Current and Emergent Suicidal Ideation in Schizophrenia: Effect of Stressful Life Events and Genome-Wide Methylation

by

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Abstract

Suicide is a serious problem, especially in patients with schizophrenia. In order to prevent suicide, it is imperative to identify those with suicidal ideation and emergent suicidal ideation. We tested the effect of childhood trauma and recent stress on current and emergent suicidal ideation. Furthermore, we identified differentially methylated probes and regions associated with current suicidal ideation, as well as methylation changes in individual sites associated with emergent suicidal ideation. We found that an increase in total stress and health-related stress were significant predictors of emergent suicidal ideation. We also report the most significant positions and regions associated with current and emergent suicidal ideation. The anticipated outcomes of these studies were to improve the understanding about risk factors for suicidal ideation, and the identification of methylation markers to monitor for suicidal ideation in psychosis.

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Contributions

Kevin Z Wang performed clinical assessments, data collection and analysis, and the writing of the thesis. However, this work could not be completed without the help of the following individuals, who are formally acknowledged:

Dr. Vincenzo De Luca (Graduate Supervisor) was responsible for the inception, planning, and design of the study, laboratory resources, and mentorship.

Dr. Ali Bani-Fatemi (Postdoctoral Fellow) assisted with the collection of clinical measures and analysis of methylation data.

Oluwagbenga Dada, Christopher Adanty, and Samia Tasmim (Graduate Students) and Sauliha Alli and Areesha Sabir (Research Assistants) performed clinical assessments and data collection.

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List of Abbreviations

5-HT	5-Hydroxytryptamine
5mC	5-Methylcytosine
AUDIT	Alcohol Use Disorders Identification Test
BHS	Beck Hopelessness Scale
BPRS	Brief Psychiatric Rating Scale
CAMH	Centre for Addiction and Mental Health
CDC	Centers for Disease Control and Prevention
CDSS	Calgary Depression Scale for Schizophrenia
CGAS	Candidate Gene Association Study
CI	Confidence Interval
CpG	Cytosine-Guanine Dinucleotide
CPZe	Chlorpromazine Equivalent
C-SSRS	Columbia- Suicide Severity Rating Scale
CTQ	Childhood Trauma Questionnaire
DAST	Drug Abuse Screening Test
DAVID	Database for Annotation, Visualization, and Integrated Discovery
DMP	Differentially Methylated Position
DMR	Differentially Methylated Region
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
EPSE	Extrapyramidal Side Effects
FGA	First-Generation Antipsychotic
FTND	Fagerström Test for Nicotine Dependence
GWAS	Genome-Wide Association Study
hGR	Human Glucocorticoid Receptor
HLA	Human Leukocyte Antigen
HPA	Hypothalamic-Pituitary-Adrenal
LAI	Long-Acting Injectable
LCU	Life Change Units
M.I.N.I.-Plus	Mini-International Neuropsychiatric Interview Plus
MHC	Major Histocompatibility Complex

MMSE	Mini-Mental State Examination
NMDAR	*N*-Methyl-D-Aspartate Receptor
OR	Odds Ratio
PCP	Phencyclidine
PSS	Perceived Stress Scale
SAI	Schedule for Assessment of Insight
SGA	Second-Generation Antipsychotic
SI-IAT	Self-Injury Implicit Association Test
SNP	Single-Nucleotide Polymorphism
SRI	Social Readjustment Index
SRRS	Social Readjustment Rating Scale
TCAG	The Centre for Applied Genomics
TRS	Treatment-Resistant Schizophrenia
WHO	World Health Organization

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Chapter 1

1 Literature Review

Schizophrenia is considered a chronic psychiatric disorder that involves a wide variety of behavioral and cognitive impairments. One of the most concerning and troubling aspects regarding patients with schizophrenia is the greatly increased risk for suicide. The following literature review will provide context for the studies completed and presented throughout this thesis. We will first present an overview of schizophrenia including risk factors, clinical features and diagnosis, and treatment. Then we will provide a summary of suicide research, with less attention to suicidal behaviors, and more focus on suicidal ideation and emergent suicidal ideation. Finally, we will combine the two topics and discuss the important considerations of suicide specifically in patients with schizophrenia. Our research in this field will attempt to improve the understanding and assist in the early identification and prevention of suicide in a vulnerable population.

1.1 Schizophrenia

1.1.1 Historical and Epidemiological Perspective

While schizophrenia has most likely been present throughout all of humankind, the first clinical reports of the disease emerged in the middle of the 19th century when European psychiatrists described a disorder of unknown origin, primarily affecting youth, and ultimately leading to chronic mental deterioration (Jablensky, 2010). It was initially widely believed that the symptoms were a direct result of being possessed by the devil or from assaults from the gods as punishment for immoral behavior (Kyziridis, 2005). Throughout history, various terms have been used to describe schizophrenia, and range from *dementia praecox* to *manic depressive insanity*; however, the term *schizophrenia* was adopted in 1911 and can be translated literally to "a mind that is torn asunder" (Lavretsky, 2008). Such descriptions relate to the historical evolution and advancement of knowledge throughout the years regarding disease pathogenesis, aetiology, and treatment (Kyziridis, 2005), as discussed below.

Schizophrenia is the most common of psychotic disorders, with the mean lifetime prevalence approximated to be 1% (APA, 2013; Kahn et al., 2015). While schizophrenia has a relatively low incidence of 15.2 per 100,000, the prevalence of 7.2 per 1,000 can be considered high due to the nature of the progression of the disease: patients typically experience symptoms in late adolescence or early adulthood and this eventually becomes chronic (Picchioni et al., 2007; Saha et al., 2005). Between genders, studies have found that schizophrenia is slightly more common in men than women with a risk ratio of 1.4 to 1 (McGrath et al., 2008; Saha et al., 2005). It should also be noted that the onset of symptoms generally occurs at a younger age in men compared to in women (Patel et al., 2014). For instance, men tend to present with symptoms during their late teenage years or early 20s, with a peak in risk between the ages of 21 and 25, while females, on the other hand, generally present with symptoms either in their late 20s and early 30s, with a peak in risk between the ages of 25 and 30, and after the age of 45 (Li et al., 2016; Schultz et al., 2007). Furthermore, it was found that men tend to have a more severe form of schizophrenia experiencing more negative symptoms, less chance of recovery, and a generally worse outcome (Jablensky, 2000; Picchioni et al., 2007).

1.1.2 Clinical Presentation and Diagnosis

The onset of schizophrenia can vary greatly between individuals in that while it usually proceeds gradually, in some cases symptoms can occur fairly abruptly (Schultz et al., 2007). Interestingly, studies have found that the non-acute onset of symptoms and longer duration of untreated psychosis were related to increased disorder severity and an overall worse long-term prognosis (Jobe et al., 2005; Kanahara et al., 2013). Approximately 75% of patients with schizophrenia reported experiencing a prodromal period prior to the development of active-phase symptoms and diagnosis that can be defined by a "heterogeneous group of behaviors temporally related to the onset of psychosis" (George et al., 2017; Keith et al., 1991). This time is marked by deterioration in the level of general functioning, and individuals may show signs of social withdrawal, poor performance at school or work, lack of personal hygiene, and other behaviors deemed unusual by friends and family, and may last for weeks, months, or even years (Larson et al., 2010; NCCMH, 2014; Schultz et al., 2007). Furthermore, these signs can also exhibit as cognitive deficits, such as memory, attention, and concentration problems, or mood changes, such as anxiety, depression, sleep disturbances, and irritability (George et al., 2017).

Active phase symptoms typically include a combination of positive and negative symptoms (Patel et al., 2014). Positive symptoms are easily identifiable as behaviors that are not present in healthy individuals, notably hallucinations and delusions (NCCMH, 2014). Hallucinations include seeing, hearing, or feeling sensations that are not present, while delusions include bizarre and unusual ideas that conflict with reality (Patel et al., 2014). For instance, patients frequently report having audible thoughts (having one's own thoughts being spoken aloud) or hearing voices carrying out a conversation or commenting on one's behavior (Larson et al., 2010). In addition, they may have normal perception followed by a delusion interpretation of events; thought insertion, withdrawal, and broadcasting; or somatic passivity, the feeling that one's emotions, impulses, or motor activity being controlled by an external force (Larson et al., 2010).

In direct contrast, negative symptoms are significantly more difficult to define and document, and can be considered major contributors to the low levels of functioning and debilitation seen in schizophrenia patients (Tandon et al., 2002). Broadly speaking, these symptoms can be categorized as *affective*, *communicative*, *conational*, and *relational* (Tandon et al., 2002). Flat affect or diminished emotional expressiveness fall under the affective category, and relate to a decreased range and intensity of expressed emotions (Holder et al., 2014; Patel et al., 2014). Communicative symptoms include reduced speech quantity (poverty of speech) and information (poverty of content), and typically manifest as a series of brief and unelaborated statements (Schultz et al., 2007; Tandon et al., 2002). Patients also tend to have conational symptoms of avolition, defined by a diminished or lack of goal-directed activities that potentially affect personal hygiene, physical activity, and the ability to follow a set schedule or routine (Holder et al., 2014; Patel et al., 2014; Tandon et al., 2002). And finally, relational symptoms refer to a decreased appeal in social activities and relationships, including friendships, family contacts, and sexual interest (Tandon et al., 2002).

It has been observed that the positive symptoms of schizophrenia tend to be relapsing and remitting, in that symptoms worsen and improve at varying times (Owen et al., 2016). However, negative symptoms appear to be continual and negatively contribute to the overall level of functioning (Owen et al., 2016). Over time, the combination of positive and negative symptoms leads to marked changes in personality, social isolation, occupational disability, cognitive impairments, and overall poor health (Millier et al., 2014).

Schizophrenia, like most psychiatric disorders, is said to be a syndromic concept and does not have a simple diagnostic test or biomarker to make a diagnosis (Owen et al., 2016). To obtain a diagnosis of schizophrenia, certain criteria need to be met. The Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DSM-V; APA, 2013) outlines the basis for a clinical diagnosis of schizophrenia. These include having two or more active phase symptoms (delusions, hallucinations, disorganized speech, disorganized behavior, and other negative symptoms) for a significant portion of time during a one-month period, with at least one symptom being delusions, hallucinations, or disorganized speech (APA, 2013). Furthermore, continuous signs of the disturbance must be present for a period of at least 6 months, including the one month of active phase symptoms, and result in a markedly reduced level of functioning with respect to work, interpersonal relationships, or self-care (APA, 2013). For a diagnosis of schizophrenia to be made, the symptoms mentioned must not have been attributed to drug use or a general medical condition (APA, 2013).

An important consideration is that patients frequently suffer from a wide range of other comorbidities. Alcohol, drug, and nicotine abuse and dependence are well documented in the context of schizophrenia and contribute to a longer duration of illness episodes, more frequent hospitalizations, and poorer overall recovery (Larson et al., 2010; Millier et al., 2014). Anxiety, depression, panic disorder, and obsessive-compulsive disorder are also commonly found in these individuals, and their associated symptoms exacerbate the symptoms already directly related to schizophrenia (Patel et al., 2014).

1.1.3 Components of a Complex Disease

1.1.3.1 Schizophrenia Hypotheses

Several hypotheses regarding the etiology of schizophrenia have been developed throughout the years. For instance, the *dopaminergic hypothesis* was initially founded as the first antipsychotic, chlorpromazine, blocked dopamine receptors in the mouse model (Carlsson et al., 1963; Stępnicki et al., 2018). Further studies reported a correlation between the affinity of antipsychotics for dopamine receptors and reduced positive symptoms in humans (Seeman et al., 1975). While these studies argued that excess dopaminergic transmission was the cause of schizophrenia, recent studies have shown that clozapine, an antipsychotic with low affinity for dopamine D_2 receptors,

also exhibited excellent clinical responses in some psychiatric patients (Meltzer et al., 1989). Ultimately, this led to the revised *dopaminergic hypothesis* of schizophrenia which suggested that positive symptoms are the result of striatal D_2 receptor hyper-stimulation whereas negative symptoms are the result of prefrontal cortex D_1 receptor hypo-stimulation (Davis et al., 1991; Lau et al., 2013). All antipsychotics currently used in the treatment of schizophrenia use the dopamine D_2 receptor as a drug target: first and second-generation antipsychotics are receptor antagonists while third-generation antipsychotics are partial agonists or biased ligands of the receptor (Stępnicki et al., 2018).

Adding to our knowledge and understanding of schizophrenia is a closely related topic of the *glutamatergic hypothesis*. Glutamate is considered the primary excitatory neurotransmitter in the brain, and disturbances in glutamate-mediated neurotransmission have been implicated in the etiology of schizophrenia (Moghaddam et al., 2012). Previous studies have showed that antagonists of the *N*-methyl-d-aspartate subtype of the glutamate receptor (NMDAR), such as phencyclidine (PCP) and ketamine, induce schizophrenia-like symptoms (Coyle, 1996; Hu et al., 2015; Javitt, 1987). Furthermore, administration of ketamine to schizophrenia patients who were not undergoing antipsychotic treatment resulted in an exacerbation of symptoms, most notably in relation to auditory hallucinations and paranoia (Malhotra et al., 1997). It is important to note that the *glutamatergic hypothesis* does not necessitate the negation of the *dopaminergic hypothesis*, rather they may play a role together. Interestingly, there are no current glutamatergic drugs to treat schizophrenia, and clinical trials of such drugs have yet to show a conclusive effect on ameliorating symptoms (Howes et al., 2015).

Serotonin, 5-hydroxytryptamine (5-HT), is a neurotransmitter that works to modulate neural activity; dysregulation of the serotonergic system has been implicated in many psychiatric and neurological disorders including schizophrenia, thus giving rise to the *serotoninergic hypothesis* of schizophrenia (Berger et al., 2009). It has been said that chronic stress-induced serotonergic activity in the cerebral cortex causes disruption of glutamate signaling, eventually leading to synaptic atrophy and grey matter loss (Aghajanian et al., 2000; Eggers, 2013). Subsequent dopamine input to the atrophied cerebral cortex result in positive symptoms, whereas hibernation in the frontal lobe results in the development of negative symptoms and cognitive impairment (Eggers, 2013). The current antipsychotic drugs, risperidone and clozapine, act as dopamine-serotonin receptor ligands to alleviate symptoms (Stępnicki et al., 2018).

1.1.3.2 Genetic and Environmental Risk Factors

Schizophrenia has many plausible risk factors, in terms of both genetic and environmental disruptions of brain development (Owen et al., 2016). The heritability of schizophrenia was estimated to be as high as 79-81%, though the specific value varies depending on the study (Hilker et al., 2018; Sullivan et al., 2003). While the lifetime risk of developing schizophrenia is estimated to be only 1%, that risk significantly increases if family or relatives have schizophrenia (Kahn et al., 2015). A series of European family studies determined the lifetime morbid risks of developing schizophrenia among relatives, depending on their biological relatedness: monozygotic twins (48%), dizygotic twins (17%), children (13%), and children of both parents with schizophrenia (46%) (McDonald et al., 2003).

A wide variety of techniques have been previously employed in molecular genetic research investigating schizophrenia, and include linkage analysis and candidate gene analysis (Henriksen et al., 2017). While several candidate genes have been identified, such as *DISC1, DTNBP1, NRG1,* and *COMT*, there has been much difficulty in replicating positive findings and understanding the pathogenetic involvement of the genes in the context of schizophrenia (Henriksen et al., 2017; Picchioni et al., 2007).

More recent studies have utilized powerful genome-wide association studies (GWAS) in a hypothesis-free approach to identify genetic variation and single-nucleotide polymorphisms (SNPs) associated with schizophrenia (Foley et al., 2017). Using this method, the Schizophrenia Working Group of the Psychiatric Genomics Consortium identified 128 SNPs associated with schizophrenia at genome-wide significance levels in a sample of 36,989 cases and 113,075 controls; several of these SNPs were related to the dopamine receptor D_2 and glutamatergic neurotransmission (2014). Interestingly, studies have also found that the most significant associations with schizophrenia lie in the major histocompatibility complex (MHC) locus, which is associated with immune functioning (Henriksen et al., 2017). Genetic variants in MHC, also known as the human leukocyte antigen (HLA), have been implicated in many complex diseases to date (Matzaraki et al., 2017). While these findings may suggest an etiological link with infectious disease or an autoimmune condition, it may be too early to draw definite conclusions since it remains unclear how genetic variability within the MHC locus lead to schizophrenia (Mokhtari et al., 2016).

Combined, GWAS studies have thus far identified approximately 150 common risk loci, yet these have only been able to explain less than 5% of the disease variance (Foley et al., 2017). While we have estimates relating to the heritability of schizophrenia, the vast majority of that heritability has yet to be adequately explained.

In addition to genetic susceptibilities as mentioned above, early and late environmental factors have also been thought to play a significant role in the development of schizophrenia. Early environmental factors involve those affecting neurodevelopment during pregnancy, and are typically related to maternal obstetric complications such as nutritional deficiencies and intrauterine growth retardation (Owen et al., 2016). Maternal infections and stress occurring during the second trimester of pregnancy were found to double the risk of offspring developing schizophrenia in adulthood (Patel et al., 2014). Interestingly, individuals born in late winter or early spring were more likely to develop schizophrenia, suggesting a possible link with intrauterine viral exposure (Picchioni et al., 2007). Systematic reviews have also suggested that patients with schizophrenia experienced perinatal hypoxia, were delivered prematurely, or had low birth weights (Picchioni et al., 2007).

In contrast, late environmental factors occur during childhood through late adolescence. Studies have suggested that schizophrenia is more common in individuals residing in cities, with increased risk for larger cities and longer duration of residence (Picchioni et al., 2007). Furthermore, coming from a minority background or immigrating to another country have also been shown to greatly increase the risk for schizophrenia, though likely due to the underlying social adversity, racial discrimination, family dysfunction, unemployment, and poor housing conditions (Selten et al., 2007). Childhood trauma or maltreatment, severe head trauma and epilepsy are also associated with an increased risk of schizophrenia (Owen et al., 2016). Apart from outright childhood abuse and neglect, the specific way parents raise children does not generally impact vulnerability for development of schizophrenia; however, individuals once diagnosed with schizophrenia who have supportive parents do far better during the course of their illness than those with critical or unaccepting parents (Picchioni et al., 2007).

An important point worth mentioning separately is the impact of illicit drug use. Stimulants such as cocaine and amphetamines have long been known to induce symptoms almost identical to paranoid schizophrenia (Picchioni et al., 2007). Furthermore, there is overwhelming evidence

suggesting that cannabis use is associated with schizophrenia, with heavy cannabis users being over-represented among new cases of schizophrenia (Hall et al., 2008; Owen et al., 2016). For instance, one study found that individuals who used cannabis by age 15 were four times more likely to develop a schizophreniform disorder by age 26, compared to non-user controls (Arseneault et al., 2002). Another prospective study found that the use of cannabis doubles the risk for schizophrenia, and concluded that cannabis is "a component cause in the development and prognosis of psychosis" (Henquet et al., 2005).

1.1.4 Treatment

The overarching goal of treating schizophrenia in the long-term involves addressing symptoms, preventing relapse, and ultimately helping to improve patient's level of functioning for eventual re-integration into their community (Patel et al., 2014). In reality, however, the first and most important milestone to achieve is simply remission, which can be defined as a period of six months with little to no symptoms that do not negatively affect or interfere with a patient's behavior (Holder et al., 2014).

Following an acute psychotic episode, pharmacological drug therapy is recommended to be administered immediately; the first line treatment of choice is an oral administration of second-generation antipsychotic (SGAs), formerly known as atypical antipsychotics, such as risperidone, olanzapine, quetiapine, or aripiprazole (Lally et al., 2015; Patel et al., 2014; Schultz et al., 2007). For the first seven days following the initial psychotic episode, the goal is to decrease the patient's hostility and return them to a normal level of functioning in terms of sleeping and eating (Patel et al., 2014). Afterwards, the focus shifts to maintenance therapy which aims to bring the patient into remission, improve mood and self-care, and increase socialization (Patel et al., 2014).

The most common obstacle involved in treatment is medication non-adherence. Rates of non-adherence are especially high in schizophrenia, and are likely attributed to the severe side effects of the prescribed antipsychotic regiment (Lally et al., 2015). First-generation antipsychotics (FGAs), such as chlorpromazine, fluphenazine, and haloperidol, were initially developed in the 1950s to treat schizophrenia and other forms of psychosis; however they were associated with severe sedation, sleep disorders, sexual dysfunction, and extrapyramidal side effects (EPSEs) (Hartling et al., 2012; Wubeshet et al., 2019). SGAs were later introduced in the 1990s- while

they were also associated with cardiometabolic abnormalities such as weight gain and dyslipidemia, they were preferred due to their lower tendency of causing EPSEs, including acute dystonia, akathisia, parkinsonism, and tardive dyskinesia (Lally et al., 2015). In addition to side effects, lack of insight and persistence of residual symptoms despite treatment also contribute to medication non-adherence (Lally et al., 2015).

