrieved from leukocyte samples of 39 depressed patients prior to antidepressant treatment demonstrated that methylation at one CpG site within the BDNF promoter IV was lower in non-responders compared to responders. (Tadic, Muller-Engling et al. 2014). From another cohort of 41 MDD patients currently being treated with pharmacotherapy, BDNF promoter I methylation was identified as a potential moderator biomarker. Increased BDNF promoter I methylation was observed in patients treated with SNRI or SSRI antidepressants compared to those treated with a combination of an antidepressant and a mood stabilizer. (D'Addario, Dell'Osso et al. 2013) . Overall, these findings suggest that differential DNA methylation of the BDNF promoter regions do have some essential role in facilitating or predicting ADR in depressed patients.

These preliminary findings from hypothesis-driven studies report significant relationships between differential DNA methylation and ADR, yet most of these findings lack replication, and are derived from small cohorts. To our knowledge, no genome wide differential methylation analyses have been completed in the context of ADR. Whole genomic approaches are critical for advancing our current understanding of ADR biomarkers, since they are unbiased in experimental methods, and capable of establishing novel biomarker candidates. (Menke and Binder 2014).

Chapter 2: Hypothesis and Objectives

To our knowledge, we performed the first genome wide DNA methylation analysis in the context of antidepressant response. We hypothesize that significantly differentially methylated CpGs assessed from peripheral blood samples of non-responders and responders to an eight-week escitalopram treatment can identify functional biomarkers which predict ADR.

We are currently preparing a manuscript presented in Chapter 3 that will be soon be submitted to Lancet Psychiatry. Additional clarifying details have been added for the purpose of this thesis. The objectives of the manuscript are outlined below:

1. Conduct genome-wide DNA methylation and genome-wide expression analysis within a well-designed, and large clinical cohort (MDD patients, n=175; healthy controls, n = 101).
2. Identify differentially methylated positions between responders and non-responders of escitalopram treatment
3. Identify differentially expressed genes between responders and non-responders of escitalopram treatment.
4. Identify differentially methylated positions that are located in regions of differentially expressed genes.
5. Validate the most significant differentially methylated positions using a targeted bisulfite sequencing approach.
6. Replicate our findings in an independent cohort (n=147).
7. Perform functional annotation analysis with DAVID to provide further functional perspectives on our differential methylation findings (Not included for our initial manuscript submission).

Chapter 3: Integrated genome wide methylation and expression analyses reveal functional predictors of response to antidepressants

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ABSTRACT

Background:

Major depressive disorder (MDD) is primarily treated with antidepressants, yet many patients fail to respond to adequate trials. Understanding who is likely to respond to antidepressant treatment and/or what mediates this response is of considerable clinical importance. As part of the Canadian Biomarker Integration Network in Depression (CAN-BIND-1) initiative, we aimed to identify differential DNA methylation marks as epigenetic predictors of antidepressant response (ADR) in MDD patients.

Methods:

Healthy participants (n=112) and depressed participants (n=211) between 18-60 years of age were recruited across six Canadian clinical centers. Eligible depressed patients with MDD by DSM-IV-TR criteria and a Montgomery-Åsberg Depression Rating Scale (MADRS) score of ≥ 24 were enrolled. Genome-wide DNA methylation analysis was conducted using the Infinium MethylationEPIC Beadchipb with DNA extracted from baseline peripheral blood samples prior to beginning an eight-week trial of escitalopram. Genome-wide mRNA expression analysis was conducted on the HumanHT-12 v4 Expression Beadchip in RNA extracted from leukocytes at baseline. Depressed patients were classified as non-responders (NRES) and responders (RES) according to changes in MADRS scores following eight weeks of treatment. Differentially methylated positions (DMPs) were identified in regions of differentially expressed genes and validated using a targeted sequencing approach. Replication was conducted with patients participating in a similar trial, the Douglas Biomarker Study. CAN-BIND-1 clinical trial was registered with the ClinicalTrials.gov identification #: NCT101655706.

Findings:

After depressed participants concluded the 8-week trial, 82 RES and 95 NRES were included in this study. Genome-wide differential DNA methylation revealed 2,572 DMPs ($p<0.05$, with FDR = 0.1). 303 DMPs were located within 271 genomic regions after applying a cut-off of two percent absolute change in β values. Differential expression of these genomic regions was assessed ($p<0.05$, FDR=0.1, logFC ≥ 0.1). Three DMPs in *CHN2 (*cg23687322, p = 0.00043 and cg06926818, p= 0.0014) and *JAK2* (cg08339825, p=0.00021) gene regions were the most significantly associated with mRNA expression changes and validated with targeted sequencing. One *CHN2* site (cg06926818) was successfully replicated in the Douglas Biomarker Study Cohort.

Interpretation:

DMPs found within *CHN2* and *JAK2* gene regions may act as predictors of ADR. Interestingly, both genes have some relevance to current theories of MDD etiology and ADR. *JAK2* encodes for a tyrosine kinase involved in specific cytokine signaling that mediates peripheral inflammation and *CHN2* codes for a GTP-ase activating protein involved in controlling axon pruning processes during neurodevelopment. Although our findings are promising, further studies are required to add clinical validity to our results.

Funding:

Canadian Institutes of Health Research, CAN-BIND (partially funded by the Ontario Brain Institute), Fonds de recherche du Québec (FRQS), and Janssen Research & Development, LLC.

Introduction

Antidepressants are an effective treatment option for major depressive disorder (MDD). However, treatment selection is clinically subjective, and response is determined by trial and error. Roughly 60% of patients fail to respond to initial interventions, whereas 20-30% of these patients do not respond despite multiple attempts. On average, four weeks are required for drug response and six weeks are required to attain remission (Kennedy, Lam et al. 2016). In addition to this time required for a clinically relevant improvement, negative side effects often lead to non-compliance. Clearly, a treatment paradigm that reliably matches patients with effective antidepressants as early as possible would minimize suffering and treatment-associated adversity. Predictive biomarkers for antidepressant response (ADR) would greatly benefit clinical practice by decreasing the evaluation period for drug efficacy (Leuchter, Cook et al. 2010).

MDD is heterogeneous in symptom presentation and treatment response, and environmental factors play an important role in onset, course and duration of illness (Friedrich 2017). As such, investigating epigenetic mechanisms as possible predictors of ADR is enticing, as they are more capable of reflecting environmental effects on the genome. The most investigated epigenetic mark in clinical studies and the best characterized mark is DNA methylation (The 2016). DNA methylation is defined by a methyl group addition, typically to cytosine bases, and predominantly, but not exclusively, at those directly followed by a guanine (CpG dinucleotide sites). It has been shown to reflect exposure to environmental factors such as drug abuse (Feng and Nestler 2013), early childhood trauma(Labonté, Suderman et al. 2012), and chronic stress (Turecki and Meaney 2016). Methylation marks are clinically suitable as biomarkers: they are unaffected by storage time, robustly detectable in peripheral tissues such as

blood, and the methods for DNA methylation analyses are relatively straightforward and available in clinical lab settings(The 2016).

Research in Context

Evidence before this study:

We searched PubMed with search terms "antidepressant response", "depression", "methylation", "genome wide" and "psychiatry" for publications from 2000 to 2018. To date, no genome-wide analysis has been conducted to identify methylation predictors of ADR. Review articles presenting an overview of ADR and epigenetic mechanisms were examined, which revealed a few studies reporting targeted analyses where CpG sites within candidate genes were proposed as possible ADR biomarkers. Decreased promoter methylation of brain derived neurotrophic factor (BDNF) (Tadić, Müller-Engling et al. 2013) and increased methylation in the serotonin transporter gene in MDD patients predicted better response to six weeks of escitalopram (Domschke, Tidow et al. 2014). Finally, lower methylation levels at two CpG sites in interleukin-11 (IL11) were associated with better ADR to escitalopram or nortriptyline within the Genome-Based Therapeutic Drugs for Depression project (Powell, Smith et al. 2013).

Added value of this study:

To our knowledge, our study represents the first genome-wide differential methylation analysis of ADR. In addition, we assess the potential function of differential methylation sites by simultaneously generating genome-wide mRNA expression data.

Implications of all the available evidence:

Standard clinical procedures used to predict ADR are subjective, and no clear biomarkers exist. Here, we demonstrate that differential methylation at CpGs within CHN2 and JAK2 are possible functional predictors of ADR. Eventually, these findings could help improve MDD treatment paradigms. Our genome wide findings were validated with a targeted sequencing approach, and some findings were replicated in an independent cohort.

Methods

Discovery Cohort Characterization: CAN-BIND-1

A detailed account of the CAN-BIND-1 clinical trial has been published elsewhere (Lam, Milev et al. 2016). Briefly, our discovery cohort consisted of participants recruited from 6 clinical centers across Canada, comprising Vancouver (Djavad Mowafaghian Centre for Brain Health), Calgary (Hotchkiss Brain Institute), Toronto (University Health Network and Centre for Addiction and Mental Health), Hamilton (St. Joseph's Healthcare Hamilton), and Kingston (Providence Care, Mental Health Services). Written consent was obtained from all participants. Healthy control participants and MDD patients ranging between 18 to 60 years of age were enrolled using exhaustive selection criteria. Specifically, healthy participants were ensured to have no psychiatric or unstable medical diagnosis, sufficiently fluent in English to complete self-assessments, and matched for sex and age distribution of MDD patients. Other exclusion criteria included the presence of other psychiatric diagnoses, in addition to the presence of psychotic features or high suicidality in MDD.

Replication Cohort Characterization

The Douglas Biomarker Study was used as our replication cohort, which had a similar design to our discovery cohort. Participants were recruited at the Depressive Disorders Program

at the Douglas Mental Health Institute, McGill University (Montreal, QC), which included an eight-week antidepressant treatment for MDD patients randomly selected to receive either densvenlafaxine (SNRI) or escitalopram (SSRI).

Evaluation of Antidepressant Response

After screening and recruitment, all participants were assessed at baseline (W0) for symptom severity using the Montgomery Åsberg Depression Rating Scale (MADRS). MDD patients were administered escitalopram (10-20mg/d) for eight weeks. At week eight (W8), MDD patients were assessed again with the MADRS. Escitalopram response was indicated by a ≥50% decrease in W8 MADRS scores relative to W0, and patients were classified as either a responder (RES) or non-responder (NRES). Hamilton Depression Rating Scale (HAM-D) scores were used instead for the Douglas Biomarker Study, where a ≥50% decrease in HAM-D scores at W8 relative to baseline denoted response. Research Ethics Boards at each recruitment site approved the study design. During screening visits, consent was obtained from all eligible participants for all procedures.

Genome wide DNA methylation analysis on the Infinium MethylationEPIC Beadchip

DNA was extracted from whole blood samples obtained from 112 healthy controls and 211 MDD patients at baseline prior to the start of the trial, using a modified version of the Qiagen FlexiGene DNA kit. Bisulfite conversion and methylation analysis was performed at the McGill University and Genome Quebec Innovation Center (GQ). The Infinium MethylationEPIC Beadchip was used to assess genome wide DNA methylation (Illumina, US). The EPIC microarray allows for single nucleotide resolution coverage of >850,000 methylation sites in coding and non-coding regions per sample (greater than twice the coverage than its earlier

version, the Infinium HumanMethylation450 Beadchip). In addition to >90% coverage of 450K Beadchip sites, the EPIC microarray allows for interrogation of CpG sites outside of CpG islands, FANTOM5 enhancer regions, ENCODE open chromatin and enhancer regions, DNase hypersensitive sites, and miRNA promoter regions (Pidsley, Zotenko et al. 2016). Thus, the EPICarray does not exclude the potential impact of methylation events in non-promoter regions. This provides a better view of the whole methylome, and accounts for recent literature that implicates the importance of gene body methylation and non-canonical relationships with gene expression (Yang, Han et al.).