Interestingly, up to 75% of patients were found to be non-adherent within the first two years following initial discharge from their psychiatric hospitalization (Leucht et al., 2006). Furthermore, other studies have suggested that the discontinuation rate for oral antipsychotics ranges from 26% to 44%, and that up to two-thirds of patients can be considered partially non-adherent at any given time (Kaplan et al., 2013). An important and effective method to counter non-adherence is the use of long-acting injectable (LAI) antipsychotics (Lally et al., 2015). Instead of taking a regiment of oral medications a few times per day that could be easily be skipped or mistakenly forgotten, patients can receive LAI antipsychotics up to once every 4 weeks (Colbert-Kaip et al., 2019). Therefore, such antipsychotics have been found to greatly prevent and reduce the number of hospitalizations and psychiatric relapse (Kishimoto et al., 2013; Patel et al., 2014).

In addition to and beyond the concept of medication non-adherence, between 10% and 30% of patients do not respond to antipsychotic treatment (Patel et al., 2014). A diagnosis of treatment-resistant schizophrenia (TRS) results from the failure to respond, or nonresponse, to two or more different antipsychotic medications at therapeutic doses, each taken for 6 to 8 weeks (Lally et al., 2015). The only medication currently known to be effective in the treatment of TRS is clozapine, though it has been found that only 60-70% of patients showed a positive response (Khan et al., 2017; Lally et al., 2016). Unfortunately, there are no other pharmacotherapies for the remaining 30-40% of TRS patients who do not respond to clozapine or chose to discontinue use due to side effects, and as such, there is much ongoing research in the field of personalized medicine to treat these patients (Lally et al., 2016).

This also brings us to the topic of suicide in schizophrenia, as clozapine significantly reduces suicidality. Suicide is highly prevalent in schizophrenia, with studies indicating that 5-13% of patients die by suicide (Hor et al., 2010; Pompili et al., 2007). In the following sections of this introductory chapter, we will discuss suicide and specifically, suicide in schizophrenia.

1.2 Suicide

1.2.1 Overview

Suicide is considered a major public health problem that accounts for over 788,000 lives lost per year worldwide (WHO, 2017). The annual global age-standardized rate for suicide was calculated to be 11.4 per 100,000 people, though specifically in Canada, that rate was slightly elevated at 12.0 per 100,000 people (StatCan, 2017; WHO, 2014). Furthermore, for every death by suicide, there were approximately 25 to 30 suicide attempts, and a minimum of 7 to 10 people profoundly affected by the suicide loss (Health Canada, 2016). Worldwide, suicide and suicide-related behaviors are ranked as the sixth and ninth leading cause of disease burden in terms of years lost to disability, ill-health, and early death, in men and women, respectively, between the ages of 15 and 44 (Klonsky et al., 2016).

It is important to clarify that the term *suicidal behavior* encompasses completed suicide, suicide attempt, and suicidal ideation (Castle et al., 2007). As defined by the Center for Disease Control and Prevention (CDC), completed suicide is "death caused by self-directed injurious behavior with an intent to die as a result of the behavior," whereas a suicide attempt is a "nonfatal, self-directed, potentially injurious behavior with an intent to die [even] if the behavior does not result in injury" (Klonsky et al., 2016). Suicidal ideation is defined as "thoughts of harming or killing oneself, [regardless] if one would [or would not] carry them out" (Arria et al., 2009; Thompson et al., 2012). The topic of suicidal ideation will be further discussed in detail in the following subsection.

A worldwide study indicated that while some variability existed in the prevalence of suicide attempt, the risk factors were fairly consistent between the different countries. From an epidemiological perspective, these risk factors included the female gender, young age, fewer years of education, and being single or unmarried (Nock et al., 2008). An interesting concept recognized for many years is the gender paradox of suicidal behavior, which states that while females have a greater rates of suicide attempt than males, males have a higher mortality rate from their suicide attempts due to more lethal means (Canetto et al., 1998). The gender paradox is statistically sound, as the World Health Organization (WHO) found that men had three times the number of completed suicides than that of women (2014).

In addition to depression, hopelessness, and impulsivity, one of the most prominent risk factors for suicide is the presence of a psychiatric disorder. In fact, psychological autopsy studies have found that over 90% of completed suicides involved individuals suffering from mental disorders at the time of their death (Bertolote et al., 2002; Brådvik, 2018). However, from an opposite perspective, the majority of individuals with psychiatric disorders do not commit suicide, with an estimated risk of only 5-8% (Brådvik, 2018). Nonetheless, considering psychiatric disorders in the context of suicide is an important aspect to keep in mind (Klonsky et al., 2016).

1.2.1.1 Theories of Suicide

Certain motivations for suicide have been suggested, and include to escape from one's environment or from an unbearable state of mind, or to even alter one's environment to communicate a point to others (Klonsky et al., 2016). For instance, among patients who attempted suicide, the primary motivations they had, looking back on their attempts, centered on autonomic intra-personal factors such as escaping from their own state of mind, and social interpersonal factors such as influencing others (Bancroft et al., 1976; Hayashi et al., 2017). Throughout the many years of suicide research, multiple theories have evolved in an attempt to explain the motivations for suicide as well as why certain individuals attempt suicide while others do not (Klonsky et al., 2016).

For instance, according to the escape theory, suicide is a means of escape from the self and surrounding world, typically beginning with events that fall short of personal standards and expectations; becoming overwhelmed with one's inadequacies eventually lead to a state where drastic measures, such as suicide, seem acceptable (Baumeister, 1990). Suicide has also been said to occur when the *psychache*, "hurt, anguish, soreness, aching, psychological pain in the [mind]," becomes too great to be bearable, though this threshold can vary between individuals explaining why only some people attempt suicide (Shneidman, 1993). The interpersonal theory proposed that the combination of thwarted belonginess, or alienation from others, and perceived burdensomeness, or the feeling of being a bothersome burden, was the most dangerous trigger of suicidal behaviors (Joiner, 2005; van Orden et al., 2010). Beyond the three most common theories, there are a multitude of other theories relating suicide to factors such as hopelessness, problem-solving, impulsivity, and interpersonal communication (Klonsky et al., 2016).

1.2.1.2 Genetics of Suicide

Family, twin, and adoption studies have concluded that suicide is highly familial, heritable, and clustered within families (Brent et al., 2005; Zai et al., 2012). The heritability of attempted and completed suicide was estimated to be approximately 43% (McGuffin et al., 2010), though other studies have proposed the heritability of, specifically, serious suicide attempts to be 55%, and suicidal ideation to be 43% (Statham et al., 1998). Each of these studies also noted that having a psychiatric disorder or experiencing some form of childhood abuse was associated with an increased risk of suicide (Zai et al., 2012). However, it was found that a family history of suicidal behavior was still associated with suicidal behavior in the proband, despite adjusting for psychiatric disorders; this suggested that the transmission of suicide is distinct and separate from that of psychiatric disorders (Brent et al., 2005).

Candidate-gene association studies (CGAS) and genome-wide association studies (GWAS) have been the most commonly utilized methods in the investigation of the genetics of suicide (Zai et al., 2012). Since it is largely recognized that single genes may not account for the full risk of developing suicide, studies in the past decade have thoroughly investigated the serotonergic and dopaminergic systems, as well as the adrenergic receptor and brain-derived neurotrophic factors (Mirkovic et al., 2016; Zai et al., 2012). While findings from these studies have been inconsistent and difficult to replicate, a recent meta-analysis found that the genetic polymorphisms most strongly associated with suicide included variants in TPH1- rs1800532, SLC6A4-5-HTTLPR, COMT-rs4680, and BDNF-rs6265 (Mirkovic et al., 2016; Zai et al., 2012). These results could be due to the incorporation of the heritability of psychiatric disorders, rather than relating to suicide alone (Kimbrel et al., 2018; Mirkovic et al., 2016; Mullins et al., 2019). Even so, these are important pieces of evidence to consider, and leads us to a brief discussion of the stress-diathesis model of suicide.

The stress-diathesis model posits that suicide is the result of a combination of environmental stressors and a susceptibility to suicidal behavior, independent of any psychiatric disorders (van Heeringen et al., 2014). Stress, albeit in the form of childhood trauma, death of a family member, or other significant events, has long been recognized as a key contributor to psychopathologies, though these stressors do not necessarily trigger suicide in all individuals (Ingram et al., 2005). Studies have acknowledged that the development of suicidal behavior involves the concept of

diathesis, an idea initially derived from the ancient Greek idea of disposition or state of health (Zuckerman, 1999). Diathesis can be seen as an underlying and distal risk factor, which predisposes individuals to suicide when stress, a proximal risk factor, is encountered later in life (Ingram et al., 2005; van Heeringen, 2012).

Given the inconsistent findings from genetic studies, it was then recently proposed that epigenetics may play a role in the diathesis and quantify the "missing heritability" of suicide (Bani-Fatemi et al., 2018). Epigenetic mechanisms are mitotically, and potentially meiotically, heritable, non-coding changes that influence gene expression, and most importantly, are sensitive to environmental factors such as stress (Gibney et al., 2010). As such, this opens the door to further studies investigating the complex interplay between stress, epigenetics, and suicide, and will be discussed in the following chapters of this thesis.

1.2.2 Suicidal Ideation

Suicidal ideation, as previously mentioned, can be broadly defined as thinking about, considering, or planning suicide (Klonsky et al., 2016). The overall lifetime prevalence of suicidal ideation was estimated to be 9.2% in the general population, and is considered especially important because it often serves as an immediate precursor to suicide attempt (Nock et al., 2008; O'Connor et al., 2013). It was found that up to 90% of individuals who completed and died from suicide saw their primary care physician within a period of a few weeks or months prior, of which 88% of these patients endorsed suicidal ideation during their last appointment (Weber et al., 2017). Stigma and the belief that they were capable of controlling their feelings were likely major barriers in these patients seeking the proper help they required; typically, over 40% of patients report being hesitant and uncomfortable discussing their suicidal thoughts due to these reasons (Weber et al., 2017).

The intensity of suicidal ideation can greatly vary between individuals, and range from fleeting thoughts of death to an "intense delusional preoccupation with self-destruction" (Goldney et al, 1989). Individuals on the mild spectrum with fleeting thoughts of death often report having a wish to die during their sleep, develop a terminal cancer, or be killed in an accident; these are characterized as passive suicidal ideation which does not directly involve the individual killing themselves (Simon, 2012). This is in contrast from individuals with active suicidal ideation who

wish and have plans to complete acts that harm themselves. However, it is important to note that passive suicidal ideation should never be considered as a negligible risk, since active suicidal ideation is inevitably present to some extent (Simon, 2012). This was evidenced in a study of 100 individuals who committed suicide in which 69% of subjects either denied suicidal ideation or reported only fleeting thoughts of death prior to their attempt (Hall et al., 1999). Therefore, suicidal ideation, at any level, should be taken seriously. Given the importance, it would be very much beneficial to openly discuss suicidal thoughts with patients and provide those individuals experiencing suicidal ideation with early intervention in the hopes of preventing future suicide (O'Connor et al., 2013).

Recent meta-analyses have reported that prior suicidal ideation, hopelessness, depression, abuse, and psychosis were significant predictors of current suicidal ideation (Franklin et al., 2017; Huang et al., 2018). Interestingly, there appears to be great similarity between the risks for suicide and suicidal ideation, though the majority of individuals who experience suicidal ideation do not eventually go on to make attempts (Klonsky et al., 2016). This suggests that suicidal ideation is a necessary factor, but is not sufficient, in predicting suicide attempts (ten Have et al., 2009). This discussion will continue in the following section. However, we will first introduce the concept of emergent suicidal ideation, which can be simply defined as the onset or worsening of suicidal thoughts.

1.2.2.1 Emergent Suicidal Ideation

While extensive research has already heavily focused on identifying those at risk for suicide and suicidal ideation, another, if not more important, aspect is the timing and determination of *when* certain individuals are at higher risk for suicide (Simon et al., 2016). Hence it is worth investigating the temporal aspect of suicidal ideation known as emergent suicidal ideation, as mentioned above. Interestingly, studies found similar predictors between first-onset suicidal ideation and suicide attempt, and these included recent negative life events and personal vulnerability indicators such as the neuroticism personality trait and childhood trauma (ten Have et al., 2009). With this in consideration, a proposition that involves the identification of individuals specifically with emergent suicidal ideation would likely be more robust in preventing suicide, rather than using suicidal ideation status alone.

Furthermore, the body of evidence surrounding the consequences of emergent suicidal ideation on suicide has been ever growing. A recent study reported visit-to-visit changes in suicidal ideation levels and found that newly emergent suicidal ideation, changing from no ideation to ideation "nearly every day," was associated with a sevenfold increased risk of suicide attempt (Simon et al., 2016). To make matters more urgent, over 60% of the transitions from suicidal ideation to suicide attempt occurred within the one-year period immediately following experiencing onset of suicidal ideation; the risk of suicide attempts then becomes greatly reduced after this one-year period has passed (Nock et al., 2008). Therefore, there is a real need to thoroughly investigate the issue of emergent suicidal ideation, and specifically aim to identify individuals at onset of ideation in an attempt to prevent suicide.

1.3 Suicide in Schizophrenia

Patients with schizophrenia have a greatly reduced life expectancy, and up to 40% of this premature mortality can be attributed to suicide and other similar unnatural deaths (Hor et al., 2010; Ventriglio et al., 2016). Current estimates have reported that about 4-5% of patients with schizophrenia die by means of suicide, and the lifetime prevalence was estimated to be ten times higher than that found in the general population (Carlborg et al., 2010; Hor et al., 2010). In fact, suicide was even historically described as "the most serious of schizophrenic symptoms" (Bleuler, 1950; Carlborg et al., 2010).

Not surprisingly, many of the risk factors for suicidal behavior in schizophrenia are similar to that of the general population, such as mood disorder, recent loss, and previous suicidal ideation or attempt (Hor et al., 2010). However, there are several differences that make it especially difficult for this vulnerable population. First and foremost, patients with schizophrenia may often fail to reach out for help when experiencing suicidal ideation and other difficulties, due to the stigma and prejudice surrounding their psychiatric disorder (Hettige et al., 2018). Especially in the psychiatric setting, patients will also deny suicidal thoughts to avoid unwanted intervention such as involuntary psychiatric holds, or to even facilitate early release from these very circumstances (Nock et al., 2008).

Additionally, factors directly relating to the symptoms of schizophrenia, itself, can complicate the process of the patient informing the clinician, and for the clinician to identify at risk patients and implement interventional measures (Hor et al., 2010). Depression is well-known to be associated with suicide across all populations (Pompli et al., 2007), though in schizophrenia, it can be easily confused with negative symptoms or side effects associated with prescribed antipsychotic medications (Carlborg et al., 2010). A similar argument can be made about the effects of psychosis (Hor et al., 2010).

To briefly summarize, multiple studies have come to a consensus of certain risks of suicide in schizophrenia, and include male gender, young age, active hallucinations or delusions, concurrent depression and hopelessness, awareness of illness, high intelligence quotient, and substance abuse (Carlborg et al., 2010; Pompili et al., 2007; Young et al., 1998). Additional risks include fear of mental disintegration, agitation, restlessness, and poor adherence to antipsychotic regiment (Hor et al., 2010). These risks can potentially be explained by the fact that schizophrenia patients are experiencing mental deterioration and have lost hope in one's life aspirations, or have lost faith in their treatment (Pompili et al., 2007). Given the high rates of suicide in patients with schizophrenia and the great difficulty in identifying those most at risk, different approaches need to be taken. As such, in this thesis, we will focus on the associations of stressful life events and epigenetic markers on suicidal ideation.

1.3.1.1 Childhood Trauma and Recent Stress

Two other major risk factors for suicide in schizophrenia patients is childhood trauma and recent stress (Hor et al., 2010), and will be the focus of our first study. Very briefly, childhood trauma encompasses both abuse and maltreatment, and essentially can be separated into the domains of emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. These aspects were found to be profoundly associated with suicide in schizophrenia patients, and has been described by numerous studies (Bahk et al, 2017; Enns et al., 2006; Mohammadzadeh et al, 2019; O'Connor et al., 2018; Roy et al., 2005). Furthermore, specifically childhood emotional abuse was also suggested to play a role in the progression of suicidal ideation to attempt in schizophrenia (Araújo et al., 2016).

In addition, several studies have also focused on the effect of recent stress on suicidal behavior, and found that generally speaking, a greater number and specific types of stressors, were very predictive of suicide (Blasco-Fontecilla et al., 2012; Foster, 2011; Funahashi et al., 2000; Hawton et al., 2005). It was found that recent life events, such as no longer living with a partner or becoming unemployed, and recent health-related problems, such as asthma, digestive disorders, chronic pain, and cardiovascular disease, were associated with emergent suicidal ideation (ten Have et al., 2009). Furthermore, our group also previously found that recent health-related stressors were predictive of emergent suicidal ideation in schizophrenia, following a three-month longitudinal study design (Tasmim et al., 2019).

1.3.1.2 Epigenetic Markers

The National Alliance for Suicide Prevention proposed the identification of peripheral biomarkers for screening and risk assessment of current and future suicide (NIMH, 2014). We adopted their approach, but also considering the fact that suicide is especially common in schizophrenia, the stress-diathesis model of suicide, and the importance of suicidal ideation. Thus, we attempted to identify epigenetic markers to predict for current and emergent suicidal ideation, specifically in the context of patients with schizophrenia.

Epigenetics is a broad field of study regarding heritable non-coding changes that affect gene activity and expression, and includes DNA methylation, post-translational modification of histone proteins and chromatin remodeling, and even RNA-based mechanisms (Gibney et al., 2010). Of these epigenetic mechanisms, DNA methylation remains the most widely studied and has been well investigated both in the context of psychiatry and other medical fields, notably cancer (Kulis et al., 2010; Wang et al., 2019); this will be the primary focus of the second and third studies presented in this thesis.

1.4 Study Objectives and Hypothesis

Suicide is a serious public health issue especially in the context of schizophrenia, though it has been shown to be immensely difficult to identify patients at risk for suicide. Suicidal ideation is considered an immediate precursor of suicide, and serves as an early warning sign for clinicians. Furthermore, recent evidence has suggested that it is imperative to examine the temporal aspect of suicidal ideation and identify at-risk individuals when they first begin experiencing suicidal thoughts. As such, our studies will focus on the current and emergent suicidal ideation phenotypes. Following this introductory background, the work compiled within this thesis was broken into three studies, each with a unique rationale, aim, and hypothesis.

CHAPTER 2

Rationale: Suicidal ideation is a known precursor of suicide, which greatly and adversely affect patients with schizophrenia. Furthermore, emergent suicidal ideation is of great concern, due to the urgency of increased suicide risk during the first year following initial onset of suicidal thoughts. Studies have previously reported that both childhood trauma and recent stressful life events are associated with suicidal behaviors, both in the general population and in patients with schizophrenia. However, it remains unclear which has a predominant effect on current and emergent suicidal ideation in schizophrenia. Elucidation of the impacts of these factors may very well aid in suicide risk assessment and help to develop effective suicide intervention strategies in this vulnerable population.

Specific Aims:

1) We aim to determine whether childhood trauma and recent stressful life events are predictive of current suicidal ideation in a cross-sectional study of patients with schizophrenia.

2) We aim to determine whether childhood trauma and changes in recent stressful life events are predictive of emergent suicidal ideation in a one-year longitudinal study of the same subjects.

Hypothesis: We hypothesize that, both, greater levels of childhood trauma and more recent stressful life events would be predictive of current suicidal ideation; also, greater levels of childhood trauma and increases in the change in recent stressful life events would be predictive of emergent suicidal ideation. We conducted a series of binary logistic regression analysis to test for these effects.

CHAPTER 3

Rationale: Family, twin, and adoption studies have long supported the notion that suicide is highly heritable. However, candidate-gene and genome-wide association studies have been unable to consistently pinpoint the observed genetic risk. Compelling evidence has suggested that epigenetic mechanisms, particularly DNA methylation, can play a role in suicide. This could explain the diathesis, or genetic vulnerability or predisposition, and in turn help quantify the "missing heritability" of suicide. Furthermore, methylation could potentially serve as biomarkers for the identification of at-risk individuals with schizophrenia.

Specific Aims:

1) We aim to identify differentially methylated positions (DMPs) throughout the genome that are associated with current suicidal ideation in patients with schizophrenia.

2) We aim to identify differentially methylated regions (DMRs) within the genome that are associated with current suicidal ideation in patients with schizophrenia.

Hypothesis: By utilizing a hypothesis-free approach, we will search for DMPs and DMRs in the white blood cell DNA profile that are associated with current suicidal ideation. To conduct this analysis, we will collect venous blood from schizophrenia patients, quantify DNA methylation, and utilize a series of advanced bioinformatic tools to identify differential methylation across the entire genome.

CHAPTER 4

Rationale: Considering the rapid transition from onset of suicidal thoughts to suicide attempt, there is an urgent need to identify patients with emergent suicidal ideation. However, patients with schizophrenia have a wide range of symptoms and side effects from their prescribed antipsychotic regimen that may hinder a clinician's ability to identify those most at risk for transitioning to suicide attempt. Considering the intra-individual stability of DNA methylation over time, studies have investigated changes in methylation in other contexts, such as psychotic experiences. However, this has not yet been investigated in emergent suicidal ideation in schizophrenia.

Specific Aims:

1) We aim to identify longitudinal methylation changes at sites throughout the genome that are associated with emergent suicidal ideation in patients with schizophrenia.

Hypothesis: As a replication analysis and follow-up to the previous study, we hypothesize that emergent suicidal ideation is also associated with changes in DNA methylation at CpG sites that were associated with current suicidal ideation. To test this hypothesis, we will collect blood from patients at two separate visits to assess for genome-wide methylation changes, and determine differences between the emergent and non-emergent suicidal ideation groups.

Chapter 2

2 Stressful Life Events and Suicidal Ideation in a One-Year Follow-Up Study

2.1 Abstract

Introduction: Up to half of patients with schizophrenia experience suicidal ideation during their illness. Transitions from ideation to suicide attempt frequently occur within the first year following onset of ideation. While studies have investigated the effect of recent stress and childhood trauma on suicide and suicidal ideation, few have examined these factors in the context of emergent suicidal ideation. Here, we aim to identify and compare the independent effects of recent stressful life events and childhood trauma on both current and emergent suicidal ideation.

Methods: A cohort of 80 patients with schizophrenia spectrum disorders was assessed at baseline and at one-year follow-up. The study was divided into two arms to test for the effect of 1) recent stress and childhood trauma on current suicidal ideation (cross-sectional) and 2) the change in recent stress and childhood trauma on emergent suicidal ideation (longitudinal). Demographic and clinical variables, where applicable, were included as covariates. The effect size, presented as an odds ratio and 95% confidence interval, was determined by logistic regression analyses.

Results: We found that 13% of subjects reported current suicidal ideation, whereas 16% reported experiencing emergent suicidal ideation at the follow-up visit. After correcting for covariates, recent stress and childhood trauma were not found to be significant predictors of current suicidal ideation. However, increases in total stress (OR = 1.093 [1.025 – 1.165], p = 0.006) and specifically health-related stress (OR = 1.300 [1.052 – 1.605], p = 0.009) at the follow-up visit were predictive of emergent suicidal ideation.

Discussion/ Conclusion: In the cross-sectional arm, the effect of recent stress and childhood trauma were diluted after considering for the severity of psychosis, depression, hopelessness, and perceived stress. In the longitudinal arm, however, increases in total stress and health-related stress were associated with emergent suicidal ideation, even in the context of age-of-onset and psychosis severity as covariates. Further studies involving larger sample sizes that investigate the interplay between several risk factors are needed.

2.2 Introduction

Suicidal ideation can be broadly defined as contemplating or planning suicide (Klonsky et al., 2016), though the intensity of suicidal ideation can be greatly varied between individuals, ranging from mere fleeting thoughts of death to an "intense delusional preoccupation with self-destruction" (Goldney et al., 1989). While the lifetime prevalence of suicidal ideation was estimated to be 9.2% in the general population (Nock et al., 2008), up to 50% of patients with schizophrenia reported experiencing suicidal ideation at any given time over the course of their illness (Kasckow et al., 2011). Suicide remains one of the leading causes of premature mortality in schizophrenia (Ventriglio et al., 2016), and the presence of suicidal ideation is considered an important early warning sign for attempted and completed suicides (Hocaoglu et al., 2009). In particular, the onset of newly emergent suicidal ideation, from no ideation to ideation "nearly every day," was found to be associated with a sevenfold increase in risk for later suicide attempt (Simon et al., 2017). Adding a sense of urgency, it also was determined that over 60% of the transitions from suicidal ideation to suicide attempt occur within the one-year period immediately following onset of ideation (Nock et al., 2008). Thus, taken together, it is imperative to identify those patients with schizophrenia who have current and emergent suicidal ideation.

There is compelling evidence that suicide is associated with recent stress. Psychological autopsies have suggested that nearly all suicide attempts in the general population are preceded by at least one adverse life event occurring within the last year (Foster, 2011), especially those pertaining to physiological illness, interpersonal conflicts, problems at work, and financial troubles (Blasco-Fontecilla et al., 2012; Kõlves et al., 2006; Heikkinen et al., 1992). Furthermore, patients with schizophrenia who committed suicide experienced more stressful life events, namely unemployment, severe illness or injury, and serious interpersonal problems in the year prior, compared to schizophrenia patients without past histories of suicide (Funahashi et al., 2000). Recent loss was also found to be a significant risk factor for suicide in schizophrenia (Hawton et al., 2005). While many previous findings, as mentioned above, utilized attempted or completed suicide as the outcome of interest, a recent study published by our group found that increased overall and, specifically, health-related stress at a 3-month follow-up visit were predictive of emergent suicidal ideation in schizophrenia (Tasmim et al., 2019).

Childhood trauma has also been recognized as a risk for suicide (Bahk et al., 2017). Childhood trauma, itself, encompasses several types of maltreatment, including physical, sexual, and emotional abuse, as well as emotional and physical neglect (Bernstein et al., 2003). Exposure to a greater number of maltreatments increases the vulnerability to suicidal ideation and behavior (Enns et al., 2006; O'Connor et al., 2018). In the context of schizophrenia, patients who attempted suicide reported higher levels of maltreatment, suggesting that childhood trauma predisposes one to future suicidal behavior (Roy, 2005). Furthermore, higher levels of, specifically, emotional abuse and physical neglect were found to be significantly associated with suicidal ideation in schizophrenia (Mohammadadeh et al., 2019). Interestingly, emotional abuse has also appeared to have further implications in playing a role in the progression and transition from suicidal ideation to attempt (Araújo et al., 2016).

While recent stressful life events and childhood trauma are the most salient risk factors associated with suicide in schizophrenia, a multitude of other factors have been identified, including male gender, young age, active hallucinations or delusions, concurrent depression and hopelessness, awareness of illness, high intelligence quotient, and substance abuse (Carlborg et al., 2010; Hor et al., 2010; Pompili et al., 2007; Young et al., 1998). Similarly, these additional risk factors could also play an important role during the period prior to suicide, i.e. current suicidal ideation and emergent suicidal ideation, as these are considered immediate precursors to attempted and completed suicides.

As an extension of our group's previous work, here we investigate the independent effects of recent stressful life events and childhood trauma on suicidal ideation. We hypothesize that both of these factors would be important predictors of current and emergent suicidal ideation. A comparison of the findings from the two analyses would allow us to gain further insight into the effects of recent stress and childhood trauma in the context of a cross-sectional and longitudinal suicidal ideation outcome. Furthermore, to consider confounding variables, we extend potential and relevant risk factors of suicide in schizophrenia to these analyses of emergent suicidal ideation. Our findings will potentially promote growth of knowledge and result in the implementation of more effective early suicide prevention and intervention strategies.

2.3 Methods

2.3.1 Participants

The present study comprised of a total of 80 eligible individuals recruited from the Centre for Addiction and Mental Health (CAMH), located in Toronto, Canada. All study participants met the inclusion criteria of being between the ages of 18 and 75 and having a diagnosis of a schizophrenia spectrum disorder confirmed by the Mini-International Neuropsychiatric Interview Plus v.6.0 (M.I.N.I.-Plus; Sheehan et al., 1998) or patient medical records if necessary. Schizophrenia spectrum disorders include schizophrenia, schizophreniform disorder, schizoaffective disorder, delusional disorder, and brief psychotic disorder. Exclusion criteria included having a history of head trauma resulting in a loss of consciousness, or having a diagnosis of an intellectual disability, major neurological disorder, or substance-induced psychosis. All participants volunteered for the study, provided written informed consent for study participation and disclosure of personal health information, and were compensated for their time and travel. The study was approved by the CAMH Research Ethics Board.

2.3.2 Study Design

All participants were evaluated at two time points: an initial assessment (baseline) and again 12 months later (one-year follow-up). This design allowed us to divide the study into two arms: A) a cross-sectional arm evaluating current suicidal ideation, and B) a longitudinal arm evaluating emergent suicidal ideation.

In Arm A, participants were grouped according to their suicidal ideation status at baseline, and categorized as either suicide ideators or non-ideators. The effects of recent stress and childhood trauma on current suicidal ideation were then examined.

In Arm B, participants were grouped on the basis of new or worsening suicidal ideation, and subsequently categorized as those with or without emergent suicidal ideation. In this arm, the effects of change in recent stress seen at follow-up and childhood trauma were investigated in relation to emergent suicidal ideation status.

2.3.3 Assessment Measures

The Columbia- Suicide Severity Rating Scale (C-SSRS) provides a robust standardized measure of suicidal ideation and behavior (Posner et al., 2011). In this study, C-SSRS scores relating to suicidal ideation in the past month, particularly severity and intensity, were utilized at both baseline and one-year follow-up visits. The severity sub-score was assessed according to the following categorical scale:

0: Absence of Suicidal Ideation,
1: Wish to be Dead,
2: Non-Specific Active Suicidal Thoughts,
3: Active Suicidal Ideation with Any Methods (Not Plan), Without Intent to Act,
4: Active Suicidal Ideation with Some Intent to Act, Without Specific Plan,
5: Active Suicidal Ideation with Specific Plan and Intent.

The intensity of suicidal ideation was also assessed for those participants reporting any form of suicidal ideation, i.e. type 1 through 5, above. A series of five questions, each scored from 1 to 5, gauged the frequency, duration, controllability, deterrents, and reasons for ideation. The overall intensity sub-score was subsequently calculated as the sum of the values from the five questions, and ranged from 5 (least intense) to 25 (most intense). In Arm A of the study, any participants with a non-zero severity sub-score were classified as suicide ideators, whereas those with a sub-score of zero were considered non-ideators. In Arm B, any participants with worsening severity at the one-year follow-up were classified as having emergent suicidal ideation. In cases of unchanged severity sub-scores between visits, emergent suicidal ideation status was assigned to those subjects with a higher intensity sub-score. The remaining subjects with lower severity or intensity sub-scores at the one-year follow-up were then considered to be without emergent suicidal ideation.

To quantify recent stress, we utilized a modified version of the Social Readjustment Rating Scale (SRRS; Holmes et al., 1967). The SRRS was administered as a self-report questionnaire at both visits, assessing for exposure to a series of 43 life events, or readjustments, occurring within the past three months. Each event was assigned a numerical weight in terms of life change units (LCU), with more weight indicative of greater stress experienced. The total score, referred to as the Social Readjustment Index (SRI), was calculated as the sum of the LCU weights

corresponding to events that participants reported experiencing (Blasco-Fontecilla et al., 2010). Individual life events, where possible, were also grouped into the domains of interpersonal, legal, health, work, or finance (**Table 2.1**); sub-scores for each domain were also calculated in a similar additive manner. In Arm A of the study, only the baseline SRI and domain sub-scores were considered, whereas, in Arm B, the change in SRI and domain sub-scores across the two time points were utilized for analyses.

Table 2.1 | List of stressors and LCU's separated into five domains. Specific life readjustments and corresponding LCU scores are adapted from the SRRS, and are separated into the domains of interpersonal, legal, health, work, and finance-related stress.

	LCU
Interpersonal	
Death of spouse	100
Divorce	73
Marital separation	65
Death of a close family member	63
Marriage	50
Marital reconciliation	45
Change in health of a family member	44
Gain of a new family member	39
Death of a close friend	37
Change in number of arguments with spouse	35
Son or daughter leaving home	29
Trouble with in-laws	29
Change in number of family get-togethers	15
Legal	
Jail term	63
Minor violation of the law	11
Health	
Personal injury or illness	53
Pregnancy	40
Sex difficulties	39
Change in sleeping habits	16
Change in eating habits	15
Work	
Fired at work	47
Retirement	45
Business readjustment	39
Change to different line of work	36
Change in responsibilities at work	29
Trouble with boss	23
Change in work hours or conditions	20
Finance	
Change in financial state	38
Mortgage over $20,000	31
Foreclosure of mortgage or loan	30
Mortgage or loan less than $20,000	17

Participants reported childhood trauma through the Childhood Trauma Questionnaire (CTQ; Bernstein et al., 1994). The CTQ comprises of 28 statements regarding childhood experiences, specifically probing for emotional, physical, and sexual abuse, as well as emotional and physical neglect. Respondents were asked to rate how well they agree with each statement (**Table 2.2**), with choices ranging from "Never True" to "Very Often True." Numerical scores were assigned for the five types of maltreatment with a total score ranging from 28 to 140, which further accounted for participants' minimization or denial of childhood experiences. Higher scores were indicative of greater severity of traumas experienced. As a retrospective measure, this scale was only completed at the baseline visit, though scores were used in both study arms.

Table 2.2 | List of questions associated with each type of abuse and neglect. These items were assessed with the CTQ to determine the severity of traumas experienced by each participant during childhood.

Emotional Abuse
People in my family called me things like "stupid," "lazy," or "ugly."
I thought that my parents wished I had never been born.
People in my family said hurtful or insulting things to me.
I felt that someone in my family hated me.
I believe that I was emotionally abused.
Physical Abuse
I got hit so hard by someone in my family that I had to go see a doctor or go to the hospital.
People in my family hit me so hard that it left me with bruises or marks.
I was punished with a belt, a board, a cord, or some other hard object.
I believe that I was physically abused.
I got hit or beaten so badly that it was noticed by someone like a teacher, neighbor, or doctor.
Sexual Abuse
Someone tried to touch me in a sexual way or tried to make me touch them.
Someone threatened to hurt me or tell lies about me unless I did something sexual with them.
Someone tried to make me do sexual things or watch sexual things.
Someone molested me.
I believe that I was sexually abused.
Emotional Neglect
There was someone in my family who helped me feel that I was important or special.
I felt loved.
People in my family looked out for each other.
People in my family felt close to each other.
My family was a source of strength and support.
Physical Neglect
I didn't have enough to eat.
I knew that there was someone to take care of me and protect me.
My parents were too drunk or high to take care of the family.
I had to wear dirty clothes.
There was someone to take me to the doctor if I needed it.
Minimization/ Denial
There was nothing I wanted to change about my family.
I had the perfect childhood.
I had the best family in the world.

To conduct a thorough and comprehensive analysis, we examined several demographic and clinical variables of interest. Data regarding age, gender, age-of-onset, and duration of illness were recorded for each participant. Several known risk factors for suicide in schizophrenia were also incorporated in our analysis of suicidal ideation. A series of psychopathological scales was administered at both the baseline visit and one-year follow-up. The Brief Psychiatric Rating Scale (BPRS; Overall et al., 1962) was utilized to assess for the severity of psychosis, the Calgary Depression Scale for Schizophrenia (CDSS; Addington et al., 1990) for concurrent depression, and the Beck Hopelessness Scale (BHS; Beck et al., 1974) for the presence of pessimism and hopelessness. The Perceived Stress Scale (PSS; Cohen et al., 1983) measured participants' appraisal and perception of non-specific stressors. The Schedule for Assessment of Insight (SAI; David et al., 1992) determined participants' degree of insight regarding their illness, and the Mini-Mental State Examination (MMSE; Folstein et al., 1975) measured general cognitive functioning. With regards to substance use, the Fagerström Test for Nicotine Dependence (FTND; Fagerström, 1978), Alcohol Use Disorders Identification Test (AUDIT; Saunders et al., 1993), and Drug Abuse Screening Test (DAST; Skinner, 1982) were each administered to assess for the degree of tobacco, alcohol, and illicit drug use, respectively. For all the scales mentioned above, scores obtained at baseline were utilized for study Arm A, whereas the changes observed at one-year follow-up were used for study Arm B.

2.3.4 Statistical Analysis

In Arm A, unadjusted binary logistic regression analyses were first performed to independently test the effect of 1) recent stress and 2) childhood trauma on current suicidal ideation. The SRI and domain sub-scores were rescaled by dividing by 10 to obtain pertinent interpretations of the effect of recent stressful events. The total CTQ score and sub-scores for each of the five types of maltreatment were included in the analyses as measures of childhood trauma. For any factors with significant effects on current suicidal ideation, we also performed adjusted binary logistic regression analysis that corrected for covariates. Demographic or clinical variables that were included as covariates in adjusted analyses were identified as having group differences between suicide ideators and non-ideators with a p-value of less than 0.1 with the Mann-Whitney U (continuous variables) and Chi-squared tests (categorical variables).

The analytic methods utilized in Arm B were similar, with both unadjusted and adjusted binary logistic regression analyses. However, here we independently tested the effect of 1) change in recent stress and 2) childhood trauma on emergent suicidal ideation. The change in SRI and domain sub-scores were calculated by subtracting baseline scores from those taken at the one-year follow-up, and re-scaled by a factor of ten, as mentioned above for Arm A. The total CTQ and sub-scores were used to quantify childhood trauma. For adjusted analyses, where applicable, changes in demographic and clinical variables with a group difference p-value less than 0.1 were included as covariates.

The effect size was described as an odds ratio (OR) with 95% confidence intervals (CI). P-values less than 0.05 were considered statistically significant for the SRI and CTQ scores. However, for specific tests, such as the stress domains (interpersonal, legal, health, work, and finance) and types of childhood maltreatment (emotional, physical, and sexual abuse, and emotional and physical neglect), only p-values less than 0.01 were considered significant, following Bonferroni correction. All analyses were conducted using IBM SPSS® Statistics v.24.0.

2.4 Results

2.4.1 Study Arm A: Current Suicidal Ideation

In our cohort of 80 participants, we found that 10 subjects, approximately 13%, reported experiencing suicidal ideation in the month prior to the baseline visit (ideators), while the remaining 70 did not report any recent suicidal ideation (non-ideators). As previously mentioned, the aim of the analyses was to examine the independent effects of recent stress and childhood trauma on current suicidal ideation status.

2.4.1.1 Unadjusted Logistic Regression

Specific stressful life readjustments, as assessed in the SRRS, were classified into the five domains of interpersonal, legal, health, work, and finance, where applicable. In the unadjusted analysis of recent stress **(Table 2.3)**, we found that ideators had a higher overall SRI than non-ideators, though it was not considered a significant predictor of current suicidal ideation (OR = 1.033 [0.980 – 1.089, p = 0.227]. Interestingly, however, increased recent health-related stress was predictive of current suicidal ideation (OR = 1.294 [1.067 – 1.570], p = 0.009).

With regards to childhood trauma **(Table 2.4)**, we found that ideators reported higher overall CTQ scores, which was predictive of current suicidal ideation (OR = 1.052 [1.019 – 1.087], $p = 0.002$). The analysis for the overall CTQ score took into account all five types of maltreatment. Specifically testing for the types of maltreatment revealed that emotional abuse (OR = 1.206 [1.051 – 1.385], $p = 0.008$), physical abuse (OR = 1.220 [1.078 – 1.381], $p = 0.002$), and physical neglect (OR = 1.343 [1.126 – 1.602], $p = 0.001$) were each significant predictors of current suicidal ideation.

Table 2.3 | Unadjusted effect of recent stress on current suicidal ideation. The effect of recent stress, in terms of total stress and domain sub-scores, on current suicidal ideation was determined with unadjusted binary logistic regression analyses.

Total ($N = 80$)	**Ideator** ($n = 10$)	**Non-Ideator** ($n = 70$)	**P-Value**	**OR [95% CI]**
SRI (Social Readjustment Index)	178.2 ± 157.8	129.6 ± 110.7	0.227	1.033 [0.980–1.089]
Interpersonal	34.9 ± 43.9	29.0 ± 49.7	0.721	1.023 [0.903–1.159]
Legal	0.0 ± 0.0	1.5 ± 7.8	0.999	1.000 [1.000–1.000]
Health	51.5 ± 39.1	22.0 ± 26.9	0.009	1.294 [1.067–1.570]
Work	27.3 ± 54.8	21.5 ± 34.6	0.647	1.039 [0.882–1.224]
Finance	11.4 ± 18.3	13.1 ± 19.5	0.793	0.953 [0.666–1.364]

*SRI scores presented as mean ± standard deviation for each group.

Table 2.4 | Unadjusted effect of childhood trauma on current suicidal ideation. The effect of childhood trauma, in terms of total trauma and domain sub-scores, on current suicidal ideation was determined with unadjusted binary logistic regression analyses.