EPIC microarray data processing and differential methylation analysis.

RES and NRES were identified using reduction in MADRS scores as described previously. Raw intensity files were received from GQ after an initial quality control assessment of methylation detection. GenomeStudio software (Illumina, USA). Ratios of the median unmethylated and methylated intensities were used to perform an additional sample quality control (QC) measurement in R (ver 3.4). Samples were removed if either intensity was below 10.5. Further bioinformatics processes were conducted in house using the Chip Analysis Methylation Pipeline (*ChAMP*) Bioconductor package. This user-friendly pipeline was designed for the purpose of analysing Illumina 450K Beadchip data, but has since been updated for the more comprehensive EPIC Beadchip array(Morris, Butcher et al. 2014). This pipeline was chosen given that it provides a straightforward workflow that incorporates multiple different Bioconductor packages to perform various steps of microarray processing. A majority of the algorithms are based on the *minfi* Bioconductor package (Aryee, Jaffe et al. 2014). Raw intensity data files were used to load the data into the R environment with the *champ.load* function, which also allows for probe QC and removal steps to occur simultaneously. Probes with low detected

signals (n=10,164), cross reactive probes (n= 44), non-CpG probes (n=2,913), probes with less than three beads in at least five percent of samples per probe (n= 170), probes that bound to SNP sites (n=150,294; removed as per Zhou et al's recommendations (Zhou, Laird et al. 2017) and additionally removed any probes that targeted known SNP sites, as this affects probe hybridization accuracy), and sex chromosome probes (n=17,245) are all considered problematic for accurate downstream methylation detection. After removing these probes, 679, 362 probes remained for downstream analysis. Although this may seem like a drastic decrease in the number of retained probes, this is normal during microarray pre-processing steps (Houtepen, Vinkers et al. 2016) (Kuan, Waszczuk et al. 2017). For each CpG, beta values were calculated as the methylation signal over the sum of unmethylated and methylated signals, which is analogous to the percent of methylation at each CpG site. Beta values were then normalized using the *champ.norm* function, specifically with the beta mixture quartile method (BMIQ function). Beta distribution graphs were analyzed to ensure that most probes fell within canonical 0-0.2 and 0.8-1 ranges suggestive of unmethylated or methylated levels, respectively. BMIQ is an intra-sample normalization method that adjusts for the type 1 and type 2 bead hybridization differences on the EPIC microarray. It transforms the type 2 probe probabilities into quantiles of type 1 probe distributions, while performing a conformal transformation of hemimethylated probes given that they do not fall under standard beta distributions (Teschendorff, Marabita et al. 2013). Next, the singular value decomposition (SVD) method was called by *champ.SVD* in order to assess the amount and significance of technical batch components, along with any potential confounding variables, in our dataset. Using the *champ.runCombat* function, Combat algorithms were applied in order to correct for our two initial submission batches, along with slide and array as technical batch components detected by SVD. Combat relies on parametric empirical Bayes frameworks

when adjusting data for batch effects in a manner suitable for larger sample sizes. (Johnson 2007)

Age and sex were corrected for as covariates after being identified as confounding biological components through SVD. Differentially methylated positions (DMPs) were identified using the function *champ.DMP*, which relies on *limma* – based linear regression methods (p-value of 0.05, FDR of 0.1)(Ritchie ME 2015). M values (log_2 transformed beta values) were used for all analyses to avoid heteroscedasticity as recommended by Du et al (Du, Zhang et al. 2010), but were reported as beta values.

Genome wide mRNA gene expression analysis on the HT-12 Beadchip

Whole blood was collected in EDTA tubes containing LeukoLOCK filters (Thermo Scientific, USA) from healthy controls and depressed patients. LeukoLOCK filters isolate leukocytes from whole blood, and eliminate the interference of globin mRNA from red blood cells (RBCs) while conducting expression analyses (Schwochow, Serieys et al. 2012). RNA was extracted as per a modified LeukoLOCK Total RNA Isolation protocol. Agilent 2200 Tapestation was used to assess RNA quality across samples, with a RNA integrity number (RIN) cutoff of six, and sent to GQ in two batches. There, further RNA QC was conducted, complementary RNA was prepared using standard Illumina Whole-Genome protocols, and was hybridized to Human HT-12 v4 Expression BeadChips (Illumina, USA). Differential gene expression analysis was conducted on the Human HT-12 v4 Expression Beadchip (Illumina, USA), which provides accurate genome wide expression coverage on up to 47,000 well-known genes, gene candidates, and splice variants. Initial quality control of raw probe signals was conducted in GenomeStudio (Illumina, USA) by GQ.

HT-12 Expression Beadchip processing:

Only the subset of samples that appeared in our DNA methylation analysis were included for whole genome expression analysis. Raw probe intensities were loaded into the R environment with the *limma* Bioconductor package, a commonly used package for analyzing differential expression on microarrays (Ritchie ME 2015). Probe signals were detected with the *propexp* function, and normalized with the *normalizeBetweenArrays* function. Probe filtering was conducted using a detection p value of < 0.01 in at least 20% of samples cut off to denote retained probes, where 16,378 gene probes were preserved for downstream analysis. Differentially expressed genes were identified through linear regression analyses, with age and sex as covariates. All analyses were conducted with $\log_2$ transformed values. Only probes with at least ± 0.1 logFC values were included in order to only include the most biologically relevant genes.

Targeted bisulfite sequencing for validation of genome wide findings

Differentially methylated CpGs with an absolute $\Delta\beta$ of at least two percent methylation and located in differentially expressed gene regions with a logFC ≥ 0.1 were selected for validation with targeted bisulfite sequencing on an Illumina MiSeq platform(Chen, Gross et al. 2017). Targeted bisulfite sequencing of our DMPs was selected as the methodology to complete our validation, given our fortunate access to an in-house Illumina MiSeq platform. We opted to use our own in-house protocol for amplicon library preparations instead of standardized library preparation biotechnologies, as it is more cost-effective, allows for better differential methylation analysis of large sample sizes, and incorporates a user-friendly multiplexed PCR-based preparation

of bisulfite DNA samples. For more detailed methodology of our targeted sequencing, please see Chen et al's paper (Chen, Gross et al. 2017). NRES and RES DNA samples were bisulfite converted using the Epitect 96 Bisulfite kit (Qiagen, USA) as per manufacturer's guidelines. Primers were designed with the Methyl Primer Express software (ThermoFisher Scientific), and all samples were ensured to have an optimal molarity of 2nM prior to being loaded onto the MiSeq platform with the V3 600 cycle kit (Illumina, US). These steps are further discussed in detail in the following sections.

Primer Design

Bisulfite sequencing primers were designed for our target amplicons with the Methyl Primer Express software (ThermoFisher Scientific). Primer sequences for probes in *CHN2* and *JAK2* are specified below:

*CHN2 (*cg23687322 and cg06926818)

- Forward sequence: ATTTTAGAGAGGAGTTTGTTAATTTTAT
- Reverse sequence: ACTTCTCAAACAAAACTTATCTAAAC

JAK2 (cg08584037 and cg08339825)

- Forward sequence: GTATTTTGATGGAAGYGATAAAATAATA
- Reverse sequence: TAAAATTCTTTTCCCAAATAATCATAAAAC

When assessing CpGs in FAM24B genomic regions, a portion of a CpG island was located within a primer sequence. Thus, CpGs in forward primers were replaced with a 50% mix of C and T, while in reverse primers, they were replaced with a 50% mix of A and G to establish equal attachment between methylated and unmethylated templates as much as possible. The second round of primers were designed by adding CS1 and CS2 sequences to our forward and reverse targeted amplicon primers respectively:

CS1: 5’ ACACTGACGACATGGTTCTACNNN 3’
CS2: 5’ TACGGTAGCAGAGACTTGGTCTNNN 3’

This allows for attachment of P5 and P7 Illumina flow-cell read primers and the Index read primer during the third PCR amplification. 3 N bases (25% mix of all 4 bases) were added between our targeted primers to increase base diversity and improve sequencing QC in early PCR amplification cycles. Our third and final round of primers were designed to target CS1 and CS2 sequences by attaching P5 and P7 Illumina flow-cell attachment sequences to our CS1 and CS2 primers:

P5: 5' AAT GAT ACG GCG ACC ACC GA 3'
P7: 5' CAA GCA GAA GAC GGC ATA CGA GAT 3'

The forward primer was designed to amplify CS1-amplicon regions, and attach the P5 flow cell clustering sequence. The reverse primer was designed to amplify CS2-amplicon regions followed by addition of a Fluidigm indexing barcode for sample identification and the P7 flow cell clustering sequence. After testing our initial amplicon primers in bisulfite converted DNA from our peripheral blood based samples, we also tested them in bisulfite converted DNA collected from post mortem brain samples to confirm that our genes are expressed in brain and blood.

Amplicon library preparations and MiSeq

Three rounds of multiplexed PCR amplification were performed using 384 well plates to prevent intra-amplicon batch effects. Using freshly bisulfite converted DNA, the first round of PCR amplification was completed using primers targeting our amplicons. The second round of PCR was performed using with first round PCR products and amplicon primers with CS1 and CS2 sequences attached. The final round of PCR reaction was performed with primers that attach P5 and P7 Illumina flow-cell binding sequences along with unique indexing barcodes per sample.

After each round of amplification, a small amount of all samples was run on a two percent agarose gel to ensure all samples were amplified correctly, followed by purification with Agencourt AMPure XP (AMPure) beads (Beckman Coulter, Cat. #: A63881). KAPA HiFi HotStart Uracil+ (Kapa Biosystems, Cat #: KK2802) was used in only the first amplification round, followed by KAPA HiFi HotStart ReadyMix (2X) (Kapa Biosystems, Cat #: KK2602) in rounds two and three. To quantify our final amplicon library's concentrations, three different methods were used (Agilent 2200 TapeStation, NanoDrop ND-1000 and qPCR) prior to MiSeq loading to ensure that our pooled samples had an optimal final molarity of 2nM. Samples were loaded onto the MiSeq platform with the V3 600 cycle kit (Illumina, Cat #: MS-102-3003).

Pre-processing of bisulfite sequencing data

Raw MiSeq read data was processed using an efficient pipeline previously established in our lab. Briefly, adaptor sequences were trimmed using Trimmomatic (v.0.35), where reads with Phred scores less than twenty were removed. Bowtie 2 (v. 2.1.0) were used to align the reads. Methylated and non-methylated signals from the CpGs were extracted to calculate the level of methylation at each target site.

Functional Analysis:

We used DAVID v6.8 to distinguish any enriched biological, molecular, cellular or pathological pathway based on a list of genes that contained differentially methylated CpG sites (Huang, Sherman et al. 2008). Only DMPs that had at least a two percent change in beta values between comparison groups were included to account for the most biologically relevant sites.

Statistical Analyses

Differential methylation analysis for both cohorts was conducted between NRES and RES using *limma*-based linear regression methods, with age and sex as covariates. For replication, antidepressant type was also accounted for as a covariate. Log_2 transformed β values were used for analyses where applicable, and reported as β values. Nominal p values < 0.05 were used to denote significant differential CpG methylation between NRES and RES groups, and corrected for genome wide multiple testing using the Benjamin-Hochberg (BH) procedure with an FDR of 0.1. A two percent change in methylation ($\Delta\beta$) was then used as a cut off value to decrease the number of significant CpGs, and to identify sites with more biologically relevant methylation differences. Targeted validation results were analyzed using one-tailed T tests with a significance threshold of 0.05. Correlation of microarray and sequencing methylation values was assessed using Pearson correlation coefficients.