Total ($N = 80$)	**Ideator** ($n = 10$)	**Non-Ideator** ($n = 70$)	**P-Value**	**OR [95% CI]**
CTQ (Childhood Trauma Questionnaire)	81.6 ± 33.1	54.3 ± 17.4	0.002	1.052 [1.019–1.087]
Emotional Abuse	15.4 ± 6.3	10.2 ± 4.6	0.008	1.206 [1.051–1.385]
Physical Abuse	13.7 ± 6.7	7.7 ± 3.9	0.002	1.220 [1.078–1.381]
Sexual Abuse	11.9 ± 7.5	7.4 ± 4.1	0.012	1.156 [1.032–1.296]
Emotional Neglect	15.6 ± 6.6	11.5 ± 4.4	0.020	1.173 [1.026–1.341]
Physical Neglect	13.5 ± 5.9	8.0 ± 3.0	0.001	1.343 [1.126–1.602]

*CTQ scores presented as mean ± standard deviation for each group.

2.4.1.2 Covariate Analysis

The demographic and clinical variables that were being considered as potential confounding variables are shown summarized in **Table 2.5**. We report that there were significant group differences between suicide ideators and suicide non-ideators in several respects. Ideators had an overall greater degree of psychotic symptoms (BPRS; $p = 0.014$), and increased severity of depression (CDSS; $p < 0.001$), hopelessness (BHS; $p = 0.002$), and perceived stress (PSS; $p = 0.002$). Since these variables each had p-values that were below the pre-determined significance threshold of $p = 0.1$, they were subsequently included as covariates in further adjusted binary logistic regression analyses.

Table 2.5 | Summary of demographic and clinical variables in current suicidal ideation. These variables were tested for group differences between suicide ideators and non-ideators. Significant group differences were included as covariates in subsequent analyses.

Total ($N = 80$)	**Ideator** ($n = 10$)	**Non-Ideator** ($n = 70$)	**P-Value**
Gender (male/ female)	3/7	40/30	0.203
Age (years)	36.5 ± 16.2	42.1 ± 13.9	0.216
Age-of-Onset (years)	21.4 ± 9.2	23.0 ± 7.2	0.122
Duration-of-Illness (years)	14.9 ± 11.6	19.4 ± 14.9	0.411
BPRS (Brief Psychiatric Rating Scale)	39.2 ± 11.5	30.1 ± 9.7	0.014
CDSS (Calgary Depression Scale for Schizophrenia)	10.4 ± 5.7	3.5 ± 3.5	< 0.001
BHS (Beck Hopelessness Scale)	9.8 ± 6.0	3.3 ± 3.5	0.002
PSS (Perceived Stress Scale)	31.7 ± 5.8	23.3 ± 7.5	0.002
SAI (Schedule for Assessment of Insight)	11.0 ± 3.7	11.1 ± 3.0	0.882
MMSE (Mini-Mental State Examination)	25.2 ± 3.1	26.4 ± 3.0	0.287
FTND (Fagerström Test of Nicotine Dependence)	0.5 ± 0.8	2.2 ± 3.0	0.189
AUDIT (Alcohol Use Disorders Identification Test)	4.3 ± 5.9	3.3 ± 4.9	0.741
DAST (Drug Abuse Screening Test)	3.6 ± 5.4	3.4 ± 4.5	0.994

*Demographic and clinical values presented as mean ± standard deviation for each group.

2.4.1.3 Adjusted Logistic Regression

The significant effect of increased recent health-related stress on current suicidal ideation in the unadjusted analyses was no longer seen following correction for psychosis, depression, hopelessness, and perceived stress (OR = 1.157 [0.856 – 1.565, p = 0.343]. Furthermore, the significant effects of increased childhood trauma were also no longer evident after inclusion of these covariates. The total CTQ score was not found to be predictive of current suicidal ideation (OR = 1.028 [0.982 – 1.075], p = 0.241), nor were any of the notable types of maltreatment initially found in the unadjusted analyses, including emotional abuse (OR = 1.067 [0.884 – 1.289], p = 0.498), physical abuse (OR = 1.166 [0.971 – 1.400], p = 0.100), and physical neglect (OR = 1.196 [0.921 – 1.552], p = 0.179).

2.4.2 Study Arm B: Emergent Suicidal Ideation

In the same cohort of 80 participants, we found that 13 subjects, approximately 16%, reported the onset or worsening of suicidal ideation in the past year (emergent suicidal ideation). The remaining 67 subjects showed the same or improved suicidal ideation levels (non-emergent suicidal ideation). Here we examined the independent effects of the change in recent stress and childhood trauma on emergent suicidal ideation status.

2.4.2.1 Unadjusted Logistic Regression

At the one-year follow-up visit, we found that participants with emergent suicidal ideation had an average SRI increase of 114.2 LCU's compared to scores obtained from the previous year, as opposed to participants without emergent suicidal ideation who showed an average decrease of 32.6 LCU's. Increased overall SRI scores were found to be predictive of emergent suicidal ideation (OR = 1.109 [1.042 – 1.181], p = 0.001) in the unadjusted logistic regression analysis (**Table 2.6**). Furthermore, health-related stress was increased by an average of 33.3 LCU's in the emergent group, versus an average of 0.3 LCU decrease in the non-emergent group. Health-related stress was also found to be a significant predictor of emergent suicidal ideation (OR = 1.354 [1.105 – 1.659], p = 0.003).

In terms of childhood trauma, we observed that participants in the emergent suicidal ideation group reported slightly higher scores in each of the five specific maltreatment categories and had a higher overall CTQ score. However, these elevated levels of childhood trauma severity were not found to be significant predictors of emergent suicidal ideation (**Table 2.7**; OR = 1.021 [0.996 – 1.047], $p = 0.104$). As a result, we did not complete adjusted logistic regression analyses on the effect of childhood trauma on emergent suicidal ideation status.

Table 2.6 | Unadjusted effect of recent stress change on emergent suicidal ideation. The effect of the change in recent stress, in terms of total stress and domain sub-scores, on emergent suicidal ideation was determined with unadjusted binary logistic regression analyses.

Total ($N = 80$)	**Emergent** ($n = 13$)	**Non-Emergent** ($n = 67$)	**P-Value**	**OR [95% CI]**
ΔSRI (Social Readjustment Index)	114.2 ± 132.4	-32.6 ± 123.7	0.001	1.109 [1.042–1.181]
Δ Interpersonal	33.4 ± 54.4	-7.7 ± 61.3	0.025	1.150 [1.018–1.299]
Δ Legal	0.0 ± 0.0	0.1 ± 12.1	0.961	0.986 [0.573–1.699]
Δ Health	33.3 ± 39.4	-0.3 ± 31.7	0.003	1.354 [1.105–1.659]
Δ Work	17.6 ± 36.9	-8.9 ± 38.8	0.029	1.233 [1.022–1.487]
Δ Finance	4.2 ± 26.7	-2.6 ± 22.4	0.331	1.129 [0.884–1.441]

* Change in SRI scores presented as mean ± standard deviation for each group.

Table 2.7 | Unadjusted effect of childhood trauma on emergent suicidal ideation. The effect of childhood trauma, in terms of total trauma and domain sub-scores, on emergent suicidal ideation was determined with unadjusted binary logistic regression analyses.

Total ($N = 80$)	**Emergent** ($n = 13$)	**Non-Emergent** ($n = 67$)	**P-Value**	**OR [95% CI]**
CTQ (Childhood Trauma Questionnaire)	67.0 ± 28.8	55.9 ± 19.8	0.104	1.021 [0.996–1.047]
Emotional Abuse	13.3 ± 5.9	10.4 ± 4.9	0.075	1.108 [0.990–1.241]
Physical Abuse	9.5 ± 5.7	8.3 ± 4.5	0.405	1.049 [0.937–1.175]
Sexual Abuse	9.5 ± 6.3	7.6 ± 4.5	0.208	1.072 [0.962–1.194]
Emotional Neglect	13.6 ± 5.8	11.7 ± 4.7	0.206	1.078 [0.959–1.212]
Physical Neglect	10.3 ± 3.0	8.3 ± 3.5	0.064	1.137 [0.993–1.303]

*CTQ scores presented as mean ± standard deviation for each group.

2.4.2.2 Covariate Analysis

The demographic variables, as well as changes in clinical variables observed at the one-year follow-up, are shown summarized in **Table 2.8**. We found differences between the emergent and non-emergent groups, with subjects with emergent suicidal ideation having a younger average age-of-onset ($p = 0.057$) and increased severity of psychosis (Δ BPRS; $p = 0.070$). While not these were not necessarily considered statistically significant, the variables did meet our pre-determined p-value threshold of 0.1 and were subsequently included as covariates in further adjusted binary logistic regression analyses.

Table 2.8 | Summary of demographic and clinical variables in emergent suicidal ideation. These variables were tested for group differences between subjects with and without emergent suicidal ideation. Significant group differences were included as covariates in subsequent analyses.

Total ($N = 80$)	**Emergent** ($n = 13$)	**Non-Emergent** ($n = 67$)	**P-Value**
Gender (male/ female)	7/6	36/31	0.766
Age (years)	41.0 ± 18.8	41.5 ± 13.3	0.620
Age-of-Onset (years)	19.7 ± 6.7	23.4 ± 7.5	0.057
Duration-of-Illness (years)	21.8 ± 17.9	18.2 ± 13.9	0.671
Δ BPRS (Brief Psychiatric Rating Scale)	5.3 ± 9.7	-0.2 ± 9.5	0.070
Δ CDSS (Calgary Depression Scale for Schizophrenia)	1.3 ± 4.3	0.1 ± 3.8	0.169
Δ BHS (Beck Hopelessness Scale)	0.1 ± 3.7	-0.1 ± 4.1	0.464
Δ PSS (Perceived Stress Scale)	0.1 ± 6.7	-0.9 ± 5.9	0.557
Δ SAI (Schedule for Assessment of Insight)	0.1 ± 3.2	0.2 ± 3.7	0.952
Δ MMSE (Mini-Mental State Examination)	0.5 ± 3.6	0.7 ± 2.8	0.901
Δ FTND (Fagerström Test of Nicotine Dependence)	0.1 ± 0.6	-0.1 ± 2.1	0.425
Δ AUDIT (Alcohol Use Disorders Identification Test)	1.3 ± 2.7	0.3 ± 3.9	0.171
Δ DAST (Drug Abuse Screening Test)	1.4 ± 5.2	-0.4 ± 2.8	0.432

*Demographic and clinical values presented as mean ± standard deviation.

2.4.2.3 Adjusted Logistic Regression

As previously mentioned, adjusted logistic regression analyses were only conducted for total and health-related stress. After correcting for age-of-onset and psychosis, we found that higher total stress remained a significant predictor of emergent suicidal ideation (OR = 1.093 [1.025 – 1.165], p = 0.006). Additionally, health-related stress also remained a strong predictor of emergent suicidal ideation (OR = 1.300 [1.052 – 1.605], p = 0.009).

2.5 Discussion

Previous studies have frequently found that recent stress and childhood trauma are highly associated with suicide. However, research focused on investigating these factors from both cross-sectional and longitudinal approaches is fairly scarce. To gain insight, the current study reports the independent effects of recent stress and childhood trauma on current and emergent suicidal ideation in a sample of schizophrenia patients.

Our hypothesis regarding recent stressful events was partially supported- we found that only health-related stress was a significant predictor of current suicidal ideation. These results are similar to those reported in a psychiatric outpatient setting, where the number of recent stressful events was not significantly associated with suicidal ideation, though being "recently hospitalized for severe medical problems" was associated (May et al., 2015). Additionally, machine learning models have also found that somatic concerns, among other factors, were strongly associated with suicidal ideation (Gradus et al., 2017). We believe that our non-significant findings pertaining to total stress and other stress domains could be partially explained. Many previous studies have found very specific stressful events to be associated with suicide, such as arguments with spouse, death of a friend or relative, loss of job, being passed over for promotion, withdrawal from school, and financial deterioration (Blasco-Fontecilla et al., 2012; Funahashi et al., 2000; Kõlves et al., 2006). These individual events can be seen as being embedded within the categories of interpersonal, work, and financial-related stress, and are therefore not always evident when considering larger domains or total stress. Furthermore, due to few participants reporting occurrences of each event on the SRRS (**Table 2.1**) resulting from our small sample size, we were unable to conduct analyses regarding the effect of specific events on suicidal ideation.

We also report that increased total stress and health-related stress at the one-year follow-up compared to baseline measures, were significant predictors of emergent suicidal ideation. These findings are partially supported by our previous work: also in a sample of patients with schizophrenia, we reported that only recent health-related stress was predictive of emergent suicidal ideation (Tasmim et al., 2019). Increased change in health-related stress being a predictor in our present analysis appears to be a logical progression, specifically with an increase in a previously identified risk for current suicidal ideation also resulting in emergent suicidal ideation. Upon closer examination of the other stress domains, we found that increased interpersonal (p = 0.025) and work (p = 0.029) -related stress were both marginally significant predictors of emergent suicidal ideation. We believe that the previously mentioned results from other groups studying the effects of recent stress (i.e. spousal arguments, unemployment) on suicide are also relevant and applicable here. Our finding that increased total stress was a significant predictor of emergent suicidal ideation could very well be attributed to the combined increases of health, interpersonal, and work-related stress.

It is also important to take note of several differences from our earlier work, as well as key features of the present study. Previously, all measures of recent stress were assessed retrospectively using the SRRS at the 3-month follow-up visit alone (Tasmim et al., 2019). However, we believe that our present study is more robust, in that we considered the changes in SRI and domain sub-scores between separate baseline and one-year follow-up visits. By adopting a truly longitudinal approach, this theoretically allowed us to exclude the impact of stressful events that were reported at both visits, while focusing only on changes that had occurred between the two time points. Furthermore, an argument can be made that it is difficult to catch current suicidal ideation and any associated recent stressful events within just a 3-month period. However, in this study assessing emergent suicidal ideation, we had the ability to assess for stressful events occurring within a greater range of time.

Childhood trauma has pervasive and long-lasting effects on individuals, including later development of maladaptive personality features, inability to regulate emotions, loss of social bonds, and impulsive behavior, among other factors (Braquehais et al., 2010). Thus, we hypothesized that childhood trauma would be a significant predictor of suicidal ideation in schizophrenia. This was partially supported in our study. We found that a greater overall severity of childhood trauma, specifically in relation to emotional abuse, physical abuse, and physical

neglect, were associated with current suicidal ideation. Our findings are consistent with other studies in schizophrenia that have found that suicide attempters have higher CTQ scores representing all maltreatment domains (Roy, 2005). Also, in a more related study investigating a suicidal ideation outcome, patients reported higher levels of emotional abuse and physical neglect (Mohammadzadeh et al., 2019), as was found in our present study. In contrast, Bahk et al. found that among the five types of childhood maltreatment covered in the CTQ, sexual abuse was the only direct predictor of suicidal ideation (2017); in our study, we found sexual abuse to be only a marginally significant predictor of current suicidal ideation ($p = 0.012$). Their study also revealed that social support mediated the relationship between neglect and suicidal ideation, whereas anxiety mediated the relationships between physical and emotional abuse, and suicidal ideation (Bahk et al., 2017). While the discrepancies in the results could be attributed to their sample being of the general population rather than specifically in schizophrenia, many features of their work, such as path analysis and the investigation of potential mediators, could and should be implemented in our future work.

With regards to childhood trauma and emergent suicidal ideation, we did not find any significant associations in our present study. Interestingly, it has been previously reported in a longitudinal population-based study that emotional abuse, physical abuse, and childhood neglect were all strongly associated with the onset of suicidal ideation and attempts (Enns et al., 2006). It should be noted however that in their study, each type of abuse or neglect was described, and while participants responded with *never*, *one time*, *sometimes*, *regularly*, *often*, or *very often*, those responses were dichotomized to reflect the occurrence or absence of each type of maltreatment (Enns et al., 2006). Whether childhood adversities were experienced is indeed a relevant factor to consider, though we argue that many times, maltreatment is not a clear black and white picture. The advantage of our study utilizing the CTQ allows us to assess the specific degree of childhood maltreatment experienced. In our sample, we report that patients with emergent suicidal ideation did indeed have elevated scores in overall childhood trauma and all maltreatment domains compared to their non-emergent counterparts, though these increases were not necessarily significant predictors of emergent suicidal ideation. We believe that our analysis provides more specificity and means for interpretation. Furthermore, we found only a weak correlation between childhood trauma and recent stress ($r = 0.245$). This suggests that collinearity would unlikely be a source of uncontrolled bias in our analyses.

Up until this point, we have only discussed the isolated effect of recent stressful events or childhood trauma on suicidal ideation and did not consider additional factors. There are several features that have been found to be associated with suicide, including gender, age, psychosis, depression, hopelessness, awareness of illness, intelligence, and substance abuse (Carlborg et al., 2010; Hor et al., 2010; Pompili et al., 2007; Young et a., 1998). For each of our two study arms examining current and emergent suicidal ideation, we probed for group differences in potential confounding variables. Any variable that we found to meet the pre-determined threshold for significance ($p < 0.1$) was subsequently included as a covariate in further adjusted logistic regression analyses.

In study Arm A, we found that current suicide ideators had significantly greater severity of psychosis ($p = 0.014$), depression ($p < 0.001$), hopelessness ($p = 0.002$), and perceived stress ($p = 0.002$) compared to non-ideators. Following correction for these covariates in an adjusted analysis, we no longer saw the significant effects of recent health-related stress, overall childhood trauma, emotional abuse, physical abuse, and physical neglect on current suicidal ideation. These findings could potentially be explained by the nature of the covariates we included. For instance, schizophrenia patients who had attempted suicide were more likely to be psychotic (De Hert et al., 2001); having positive symptoms such as auditory hallucinations and delusions was associated with an increased suicide risk (Hor et al., 2010). Depression, whether considered from a mood or syndrome perspective, is a significant risk factor for suicide across all populations (Pompili et al., 2007), and has even been suggested to serve as a trigger for suicide attempts (Harkavy-Friedman et al., 1999). In schizophrenia, hopelessness has been found to be a predictor of suicide attempt (Nordentoft et al., 2002), and interestingly, one study found that the relationship between depression and suicide disappears when accounting for hopelessness (Drake et al., 1986). Perceived stress refers to the degree of non-specific stress experienced in coping with encountered stressful events, and higher levels were associated with the development of suicidal ideation and other suicidal behaviors (Chen et al., 2019). We believe that in our study, the loss of significant effects in the adjusted logistic regression can be attributed to compelling differences in regard to the covariates. This suggests that the combined effect of psychosis, depression, hopelessness, and perceived stress on current suicidal ideation is greater than that of each individual predictor we tested.

In study Arm B, we identified that subjects with emergent suicidal ideation generally had a younger age-of-onset ($p = 0.057$) and an increase in psychosis compared to the baseline visit ($p = 0.070$). While these group differences may not necessarily be considered statistically significant, they did meet our p-value threshold and were included as covariates. In addition to the evidence for psychosis previously mentioned in the context of current suicidal ideation, a recent longitudinal study found that psychotic experiences, an important but under-recognized marker, increase the risk for persistent suicidal ideation in young adolescents (Kelleher et al., 2014). Furthermore, it has been suggested that having a later age-of-onset of psychotic symptoms is consistent with "having high personal expectations and hopes," and "having an understanding that life's expectations and hopes are not likely to be met," thus leading to increased suicide risk (Sher et al., 2019). There are, however, conflicting findings, with other studies reporting that an earlier age-of-onset is a risk factor for suicide (Ventriglio et al., 2016). Nevertheless, after correcting for these two covariates, we found that in our sample, higher recent total (OR = 1.093, $p = 0.006$) and health-related stress (OR = 1.300, $p = 0.009$) remained strong predictors of emergent suicidal ideation. This indicates that even after correcting for psychosis and age-of-onset, we expect to see a 9.3% increase in the odds of developing emergent suicidal ideation for every 10 LCU increase in total stress change, and a 30% increase in the odds of emergent suicidal ideation with every 10 LCU increase in health-related stress change. While the effect of total stress change is less than health-related stress change, it should be noted that the total stress change accounts for other domains that were not found to have significant effects on emergent suicidal ideation.

Considering the vast realm of knowledge surrounding suicidal ideation and behaviors, we specifically aimed to test the independent effects of recent stress and childhood trauma on both current and emergent suicidal ideation. Based on the combination of our findings, childhood trauma is a stronger predictor of current suicidal ideation compared to emergent suicidal ideation. Logically, maltreatments experienced as a child would be expected to affect current suicidal ideation, though markedly less on emergent ideation. In future extensions of our study, it would be beneficial to investigate mediating or moderating interplay among childhood trauma, psychosis, depression, hopelessness, and perceived stress, all in an increased sample size. Furthermore, increased recent total and health-related stress appeared to be stronger predictors of emergent suicidal ideation in the context of covariates, compared to recent stress on current suicidal ideation.