To assess differential expression, *limma*-based linear regression analyses on log_2 transformed values was performed with age and sex accounted for as covariates. Genes that contained differentially methylated CpGs with absolute $\Delta\beta$ values greater than two percent, and that appeared in our differential expression analysis were identified. For these genes, significant differential expression was denoted with a p-value of 0.05, and an FDR of 0.1 to account for multiple testing corrections with the BH method.

To address the possibility of confounding effects of blood cell composition, complete blood cell counts were obtained from each patient during the trial. One way ANOVA tests were used analyze all three comparison groups for any effects of blood cell proportions on our main results, with a p value significance threshold of 0.05.

RESULTS

Differential methylation analysis

After completing all quality control procedures see methods and Table 1), we included in this study 101 controls and 177 depressed (95 NRES and 82 RES) samples study. Raw EPIC microarray data were pre-processed with the ChAMP pipeline and 686,006 CpG probes were retained for differential methylation analysis. Linear regression analyses were performed on log-transformed β values for NRES and RES. We identified 2,572 significantly differentially methylated positions (DMPs) ($p < 0.05$, FDR = 0.1); however, this includes DMPs with very low differences in methylation (i.e. $\Delta\beta$ below 0.5%). Therefore, we applied a $\Delta\beta$ value cutoff of two percent to identify 303 DMPs with methylation changes that are more likely to be biologically relevant (Supplementary Table 1).

Differential mRNA expression analysis

mRNA expression data from the subjects included in this study were generated using the HT-12 expression microarray. After pre-processing raw HT-12 microarray data with the limma Bioconductor package, 16,378 mRNA probes were retained and assessed for differential mRNA expression with linear regression analyses. We applied a logFC cutoff of 0.1 to eliminate gene probes with low levels of differential expression, which resulted in 2009 retained probes. The expression probes were overlapped with DMP probes to identify differentially methylated positions that are more likely to affect *cis* gene expression.

Target selection and validation

We overlapped the list of genes identified from our 303 significant DMPs with genes targeted by 2009 HT-12 probes to select DMPs to validate. Sixteen DMPs (Table 2) were located within genes that appeared on our list of 2009 expression probes, and all but two DMP probes overlapped with unique genes (Table 3). Of these sixteen sites, *CHN2* and *JAK2* were the most significant differentially expressed genes (FDR = 0.05). Thus, cg23687322 (*CHN2;* $p = 1.93 \times 10^{-4}$, FDR = 0.08, $\Delta\beta = -0.05$), cg06926818 (*CHN2*; $p = 9.67 \times 10^{-5}$, FDR = 0.07, $\Delta\beta = -0.04$) and cg08584037 (*JAK2;* $p = 3.14 \times 10^{-4}$, FDR = 0.09, $\Delta\beta = -0.02$) in *JAK2* were selected for targeted validation. All three probes were located in promoter regions, and responders were observed to have a decrease in methylation compared to non-responders.

Validation was conducted with 92 NRES and 83 RES samples. We performed targeted bisulfite sequencing of CpG probes within *CHN2 (*cg23687322, p = 0.0016 and cg06926818, p = 0.0058) and *JAK2* (cg08584037, p=0.0009) (Figure 1; A-C and Table 4). The level of CpG methylation assessed by targeted bisulfite sequencing and microarray methods were significantly correlated ($p < 0.0001$) with relatively high Pearson correlation coefficients for all three CpG probes (Figure 1; D-F).

Differential Methylation when comparing groups with psychiatrically healthy controls

We compared differential methylation at cg06926818, cg23687322, and cg08584037 between depressed cases and psychiatrically healthy controls to ascertain whether our findings were specific to antidepressant response. No significant differential methylation was observed between healthy controls and NRES groups for all three probes (*CHN2;* cg23687322, p = 0.11 and cg06926818, p = 0.33. *JAK2;* cg08584037, p = 0.73). Significant differential methylation

was observed between healthy controls and RES (*CHN2;* cg23687322, p = 0.03 and cg06926818, p = 0.004. *JAK2;* cg08584037, p = 0.001). Overall, these findings suggest that, among depressed patients, differential methylation in these loci may predict response to antidepressant treatment.

Blood Cell Heterogeneity:

Heterogeneity of white blood cell types has potential confounding effects on DNA methylation measurements based in peripheral blood samples. We used complete blood cell count data of all participants collected at the beginning of the trial to rule out any effects of cellular composition. No significant differences were found between groups in the levels or proportions of white cell types (Supplementary Table 2).

DAVID gene enrichment and pathway analyses:

DAVID gene enrichment and pathway analyses 271 unique genes were identified from our list of 303 DMPs with greater than two percent change in methylation between NRES and RES. Functional annotation clustering analysis conducted using DAVID revealed a significant enrichment of these genes in cellular development, neuron differentiation, neuron generation, nervous system development, neurogenesis, neuron development, cell projection organization, and GTPase mediated signalling, along with regulation of these processes (FDR < 0.05, Table 5).

Replication in the Douglas Biomarker Study Cohort

We used 32 HC, 76 NRES, and 71 RES (Table 1) samples from the Douglas Biomarker Study cohort to increase external validity of our main findings. Results are summarized in Table

4. In the *CHN2* gene, we replicated differential methylation at cg06926818 ($p = 0.027$, $\Delta\beta = -0.03$). Although of similar magnitude and direction, differential methylation at cg23687322 did not reach significance in this cohort ($p = 0.17$, $\Delta\beta = -0.03$). We did not replicate the cg08584037 position in *JAK2* ($p = 0.59$, $\Delta\beta = -0.003$).

DISCUSSION

To our knowledge, this study represents the first genome-wide methylation analysis of ADR. Particularly for biomarker studies, genome wide analyses reveal novel, potentially relevant candidates for further functional investigations. We incorporated differential gene expression data to guide selection of potentially functional DMPs, and validated the level of methylation at these sites with a targeted bisulfite sequencing approach. Two CpGs, and one CpG located in *CHN2* and *JAK2* respectively were found to be candidate predictors of ADR. We additionally compared DNA methylation levels at these three CpGs in NRES and RES groups to healthy controls. Differential methylation was observed between RES and NRES, and RES and healthy controls, but not between NRES and healthy controls for all three probes. One probe located in *CHN2* was successfully replicated.

In the brain, *CHN2* observed to have a role in neurodevelopmental hippocampal axon pruning. Interestingly, adult hippocampal neurogenesis has been observed to be stimulated by antidepressant administration in rats (Malberg, Eisch et al. 2000) and non-human primates (Perera, Coplan et al. 2007). Multiple animal models demonstrate that all major antidepressants types require the presence of hippocampal neurogenesis in order to produce behavioural response (Santarelli, Saxe et al. 2003). Differential *CHN2* methylation has not been directly assessed in the context of ADR or MDD, but has been implicated in disorders that are often occur with

MDD or depressive symptoms, such as substance abuse(Hao, Luo et al. 2017), ADHD (Elia, Gai et al. 2010), and psychosis (Yuan, Miller et al. 1995). *CHN2,* or β2-chimaerin, maps to chromosome 7p15.3 and encodes for a GTP-ase activating protein predominantly expressed in the pancreas and brain (Yuan, Miller et al. 1995). Our results of differential baseline methylation levels could imply that molecular processes required for ADR, such as those involved in hippocampal neurogenesis, may be more likely to predict responders to escitalopram. However, this remains to be proven.

JAK2, or Janus kinase 2, encodes for a tyrosine kinase that initiates downstream effects through a family of signal transducer and activator of transcription (STAT) molecules. *JAK2* has a non-redundant role in cytokine receptor signaling pathways which mediate components of innate and adaptive immunity (Peña, Cai et al. 2010). In the context of MDD, increased inflammation is thought to be associated with poorer ADR (Köhler, Krogh et al. 2016). JAK/STAT pathways are implemented in both central and peripheral inflammation, but particularly in the latter. Inflammatory biomarkers that interact with JAK/STAT signaling(Peña, Cai et al. 2010) (i.e. *IL-6* and *CRP*) have been implicated as biomarkers of predicting of ADR (Raison, Rutherford et al. 2013). Differential methylation in *JAK2* may be indicative of differing levels of inflammatory molecules which in turn predict escitalopram response. Furthermore, serotonin and norepinephrine moderate differential production of cytokines, and a chronic imbalance of these neurotransmitters shifts the balance of different types of peripheral cytokines. Use of SSRI or SNRI antidepressants are also associated with changes in levels of inflammation(Blier 2001). These differential effects of neurotransmitter levels on inflammation may be related to why our *JAK2* probe was not indicative of ADR in the replication cohort which involved both SSRI and SNRI treatment. Perhaps *JAK2* is solely predictive of SSRI ADR, but

not SNRI ADR, given due to norepinephrine's effects on cytokine production. Similarly, to *CHN2*, differential methylation at *JAK2* may also have functional effects on neurogenesis that could be associated with ADR. Ketamine, an acutely acting antidepressant, reverses stress-induced learning deficits in rats and increases *Arc* levels (a synaptic plasticity consolidating protein) only in the presence of *JAK2*. Furthermore, phosphorylated JAK2 colocalizes with Arc in dendritic spines, showing evident *JAK2/STAT* signaling during synaptic plasticity events (Patton, Lodge et al. 2016).

Our differential methylation analysis was conducted with peripheral whole blood samples, a preferred biological material for biomarker studies. However, peripheral tissue sampling methods make it difficult to fully distinguish specific mechanisms involved, particularly from other organs such as the brain. This is a general limitation for functional investigation of neurogenesis-based ADR theories. There are also concerns for blood cell types as a potentially confounding factor. However, our CpGs of interest were chosen partially based on our differential expression analysis conducted solely in leukocyte*s,* and we found no significant differences in white blood cell type counts between groups.

When comparing the relationship between differential gene expression and methylation data for *CHN2* and *JAK2*, a canonical inverse relationship was not detected. This could be potentially due to effects of 3D chromatin structure affecting how epigenetic molecules bind to regulate expression, the presence of hydroxymethylation, or the activity of other regulatory molecules that influence expression in addition to DNA methylation.

A number of specific limitations may apply to our study. Firstly, the differential methylation levels between comparison groups are relatively low, which may decrease biological significance of our findings. However low fold changes are expected in complex and

multifactorial illnesses such as MDD, and are commonly reported in psychiatry research (Dalman, Deeter et al. 2012, Mai Sakaia 2015, Hachiya, Furukawa et al. 2017). Ultimately, we were able to validate our EPICmicroarray results with a targeted approach. Secondly, the EPICarray is designed to target a large portion of well-known CpGs and non-CpG sites, yet this still leaves some potentially important methylation sites undetected. Thirdly, our methods used for differential methylation analyses do not distinguish between hydroxymethylated cytosines and methylated cytosines. However, hydroxymethlyated cytosines are often found in gene bodies, and all three CpGs of interest were located in gene promoter regions (Shi, Ali et al. 2017), decreasing the likelihood that our main findings are affected by this limitation.

Several follow-up analyses would be valuable to conduct based on our preliminary results. These include replication of our exploratory findings in another independent cohort to increase clinical validity; exploration of *JAK2* as a predictor of response in an independent SSRI clinical trial. In general, more genome-wide investigations, with even greater sample sizes and more consideration of sociodemographic factors related to DNA methylation (i.e. smoker status, family history of mental illness), are required. Furthermore, different types of antidepressant monotherapies or combinational therapies could be assessed, across multiple time points. Data from these analyses could potentially reveal different types of biomarkers for various types of antidepressants and other treatment modalities, provide insight into differing patient response rates, and increase our functional understanding of ADR biomarkers. Additionally, the use of a panel of biomarkers rather than one singular molecule is likely more clinically effective for diseases that manifest heterogeneously across patients. As more data is generated on predictive ADR biomarkers, a composite measure of all their levels can be considered. This is also partially

why it is important to consider other epigenetic regulators in future ADR biomarker analyses as well, as epigenetic mechanisms rarely act independently.

In conclusion, our study represents the first genome-wide methylation analysis for antidepressant response, and emphasizes the importance of additional explorations to identify robust biomarkers, along with any possible functional mechanisms related to differential response.

Tables

	Discovery Cohort *CAN-BIND-1*			**Replication Cohort** *Douglas Biomarker Study*		
	HC	**NRES**	**RES**	**HC**	**NRES**	**RES**
n	101	95	82	32	76	71
Male	38	39	28	16	27	28
Female	63	56	54	16	49	43
Female%	62.4%	58.9%	65.4%	50%	64.5%	60.6%
Age	32.7 ± 10.4	36 ± 13.10	35.2 ± 12.17	47 ±13.91	41 ± 12.47	39.2 ± 11.54
MADRS/HAM-D T0	0.9 ± 1.73	30.5 ± 5.40	29.3 ± 5.45	0.75 ± 0.99	33.5 ± 6.29	31.3 ± 6.84
MADRS/HAM-D T8	1.1 ± 2.19	23.9 ± 7.26	7.9 ± 4.96	1.6 ± 1.97	25.2 ± 7.46	8.6 ± 5.60

Table 1: Demographic characteristics of discovery and replication cohorts. In our discovery cohort, out of the 211 depressed patients initially recruited, 31 did not continue the trial until Week 8. From the 180 that completed the trial, 1 NRES and 2 RES samples were removed after failing quality control assessments at Genome Quebec. 1 healthy control sample was removed due to poor methylation signal detection QC. Age, MADRS T0, and MADRS T8 averages are followed by standard deviation values. For assessment of symptom severity, MADRS scores and HAM-D scores were used for our discovery and replication cohorts respectively.
(NRES = non-responder, RES = responder, MADRS = Montgomery Asberg Depression Rating Scale, HAM-D = Hamilton Depression Scale).

Probe ID	Gene	CHR	Location	Feature	Cgi	p value	FDR	ΔBeta
cg02745111	ATMIN	16	81070647	Body	shore	9.60E-05	0.07	-0.02
cg13104274	ATP1B1	1	169078316	Body	shore	4.77E-05	0.06	-0.02
cg26009832	ATP1B1	1	169081894	Body	opensea	9.50E-05	0.07	-0.02
cg19677267	CD52	1	26645161	Body	opensea	2.10E-04	0.08	0.03
cg12001491	CD52	1	26645487	Body	opensea	3.50E-04	0.10	0.04
cg23687322	CHN2	7	29523056	TSS1500	opensea	1.93E-04	0.08	-0.05
cg06926818	CHN2	7	29523160	TSS1500	opensea	9.67E-05	0.07	-0.04
cg06279274	FAM24B	10	124635805	5'UTR	shelf	1.31E-04	0.07	-0.03
cg06705237	FBP1	9	97402555	TSS200	shore	2.47E-04	0.09	-0.02
cg14422240	FTSJD2	6	37425031	Body	opensea	7.06E-05	0.06	-0.03
cg08584037	JAK2	9	4984071	TSS1500	shore	3.14E-04	0.09	-0.02
cg22200736	KRT72	12	52995358	TSS200	shore	3.20E-04	0.10	-0.02
cg04453169	MAP7	6	136680760	Body	shore	3.94E-05	0.05	-0.04
cg18581616	NLRP8	19	56478019	Body	opensea	4.25E-06	0.03	0.03
cg03485252	NRG1	8	31503975	Body	opensea	1.05E-05	0.04	0.02
cg21870668	SNRPN	15	25123731	5'UTR	shore	3.92E-05	0.05	-0.03
cg22707675	WDR43	2	29116967	TSS1500	shore	7.38E-05	0.06	0.03

Table 2: Significant DMPs with at least a two percent differential methylation between NRES and RES located within differential expressed gene regions.

NRES = average NRES β values at a CpG site. RES = average RES β values at a CpG site. Δ β = change in methylation relative to responders. For a detailed description of terms in the table, please see Supplementary Table 1.

Probe_ID	Gene	AveExpr	t	p.val	FDR	logFC
ILMN_1772540	ATMIN	5.694	-2.338	0.020	0.08	-0.105
ILMN_2223720	ATMIN	7.142	1.749	0.081	0.11	0.150
ILMN_1730291	ATP1B1	5.207	2.141	0.033	0.08	0.113
ILMN_3244172	CD52	11.269	-2.231	0.027	0.08	-0.161
ILMN_2403237	CHN2	6.391	-2.775	0.006	0.05	-0.139
ILMN_1774110	CHN2	5.326	-2.094	0.037	0.08	-0.112
ILMN_2140799	FAM24B	5.469	-2.113	0.036	0.08	-0.109
ILMN_1728799	FBP1	7.394	-1.747	0.082	0.11	-0.107
ILMN_3246953	FTSJD2	6.601	-2.046	0.042	0.08	-0.101
ILMN_1683178	JAK2	7.110	-2.754	0.006	0.05	-0.126
ILMN_2055760	KIAA1715	5.959	0.001	0.999	1.00	0.000*
ILMN_1695812	KRT72	5.715	-0.988	0.324	0.34	-0.137
ILMN_2216815	MAP7	5.656	1.718	0.087	0.11	0.127
ILMN_2075794	NLRP8	11.263	1.478	0.141	0.16	0.205
ILMN_1737252	NRG1	5.664	-1.476	0.141	0.16	-0.169
ILMN_1693341	SNRPN	7.368	1.935	0.054	0.09	0.122
ILMN_1671442	WDR43	5.423	-2.380	0.018	0.08	-0.102

Table 3: Sixteen gene probes identified in our differential expression analysis that contains significant DMPs with least a two percent change in methylation. There were fourteen unique genes that overlapped between our differential methylation and differential expression analyses. The fold change (FC) is in reference to RES.

		Validation		Replication	
Probe ID	**Gene**	**p value**	**ΔBeta**	**p value**	**ΔBeta**
cg23687322	CHN2	4.33E-04	-0.05	0.17	-0.03
cg06926818	CHN2	1.43E-03	-0.04	0.03	-0.03
cg08584037	JAK2	9.47E-04	-0.02	0.59	-0.003

Table 4: Validation and Replication values at CpG probes in *CHN2* and *JAK2*. All probes were successfully validated, and correlated well with methylation values assessed by the EPIC microarray within the CAN-BIND-1 cohort. cg06926818 was the only probe that replicated in the Douglas Biomarker Study Cohort.

Biological Function Term	# of Genes	%	p value	FDR
Cellular development	*93*	*17.4*	*9.70E-08*	*4.80E-04*
Neuron differentiation	*63*	*11.8*	*1.40E-06*	*3.50E-03*
Neuron generation	*67*	*12.5*	*2.40E-06*	*3.90E-03*
Nervous system development	*94*	*17.5*	*3.80E-06*	*4.80E-03*
Neurogenesis	*69*	*12.9*	*5.20E-06*	*5.20E-03*
Neuron development	*51*	*9.5*	*8.40E-06*	*6.90E-03*
Cell projection organization	*63*	*11.8*	*9.40E-06*	*6.70E-02*
Regulation of cellular component organization	*96*	*17.9*	*2.10E-05*	*1.30E-02*
Regulation of small GTPase mediated signal transduction	*23*	*4.3*	*2.40E-05*	*1.30E-02*
Positive regulation of cellular component organization	*57*	*10.6*	*3.20E-05*	*1.60E-02*
Regulation of nervous system development	*41*	*7.6*	*1.00E-04*	*2.90E-02*
Positive regulation of nervous system development	*28*	*5.2*	*1.00E-04*	*4.20E-02*
Positive regulation of cell differentiation	*43*	*8*	*1.10E-04*	*4.00E-02*
Regulation of neurogenesis	*37*	*6.9*	*1.10E-04*	*4.00E-02*
Positive regulation of GTPase activity	*35*	*6.5*	*1.30E-04*	*4.20E-02*
Regulation of GTPase activity	*37*	*6.9*	*1.40E-04*	*4.40E-02*
Regulation of neuronal differentiation	*32*	*6*	*1.50E-04*	*4.20E-02*
Negative regulation of kinase activity	19	3.5	2.10E-04	5.60E-02
Negative regulation of protein kinase activity	18	3.4	2.70E-04	6.80E-02
Regulation of signal transduction	103	19.2	3.30E-04	8.00E-02
Regulation of cellular component biogenesis	40	7.5	3.60E-04	8.30E-02
Neuron projection development	40	7.5	4.60E-04	9.90E-02
Axon development	26	4.9	5.10E-04	1.10E-01

Table 5: DAVID functional annotation results

Functional annotation clustering analysis conducted using DAVID revealed a significant enrichment in multiple terms (FDR < 0.05; italicized).

FDR = false discovery rate calculated with the BH.

Figures

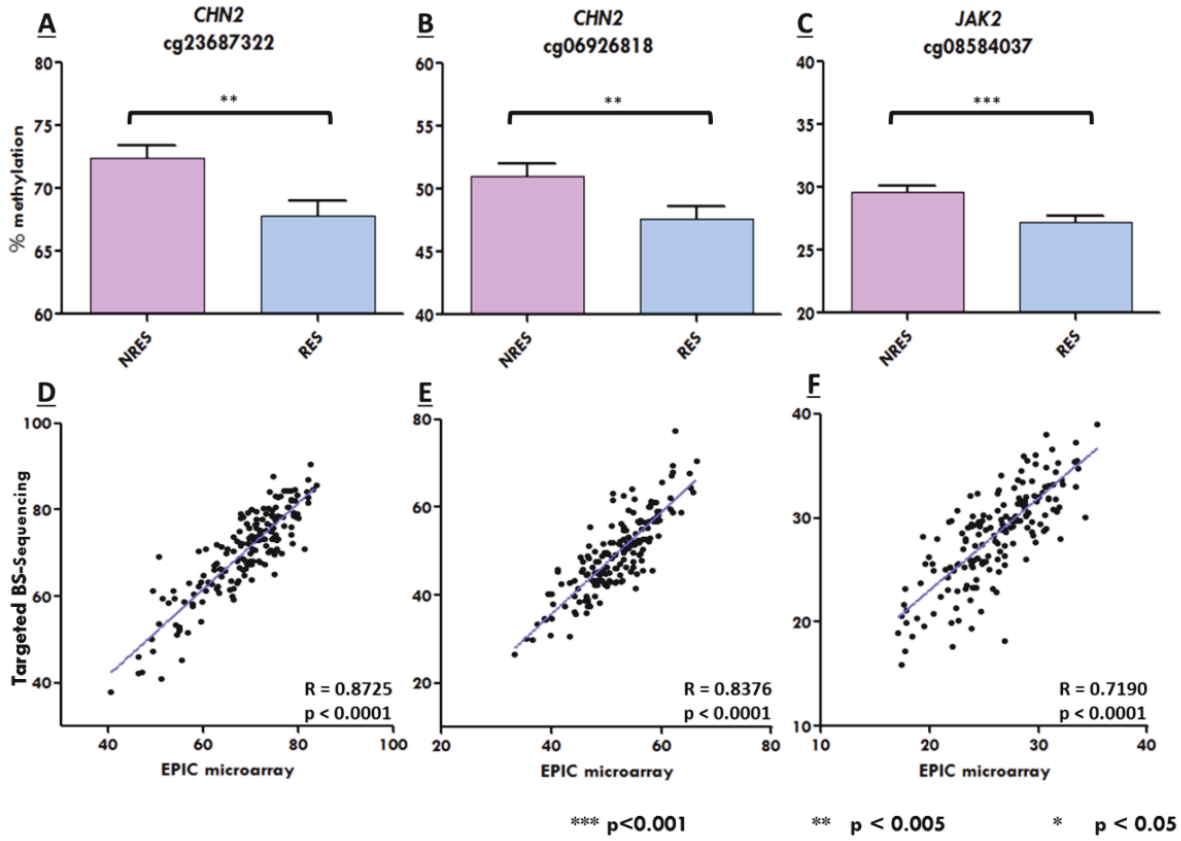

Figure 1 (A-C): Bar graphs showing % methylation of NRES and RES detected by targeting bisulfite sequencing

(A) cg23687322 (p = 0.0009), (B) cg06926818 (p = 0.0058) and (C) cg08584037 (p = 0.0009).