Applying research findings to the clinical setting would entail further consideration of other factors surrounding suicide. One of the major barriers in suicide prevention is that individuals will often deny ideation to avoid unwanted intervention, such as involuntary psychiatric holds, or even to facilitate release from such circumstances (Nock et al., 2007). Depression is a well-known risk factor for suicide across all populations (Pompili et al., 2007), though in schizophrenia, it can be easily confused with negative symptoms or side effects of antipsychotic medications (Carlborg et al., 2010). To date, there is a lack of a highly effective, gold-standard method for the identification and treatment of at-risk patients (Klonsky et al., 2016). Theoretically if we were to directly implement our findings from this study, it would likely involve the administration of the SRRS to assess for recent stressful events at given time points. Any significant increases in stressful events experienced, specifically those related to health, between visits would be a preliminary indicator for clinicians to probe for suicide risk. Outside of this study, one promising assessment technique used to predict suicidal ideation and attempt is the Self-Injury Implicit Association Test (SI-IAT), and it is described as a "reaction-time measure of implicit associations between self-injury and oneself" (Nock et al., 2008). Additional work is warranted to better predict and prevent suicide.

2.6 Conclusion

In summary, this one-year longitudinal study found that recent stressful life events, and specifically those health-related, were strongly associated with emergent suicidal ideation in a population of patients with schizophrenia. Our results also suggested that health-related stressful life events and childhood trauma were indicative of current suicidal ideation, though these findings should be considered in the context of other risk factors. These findings may have important implications on current suicide research, though further studies with larger sample sizes should focus on the effect of specific types of life events and the complex interplay among recent stress, childhood trauma, and other known risk factors.

Chapter 3

3 Genome-Wide Methylation Analysis of Suicidal Ideation in Schizophrenia

3.1 Abstract

Introduction: There are a multitude of factors that make it difficult to identify those at risk for suicide, especially schizophrenia patients with suicidal ideation. Suicide cannot be explained by genetics alone, therefore epigenetic mechanisms including DNA methylation are thought to play a role. DNA methylation can prove to be a valuable tool in helping predict those at-risk individuals.

Methods: This cross-sectional study comprised of 113 subjects diagnosed with a schizophrenia spectrum disorder, and were grouped according to the presence of suicidal ideation. DNA methylation across the genome was measured with the Infinium® MethylationEPIC BeadChip. We utilized the *dmpFinder* and *bumphunter* functions within the Bioconductor minfi package to identify differentially methylated positions (DMPs) and differentially methylated regions (DMRs), respectively.

Results: Following quality control, we removed one sample from the analysis and reported the most significant DMPs and DMRs associated with suicidal ideation. All positions and regions identified in this analysis were only found to have suggestive levels of significance at the genome-wide level.

Discussion/ Conclusion: The present study was one of the first to investigate genome-wide methylation in suicidal ideation. While there were many strengths of our study, including investigating both differentially methylated positions and regions, further larger-scale studies are necessary to consider for covariates to replicate, support, and validate our findings presented here.

3.2 Introduction

Suicidal behavior has consistently been ranked as one of the leading causes of injury and death worldwide, accounting for over 788,000 lives lost per year (WHO, 2014; WHO, 2017). Furthermore, patients with schizophrenia have a tenfold increase in suicide risk compared to that of the general population, with an estimated 25-50% of these individuals making at least one suicide attempt during the course of their lifetime (Carlborg et al., 2010; Meltzer, 2001). Suicidal ideation, as reported by patients themselves, greatly increases the risk for later suicide attempt and can serve as an important early warning sign for family, caregivers, and clinicians alike (Hocaoglu et al., 2009; Simon et al., 2017).

It is also important to note that the symptoms of schizophrenia often complicate the ability to accurately predict those most at risk. For instance, positive symptoms including auditory hallucinations and delusions, are known to increase the risk for suicide, yet these same symptoms frequently hinder proper and adequate communication with the patient (Hor et al., 2010). Depression, also strongly associated with suicide, can easily be confused with the negative symptoms of schizophrenia, such as apathy and reduced expression, or can even be attributed to the side effects of prescribed antipsychotic regiments (Carlborg et al., 2010; Klaus et al., 2018). In light of these considerations, it becomes necessary to consider other factors to better predict and prevent suicide. The National Alliance for Suicide Prevention proposed the identification of peripheral biomarkers for screening and risk assessment (NIMH, 2014). We, in turn, adopted this approach to predict for current and emergent suicidal ideation, specifically in the context of patients with schizophrenia.

Family studies have long supported the notion that suicidal behaviors, including both suicide attempt and completion, have a strong genetic component (Zai et al., 2012), with the heritability of suicide attempt estimated to be approximately 43% (McGuffin et al., 2010). However, genome-wide association studies (GWAS) have thus far been unable to consistently and reliably identify risk associated with DNA sequence variants alone, with only two studies reporting genome-wide significant associations (Mirkovic et al., 2016; Perlis et al., 2010; Willour et al., 2012). As such, in recent years, epigenetic mechanisms have been suggested to play a role in “quantifying the missing heritability” of suicide in schizophrenia (Bani-Fatemi et al., 2018).

Epigenetic modifications are heritable, non-coding changes that play a significant role in gene expression regulation (Jang et al., 2017). DNA methylation, the most common epigenetic mechanism, uses DNA methyltransferases to add a methyl group (-CH_3) to the fifth carbon of the pyrimidine ring of cytosine to form 5-methylcytosine (5mC) (Jang et al., 2017). Generally, the incorporation of 5mC either recruits proteins involved in the gene repression process or works to inhibit binding of transcription factors to the DNA (Moore et al., 2013). The vast majority of 5mC's are located within cytosine-guanine dinucleotide (CpG) sites, though they can also be found at non-CpG sites such as CpA, CpT, and CpC sites (Jang et al., 2017). Furthermore, CpG islands are short interspersed DNA sequences of approximately 500-1500 base pairs in length that play an integral role in transcription initiation, with an elevated composition of cytosine and guanosine residues and are rich in terms of the number of CpG sites (Deaton et al., 2011). DNA methylation has been found to play an important key role in the development and progression of many human pathologies (Li et al., 2015), of which includes suicidality.

DNA methylation and suicide have been studied extensively in relation to dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis. As a brief introduction, the HPA axis is a neuroendocrine system involved in the adaptive response to stress, allowing one to rapidly respond to stressful events when necessary and subsequently return to basal levels once the stressor has been resolved (Smith et al., 2006). The most compelling evidence implicating the involvement of DNA methylation in suicide involves the human glucocorticoid receptor (hGR), an integral part of the negative feedback to attenuate the stress response (Gjerstad et al., 2018), and the spindle and kinetochore-associated protein 2 (SKA2), a key chaperone involved in the transport of the hGR (Yin et al., 2016).

It has long been reported that maternal care has the ability to affect the HPA axis and stress response in non-human primates (Higley et al., 1991) and in rodents specifically by epigenetic programming of GR expression (Meaney, 2001). Recently, it was also discovered that there were lower levels of hGR mRNA in postmortem hippocampal samples of psychiatric suicide victims with a history of childhood abuse, compared to psychiatric controls who died of unrelated accidental causes (McGowan et al., 2009). The decreased gene expression was subsequently found to be attributed to hypermethylation of individual CpG sites within the hGR1_B, 1_C, and 1_F promoter sequences of the GR gene *NR3C1* (Labonté et al., 2012; McGowan et al., 2009).

Genome-wide screens for variation in DNA methylation levels in postmortem brain tissue associated with suicide found that elevated methylation levels at CpG site cg13989295 located within the *SKA2* gene were predictive of higher rates of suicidal ideation and behaviors (Guintivano et al., 2014; Sadeh et al., 2016). Another study later reported that both the protein and gene expression of *SKA2* were both significantly lower in the prefrontal cortex of psychiatric suicide victims compared to non-suicidal psychiatric patients; similar expression levels were observed between non-psychiatric controls and non-suicidal psychiatric subjects, suggesting that the observed decrease in *SKA2* expression was specific to suicide, and independent of the psychiatric diagnosis (Pandey et al., 2016).

Similar methylation markers in white blood cells have also been reported, thereby indicating that peripheral tissues may serve as a proxy for the brain (Clive et al., 2016; Perroud et al., 2011). Combined, the evidence from the *NR3C1* and *SKA2* genes suggest that these epigenetic biomarkers can prove to be invaluable in investigating suicide attempt and completed suicides. However, predicting suicidal ideation may very well yield differing results from those studies utilizing suicidal behaviors as the outcome variable (Le-Niculescu et al., 2013). As such, it is necessary to replicate these studies in the context of suicidal ideation. The present study will investigate genome-wide methylation status in association with current suicidal ideation. The following chapter will be a continuation, and investigate genome-wide methylation changes with respect to emergent suicidal ideation.

3.3 Methods

3.3.1 Participants

The current study included 113 individuals having a diagnosis of a schizophrenia spectrum disorder, recruited from the Centre for Addiction and Mental Health (CAMH) in Toronto, Canada. Each subject was between the ages of 18 and 75, and did not have a past history of head trauma with loss of consciousness, or diagnosis of an intellectual disability, major neurological disorder, or substance-induced psychosis. These participants were a part of a larger scale study. This study was approved by the CAMH Research Ethics Board.

As the present study was cross-sectional in design, participants were only assessed at a single time point. The Columbia- Suicide Severity Rating Scale (C-SSRS; Posner et al., 2011) was administered to determine whether subjects were experiencing suicidal ideation at the time of the visit, and if so, the severity of ideation.

3.3.2 Sample Collection and DNA Methylation

From each participant, we collected approximately 8 mL of venous blood in BD Vacutainer® Plus EDTA tubes (Becton, Dickinson, and Company). We then extracted genomic DNA from white blood cells using the QIAamp® DNA Blood Maxi Kit (QIAGEN Inc.). Samples were sent to The Centre for Applied Genomics (TCAG) at the Hospital for Sick Children for further processing. There, 500 ng of DNA was treated with sodium bisulfite using the EZ DNA Methylation Kit (Zymo Research). The process of bisulfite conversion involved the deamination of unmethylated cytosine residues to uracils, while leaving the remaining methylcytosines unaltered (Zhang et al., 2009). Genome-wide DNA methylation was quantified using the Infinium® MethylationEPIC BeadChip array (Illumina) to interrogate over 850,000 CpG loci at single-nucleotide resolution. The confocal laser scanning system, iScan® (Illumina), utilized a pair of fluorescent dyes to recognize the bisulfite-converted DNA and outputted summary intensities for each sample as IDAT file formats, short for “intensity data file” (Li et al., 2015; Smith et al., 2013).

3.3.3 Identification of Differentially Methylated Positions and Regions

All analyses were conducted with the minfi Bioconductor package, run in the R- 3.5.1 (64 bit) statistical analysis environment on the CAMH Specialized Computing Cluster. The minfi package began by reading the raw methylation intensity data for each sample, in the form of IDAT files, as well as phenotype data indicating the suicidal ideation status for each corresponding subject. The *preprocessRaw* function was first implemented to convert IDAT data into methylation levels (β-values) without normalization; we then generated a probe intensity scatterplot and β-value density plot for quality control purposes (Aryee et al., 2014; Hansen et al., 2012). Following quality control assessments, samples with poor quality with excluded from further analyses. The *preprocessFunnorm* function was then utilized for functional normalization to remove biological or technical variation (Fortin et al., 2014). We proceeded to apply a series of intricate

bioinformatic functions to identify differentially methylated positions (DMPs) and differentially methylated regions (DMRs).

To identify DMPs, the *dmpFinder* function was used to test individual CpG sites for associations between methylation level and current suicidal ideation phenotype. We utilized both a binary phenotype indicating the presence or absence of suicidal ideation, and a continuous phenotype rating the severity of suicidal ideation from zero to five according to the C-SSRS. On the other hand, DMRs were identified through the implementation of the *bumphunter* function. The utility of bump hunting allows us to consider correlations of methylation levels between nearby CpG sites, and hence allow for the identification of regions that are differentially methylated (Jaffe et al., 2012; Li et al., 2015). For bump hunting, we applied a methylation differential cutoff of 0.1, or 10%, as was done in Kebir et al. (2017). The specific innerworkings of the *dmpFinder* and *bumphunter* functions are statistically complex, and are not discussed in this thesis. In addition, only autosomal positions and regions were included in the analyses, as it is well known that there are differing methylation profiles on sex chromosomes between the male and female sex.

3.4 Results

3.4.1 Demographic and Clinical Characteristics

Among our cohort of 113 participants, we found that 19 subjects, or approximately 17%, reported experiencing recent suicidal ideation, while the remaining 94 subjects did not report suicidal ideation. The demographic and clinical variables from participants are summarized in **Table 3.1**. We also calculated p-values for group differences between ideators and non-ideators with the Mann-Whitney U test for continuous variables and Chi-squared tests for categorical variables. Interestingly, these results were similar to those found from the previous clinical chapter, in that there were significant differences between groups in terms of psychosis severity (BPRS), depression (CDSS), hopelessness (BHS), and perceived stress (PSS).

Table 3.1 | Demographic and clinical variables in the current suicidal ideation methylation cohort. These variables were tested for group differences between subjects with and without current suicidal ideation.

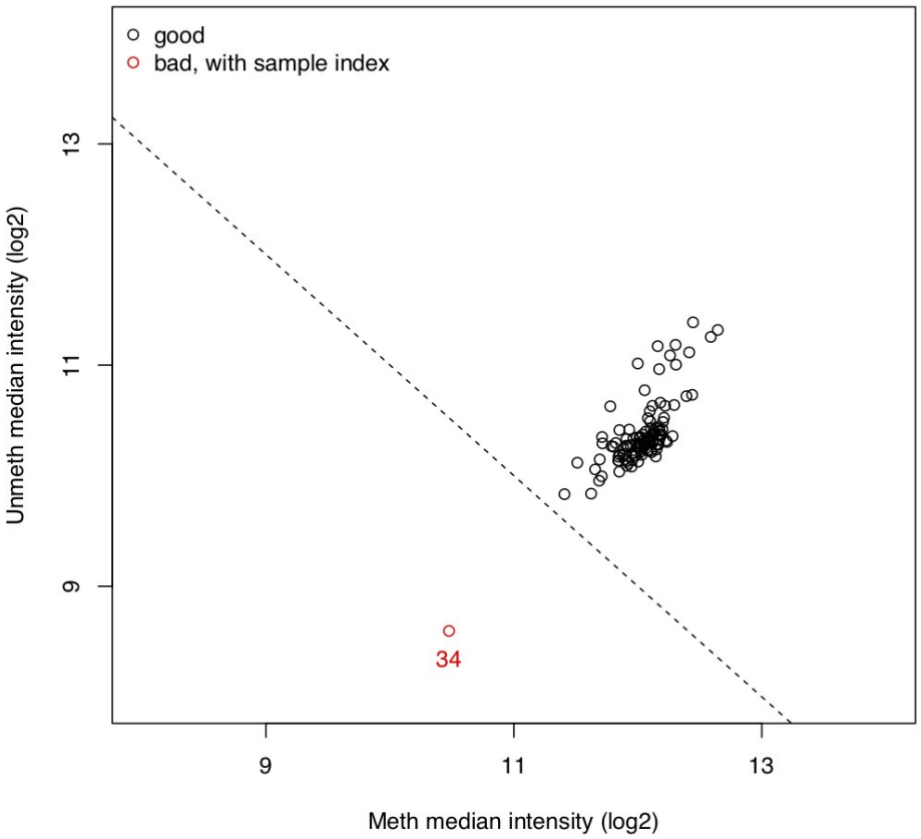
Total (N = 113)	**Ideator** (n = 19)	**Non-Ideator** (n = 94)	**P-Value**
Gender (male/ female)	11/8	59/35	0.689
Age (years)	44.7 ± 9.3	45.1 ± 13.0	0.890
Age-of-Onset (years)	21.2 ± 6.1	22.8 ± 6.7	0.341
Duration-of-Illness (years)	23.1 ± 9.9	21.8 ± 13.5	0.698
BPRS (Brief Psychiatric Rating Scale)	33.2 ± 7.8	28.4 ± 6.6	0.017
CDSS (Calgary Depression Scale for Schizophrenia)	6.7 ± 5.3	2.9 ± 3.3	0.002
BHS (Beck Hopelessness Scale)	7.6 ± 6.8	4.1 ± 4.2	0.004
PSS (Perceived Stress Scale)	31.2 ± 6.6	24.2 ± 6.7	0.013
SAI (Schedule for Assessment of Insight)	11.1 ± 2.6	11.5 ± 3.0	0.740
MMSE (Mini-Mental State Examination)	27.5 ± 2.8	27.3 ± 2.9	0.911

*Demographic and clinical values presented as mean ± standard deviation.

3.4.2 Quality Control Assessments

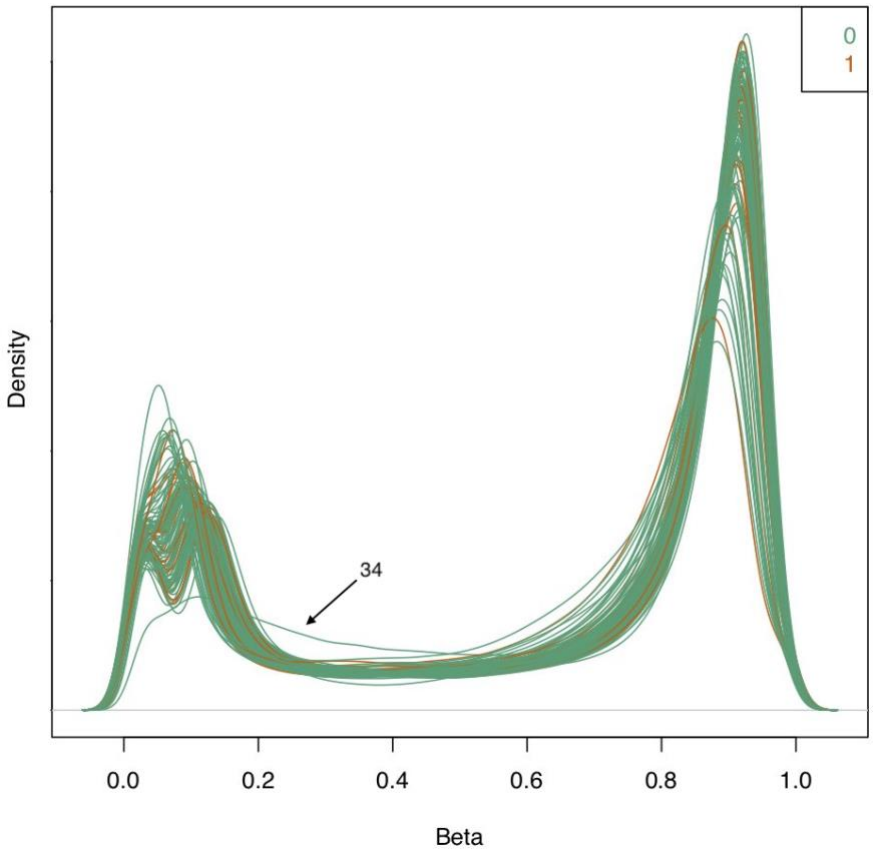
Following the conversion of raw IDAT data into methylation levels, otherwise known as β-values, we examined various quality control assessments. The probe intensity scatterplot, shown in **Figure 3.1**, in essence, plots the logarithm of the median intensity of the methylated signal against the logarithm of the median intensity of the unmethylated signal. Typically what is seen is that 'good' samples cluster together with high median methylated and unmethylated intensities, whereas 'bad' samples are located separate from the main cluster at lower medians (Fortin et al., 2015). Our results indicated that one sample, at index 34 in our list of subjects, was considered a 'bad' sample with both low methylated and unmethylated signal intensities.

Figure 3.1 | Probe intensity scatterplot quality control assessment. The log median methylated and unmethylated signal intensities were plotted. Samples of good quality appear to cluster with high signal intensities, whereas samples with bad quality are located separate from the main cluster with lower signal intensities. One sample with bad quality was identified and indicated in red with the sample index.



The β-value density plot is a visual representation that also allows for the identification of sample outliers with poor quality. Each line on the β-value density plot, shown in **Figure 3.2**, represents the density distribution of β-values throughout the genome. The two peaks in the distribution curve that are seen near 0.0 and 1.0 refer to the theoretical states of CpG sites being completely methylated or unmethylated. The number '0' and the associated green lines represent samples from subjects not reporting suicidal ideation. The number '1' and red lines correspond to those subjects with current suicidal ideation. We observed that there was an overall level of consistency in the density lines, though one sample showed poor quality. This was found to be from a subject without suicidal ideation and was the same identified from the previous quality control assessment. As a result, the sample at index 34 was automatically removed from analysis by subsequent functions to identify DMPs and DMRs.

Figure 3.2 | β-value density plot quality control assessment. The density distribution of methylation β-values throughout the genome-wide CpG sites are plotted. The number '0' and associated green lines correspond to subjects not reporting suicidal ideation, whereas the number '1'and associated red lines correspond to those reporting suicidal ideation. One sample showed inconsistency and was found to be at the same index as identified in previous quality controls.