(D-F) Scatterplots showing average sample % methylation correlation levels between EPIC microarray and targeted bisulfite sequencing platforms

(D) cg23687322, (E) cg08339825 and (F) cg08584037.

R = Pearson correlation coefficient.

Supplementary Data

	Gene	chr	bp	feature	cgi	p val	FDR	NRES	RES	Δbeta
cg11249728	ACSL3	2	223800611	Body	opensea	2.04E-04	8.43E-02	0.608	0.584	-0.024
cg03261737	ADGRG1	16	57666593	5'UTR	opensea	3.97E-05	5.19E-02	0.581	0.557	-0.024
cg10772169	AGL	1	100315213	TSS1500	shore	1.62E-06	2.27E-02	0.527	0.505	-0.021
cg15720535	AGPAT2	9	139582585	TSS1500	island	2.35E-04	8.77E-02	0.529	0.486	-0.043
cg12461099	ALDH4A1	1	19217794	TSS1500	opensea	1.82E-04	8.16E-02	0.578	0.558	-0.021
cg14344550	ALK	2	29516596	Body	opensea	8.81E-06	3.65E-02	0.199	0.241	0.042
cg08166588	AMIGO3	3	49757438	TSS1500	shore	7.00E-07	1.93E-02	0.627	0.599	-0.028
cg15486224	ANKRD46	8	101527561	Body	opensea	1.93E-06	2.39E-02	0.385	0.326	-0.059
cg03497652	ANKS3	16	4751569	Body	opensea	1.40E-04	7.44E-02	0.597	0.574	-0.022
cg09950162	ANO4	12	101480520	Body	opensea	9.05E-06	3.65E-02	0.594	0.559	-0.035
cg07737560	ANO4	12	101470827	Body	opensea	1.85E-04	8.18E-02	0.500	0.530	0.030
cg16362232	ANO9	11	430036	Body	shore	3.27E-04	9.58E-02	0.755	0.728	-0.028
cg12655260	ARHGAP26	5	142562569	Body	opensea	1.25E-04	7.25E-02	0.361	0.304	-0.057
cg17140497	ARHGAP26	5	142563177	Body	opensea	8.53E-05	6.42E-02	0.210	0.186	-0.024
cg11710969	ARMC3	10	23217173	5'UTR	opensea	1.90E-04	8.28E-02	0.280	0.256	-0.024
cg03493768	ART3	4	76996690	5'UTR	opensea	1.06E-04	6.80E-02	0.645	0.620	-0.025
cg09868768	ASPRV1	2	70188605	1stExon	island	2.33E-04	8.75E-02	0.630	0.609	-0.021
cg25337691	ATG9B	7	150717629	Body	shore	3.19E-04	9.51E-02	0.581	0.561	-0.020
cg02745111	ATMIN	16	81070647	Body	shore	9.60E-05	6.62E-02	0.351	0.328	-0.023
cg20182111	ATP13A4	3	193217253	Body	opensea	3.01E-04	9.35E-02	0.615	0.570	-0.044
cg13104274	ATP1B1	1	169078316	Body	shore	4.77E-05	5.56E-02	0.790	0.770	-0.020
cg26009832	ATP1B1	1	169081894	Body	opensea	9.50E-05	6.62E-02	0.673	0.653	-0.020
cg21187669	ATPAF2	17	17929033	Body	opensea	2.61E-04	8.98E-02	0.573	0.551	-0.022
cg00195322	B4GALT5	20	48278559	Body	opensea	1.06E-04	6.80E-02	0.425	0.401	-0.024
cg15812976	BAG1	9	33256910	ExonBnd	opensea	4.85E-05	5.58E-02	0.748	0.725	-0.023
cg08500171	BAT2	6	31590674	Body	shore	3.78E-05	5.16E-02	0.705	0.685	-0.021
cg23202887	BCL11B	14	99691372	Body	opensea	2.16E-04	8.54E-02	0.507	0.529	0.022
cg11519176	BGLAP	1	156211801	TSS200	shelf	3.35E-04	9.65E-02	0.393	0.368	-0.025
cg19749188	BICD1	12	32351549	Body	opensea	1.00E-04	6.72E-02	0.809	0.788	-0.021
cg16053902	BRD1	22	50181906	Body	shelf	3.44E-04	9.75E-02	0.809	0.774	-0.035
cg12096447	BRF1	14	105714400	TSS200	shore	1.48E-04	7.68E-02	0.690	0.713	0.023
cg17226676	BSPRY	9	116126574	Body	opensea	1.34E-04	7.33E-02	0.534	0.501	-0.032
cg18039797	BUB1B	15	40509518	Body	opensea	8.43E-06	3.65E-02	0.491	0.464	-0.027
cg08021797	C16orf74	16	85785130	TSS1500	island	6.72E-05	6.02E-02	0.481	0.459	-0.022
cg24766229	C17orf99	17	76151124	Body	opensea	3.57E-04	9.87E-02	0.400	0.378	-0.022
cg17234962	C1R	12	7241782	Body	opensea	5.68E-06	3.54E-02	0.630	0.605	-0.025
cg07959070	C22orf34	22	50026188	Body	island	7.62E-06	3.65E-02	0.775	0.685	-0.090

cg18527739	C22orf34	22	49946312	Body	opensea	8.34E-06	3.65E-02	0.820	0.761	-0.059
cg04138436	C22orf34	22	49822980	Body	opensea	2.76E-04	9.12E-02	0.729	0.692	-0.038
cg03814063	C22orf34	22	50025915	Body	island	3.30E-05	4.93E-02	0.816	0.785	-0.031
cg00783170	C22orf34	22	49861705	Body	opensea	1.73E-05	4.12E-02	0.885	0.855	-0.030
cg06355422	C22orf34	22	50013649	Body	shelf	7.06E-05	6.12E-02	0.844	0.816	-0.028
cg01681680	C22orf34	22	49855255	Body	opensea	8.60E-06	3.65E-02	0.884	0.859	-0.025
cg15823183	C3orf56	3	126911942	TSS200	opensea	1.31E-04	7.31E-02	0.843	0.865	0.022
cg24642844	C7orf50	7	1081250	Body	shore	2.57E-04	8.94E-02	0.799	0.766	-0.033
cg02210115	CAB39L	13	49990767	5'UTR	opensea	3.57E-05	5.11E-02	0.617	0.592	-0.025
cg06831653	CACNA1E	1	181748219	Body	opensea	1.05E-04	6.80E-02	0.819	0.798	-0.022
cg13532410	CACNA2D3	3	54732582	Body	opensea	1.81E-04	8.15E-02	0.745	0.768	0.023
cg20106684	CACNG3	16	24269504	Body	shore	1.67E-04	7.99E-02	0.603	0.636	0.033
cg22536580	CALCB	11	15095822	5'UTR	island	2.98E-05	4.76E-02	0.165	0.139	-0.025
cg14551034	CAPRIN1	11	34094566	Body	opensea	4.54E-06	3.37E-02	0.484	0.453	-0.030
cg21646082	CCDC21	1	26603970	3'UTR	shelf	5.28E-07	1.93E-02	0.810	0.790	-0.020
cg25215890	CD48	1	160651452	Body	opensea	3.73E-04	9.99E-02	0.474	0.451	-0.023
cg07060948	CD48	1	160651479	Body	opensea	1.98E-04	8.36E-02	0.211	0.191	-0.020
cg19677267	CD52	1	26645161	Body	opensea	2.10E-04	8.49E-02	0.253	0.288	0.035
cg12001491	CD52	1	26645487	Body	opensea	3.50E-04	9.80E-02	0.356	0.396	0.040
cg26117104	CDK5RAP1	20	31975140	ExonBnd	opensea	4.40E-06	3.37E-02	0.821	0.781	-0.040
cg23687322	CHN2	7	29523056	TSS1500	opensea	1.93E-04	8.28E-02	0.715	0.667	-0.048
cg06926818	CHN2	7	29523160	TSS1500	opensea	9.67E-05	6.62E-02	0.537	0.499	-0.038
cg11803859	CHST15	10	125770124	Body	opensea	2.33E-04	8.75E-02	0.558	0.530	-0.028
cg19236247	CIB4	2	26835353	Body	opensea	9.68E-06	3.65E-02	0.482	0.434	-0.049
cg04471485	CLIC3	9	139889789	Body	island	8.08E-05	6.36E-02	0.549	0.526	-0.024
cg06682371	CMTM8	3	32291082	Body	opensea	3.10E-04	9.42E-02	0.269	0.243	-0.025
cg04269043	CNGB1	16	57918043	3'UTR	island	2.04E-04	8.43E-02	0.302	0.279	-0.023
cg13904574	CNTFR	9	34586050	5'UTR	shelf	8.40E-06	3.65E-02	0.227	0.250	0.024
cg17444747	COL23A1	5	177915909	Body	opensea	2.51E-04	8.88E-02	0.516	0.480	-0.035
cg21871330	COL23A1	5	177882221	Body	opensea	2.17E-04	8.56E-02	0.775	0.754	-0.021
cg09001527	COL23A1	5	177942856	Body	shelf	2.04E-04	8.43E-02	0.764	0.743	-0.021
cg07427642	COL23A1	5	177944298	Body	shore	2.03E-04	8.42E-02	0.583	0.562	-0.021
cg15301006	CPLX2	5	175267609	5'UTR	opensea	8.85E-05	6.48E-02	0.611	0.651	0.040
cg19421526	CRTAC1	10	99734513	Body	opensea	1.79E-05	4.18E-02	0.235	0.199	-0.036
cg25758699	CRYGS	3	186257301	Body	opensea	1.12E-04	6.94E-02	0.633	0.608	-0.025
cg05492904	CYP19A1	15	51604503	5'UTR	opensea	2.80E-04	9.14E-02	0.497	0.468	-0.030
cg16170087	CYP1B1-AS1	2	38368819	Body	opensea	6.22E-05	5.94E-02	0.480	0.439	-0.041
cg18148659	DENND4A	15	65953468	3'UTR	opensea	3.58E-04	9.88E-02	0.679	0.659	-0.020
cg07373298	DISC1FP1	11	90434175	Body	opensea	3.19E-04	9.51E-02	0.859	0.839	-0.020
cg02209770	DLGAP4	20	35062903	Body	shore	3.18E-04	9.51E-02	0.504	0.482	-0.022
cg20109856	DLX6AS	7	96643454	TSS200	shelf	1.66E-06	2.27E-02	0.469	0.430	-0.039

cg08180998	DNAJC15	13	43663213	Body	opensea	6.23E-06	3.54E-02	0.734	0.712	-0.022
cg27209571	DNER	2	230563222	Body	opensea	1.66E-04	7.99E-02	0.691	0.712	0.021
cg08549898	DOCK8	9	296755	Body	opensea	1.79E-04	8.13E-02	0.703	0.678	-0.024
cg22340526	DPP6	7	153586207	Body	shore	1.05E-04	6.80E-02	0.781	0.760	-0.020
cg21989229	DSE	6	116608164	5'UTR	opensea	1.02E-04	6.77E-02	0.605	0.575	-0.030
cg25700077	DTX2P1-UPK3BP1-PMS2P11	7	76637488	Body	opensea	3.32E-05	4.94E-02	0.777	0.744	-0.034
cg26739697	DTX2P1-UPK3BP1-PMS2P11	7	76637493	Body	opensea	1.13E-05	3.67E-02	0.848	0.820	-0.029
cg19689387	EIF5	14	103807182	Body	opensea	3.02E-04	9.35E-02	0.495	0.468	-0.027
cg18892446	ENC1	5	73938574	TSS1500	shore	3.72E-04	9.99E-02	0.561	0.534	-0.026
cg24343097	ENTPD5	14	74486664	TSS1500	shore	6.67E-05	6.00E-02	0.416	0.395	-0.021
cg03502625	EPB41	1	29212825	TSS1500	shore	2.15E-04	8.52E-02	0.568	0.544	-0.024
cg22056241	EPHA1	7	143107282	TSS1500	opensea	5.42E-05	5.72E-02	0.701	0.679	-0.022
cg07068406	EPHB1	3	134647778	Body	opensea	5.99E-05	5.86E-02	0.507	0.387	-0.119
cg10785929	EPHB1	3	134650410	Body	opensea	8.90E-05	6.50E-02	0.817	0.739	-0.078
cg04425710	ESCO2	8	27630920	TSS1500	shore	2.26E-04	8.65E-02	0.693	0.717	0.023
cg21025681	EXOSC10	1	11134131	Body	opensea	9.71E-06	3.65E-02	0.689	0.666	-0.023
cg06279274	FAM24B	10	124635805	5'UTR	shelf	1.31E-04	7.31E-02	0.739	0.713	-0.026
cg15355800	FAM45A	10	120895864	Body	opensea	2.55E-05	4.59E-02	0.615	0.583	-0.033
cg24508168	FAM83F	22	40405832	Body	opensea	2.35E-05	4.56E-02	0.262	0.293	0.031
cg06705237	FBP1	9	97402555	TSS200	shore	2.47E-04	8.87E-02	0.561	0.537	-0.024
cg15621260	FIBIN	11	27015813	5'UTR	opensea	4.79E-05	5.57E-02	0.278	0.245	-0.034
cg00700214	FMO2	1	171154802	5'UTR	opensea	1.43E-04	7.55E-02	0.727	0.702	-0.025
cg14422240	FTSJD2	6	37425031	Body	opensea	7.06E-05	6.12E-02	0.690	0.661	-0.028
cg03149245	GALNT9	12	132703598	Body	opensea	3.67E-05	5.14E-02	0.301	0.329	0.028
cg05521767	GDPD5	11	75230135	5'UTR	opensea	1.43E-04	7.56E-02	0.505	0.485	-0.020
cg07805029	GFI1	1	92953256	TSS1500	shore	2.98E-04	9.33E-02	0.607	0.580	-0.028
cg26393275	GPD2	2	157435476	Body	opensea	9.36E-05	6.60E-02	0.601	0.579	-0.022
cg00081729	GREM2	1	240656737	Body	shore	2.51E-04	8.88E-02	0.698	0.663	-0.035
cg20716668	GRK5	10	121043687	Body	opensea	3.31E-04	9.60E-02	0.543	0.566	0.023
cg14479617	GSK3B	3	119542274	3'UTR	opensea	1.80E-04	8.15E-02	0.736	0.714	-0.023
cg25210835	GSTM5	1	110254828	TSS200	opensea	1.78E-04	8.13E-02	0.321	0.241	-0.080
cg24467349	GSTM5	1	110254835	TSS200	opensea	2.15E-04	8.52E-02	0.353	0.277	-0.076
cg14377951	GSTM5	1	110254896	1stExon	opensea	2.47E-04	8.87E-02	0.350	0.287	-0.063
cg25535106	GTF3C2	2	27549046	3'UTR	opensea	3.15E-05	4.82E-02	0.687	0.666	-0.020
cg11987759	GUSB	7	65425863	3'UTR	opensea	9.68E-05	6.62E-02	0.847	0.816	-0.031
cg26214742	H2AFY	5	134735914	TSS1500	shore	2.84E-05	4.71E-02	0.436	0.410	-0.026
cg00057840	HDAC4	2	240076109	Body	opensea	2.27E-04	8.69E-02	0.461	0.435	-0.026
cg18564053	HES3	1	6303793	TSS1500	shore	1.10E-04	6.91E-02	0.201	0.177	-0.023
cg21718051	HIVEP1	6	12071462	Body	opensea	2.94E-04	9.30E-02	0.565	0.540	-0.026
cg02549973	HMHA1	19	1076202	TSS1500	shore	2.56E-05	4.59E-02	0.699	0.725	0.026
cg24987751	HPSE2	10	100276194	Body	opensea	5.94E-05	5.86E-02	0.664	0.684	0.020

cg20464360	HSF5	17	56564855	Body	island	1.39E-05	3.88E-02	0.512	0.541	0.029
cg22922494	IL12A-AS1	3	159647642	Body	opensea	1.81E-04	8.15E-02	0.323	0.296	-0.026
cg21593409	IL17C	16	88706389	Body	island	2.50E-04	8.88E-02	0.507	0.479	-0.028
cg07794885	IL17C	16	88703611	TSS1500	shelf	1.40E-04	7.44E-02	0.540	0.512	-0.028
cg10479672	IL1F8	2	113810641	TSS1500	shore	3.91E-05	5.19E-02	0.694	0.718	0.023
cg00756845	IPCEF1	6	154678593	TSS1500	opensea	9.31E-05	6.59E-02	0.653	0.623	-0.030
cg03615426	IQCK	16	19777410	Body	opensea	2.75E-04	9.11E-02	0.304	0.396	0.092
cg21951975	IRF6	1	209979733	TSS1500	shore	6.08E-05	5.87E-02	0.182	0.144	-0.038
cg25192855	IRF6	1	209979283	5'UTR	shore	8.52E-05	6.42E-02	0.228	0.207	-0.021
cg16134369	IRX4	5	1888009	TSS1500	shore	2.52E-04	8.88E-02	0.367	0.347	-0.020
cg02287260	ITPK1	14	93510671	Body	opensea	1.79E-04	8.14E-02	0.745	0.721	-0.023
cg12534855	ITPR1	3	4735740	Body	opensea	2.42E-04	8.85E-02	0.626	0.595	-0.030
cg06287611	ITPR2	12	26624359	Body	opensea	3.22E-04	9.52E-02	0.424	0.399	-0.025
cg08584037	JAK2	9	4984071	TSS1500	shore	3.14E-04	9.45E-02	0.268	0.247	-0.021
cg10975897	JARID2	6	15504844	Body	opensea	3.63E-04	9.93E-02	0.515	0.493	-0.022
cg11738976	JPH2	20	42744590	Body	island	8.13E-06	3.65E-02	0.345	0.320	-0.025
cg17238677	KCNN3	1	154736223	Body	shelf	9.56E-08	9.37E-03	0.756	0.735	-0.021
cg20204316	KCNQ3	8	133460603	TSS1500	opensea	1.32E-04	7.31E-02	0.633	0.612	-0.021
cg00399027	KIAA0182	16	85676861	5'UTR	shore	2.10E-04	8.49E-02	0.500	0.479	-0.022
cg03182584	KIAA0895	7	36364854	3'UTR	opensea	1.10E-04	6.91E-02	0.600	0.578	-0.022
cg02881189	KIAA1539	9	35111032	5'UTR	shore	4.69E-05	5.56E-02	0.462	0.437	-0.024
cg00620464	KIAA1715	2	176868389	TSS1500	shore	4.94E-05	5.60E-02	0.726	0.698	-0.028
cg07149296	KIAA1755	20	36889389	TSS1500	island	2.32E-04	8.75E-02	0.366	0.340	-0.026
cg26856575	KIF1B	1	10291784	5'UTR	opensea	1.63E-05	4.04E-02	0.681	0.660	-0.020
cg04382643	KLC3	19	45849853	Body	island	2.21E-05	4.49E-02	0.555	0.515	-0.040
cg26299044	KRT12	17	39021588	Body	shore	4.61E-05	5.56E-02	0.568	0.538	-0.030
cg22200736	KRT72	12	52995358	TSS200	shore	3.20E-04	9.51E-02	0.428	0.406	-0.022
cg12693179	LGR5	12	71863439	Body	opensea	1.49E-05	3.94E-02	0.738	0.716	-0.022
cg12398777	LINC00968	8	57472469	TSS200	opensea	2.13E-04	8.52E-02	0.327	0.300	-0.027
cg14684596	LINC01268	6	114191904	Body	opensea	3.67E-04	9.95E-02	0.759	0.735	-0.024
cg08153693	LINC01289	8	64680636	TSS1500	opensea	8.49E-05	6.42E-02	0.529	0.552	0.023
cg00117532	LINGO1	15	78098084	5'UTR	opensea	3.02E-04	9.35E-02	0.368	0.345	-0.023
cg09754549	LOC100130274	8	144790656	TSS1500	island	1.02E-04	6.77E-02	0.766	0.795	0.030
cg03431084	LOC100131496	20	45948853	Body	opensea	1.93E-04	8.29E-02	0.632	0.610	-0.021
cg02272576	LOC100132354	6	43868964	Body	opensea	8.39E-06	3.65E-02	0.611	0.561	-0.050
cg08627981	LOC100289473	20	1757237	Body	shore	5.57E-05	5.74E-02	0.219	0.185	-0.034
cg18270009	LOC100506869	12	59198886	Body	opensea	3.58E-04	9.87E-02	0.329	0.356	0.028
cg12234768	LOC101928371	2	88862824	Body	opensea	3.20E-04	9.51E-02	0.681	0.653	-0.028
cg00967229	LOC101928978	4	85180506	Body	opensea	2.55E-04	8.92E-02	0.388	0.367	-0.021
cg06686396	LOC101928989	11	82026140	Body	opensea	8.77E-05	6.48E-02	0.315	0.351	0.036
cg18950481	LOC149134	1	246952889	TSS200	shore	4.77E-05	5.56E-02	0.825	0.805	-0.020