3.4.3 Differentially Methylated Position (DMPs)

3.4.3.1 Binary Phenotype: Presence or Absence of Suicidal Ideation

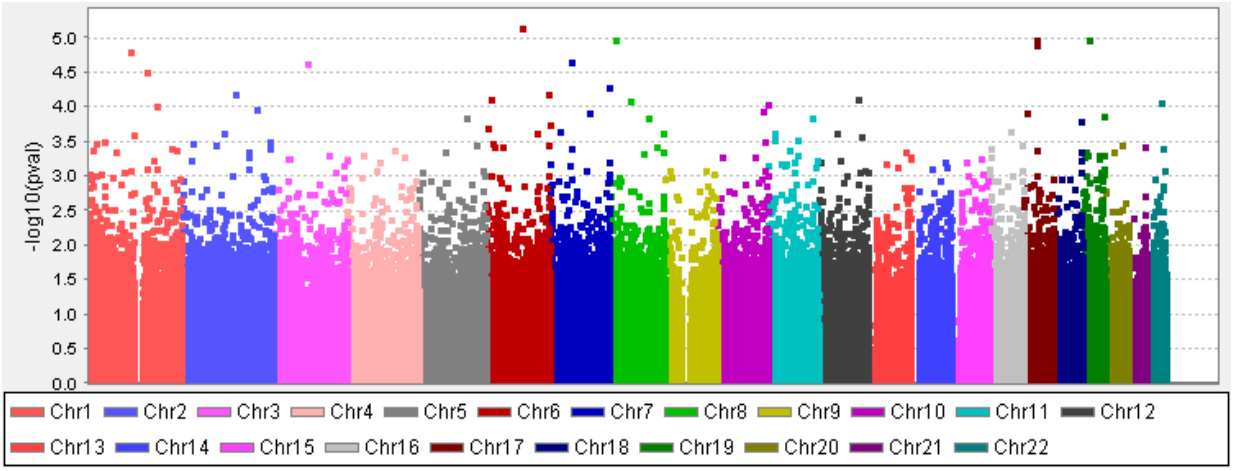
In our identification of DMPs with the *dmpFinder* function, we report the 20 most significant CpG sites that were found to be differentially methylated between subjects with and without suicidal ideation using the binary phenotype (**Table 3.2**). Furthermore, the association p-values for all CpG sites across the genome are represented graphically in a Manhattan plot depicted in **Figure 3.3**.

Table 3.2 | Top 20 DMPs associated with current suicidal ideation using a binary phenotype. These sites were found to be the most differentially methylated positions between subjects with and without current suicidal ideation. For each CpG site identified, the chromosome number, position, and gene, if applicable, were also included.

Chr	Position	CpG Site	Gene	P-Value	*β Coefficient*
6	97285662	cg14723344	*GPR63*	6.87E-06	-0.904
17	37774242	cg06074143	IGR	1.01E-05	-0.409
19	19036805	cg12315994	*DDX49*	1.04E-05	-0.663
8	17658561	cg20517154	*MTUS1*	1.05E-05	-0.911
17	33772796	cg00888402	*SLFN13*	1.26E-05	-1.547
1	113392580	cg27077219	*LINC01356*	1.55E-05	-1.042
7	55594131	cg19425773	*VOPP1*	2.22E-05	-0.851
3	94243571	cg16274205	IGR	2.33E-05	-0.808
1	153044071	cg13950674	*SPRR2B*	3.11E-05	-1.639
7	158330926	cg04064735	*PTPRN2*	5.13E-05	-1.166
2	143792380	cg03184584	*KYNU*	6.35E-05	0.479
6	168629778	cg01801443	IGR	6.49E-05	-0.573
12	107716576	cg07213698	*BTBD11*	7.51E-05	-0.771
6	12075367	cg15446704	*HIVEP1*	7.66E-05	-0.953
8	55380008	cg17993900	IGR	7.79E-05	-0.373
22	39408880	cg11413071	*APOBEC3C*	8.18E-05	-0.514
10	134511404	cg02250553	*INPP5A*	8.75E-05	0.740
1	180204221	cg05719164	*LHX4*	9.25E-05	-0.739
2	201399802	cg14551984	*SGOL2*	1.03E-04	0.645
10	124668751	cg09557726	*FAM24A*	1.11E-04	-0.393

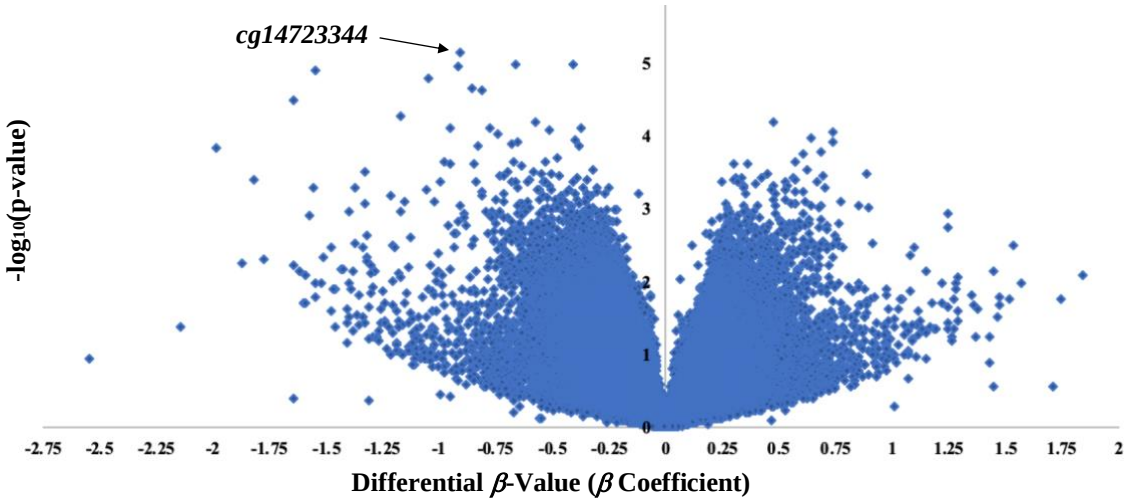
* Chr = chromosome number; Position = base-pair coordinate of the CpG site; P-Value = significance of the differentially methylated position associated with current suicidal ideation; β Coefficient = differential methylation β-value; IGR denotes an intergenic region located between genes.

Figure 3.3 | Manhattan plot of DMPs associated with current suicidal ideation using a binary phenotype. Scatterplot representation of the association p-values for genome-wide CpG sites that were differentially methylated, arranged in order based on chromosome number and position. The y-axis represents the negative logarithm of p-values.



The direction of methylation at individual CpG sites was determined by the differential methylation β-value, denoted as the β coefficient (Xie et al., 2019). Negative β coefficients were indicative of hypomethylation, whereas positive β coefficients represented hypermethylation in subjects with current suicidal ideation. A graphical, illustrative representation of the genome-wide sites is shown in the volcano plot in **Figure 3.4**; this type of scatterplot allows for the clear identification of methylation directionality.

Figure 3.4 | Volcano plot of DMPs associated with current suicidal ideation using a binary phenotype. This type of scatterplot also represents the association p-values for genome-wide CpG sites, though it also indicates the directionality of methylation. The arrow points to the most significant DMP that we identified.



Considering a binary phenotype, we determined that cg14723344, located within the *GPR63* gene on chromosome 6, was hypomethylated in subjects with current suicidal ideation. Furthermore, it appeared that the majority of the most significant DMPs were also hypomethylated in subjects experiencing suicidal ideation.

3.4.3.2 Continuous Phenotype: Suicidal Ideation Severity Score

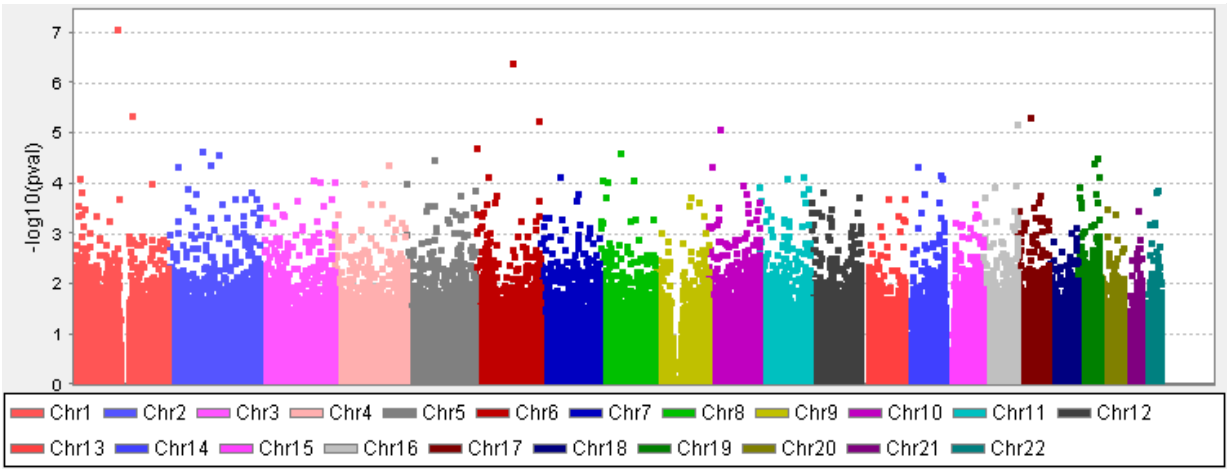
In addition to using a binary phenotype, we repeated the analysis with the *dmpFinder* function to identify DMPs between subjects with and without suicidal ideation considering their suicidal ideation severity scores. This continuous phenotype ranged from a score of zero to five, with increasing severity. The 20 most significant CpG sites that we identified using this approach are reported in **Table 3.3**. The association p-values for all CpG sites across the genome are also represented graphically in a Manhattan plot depicted in **Figure 3.5**.

Table 3.3 | Top 20 DMPs associated with current suicidal ideation using a continuous phenotype. These sites were found to be the most differentially methylated positions between subjects with and without current suicidal ideation. For each CpG site identified, the chromosome number, position, and gene, if applicable, were also included.

Chr	Position	CpG Site	Gene	P-Value	*β Coefficient*
1	113392580	cg27077219	*LINC01356*	7.85E-08	-0.600
6	97285662	cg14723344	*GPR63*	3.85E-07	-0.478
1	153044071	cg13950674	*SPRR2B*	4.08E-06	-0.851
17	33772796	cg00888402	*SLFN13*	4.54E-06	-0.766
6	168629778	cg01801443	IGR	5.47E-06	-0.306
16	89299756	cg27334271	IGR	6.39E-06	0.574
10	30692613	cg02903852	IGR	7.65E-06	0.302
6	5951562	cg12116564	IGR	1.92E-05	-0.257
2	88355002	cg06459916	*KRCC1*	2.20E-05	-0.305
8	55380008	cg17993900	IGR	2.37E-05	-0.188
2	132054158	cg05978071	*LOC440910*	2.43E-05	0.404
19	50411521	cg06477444	*NUP62*	2.86E-05	0.313
5	73112528	cg10572670	*RGNEF*	3.18E-05	0.161
19	44952798	cg17612420	*ZNF229*	3.85E-05	-0.283
4	143633738	cg06176987	*INPP4B*	3.98E-05	-0.413
2	113404678	cg06121808	*SLC20A1*	4.15E-05	0.160
10	8094860	cg00407546	*FLJ45983*	4.23E-05	-0.448
2	27232783	cg08398556	*MAPRE3*	4.32E-05	0.474
14	36003528	cg08226111	*INSM2*	4.51E-05	-0.288
14	94638510	cg19701560	IGR	6.54E-05	-0.241

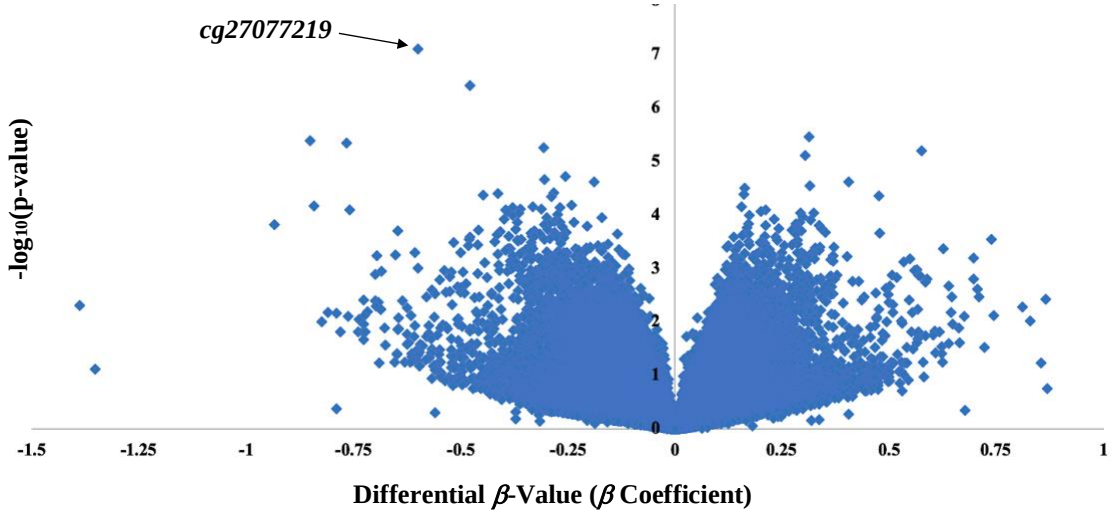
* Chr = chromosome number; Position = base-pair coordinate of the CpG site; P-Value = significance of the differentially methylated position associated with current suicidal ideation; β Coefficient = differential methylation β-value; IGR denotes an intergenic region located between genes.

Figure 3.5 | Manhattan plot of DMPs associated with current suicidal ideation using a continuous phenotype. Scatterplot representation of the association p-values for genome-wide CpG sites that were differentially methylated, arranged in order based on chromosome number and position. The y-axis represents the negative logarithm of p-values.



A graphical representation of the DMPs identified considering a continuous phenotype is shown in the volcano plot in **Figure 3.6**. We determined that cg27077219, located within the *LINC01356* gene on chromosome 1, was hypomethylated in subjects experiencing current suicidal ideation.

Figure 3.6 | Volcano plot of DMPs associated with current suicidal ideation using a continuous phenotype. This type of scatterplot also represents the association p-values for genome-wide CpG sites, though it also indicates the directionality of methylation. The arrow points to the most significant DMP that we identified.



3.4.4 Differentially Methylated Regions (DMRs)

In our investigation of DMRs (*bumphunter*), we identified 575 regions that were differentially methylated and associated with current suicidal ideation. A list of the 20 most significant DMRs are shown in **Table 3.4**. The methylation difference value represents the percent difference in methylation at a particular DMR between subjects with and without suicidal ideation. Positive methylation differences were indicative of a particular site being hypermethylated, and negative differences indicated hypomethylation of the DMR in subjects with current suicidal ideation. We report that a DMR located in chromosome 10 with a start position at 79655482 was hypermethylated in subjects with current suicidal ideation.

Table 3.4 | Top 20 DMRs associated with current suicidal ideation. These regions were found to be the most differentially methylated regions between subjects with and without current suicidal ideation. For each region identified, the chromosome and start position are also included.

Chr	Position	Methylation Difference (%)	P-Value
10	79655482	27.78	1.04E-03
1	2100232	27.65	1.14E-03
12	49074303	-26.56	1.66E-03
22	43168851	-23.77	4.89E-03
11	118022607	-23.54	5.41E-03
13	103423502	-23.53	5.41E-03
9	135937572	-22.90	7.59E-03
1	152572665	-15.32	7.80E-03
12	123757860	21.95	9.88E-03
1	152586240	-21.63	1.10E-02
2	12695930	21.56	1.12E-02
10	8952817	-21.55	1.12E-02
2	172374119	-21.57	1.12E-02
11	132992485	21.23	1.24E-02
12	31148661	-21.11	1.27E-02
1	153044071	-20.93	1.37E-02
8	125942777	-20.49	1.63E-02
9	140247365	20.25	1.76E-02
6	32774788	20.20	1.82E-02
6	158390146	20.18	1.83E-02

* Chr = chromosome number; Position = base-pair coordinate of the beginning of the DMR; Methylation Difference = difference in the methylation levels (%) between subjects with and without suicidal ideation; P-Value = significance of the differentially methylated region associated with current suicidal ideation.

3.5 Discussion

In the present study, we assessed differential DNA methylation across the genome at the level of individual positions (DMPs) and regions (DMRs). To the best of our knowledge, this study is the first to investigate genome-wide methylation in relation to suicidal ideation in schizophrenia. We identified several DMPs and DMRs associated with suicidal ideation. However, we were unable to conclude that these findings were significant at the genome-wide level. When applying a Bonferroni correction for 850,000 CpG sites, as in the DMP test, genome-wide significance would require a $p < 5.8E-08$. For the DMRs, the threshold would be less stringent. Considering that the human genome has approximately 30,000 CpG islands (Jeziorska et al., 2017), the genome-wide significance would require a $p < 1.6E-06$. Our top DMPs and DMRs were therefore only found to have suggestive levels of significance at the genome-wide level.

Despite these results, our study had several strengths. From a technical standpoint, we utilized the latest Infinium® MethylationEPIC BeadChip array with the most comprehensive coverage of 850,000 CpG sites, compared to previous generations of methylation arrays which covered only 450,000 and 27,000 sites. The MethylationEPIC array removed approximately 10% of CpG sites found in the 450K chip due to poor performance, and among others, added 333,265 CpG sites located on intergenic and gene enhancer regions (Solomon et al., 2018; Zaimi et al., 2018). Prior to the identification of DMPs and DMRs, we conducted an array of quality control assessments to identify and remove samples of poor quality, as well as extra steps to process and normalize methylation measures.

Furthermore, our study design considered both differentially methylated positions (DMPs) and regions (DMRs) in an annotation-free approach. We were not the first to utilize these methods, with another study identifying both DMPs and DMRs associated with psychotic experiences (Roberts et al., 2019). Nonetheless, it is a strength of this study. In fact, it was even suggested that both approaches be run in tandem, since individual DMPs are not necessarily evenly spaced across the genome, and in many cases are not located within 1 kbp of a neighboring site (Wright et al., 2016). While the identification of DMPs is certainly of interest, the region analysis is generally considered more robust than individual probes. Bump hunting for regions that are differentially methylated is more likely to identify differentially expressed genes than probing for individual CpG sites within the genome (Aryee et al., 2014). These would then have the potential to lead to

identification of downstream associated pathways and greater understanding of etiological factors contributing to suicidal ideation.

Our analyses should also be considered in light of limitations. Methylation levels are known to be influenced by several factors, including age, biological sex, smoking, and medication use, and it is therefore important to adjust for such covariates (Dunbar et al., 2019; Perrier et al., 2018). Furthermore, studies that are cross-sectional in nature are more likely to be confounded by these effects as opposed to longitudinal studies (Roberts et al., 2019). In the present analysis, we did not consider the effects of covariates due to limitations we experienced during statistical processing. However, since it is well understood that biological sex greatly affects the methylation profile on the X and Y sex chromosomes, only DMPs and DMRs located on autosomes were included. This, in our opinion, served as the biggest limitation of the present study.

While our study had a reasonable sample size, it has been suggested that for improved interpretation of genome-wide results, the sample size should be increased (Tsai et al., 2015). Furthermore, in the investigation of psychotic experiences mentioned above, the authors utilized a sample size of a total of 845 participants (Roberts et al., 2019). Further studies with increased sample sizes and appropriate incorporation of covariates are thus required.

3.6 Conclusion

We investigated individual sites and regions across the genome that were differentially methylated between subjects with and without suicidal ideation. While the present hypothesis-free study did not determine any positions or regions differentially methylated that were significant at the genome-wide level, our findings suggest trends toward significance. While epigenome-wide association studies are still in their infancy, further work is required to replicate, support, and validate our findings presented here. Furthermore, in this chapter, we describe a methodological stepwise approach to identify differentially methylated markers of suicidal ideation using the Bioconductor package minfi.

Chapter 4

4 Longitudinal DNA Methylation Changes in Emergent Suicidal Ideation

4.1 Abstract

Introduction: While suicidal ideation is known to precede suicide attempt, the new onset of suicidal ideation, or emergent suicidal ideation, greatly increases the risk. Furthermore, the transitions from ideation to attempt often occur within the year following onset of ideation. As an extension of the previous chapter, here we examine the association between longitudinal methylation change and emergent suicidal ideation.