cg17429662	LOC149373	1	231323501	TSS200	opensea	1.40E-05	3.88E-02	0.568	0.599	0.031
cg24587835	LOC339166	17	5674234	TSS1500	opensea	4.29E-06	3.37E-02	0.391	0.523	0.132
cg15252215	LOC399815	10	124639110	TSS200	island	1.58E-04	7.88E-02	0.205	0.230	0.026
cg11216554	LOC399815	10	124638983	TSS200	island	1.61E-04	7.96E-02	0.414	0.451	0.037
cg01091620	LOH12CR1	12	12561471	Body	opensea	2.85E-04	9.18E-02	0.235	0.211	-0.024
cg24845165	LPIN3	20	39968231	TSS1500	shore	7.24E-05	6.16E-02	0.630	0.607	-0.023
cg13816228	LRRC8B	1	90022780	TSS1500	opensea	1.67E-04	7.99E-02	0.530	0.482	-0.048
cg09272338	LRRC8B	1	90013382	5'UTR	opensea	2.12E-05	4.43E-02	0.661	0.636	-0.024
cg15791719	LRRC8D	1	90354310	5'UTR	opensea	1.14E-04	7.01E-02	0.659	0.630	-0.029
cg05542101	MACC1	7	20186624	Body	opensea	3.08E-05	4.79E-02	0.622	0.594	-0.028
cg10153341	MAN1A1	6	119665694	Body	shelf	3.85E-05	5.19E-02	0.664	0.632	-0.032
cg13950452	MAP1B	5	71463696	Body	opensea	2.18E-05	4.49E-02	0.769	0.748	-0.021
cg04453169	MAP7	6	136680760	Body	shore	3.94E-05	5.19E-02	0.501	0.466	-0.036
cg21099759	MARCH7	2	160567993	TSS1500	shore	2.39E-04	8.80E-02	0.568	0.545	-0.024
cg10639811	MBNL1	3	152083133	Body	opensea	2.95E-04	9.32E-02	0.578	0.553	-0.025
cg20553766	MC3R	20	54824583	1stExon	island	1.35E-05	3.88E-02	0.448	0.493	0.045
cg06269415	MCC	5	112602827	Body	opensea	6.69E-05	6.01E-02	0.460	0.440	-0.021
cg10230190	MCOLN2	1	85405081	Body	opensea	3.52E-04	9.81E-02	0.553	0.524	-0.029
cg22676212	MEIS3	19	47910108	Body	island	6.27E-05	5.94E-02	0.554	0.513	-0.041
cg19273694	MFSD2B	2	24233923	Body	shore	1.58E-05	3.99E-02	0.731	0.708	-0.023
cg21356710	MFSD2B	2	24234017	Body	shore	2.26E-04	8.65E-02	0.552	0.530	-0.023
cg00784161	MLPH	2	238406432	Body	opensea	6.30E-05	5.94E-02	0.802	0.781	-0.022
cg09805466	MOGAT1	2	223566483	Body	opensea	2.07E-04	8.46E-02	0.433	0.457	0.024
cg08663592	MUC16	19	8989084	Body	opensea	2.65E-04	9.01E-02	0.421	0.446	0.025
cg09271052	MUC22	6	30977529	5'UTR	opensea	4.65E-05	5.56E-02	0.759	0.783	0.025
cg23738210	MYO18B	22	26253182	Body	opensea	2.69E-04	9.05E-02	0.570	0.592	0.023
cg09269848	MYO1C	17	1396074	TSS200	shore	1.89E-04	8.24E-02	0.342	0.314	-0.028
cg22795769	MYO1C	17	1396123	TSS200	shore	1.32E-04	7.31E-02	0.754	0.733	-0.021
cg15699693	MYOZ3	5	150054944	Body	shelf	2.99E-04	9.33E-02	0.549	0.517	-0.032
cg25457884	MYT1	20	62796136	5'UTR	opensea	1.83E-04	8.16E-02	0.785	0.810	0.025
cg04287574	NAV1	1	201619622	Body	island	3.27E-04	9.58E-02	0.343	0.288	-0.055
cg19095920	NBLA00301	4	174458819	Body	shore	1.75E-04	8.08E-02	0.217	0.192	-0.025
cg12778228	NCRNA00188	17	16341601	TSS1500	shore	1.38E-04	7.42E-02	0.549	0.525	-0.023
cg19282259	NCRNA00200	10	1205611	TSS200	island	2.85E-04	9.18E-02	0.865	0.839	-0.025
cg21773245	NDNF	4	121983226	5'UTR	opensea	3.15E-04	9.48E-02	0.571	0.543	-0.028
cg07623113	NDUFB10	16	2008723	TSS1500	shore	5.49E-05	5.74E-02	0.720	0.686	-0.033
cg18581616	NLRP8	19	56478019	Body	opensea	4.25E-06	3.37E-02	0.816	0.845	0.029
cg18496287	NRD1	1	52259574	Body	opensea	3.81E-05	5.18E-02	0.548	0.525	-0.023
cg03485252	NRG1	8	31503975	Body	opensea	1.05E-05	3.67E-02	0.721	0.744	0.023
cg13610659	OBFC2B	12	56622608	Body	opensea	4.95E-05	5.60E-02	0.663	0.638	-0.025
cg11359094	OPRD1	1	29172578	Body	opensea	1.05E-05	3.67E-02	0.604	0.576	-0.028

cg17212470	OR1J4	9	125280584	TSS1500	opensea	1.23E-04	7.25E-02	0.577	0.603	0.026
cg20576955	OR52I2	11	4606701	TSS1500	opensea	5.96E-06	3.54E-02	0.785	0.807	0.022
cg13816428	OR5AU1	14	21625173	TSS1500	opensea	1.33E-04	7.31E-02	0.502	0.534	0.032
cg11320244	OSBPL1A	18	21795073	Body	opensea	3.58E-04	9.87E-02	0.622	0.589	-0.032
cg21449673	PAAF1	11	73618548	Body	opensea	2.65E-04	9.01E-02	0.344	0.302	-0.042
cg12575659	PACRG-AS1	6	163746319	TSS1500	opensea	5.65E-05	5.79E-02	0.229	0.206	-0.023
cg27000690	PACSIN1	6	34437227	5'UTR	shelf	1.11E-04	6.93E-02	0.624	0.601	-0.022
cg24025782	PACSIN2	22	43327600	5'UTR	opensea	3.21E-04	9.52E-02	0.307	0.279	-0.028
cg14353649	PAOX	10	135191496	TSS1500	shore	2.85E-04	9.18E-02	0.459	0.438	-0.021
cg09685257	PARD3	10	34686368	Body	opensea	3.72E-04	9.99E-02	0.619	0.597	-0.021
cg04276953	PAX7	1	18980700	Body	opensea	1.27E-04	7.28E-02	0.450	0.424	-0.026
cg12080079	PAX7	1	19007925	Body	opensea	5.23E-07	1.93E-02	0.742	0.783	0.040
cg21805940	PCCA	13	101174420	Body	opensea	3.43E-04	9.74E-02	0.465	0.436	-0.029
cg12526318	PCDHB17	5	140535392	TSS200	shore	2.64E-04	9.00E-02	0.186	0.208	0.022
cg16179521	PCSK6	15	102009894	Body	opensea	3.47E-04	9.77E-02	0.289	0.265	-0.024
cg11229771	PDE6B	4	640503	Body	opensea	9.47E-05	6.62E-02	0.736	0.766	0.030
cg26693817	PDE6B	4	640348	Body	opensea	6.00E-05	5.86E-02	0.662	0.705	0.042
cg23954416	PDPN	1	13909161	TSS1500	shore	4.51E-07	1.93E-02	0.778	0.740	-0.038
cg06452518	PEPD	19	33923307	Body	opensea	1.53E-04	7.75E-02	0.496	0.470	-0.025
cg00846554	PHACTR1	6	12748001	Body	shore	3.62E-05	5.12E-02	0.436	0.413	-0.023
cg00275896	PHACTR3	20	58251756	1stExon	opensea	3.49E-05	5.05E-02	0.704	0.734	0.030
cg05688588	PI4K2B	4	25237268	Body	shore	7.29E-05	6.18E-02	0.304	0.342	0.038
cg17865045	PILRA	7	99994933	Body	opensea	1.25E-05	3.80E-02	0.440	0.408	-0.032
cg18319102	PIWIL1	12	130822256	TSS200	shore	1.32E-05	3.86E-02	0.422	0.357	-0.065
cg24838063	PIWIL1	12	130822603	TSS200	island	2.71E-04	9.06E-02	0.742	0.681	-0.061
cg24229701	PIWIL1	12	130821962	TSS1500	shore	1.43E-04	7.56E-02	0.627	0.576	-0.052
cg09858226	PKNOX1	21	44401549	5'UTR	opensea	1.27E-05	3.80E-02	0.800	0.778	-0.023
cg08385266	PLB1	2	28769125	Body	opensea	5.33E-05	5.69E-02	0.475	0.442	-0.033
cg22412747	PLB1	2	28768014	Body	opensea	2.44E-05	4.57E-02	0.343	0.311	-0.032
cg22784187	PON3	7	95025407	Body	shore	2.02E-04	8.41E-02	0.496	0.472	-0.024
cg25844590	PPFIBP2	11	7621556	Body	opensea	3.28E-04	9.58E-02	0.566	0.530	-0.036
cg03186149	PPP5C	19	46877200	Body	opensea	1.58E-04	7.88E-02	0.593	0.568	-0.025
cg05257528	PRTN3	19	846179	Body	island	1.83E-04	8.16E-02	0.581	0.557	-0.024
cg17144383	PTPDC1	9	96868748	Body	opensea	8.35E-05	6.42E-02	0.721	0.699	-0.023
cg11055991	PTPRN2	7	158280410	Body	shore	1.59E-04	7.90E-02	0.619	0.591	-0.028
cg09865698	PVRL1	11	119597471	Body	shore	2.94E-05	4.74E-02	0.516	0.496	-0.021
cg21602651	RAB3GAP2	1	220397618	Body	opensea	6.63E-05	6.00E-02	0.466	0.434	-0.032
cg00227342	RAB5C	17	40284353	5'UTR	opensea	5.60E-06	3.54E-02	0.382	0.354	-0.028
cg15975554	RABGAP1L	1	174959074	Body	opensea	5.39E-06	3.49E-02	0.739	0.713	-0.026
cg13580105	RAP1GAP	1	21975062	Body	shelf	1.13E-04	6.94E-02	0.609	0.574	-0.035
cg04062715	RASGEF1C	5	179632567	5'UTR	shelf	2.70E-05	4.68E-02	0.698	0.678	-0.020

cg00009085	RBM33	7	155473359	Body	opensea	5.74E-06	3.54E-02	0.633	0.611	-0.022
cg10527482	RELL1	4	37676204	Body	opensea	2.57E-04	8.94E-02	0.672	0.646	-0.026
cg08047233	RERE	1	8578167	Body	opensea	2.15E-04	8.52E-02	0.652	0.629	-0.023
cg11505048	RGL1	1	183622726	5'UTR	opensea	3.27E-04	9.58E-02	0.632	0.611	-0.021
cg11283152	RHOBTB1	10	62749211	Body	opensea	1.03E-04	6.77E-02	0.761	0.704	-0.057
cg10180052	RNF151	16	2018558	Body	island	1.24E-04	7.25E-02	0.708	0.680	-0.028
cg16738194	RNF212	4	1076636	Body	opensea	2.03E-06	2.39E-02	0.579	0.559	-0.020
cg20980321	RNF5	6	32144667	TSS1500	opensea	2.77E-04	9.12E-02	0.448	0.425	-0.024
cg10929784	ROBO1	3	79773602	5'UTR	opensea	2.90E-04	9.25E-02	0.663	0.643	-0.020
cg24315876	RPTOR	17	78913111	Body	shelf	2.66E-04	9.01E-02	0.855	0.881	0.026
cg08328225	RREB1	6	7183048	Body	opensea	1.32E-04	7.31E-02	0.656	0.631	-0.025
cg10752745	SCHIP1	3	158991106	5'UTR	opensea	1.69E-04	8.02E-02	0.438	0.477	0.040
cg09108429	SDCCAG8	1	243451540	Body	opensea	3.52E-04	9.81E-02	0.549	0.528	-0.021
cg25927227	SFRP1	8	41127218	Body	opensea	3.25E-04	9.57E-02	0.590	0.567	-0.022
cg08572336	SHANK1	19	51165404	Body	island	6.56E-05	6.00E-02	0.818	0.789	-0.028
cg18528054	SLC22A20	11	64982895	Body	opensea	2.62E-04	8.98E-02	0.422	0.397	-0.025
cg15298607	SLC25A25	9	130865618	Body	opensea	3.12E-05	4.82E-02	0.678	0.658	-0.020
cg02272859	SLC34A2	4	25656514	TSS1500	shore	3.19E-05	4.86E-02	0.679	0.652	-0.028
cg23750338	SLC45A4	8	142222470	Body	shore	2.47E-04	8.87E-02	0.753	0.730	-0.023
cg02591213	SLC5A11	16	24857208	TSS1500	opensea	7.93E-06	3.65E-02	0.417	0.388	-0.029
cg20717474	SLC5A11	16	24857188	TSS1500	opensea	1.21E-05	3.77E-02	0.526	0.502	-0.024
cg07099998	SLC5A11	16	24856891	TSS1500	opensea	2.42E-06	2.46E-02	0.655	0.633	-0.023
cg01829163	SLC7A5	16	87871160	Body	opensea	2.21E-05	4.49E-02	0.804	0.774	-0.031
cg11117131	SLN	11	107582818	TSS200	opensea	6.32E-05	5.94E-02	0.758	0.781	0.022
cg17567562	SMARCC1	3	47687980	Body	opensea	2.17E-04	8.55E-02	0.566	0.533	-0.033
cg21870668	SNRPN	15	25123731	5'UTR	shore	3.92E-05	5.19E-02	0.502	0.474	-0.028
cg23999078	SNX9	6	158314486	Body	opensea	1.68E-04	8.01E-02	0.744	0.768	0.024
cg14578284	SOHLH1	9	138592347	TSS1500	shore	5.84E-06	3.54E-02	0.827	0.805	-0.021
cg10534788	SORCS2	4	7245919	Body	opensea	6.35E-05	5.94E-02	0.635	0.655	0.020
cg03819945	SPAG16	2	215243717	Body	opensea	3.61E-04	9.91E-02	0.626	0.650	0.024
cg10574494	SPATA18	4	52918457	Body	shore	3.64E-04	9.93E-02	0.221	0.194	-0.027
cg15706621	SPTBN1	2	54861447	Body	opensea	1.85E-04	8.18E-02	0.630	0.653	0.023
cg24259228	SSC5D	19	55999593	TSS1500	shore	1.95E-04	8.29E-02	0.537	0.512	-0.025
cg20881311	STK38L	12	27457989	Body	opensea	2.57E-05	4.59E-02	0.667	0.637	-0.030
cg16096646	SYNPO2	4	119771931	5'UTR	opensea	2.95E-04	9.32E-02	0.361	0.406	0.045
cg11368628	SYT8	11	1856183	Body	opensea	8.21E-05	6.37E-02	0.402	0.379	-0.023
cg12958046	TBC1D1	4	38019409	Body	shelf	9.36E-06	3.65E-02	0.691	0.656	-0.035
cg19437917	TBCD	17	80865872	Body	shelf	2.68E-04	9.03E-02	0.794	0.816	0.023
cg03563169	TBX18	6	85445250	3'UTR	opensea	8.77E-05	6.48E-02	0.801	0.779	-0.021
cg05555876	TCEA1	8	54935915	TSS1500	shore	9.62E-06	3.65E-02	0.688	0.659	-0.028
cg19933954	TCFL5	20	61494242	TSS1500	shore	2.79E-04	9.14E-02	0.575	0.547	-0.028

cg05591105	TENM4	11	78509989	Body	opensea	1.36E-05	3.88E-02	0.526	0.497	-0.029
cg13183732	TMEM132D	12	130091846	Body	opensea	1.78E-04	8.13E-02	0.697	0.718	0.021
cg03992323	TP53BP2	1	224023297	Body	opensea	2.88E-04	9.23E-02	0.414	0.380	-0.034
cg12268562	TP73	1	3625409	Body	shore	1.36E-04	7.35E-02	0.481	0.455	-0.026
cg16407924	TPO	2	1452260	Body	opensea	8.78E-06	3.65E-02	0.651	0.626	-0.024
cg14931486	TRAPPC4	11	118892305	Body	shelf	1.79E-04	8.13E-02	0.541	0.515	-0.026
cg05958922	TRIM67	1	231319825	Body	opensea	2.84E-04	9.18E-02	0.532	0.558	0.026
cg12547959	TRIO	5	14326153	Body	opensea	6.28E-05	5.94E-02	0.444	0.408	-0.036
cg10956093	TRPM1	15	31341548	Body	opensea	2.90E-04	9.25E-02	0.368	0.331	-0.036
cg07875873	TSKU	11	76493379	TSS1500	shore	8.90E-05	6.50E-02	0.576	0.552	-0.023
cg12958315	TTBK1	6	43233455	Body	shelf	2.87E-04	9.21E-02	0.510	0.488	-0.022
cg11321181	UNC13A	19	17721489	Body	shelf	1.22E-04	7.21E-02	0.381	0.337	-0.044
cg11970806	VEZF1	17	56066485	TSS1500	shore	7.94E-05	6.36E-02	0.672	0.648	-0.025
cg19317830	VGLL4	3	11675660	Body	opensea	3.03E-05	4.79E-02	0.212	0.184	-0.027
cg22707675	WDR43	2	29116967	TSS1500	shore	7.38E-05	6.18E-02	0.547	0.575	0.028
cg04276715	WDR46	6	33254460	Body	shelf	1.09E-05	3.67E-02	0.702	0.678	-0.024
cg16020118	WDR88	19	33622600	TSS1500	shore	2.54E-04	8.89E-02	0.620	0.599	-0.022
cg20341251	WNT5B	12	1754156	Body	shore	2.83E-05	4.71E-02	0.756	0.720	-0.036
cg03995300	ZNF232	17	5019989	5'UTR	shore	2.84E-04	9.18E-02	0.387	0.339	-0.048
cg00601727	ZNF783	7	148989733	Body	shore	7.91E-05	6.36E-02	0.704	0.680	-0.024

Supplementary Table 1: 303 DMPs in genomic regions with at least ±2% Δbeta values.

CpG probe = probe ID/unique identified of the CpG site as per the Illumina CG database.

Gene = target gene name(s) as per the UCSC genome database.

Chr = chromosome containing the CpG, Bp = base pairs; the CpG's location on the chromosome (GrCh37/hg19).

Feature = the type of gene region where the CpG is located

TSS200 = 0–200 bp upstream of the TSS, the transcriptional start site.

TSS1500 = 200–1500 bp upstream of the TSS.

5'UTR = 5' untranslated region, between the TSS and the start codon.

Body = Gene body; defined as between the start and stop codon (regardless of introns, exons, TSS, or promoter regions).

ExonBnd = boundary between an exon and an intron.

3'UTR = 3' untranslated region, between the stop codon and the poly A tail.

Cgi: denotes the location of a particular CpG relative to the closest CpG island.

Island: located within the CpG island.

Shore: 0-2kb from the CpG island.

Shelf: 2-4kb from the CpG island.

Opensea: Isolated CpGs in the genome

	% Lymphocytes	% Monocytes	% Neutrophils	% Eosinophils	% Basophils
HC	33.50 ± 7.55	8.05 ± 2.13	55.14 ± 9.12	2.72 ± 2.04	0.48 ± 0.64
NRES	31.18 ± 7.62	7.87 ± 2.38	57.60 ± 9.18	2.69 ± 3.50	0.43 ± 0.64
RES	31.39 ± 8.29	7.60 ± 2.05	58.21 ± 9.27	2.28 ± 1.90	0.40 ± 0.51
F value	2.22	0.82	2.53	0.7	0.36
P value	0.11	0.44	0.081	0.5	0.7

Supplementary Table 2: Blood cell count comparison between NRES, RES and HC. No confounding effects of white blood cell types were observed. Cell type percentage averages are shown for all three groups, followed by standard deviation values.

NRES = non-responder, RES = responder, HC = healthy controls.

Chapter 4: Conclusion

This thesis investigated current mechanisms underlying depression and antidepressant response. Specifically, existing neurobiological and psychosocial theories of MDD were characterized, along with an overview of biomarkers, epigenetic regulation (with an emphasis on DNA methylation), and differential DNA methylation as a possible biomarker of ADR. I focused on differential DNA methylation biomarkers of ADR given the evidence suggesting that epigenetic mechanisms better reflect environmental effects on the genome. We performed the first novel genome wide methylation analysis to compare differential methylation between non-responders and responders to escitalopram treatment. Using associated genome wide expression data, we identified the most significant differentially methylated positions within differentially expressed genes as candidate biomarkers. Our results suggested that differential methylation at 3 CpG sites, located in *CHN2* and *JAK2*, could act as potential predictors of ADR to escitalopram. These two genes are involved in neurogenesis and immune response pathways respectively, both of which have been implicated in MDD etiology and antidepressant response. Finally, our functional annotation results conducted with genes identified from a subset of significant DMPs revealed an enrichment in pathways such as neuron differentiation, neuron generation, nervous system development, and neurogenesis.

MDD pathophysiology and inconsistent levels of antidepressant response are complicated events that are unable to be explained by any single mechanism. It is more likely that multiple mechanisms are involved in the etiology of varying phenotypes of disease and treatment response seen across MDD patients, and future studies are required for accurate characterization. Firstly, our results were based in baseline patient samples and solely focused on identifying predictors of ADR. It would be interesting to analyze week eight samples of patients to look for

whether methylation levels at any of the 3 CpGs we identified change following escitalopram treatment. Secondly, it would be informative to also look into other types of epigenetic regulation that can distinguish between two response phenotypes as well. Thirdly, aside from differential molecular markers, it could be helpful to combine these findings with multi-disciplinary data (i.e. neuroimaging and clinical biomarkers of antidepressant response) to propose a panel of biomarkers for treatment response. Fourthly, although we replicated one of our CpG sites in the Douglas Biomarker study cohort, additional replication of our results in preferably even larger cohorts are required to increase clinical validity of these CpG sites as biomarker candidates. Fifthly, other types of biological samples can be investigated to identify biomarkers with functional components to elucidate how communication between the brain and the periphery varies in MDD and ADR phenomena (i.e. exosomes). Sixthly, microarray technologies only interrogate pre-determined CpGs. Future analyses should attempt to utilize WGBS to truly investigate the entire methylation, and explore sites that are less represented on microarrays (i.e. non-CpG methylation). Additionally, more efficient assays that differentiate between DNA methylation and hydroxymethylation marks are likely to be developed, which can be utilized to increase specificity of methylation-based findings in future investigations. Finally, as robust biomarkers of MDD and/or ADR are introduced to clinical practice, this will allow for quicker and more objective decision making for diagnostic and treatment dispositions. Resultantly, the discovery of objective clinical measures will also decrease the social stigma surrounding MDD and psychiatric disorders overall.

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