Methods: A group of 8 subjects diagnosed with a schizophrenia spectrum disorder was assessed at a baseline visit and 3-month follow-up. The grouping variable in this study was the presence or absence of emergent suicidal ideation at the follow-up visit. Genome-wide DNA methylation was assessed with the Infinium® MethylationEPIC BeadChip, and methylation levels at over 850,000 CpG sites were estimated using GenomeStudio and minfi analysis tools. The change in methylation levels over the 3-months at individual CpG sites was calculated, and tested for association with emergent suicidal ideation status.

Results: We report that the estimation of methylation levels using GenomeStudio and minfi yielded similar results. Furthermore, the methylation change in several CpG sites, including cg06371916 and cg03759077 located within the coding regions of the ADK and ARL6IP6 genes, respectively, was associated with emergent suicidal ideation.

Discussion/ Conclusion: This preliminary study is exploratory in nature, yet it provides proof-of-concept to an epigenetic method of identifying schizophrenia patients with emergent suicidal ideation. Further large-scale studies investigating a wider range of factors are needed to provide improved interpretation of methylation changes, and have the potential to yield insight into specific pathways related to suicidality and potential links with stress.

4.2 Introduction

As previously mentioned, this is an extension of the previous chapter in that we investigated longitudinal methylation changes in association with emergent suicidal ideation. There is a great urgency to consider emergent suicidal ideation. While having ideation is indeed a risk factor for future suicide, not everyone who has thoughts of suicide will proceed to make a suicide attempt (Klonsky et al., 2016). However, when individuals who have not previously experienced suicidal ideation report that they experience ideation "nearly every day," it was found that they had a sevenfold increase in risk for suicide (Simon et al., 2017). Furthermore, it was determined that over 60% of the transitions from suicidal ideation to suicide attempt occurred within the first year following onset of ideation (Nock et al., 2008). Therefore, emergent suicidal ideation is of especially grave concern, and as such, it is imperative to identify those schizophrenia patients most at risk for suicide.

Regarding methylation- in the previous chapter, we investigated differentially methylated positions and regions that were associated with current suicidal ideation status. By the same logic, we aim to extend those findings and assess longitudinal changes in DNA methylation that are associated with emergent suicidal ideation. A recent study examining the variation in DNA methylation over a one-year period found that the intra-individual similarity in methylation was statistically significant, and the methylation profiles measured a year later clustered tightly with baseline measures (Zaimi et al., 2018). With these findings in consideration and assuming all other factors remain the same, we would theoretically be able to identify changes in methylation that can be attributed directly to the development of emergent suicidal ideation.

Studies have investigated longitudinal changes in DNA methylation during the onset of psychosis and time preceding psychotic experiences (Kebir et al., 2017; Roberts et al., 2019), and have identified several associated CpG sites that have altered methylation. However, to the best of our knowledge, this study will be the first to investigate longitudinal methylation changes associated with emergent suicidal ideation. The identification of CpG sites that could potentially serve as biomarkers for the onset of suicidal ideation would translate to clinical utility in the early identification of at-risk schizophrenia patients.

4.3 Methods

4.3.1 Participants

The following study comprised of a cohort of 8 individuals, part of a larger scale study at the Centre for Addiction and Mental Health (CAMH). The same inclusion and exclusion criteria mentioned in previous chapters applied and are briefly summarized. All participants were between the ages of 18 and 75 and had a diagnosis of a schizophrenia spectrum disorder, but were excluded if they had a history of head trauma with loss of consciousness, or had a diagnosis of an intellectual disability, major neurological disorder, or substance-induced psychosis. This study was approved by the CAMH Research Ethics Board.

Emergent suicidal ideation status was the grouping phenotype in this study, and was assessed longitudinally at a baseline visit and 3-month follow-up using the Columbia- Suicide Severity Rating Scale (C-SSRS; Posner et al., 2011). Subjects with new or worsening suicidal ideation at the follow-up visit were categorized as having emergent suicidal ideation. The remaining subjects, without new or worsening suicidal ideation, will hereinafter be referred to as the 'non-emergent' group. Data regarding age and sex were also collected.

4.3.2 Sample Collection and DNA Methylation

For each participant, approximately 8 mL of venous blood was collected in BD Vacutainer® Plus EDTA tubes (Becton, Dickinson, and Company) at both the baseline and follow-up visit. Genomic DNA was extracted from white blood cells with the QIAamp® DNA Blood Maxi kit (QIAGEN Inc.), and 500 ng of DNA was treated with sodium bisulfite using the EZ DNA Methylation Kit (Zymo Research). The process of bisulfite treatment specifically involves the conversion of non-methylated cytosine residues to uracil, while maintaining the methylcytosines and hence, original methylation state for further assays (Zhang et al., 2009). Genome-wide DNA methylation was then quantified with the Infinium® MethylationEPIC BeadChip (Illumina), interrogating over 850,000 CpG loci with single-nucleotide resolution. The iScan® System (Illumina) used a pair of fluorescent dyes to recognize the bisulfite-converted DNA, and outputted the summary intensities for each sample as IDAT files (Li et al., 2015; Smith et al., 2013). The bisulfite conversion and MethylationEPIC array were conducted by The Centre for Applied Genomics (TCAG) at the Hospital for Sick Children.

4.3.3 Methylation Data Preprocessing

The MethylationEPIC array data ultimately allows one to estimate the β-value, which can be loosely interpreted as the percentage of cells that are methylated at a particular CpG site (Perrier et al., 2018). These values can theoretically range from 0, when all copies of a particular CpG site are unmethylated, to 1, when all copies are methylated. The β-value at a particular CpG site can be calculated as the methylated probe intensity divided by the sum of the methylated and unmethylated intensities. From a more mathematical perspective, this can be calculated as:

$$\beta = \frac{\max(y_{i,methy}, 0)}{\max(y_{i,methy}, 0) + \max(y_{i,unmethy}, 0) + \alpha}$$

where the terms $y_{i,methy}$ and $y_{i,unmethy}$ represent the intensities of the i_{th} methylated and unmethylated probes, respectively, and α, usually set to 100, is a constant offset value to stabilize β when both methylated and unmethylated intensities are low (Du et al., 2010). The specific probe intensities used for these calculations are located within the IDAT files associated with each sample. Furthermore, before downstream statistical analyses, β-values are typically preprocessed using a wide variety of recognized methods (Li et al., 2015). For the present study, we utilized β-values generated from the GenomeStudio® Methylation Module v.1.8 (Illumina) and the minfi Bioconductor package run within the R-3.5.1 (64 bit) statistical analysis environment. Due to the nature of the different analytical methods, it was expected that there were slight differences between the β-values for the interrogated CpG loci.

Here, we will briefly summarize the preprocessing methods utilized in this study. GenomeStudio implemented a background subtraction method and a step for normalization to internal controls. The average signal of a series of built-in negative controls was subtracted from all individual probe intensities across the genome, thus minimizing the amount of variation in background signals (Illumina, 2010). To reduce non-biological variation (i.e. those related to experimental artifacts, random noise, and technical variation inherently present in microarray technology), a normalization factor, determined by 90 pairs of normalization control probes in a reference sample, was incorporated into all CpG loci probe intensity values (Illumina, 2010; Wilhelm-Benartzi et al., 2013).

The preprocessing approach conducted in the minfi package was significantly more complex, and involved a stratified quantile normalization method using the *preprocessQuantile* function (Aryee et al., 2014; Touleimat et al., 2012). While beyond the scope of this study, the Illumina platform, by design, consisted of Type I and Type II probes for each interrogated CpG site. Type I probes had two probe sequences to represent methylated and unmethylated sites, while Type II probes only had one probe sequence per CpG site (Pidsley et al., 2016). Minfi preprocessing involved quantile normalizing Type II probes across samples, and subsequently estimating a reference distribution to normalize Type I probes (Touleimat et al., 2012). This approach was chosen over other preprocessing approaches available in the minfi package, due to greatly improved performance with respect to bias correction and methylation signal estimation (Touleimat et al., 2012). Both analytical methods each resulted in an estimation of β-values for the approximately 850,000 genome-wide CpG loci for 16 samples, taking into account two visits for each of the 8 subjects.

4.3.4 Statistical Analysis

β-values generated from both GenomeStudio and minfi preprocessing were separately subjected to the following series of statistical analyses. In order to examine the longitudinal changes in DNA methylation for each subject, we calculated the difference between methylation levels between the baseline and 3-month follow-up visit (baseline measurement subtracted from that of the 3-month follow-up). We then used an independent samples *t*-test, a commonly used method in genomic data analysis, to identify equivalence of means in β-value change between the emergent and non-emergent suicidal ideation groups (Li et al., 2015). We used the open-source Haploview v.4.2 software package to visualize population haplotype patterns in the form of Manhattan plots (Barrett et al., 2005); this allowed us to graphically represent all CpG loci covered in the MethylationEPIC array and their location throughout the genome, along with their relevant levels of significance.

Due to the exploratory nature of the present study, a genome-wide statistical significance determined with a Bonferroni correction for 850,000 CpG sites would be considered too conservative ($p < 5.8 \times 10^{-8}$). Instead, we allowed tests with a $p < 5.0 \times 10^{-5}$ to be indicative of reaching levels of significance, as was suggested in another recent longitudinal methylation study

(Roberts et al., 2019). Furthermore, to graphically represent the deviation of observed p-values in the association of longitudinal DNA methylation changes and emergent suicidal ideation, compared to expected p-values determined from a theoretical $\chi 2$-distribution (Ehret, 2010), we created quantile-quantile (Q-Q) plots in the R- 3.5.1 environment.

4.4 Results

4.4.1 Demographic and Clinical Variables

In the present study, we included 4 subjects with and 4 subjects without emergent suicidal ideation. The summary of demographic and clinical variables of these subjects are reported in **Table 4.1**. P-values were calculated to identify group differences between those with and without emergent suicidal ideation, using the Mann Whitney U test for continuous variables and the Chi-square test for categorical variables. The change in β-values in the approximately 850,000 genome-wide CpG loci were then tested for associations with emergent suicidal ideation.

Table 4.1 | Demographic and clinical variables of subjects in the emergent suicidal ideation methylation cohort. These variables were tested for group differences between subjects with and without emergent suicidal ideation.

Total (N = 8)	**Emergent** (n = 4)	**Non-Emergent** (n = 4)	**P-Value**
Gender (male/ female)	1/3	2/2	0.465
Age (years)	45.7 ± 5.6	38.2± 9.5	0.224
Age-of-Onset (years)	25.2 ± 7.5	21.5 ± 5.4	0.449
Duration-of-Illness (years)	20.5 ± 9.9	16.7 ± 12.2	0.652
Δ BPRS (Brief Psychiatric Rating Scale)	0.0 ± 6.2	1.0 ± 5.0	0.811
Δ CDSS (Calgary Depression Scale for Schizophrenia)	1.2 ± 2.0	-0.5 ± 4.9	0.536
Δ BHS (Beck Hopelessness Scale)	1.2 ± 3.6	-1.0 ± 0.8	0.278
Δ PSS (Perceived Stress Scale)	-0.2 ± 9.3	-5.7 ± 6.3	0.369
Δ SAI (Schedule for Assessment of Insight)	0.2 ± 1.5	-0.5 ± 1.0	0.437
Δ MMSE (Mini-Mental State Examination)	-0.5 ± 3.1	-0.2 ± 2.0	0.897

*Demographic and clinical values presented as mean ± standard deviation.

4.4.2 GenomeStudio

Using the β-values generated from the GenomeStudio preprocessing method, we found 46 CpG sites that had significant differences in methylation change between the emergent and non-emergent groups. The 10 most significant CpG sites are listed in **Table 4.2**. We report that a decrease in methylation level at CpG cg06371916, located on chromosome 10, was associated with the development of emergent suicidal ideation at the 3-month follow-up (p = 6.79 E-07). This CpG site was located within the coding region of the *ADK* gene.

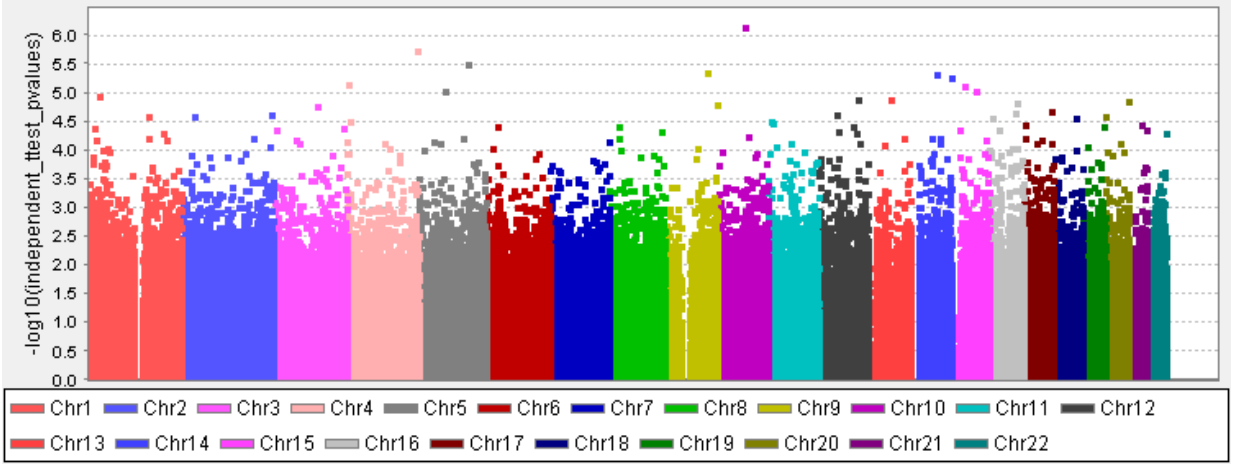
Our results using GenomeStudio methylation estimates are visually represented in the Manhattan plot shown in **Figure 4.1**. CpG sites located within the 22 autosomes are arranged on the x-axis in accordance with their respective chromosomal positions, while the y-axis indicates the negative logarithm to base 10 of the p-values calculated from our independent samples t-test. Individual points on the Manhattan plot represent single CpG sites. Considering our established threshold of $p < 5.0 \times 10^{-5}$ for statistical significance, any points exceeding 4.3 seen on the y-axis would be considered significant. As previously mentioned, we found 46 CpG sites that met this criterion, and are plotted accordingly.

Table 4.2 | Top 10 CpG sites with methylation change significantly associated with emergent suicidal ideation using the GenomeStudio preprocessing method. Emergent suicidal status was determined at a 3-month follow-up visit.

Chr	Position	CpG Site	Gene	Emergent Δ%	Non-Emergent Δ%	P-Value
10	76070313	cg06371916	*ADK*	-0.489	1.562	6.79 E-07
4	186953239	cg00953728	IGR	-1.962	0.659	1.82 E-06
5	131983498	cg20561758	*TH2LCRR*	-1.580	1.307	3.04 E-06
9	116856199	cg13833988	*KIF12*	0.910	-2.015	4.22 E-06
14	69454981	cg06881432	IGR	2.531	-2.244	4.67 E-06
14	107131940	cg09441852	IGR	3.893	0.017	5.27 E-06
4	2819707	cg01710886	*SH3BP2*	-1.929	1.896	7.01 E-06
15	34807143	cg12600289	IGR	-2.651	0.549	7.54 E-06
5	72742787	cg23454038	*FOXD1*	-2.174	0.818	9.22 E-06
15	68498251	cg05338167	*CALML4*	-3.048	1.775	9.24 E-06

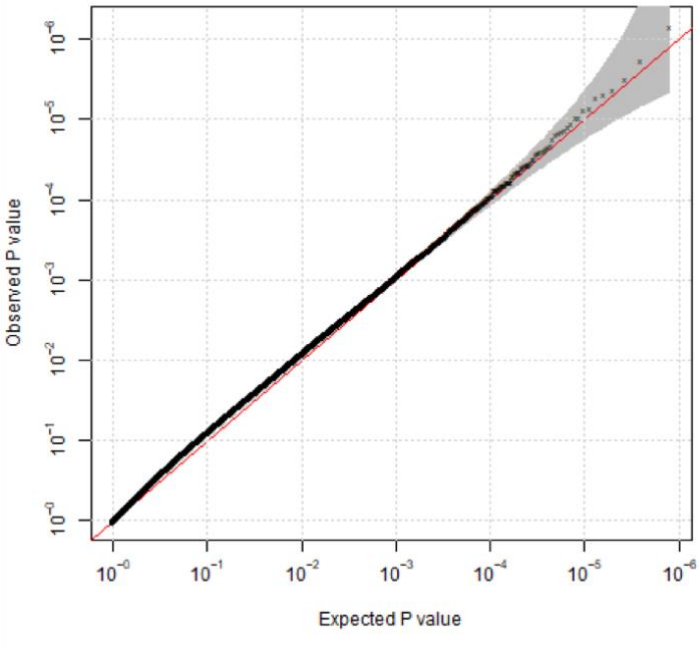
* Chr = chromosome number; Position = base-pair coordinate of the CpG site; Emergent Δ% = percent change in methylation after 3 months in subjects with emergent suicidal ideation; Non-Emergent Δ% = percent change in methylation after 3 months in subjects without emergent suicidal ideation; P-Value = significance of the association with emergent suicidal ideation; IGR denotes an intergenic region located between genes.

Figure 4.1 | Manhattan plot of genome-wide methylation changes, estimated with GenomeStudio. Scatterplot representation of the association p-values between subjects with and without emergent suicidal ideation, arranged in order based on chromosome number and position. The y-axis represents the negative logarithm of p-values.



The Q-Q plot of the observed quantiles of p-values for each CpG site association test, against the distribution of expected p-values is shown in **Figure 4.2**. Graphically, we see that the observed distribution of p-values corresponded well to expected values, without early separation from the line $y = x$, representing the null hypothesis that the data are normal. This indicates that the significant CpG sites we identified, shown as individual points to the left of the line, can be accurately considered true positives.

Figure 4.2 | Q-Q plot of genome-wide methylation changes, estimated with GenomeStudio. Association test between subjects with and without emergent suicidal ideation.



4.4.3 Minfi

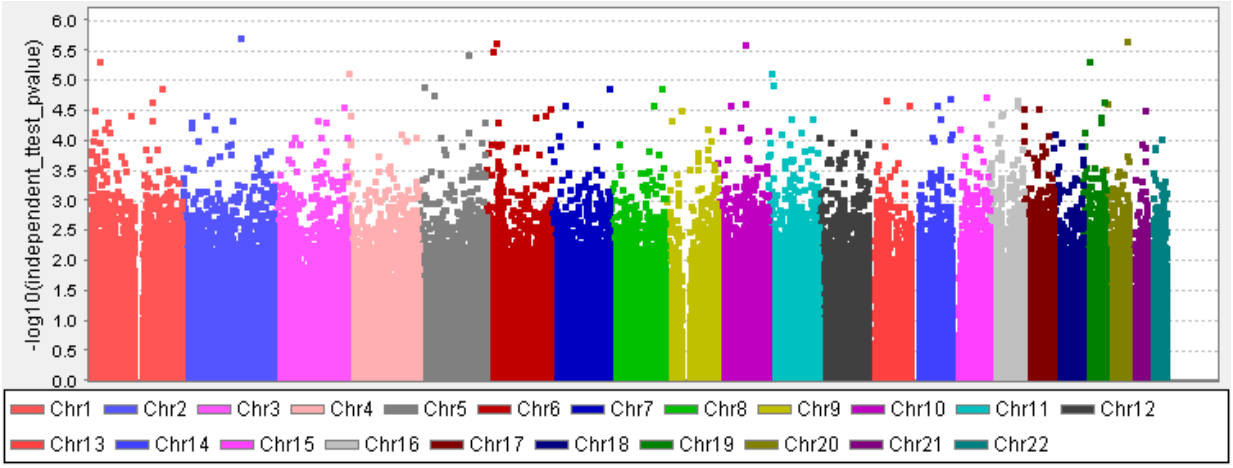
In comparison, when we utilized methylation β-values generated by minfi preprocessing, we found 59 CpG sites that had significant differences in methylation change between emergent and non-emergent groups. The 10 most significant CpG sites are listed in **Table 4.3**. We found that a decrease in methylation level at cg03759077, located on chromosome 2, was associated with the development of emergent suicidal ideation at the 3-month follow-up (p = 1.861 E-06). This CpG site was located in the coding region of the *ARL6IP6* gene. **Figure 4.3** shows the Manhattan plot of the results based on methylation levels estimated by minfi.

Table 4.3 | Top 10 CpG sites with methylation change significantly associated with emergent suicidal ideation using the minfi preprocessing method. Emergent suicidal status was determined at a 3-month follow-up visit.

Chr	Position	CpG Site	Gene	Emergent Δ%	Non-Emergent Δ%	P-Value
2	153575599	cg03759077	*ARL6IP6*	-0.331	0.992	1.86 E-06
20	60531577	cg24959147	IGR	-0.575	3.847	2.08 E-06
6	26233442	cg17866778	IGR	1.631	-3.447	2.17 E-06
10	76070313	cg06371916	*ADK*	-0.615	1.888	2.41 E-06
6	16450762	cg02052569	*ATXN1*	1.109	-1.469	3.13 E-06
5	131983498	cg20561758	*TH2LCRR*	-1.842	1.599	3.45 E-06
19	15083818	cg10385290	*SLC1A6*	1.457	-0.927	4.45 E-06
1	26825530	cg23731663	IGR	-2.306	1.112	4.62 E-06
11	11862470	cg24787405	*USP47*	-1.841	1.806	7.25 E-06
4	2819707	cg01710886	*SH3BP2*	-1.564	1.397	7.37 E-06

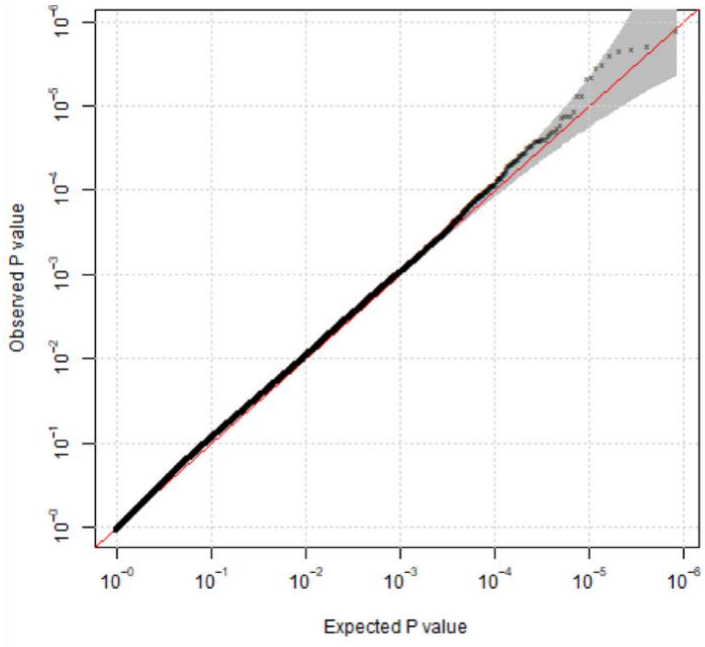
* Chr = chromosome number; Position = base-pair coordinate of the CpG site; Emergent Δ% = percent change in methylation after 3 months in subjects with emergent suicidal ideation; Non-Emergent Δ% = percent change in methylation after 3 months in subjects without emergent suicidal ideation; P-Value = significance of the association with emergent suicidal ideation; IGR denotes an intergenic region located between genes.

Figure 4.3 | Manhattan plot of genome-wide methylation changes, estimated with minfi. Scatterplot representation of the association p-values between subjects with and without emergent suicidal ideation, arranged in order based on chromosome number and position. The y-axis represents the negative logarithm of p-values.



The Q-Q plot of the observed quantiles of p-values for each CpG site association test, against the distribution of expected p-values is shown in **Figure 4.4**. Similar to what was seen for the Q-Q plot for GenomeStudio, we see that the observed distribution of p-values corresponded well to expected values. Again, we can accept the null hypothesis that the data represent a normal distribution. Significant CpG sites that we identified, shown as individual points to the left of the line, can accurately be considered true positives.

Figure 4.4 | Q-Q plot of genome-wide methylation changes, estimated with minfi. Association test between subjects with and without emergent suicidal ideation.



4.5 Discussion

In the present study, we reported longitudinal methylation changes in individual CpG sites that were associated with emergent suicidal ideation. In addition, we utilized two bioinformatic tools, GenomeStudio and minfi, to handle and preprocess raw methylation data. To briefly summarize our results, we found that decreases in methylation in cg06371916 located within the *ADK* gene (GenomeStudio) and cg03759077 located with the *ARL6IP6* gene (minfi) were significantly associated with the development of emergent suicidal ideation. Furthermore, we found 3 CpG sites that were significant using both analytical methods. Upon examining the Q-Q and Manhattan plots, graphically we did not notice any observable differences or bias in the results presented by GenomeStudio and minfi, though specific statistical tests would be required to confirm. It is also important to acknowledge several factors impacting our current study, associated limitations, future study approaches, and interpretations of findings in a broader context.

Regarding the genes that the CpG sites were located within- previous studies have not identified *ADK* and *ARL6IP6* to be associated with suicidality. The *ADK* gene codes for adenosine kinase, a purine salvage enzyme in eukaryotes that works by catalyzing the phosphorylation of adenosine to adenosine monophosphate (Lu et al., 2009). The *ARL6IP6* gene encodes the ADP-ribosylation-like factor 6 interacting protein 6 and was previously found to be differentially expressed between radiosensitive and radioresistant cancers, though the specific function of the gene is currently unknown (Hou et al., 2014). In light of our 'novel' findings, the significance of CpG site methylation within these genes should be taken cautiously. Due to the exploratory nature of the current study, we anticipated that CpG sites would not reach the level of genome-wide significance, $p < 5.8 \times 10^{-8}$, when applying a Bonferroni correction. However, we did find a number of CpG sites that exceeded a more lenient threshold suggested by Roberts et al. (2019). Nonetheless, these findings would prove worthwhile in the investigation of specific pathways with gene enrichment analysis, in relation to emergent suicidal ideation.

There are many factors that are known to influence DNA methylation measurements in individuals, including, but not limited to age, sex, smoking, medication use, and other environmental exposures (Perrier et al., 2018). For instance, studies have found an overall genome-wide decrease in methylation during aging, and increased variability at certain CpG sites were found to be associated with age (Horvath et al., 2012; Wang et al., 2018). Biological

speaking, males were shown to have slightly higher overall methylation levels than females, despite considering the effect of hypermethylation on the X-chromosome involved in female X-chromosome inactivation (El-Maarri et al., 2007). Smoking is widely known to be associated with an overall reduced methylation profile (Tsai et al., 2018). The use of antipsychotics and antidepressants, such as clozapine and citalopram, have been shown to alter the methylation status at many CpG sites across the entire genome (Kanherkar et al., 2018; Kinoshita et al., 2017). Furthermore, other environmental factors, including early life stressors, are known to influence DNA methylation and have lasting effects that persist into adulthood (McGowan et al., 2009; Vidrascu et al., 2019). In the present study, we attempted to correct for the covariate effect of sex, and excluded CpG sites found on sex chromosomes from analyses. Further covariates should also be considered, specifically in relation to smoking, medications, and body mass index. The prevalence of smoking is considerably higher in patients with schizophrenia (Šagud et al., 2009), and can be measured with the Fagerström Test for Nicotine Dependence (FTND; Fagerström, 1978). Antipsychotic medications are often prescribed for the management of symptoms in schizophrenia, and current medications can be standardized and quantified in terms of chlorpromazine equivalents (CPZe; Gardner et al., 2010). Additionally, various types of stressors, including childhood trauma measured through the Childhood Trauma Questionnaire (CTQ; Bernstein et al., 1994) and recent stress measured with the Social Readjustment Rating Scale (SRRS; Holmes et al., 1967), could also be taken into account in future studies.

One of the biggest strengths of our work is the longitudinal prospective nature of the study. The majority of studies investigating psychotic disorders and methylation thus far have taken a cross-sectional approach, which allows for increased likelihood of confounding effects on methylation (Roberts et al., 2019). Intra-individual longitudinal variations can be seen as more suitable for reflecting the dynamic nature of methylation, rather than cross-sectional analyses (Kebir et al., 2017).

Much previous research has focused largely on the dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is a neuroendocrine system involved in the adaptive response to stress, allowing one to rapidly respond to stressful events when required (Smith et al., 2006). After the given stressor has been resolved, the glucocorticoid receptor, encoded by the Nuclear Receptor Subfamily 3 Group C Member 1 (*NR3C1*) gene, exerts negative feedback regulation to

reduce the stress response accordingly to basal levels (Stephens et al., 2012). However, hyperactivity of the HPA axis has been linked to a 4.5-fold increase in suicide risk (Mann et al., 2007). Therefore, in addition to the evidence of early stressors impacting DNA methylation patterns, we should also investigate the effect of recent stress in relation to methylation and suicidality. Statistical mediation analysis would help to better understand the relationship among methylation, stress, and suicidality.

Finally, one of the most obvious limitations to this study was the small sample size, with 4 subjects in each of the emergent and non-emergent groups. Despite the high costs associated with genome-wide methylation arrays, it has been suggested that larger scale studies are required for appropriate design and interpretation of genome-wide results (Tsai et al., 2015).

4.6 Conclusion

This genome-wide study found that longitudinal changes in methylation at certain CpG sites were associated with emergent suicidal ideation. However, the results of our present study should be taken with caution, especially considering the small sample size. Further work can be done to include additional relevant covariates, investigate specific pathways, conduct mediation analyses with stress, and increase the sample size to achieve the level of genome-wide significance. However, this preliminary and exploratory study provides a feasible, methodological approach to identify epigenetic biomarkers that can have the potential to identify subjects at future risk of developing suicidal ideation in psychosis.

Furthermore, this longitudinal methylomic approach can be useful for a better understanding of possible molecular mechanisms related to the development of suicidal ideation in psychosis and to monitor proof-of-concept interventions aiming at reducing suicidal ideation in schizophrenia.

Chapter 5

5 General Discussion

5.1 Discussion

Suicide is one of the leading causes of premature mortality in patients with schizophrenia (Ventriglio et al., 2016). While suicidal ideation is considered an important early warning sign of future suicide attempts, emergent suicidal ideation, although not always apparent, is cause for even greater concern (Hocaoglu et al., 2009). For instance, a study found that approximately 60% of the transitions from suicidal ideation to attempt occurred within one year immediately following onset of ideation (Nock et al., 2008). As such, it is imperative to identify patients with current and emergent suicidal ideation.

This thesis has been organized in a way to first introduce the clinical factors related to suicide and suicidal ideation. This included a thorough investigation regarding the effects of certain stressors such as childhood trauma and recent stressful life events on current and emergent suicidal ideation. Afterwards, we attempted to identify epigenetic markers of suicidal ideation through cross-sectional and longitudinal approaches.

To summarize our findings, we reported that health-related stress and childhood trauma (emotional abuse, physical abuse, and physical neglect) were each significant predictors of current suicidal ideation. However, after adjusting for psychosis, depression, hopelessness, and perceived stress as covariates, the significance of each predictor was no longer evident. This implied that the combined effect of the covariates was greater than each of the individual predictors we tested. With regards to emergent suicidal ideation at the one-year follow-up, we found that the increases in both total and health-related stress were significant predictors of emergent suicidal ideation. After correcting for age-of-onset and psychosis severity as covariates, these predictors remained significant. Direct interpretation of our results was that for every 10 LCU increase on the SRRS scale for total stress, there was a 9.3% increase in the odds of developing emergent suicidal ideation, whereas for every 10 LCU increase in health-related stress, it increased the odds of emergent suicidal ideation by 30%.

With regards to the series of methylation analyses, we identified several differentially methylated positions (DMPs) and regions (DMRs) associated with current suicidal ideation. However, we found that none of the DMPs and DMRs reached levels of genome-wide significance, but had suggestive levels of significance. In a longitudinal analysis, we found that changes in DNA methylation at several CpG sites were significantly associated with emergent suicidal ideation. However, those sites were located within genes that were not previously known to be associated with suicidal behavior.

While detailed findings and a thorough discussion are included after each individual study in the above chapters, we would like to focus attention to several points in the general study. These points will be separately discussed below, as many times, there is overlap between the three studies.

5.1.1 Disclosure of Suicide and Suicidal Thoughts

Measures to prevent suicide are often dependent on the willingness or ability of individuals to disclose suicidal thoughts (Mérelle et al., 2018). From a clinical perspective, there are a variety of factors, motivations, and stigma that can influence an individual's decision or ability whether to disclose information relating to suicide and ongoing suicidal thoughts. For instance, people from countries and cultures where suicide is prohibited may underreport suicide and suicidal ideation and be less likely to seek help due to the fear of persecution or even being stigmatized for feeling suicidal (Klonsky et al., 2016). Especially relevant in the psychiatric setting is the fact that patients will often attempt to deny suicidal thoughts, albeit mild, to avoid unwanted interventions, such as involuntary psychiatric holds, or to facilitate an early discharge from the hospital (Blanchard et al., 2018; Nock et al., 2007). Another factor that was significantly associated with non-disclosure was social loneliness (Mérelle et al., 2018).

In other unintentional cases, individuals may not necessarily be aware of the thoughts and feelings associated with suicidality, and lack the ability to inform others and seek help (Nock et al., 2007). Suicidal ideation can range in intensity from fleeting thoughts of death to an "intense delusional preoccupation with self-destruction" (Goldney et al, 1989). It could be that during clinical interviews, patients do not experience ideation, though those feelings resurface upon release from the hospital (Nock et al., 2007). Interestingly, it was found that suicide risk was elevated during

the one week following discharge from a psychiatric hospital (Carlborg et al., 2010; Prinstein et al., 2008).

These issues make it significantly more difficult to address suicide in patients who do not disclose ideation, but in reality, are at high risk and need the help. In this study, we investigate alternative methods of determining and predicting an individual's current or emergent suicidal ideation status though an examination of stressors encountered as a child or recent, as well as DNA methylation markers. At the bare minimum, those predicted to be at higher risk would be further evaluated to ensure individual's safety and well-being.

5.1.2 DNA Methylation as a Peripheral Biomarker

DNA methylation has been commonly discussed in the context of cancer. In fact, there are already two approved epigenetic drugs, azacytidine and decitabine, that work by removing DNA methylation marks and re-activating tumor suppressor genes (Li et al., 2015). While the development of a drug that alters DNA methylation patterns to reduce suicide and suicidal ideation is too futuristic at the current state of research, DNA methylation could serve as a biomarker to identify those individuals most at risk.

Much research has been conducted in the field of DNA methylation and suicide. Studies have found that the levels of the glucocorticoid receptor gene NR3C2 mRNA in postmortem human hippocampal samples were lower in psychiatric suicide victims compared to psychiatric controls who died of unrelated accidental causes (McGowan et al., 2009). The decreased mRNA levels were attributed to hypermethylation at several CpG sites within the promoter region of the gene (Labonté et al., 2012; McGowan et al., 2009).

Furthermore, a genome-wide screen of DNA methylation profiles revealed, also in postmortem brain tissue, that elevated levels of a CpG site located within the *SKA2* gene promoter was associated with suicide (Guintivano et al., 2014). The trend in previous work has been the study of completed suicide attempt in postmortem human brain tissue. To assess for suicidal ideation and emergent suicidal ideation in living patients, it certainly would not be feasible to collect a brain biopsy. An alternative is the identification of peripheral biomarkers in the blood, yet this brings up the question of whether DNA methylation markers in the brain are similar to that found in blood.

A study conducted by Braun et al., (2019) determined that the overall levels of genome-wide methylation correlated well between the blood and brain (r = 0.86). Furthermore, using a website they developed from their study, methylation in the blood and brain for the gene *NR3C1* (r = 0.77) and *SKA2* (r = 0.92) were identified to be fairly highly correlated (Braun et al., 2019). These findings indicate that methylation profiles in the blood has the potential to serve as an acceptable surrogate for methylation in brain tissue (Braun et al., 2019).

5.1.3 Longitudinal DNA Methylation and Confounding Variables

The vast majority of current studies on DNA methylation and psychiatric disorders have utilized a cross-sectional approach (Roberts et al., 2019). However, two noteworthy studies have adopted a longitudinal DNA methylation approach to investigate changes preceding psychotic experiences (Roberts et al., 2019) and changes during the conversion to psychosis (Kebir et al., 2017). It has been observed that the use of these longitudinal study designs, especially with psychotic patients, is less likely to include confounding variables, notably antipsychotic regimen (Roberts et al., 2019).

First and foremost, there are many factors that affect DNA methylation, and include age, sex, smoking, and medication use, among others (Perrier et al., 2018). Age is known to be associated with an overall genome-wide decrease in methylation and increased variability at certain CpG sites (Horvath et al., 2012; Wang et al., 2018). In terms of sex, males have a slightly higher overall level of methylation, though females have hypermethylation of the X chromosome due to X-chromosome inactivation (El-Maarri et al., 2007). Smoking is also known to reduce the overall methylation profile (Tsai et al., 2018). The most notable and directly applicable in a population of psychotic patients, is the use of antipsychotics which alter the numerous CpG sites across the entire genome (Kanherkar et al., 2018; Kinoshita et al., 2017).

In both of our studies relating to methylation, the largest limitation was that we failed to correct for these confounding variables. This was especially applicable to the cross-sectional analysis for current suicidal ideation, since patients were only assessed at a single time. For longitudinal studies (i.e. emergent suicidal ideation), however, we believe that the effect of covariates would be reduced. If there were no factors relating to a patient's lifestyle or antipsychotic dosage that

had changed over the course of the 3-month follow-up, we would be able to cautiously assume that the effect of the confounding variables was negated.

Furthermore, a recent study evaluated the variation in DNA methylation in human blood over a one-year period, and found statistically significant intra-individual similarity in methylation ($p < 0.001$) (Zaimi et al., 2018). This can be interpreted as additional support for our longitudinal study design. Assuming in a perfect scenario where nothing in an individual's life changed within the longitudinal evaluation period, we would expect to see minimal baseline variation. However, if an individual developed emergent suicidal ideation, we could theoretically identify those changes and attempt to associate them with the change in ideation. One of the biggest strengths of our study is that we considered methylation from both a cross-sectional and longitudinal approach.

5.1.4 Mediation and Moderation Models

As previously alluded to with the *NR3C1* gene and HPA axis, stress is an important factor associated with emergent suicidal ideation. Furthermore, in our longitudinal methylation analysis, we identified several significant CpG sites in which the change in methylation was associated with emergent suicidal ideation. These findings set the stage for potential mediation or moderation analysis in our future work.

In a statistical mediation model, the goal is to identify and explain the mechanism by which an exposure (X) operates via another explanatory variable, known as the mediator variable (M), to affect an outcome of interest (Y) (Wu et al., 2018). Specifically in our longitudinal study, this involved stress (X) affecting emergent suicidal ideation status (Y), by means of a methylation mediator variable (M).

In contrast, a moderation model, also known as an interaction model, utilizes methylation as the moderation variable to determine the form and strength of the relation between recent stress and emergent suicidal ideation (MacKinnon, 2011).

In order to complete a whole picture and claim a mediating or moderating role of DNA methylation changes in response to stress exposure in conferring risk for emergent suicidal ideation, it would be required to conduct these analyses. However, we found that our current

sample was too small to conduct such analyses. Larger scale studies are thus required for appropriate design and interpretation of results (Tsai et al., 2015).

5.2 Conclusion

The studies included in this thesis investigated the effects of childhood trauma and recent stressful events on current and emergent suicidal ideation. Our hypothesis was partially supported in that only increased total and health-related stress was predictive of emergent suicidal ideation, after correcting for covariates. We then attempted to identify methylation markers of current and emergent suicidal ideation. Again, our hypothesis was only partially supported in that we only found CpG sites that were suggestive of significance at the genome-wide level for current suicidal ideation. Longitudinally, we found several CpG sites in which the changes were associated with emergent suicidal ideation, in support of our hypothesis.

The anticipated outcomes of these studies were to gain an improved understanding of the effects of various stressors on suicidal ideation, as well as the identification of methylation markers that potentially could be used to monitor suicidal ideation in psychosis.

5.3 Future Directions

We previously described the addition of mediation and/or moderation model to enhance our understanding of the complex interplay among stress, DNA methylation, and suicidal ideation. This would be considered the most direct and immediate analyses to perform. If we are able to show that methylation changes induce suicidal ideation, as a result of recent stress, we would be able to offer novel insights into the molecular mechanisms of suicide.

In addition, we propose two additional future studies that may prove to be of great benefit in suicide research.

5.3.1 Pathway Analysis

We propose that the differentially expressed positions (DMPs) and regions (DMRs) associated with current suicidal ideation, as well as the individual CpG sites with changes in methylation associated with emergent suicidal ideation, undergo analysis for gene ontology enrichment. This process would be completed in the Database for Annotation, Visualization, and Integrated

Discovery (DAVID) resource, and would allow us to annotate the genes, and split them into functional groups (Pomaznoy et al., 2018).

5.3.2 Imaging Epigenetics

The field of imaging epigenetics is an ever-increasing useful technique that involves the use of neuroimaging and epigenetics to assess the impact of epigenetic variation on function and structure (Hashimoto et al., 2015). Previous studies have found very specific features related to schizophrenia suicide attempters, such as a reduced gray matter density in the left superior temporal lobe and left orbitofrontal cortex (Aguilar et al., 2008). It appears likely to be able to identify epigenetic and imaging markers in schizophrenia patients who are at high risk for suicide, and thus makes for an excellent series of future studies.

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