GENES AND THE ENVIRONMENT IN DEVELOPMENTAL PSYCHOPATHOLOGY LEADING TO SEVERE MENTAL ILLNESS

by

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ABSTRACT

Severe mental illness refers to mental disorders that cause functional impairment and interfere with major life activities. Currently, the strongest predictor of severe mental illness is a positive family history. However, most individuals who become ill do not have a family history of severe mental illness. I sought to examine genetic and developmental psychopathology factors that may be used to complement family history information when predicting risk of severe mental illness among youth. First, I examined associations between family history of severe mental illness and two phenotypes that are identifiable early in life: affective lability and basic symptoms. I found that affective lability is associated with a family history of major mood disorders, suggesting that this phenotype is an indicator of familial liability to mood disorders. I also found that basic symptoms are transdiagnostically associated with parental illness severity, suggesting that basic symptoms during childhood are a marker of familial risk of psychopathology. Next, I examined whether genetic scores indexing disposition to intelligence and attention-deficit/hyperactivity disorder (ADHD) predicted exposure to adversity during childhood and adolescence. I found that genetic disposition to ADHD strongly predicted exposure to adversity. However, there was no significant relationship between genetic disposition to intelligence and adversity. This finding suggests that genetic liability to ADHD may be an important early predictor of adverse life experiences. Finally, I described a genetic counselling-based intervention that uses genetic information to communicate risk of developing SMI, depending on whether or not individuals choose to use cannabis. The results of this intervention will provide insight into the acceptability and efficacy of genetic counselling among young people who are not seeking treatment. The findings presented in my thesis will contribute to a better understanding of early risk factors of severe mental illness and will inform future early preventative interventions.

LIST OF ABBREVIATIONS USED

ADHD	Attention-deficit/hyperactivity disorder
AL	Affective lability
ASD	Autism spectrum disorder
BMI	Body mass index
BS	Basic symptoms
CALS	Children's Affective Lability Scale
CALS-C	Children's Affective Lability Scale Child report
CALS-P	Children's Affective Lability Scale Parent report
CBCL	Child Behaviour Checklist
CBCL-C	Child Behaviour Checklist – Child report
CBCL-P	Child Behaviour Checklist – Parent report
CD	Conduct disorder
CECA	Childhood Experiences of Care and Abuse
CEQ	Cannabis Experience Questionnaire
COGDIS	Cognitive Disturbances
COPER	Cognitive-Perceptive
DNA	Deoxyribonucleic acid
DSM	Diagnostic and Statistical Manual
DUSI	Drug Use Screening Inventory
FIGS	Family Interview Guide for Genetic Studies
FORBOW	Families Overcoming Risks and Building Opportunities for Well-being

FSIQ	Full-scale intelligence quotient
G × E	Gene-environment interaction
GWAS	Genome-wide association study
HWE	Hardy-Weinberg equilibrium
IQ	Intelligence quotient
JVQ	Juvenile Victimization Questionnaire
JVQ-C	Juvenile Victimization Questionnaire Child report
JVQ-P	Juvenile Victimization Questionnaire Parent report
KSADS-PL	Kiddie Schedule for Affective Disorders and Schizophrenia - Present and Lifetime Version
MAF	Minor allele frequency
NSMD	Non-severe mood disorder
ODD	Oppositional defiant disorder
PCR	Polymerase chain reaction
PGS	Polygenic score
rGE	Gene-environment correlation
SADS	Schedule for Affective Disorders and Schizophrenia
SCID-5	Structured Clinical Interview for DSM-5
SD	Standard deviation
SES	Socioeconomic status
SMI	Severe mental illness
SNP	Single nucleotide polymorphism
SPI-CY	Schizophrenia Proneness Instrument – Child and Youth Version

THC	$^{\Delta 9}$-tetrahydrocannabinol
TOF	Test Observation Form
TwiC	Trial-within-cohort
WASI-II	Wechsler Abbreviated Scale of Intelligence – Second Edition

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CHAPTER 1
INTRODUCTION

Contribution Statement

I conducted the literature search and drafted the manuscript that was used as the basis for this chapter. Drs. Rudolf Uher and Eileen Denovan-Wright guided provided guidance and suggestions.

1.1 Overview

Severe mental illness (SMI) refers to mental disorders that cause functional impairment and substantially interfere with one or more major life activities. Most SMI cases are accounted for by major mood and psychotic disorders, including major depressive disorder, bipolar disorder, and psychosis spectrum disorders. Mental disorders are a leading cause of disability worldwide.[1] SMI tends to emerge in early adulthood and is associated with physical morbidity and premature death.[2] A variety of risk factors for SMI have been identified, but the etiology of SMI is still far from being understood. SMI is typically preceded and predicted by early manifestations of psychopathology that do not specifically predict any single adult diagnosis.[3] This thesis explores specific hypotheses related to family history of SMI, molecular genetic liability to psychopathology, environmental risk factors for SMI, and developmental antecedents to SMI.

1.2 Genetic factors in the etiology of severe mental illness

1.2.1 Family, adoption, and twin studies

The strongest predictor of SMI is having a close, biological relative who is affected.[4] Approximately one out of every three offspring of a parent with a major mood or psychotic disorder will develop SMI by adulthood.[5] Twin and adoption studies suggest that the familial clustering of SMI is due largely to genetic factors. Among individuals with schizophrenia who were adopted at birth, rates of schizophrenia were elevated among their biological relatives but not among their adoptive families.[6] The results of adoption studies of mood disorders are also consistent with genetic transmission of disease risk.[7,8] Additionally, twin studies show that monozygotic twins, who are genetically identical, are

more similar in their propensity to develop psychopathology than are dizygotic twins, who share half of their genetic material. Higher twin study-derived heritability estimates have been found for mental disorders that are less common and more severe (*e.g.*, schizophrenia, bipolar disorder) than for disorders that are more common and less severe (*e.g.*, major depressive disorder, anxiety).[9] These findings converge to suggest that genetic factors contribute substantially to the risk of SMI.

1.2.2 Candidate gene studies

SMI is a complex family of disorders and no single gene or genetic variant has been implicated as a necessary and sufficient causal factor. Early investigations into genetic contributors to SMI focused on variants within genes thought to be involved in leading etiological hypotheses, commonly referred to as candidate gene studies. Many early investigations into the genetic underpinnings of SMI were focused on schizophrenia, due to its high estimated heritability.[10] This methodology led to the identification of associations between biologically plausible genes and SMI. However, initial reports did not reliably replicate.[10] One consistent finding that was identified via the candidate gene approach is the association between a single nucleotide polymorphism (SNP) within the calcium channel encoding gene, *CACNA1C,* and multiple forms of psychopathology, including bipolar disorder, schizophrenia, depression, autism, and anxiety.[11–13] However, many other results were uncertain and the candidate gene era culminated in the finding that case-control differences in allele frequencies at SNPs in leading candidate genes previously reported to be associated with schizophrenia were consistent with chance expectation in the largest sample available at the time.[14]

1.2.3 Rare and structural variants

Individual rare variants can have large effects on SMI risk. Copy number variants and disruptive mutations are enriched among individuals with SMI compared to controls.[15,16] Interestingly, SMI-associated copy number variants are enriched within genes associated with synaptic function and neurobehavioural phenotypes in mice.[16] Additionally, a specific large deletion on chromosome 22, leading to 22q11.2 deletion syndrome is associated with particularly increased risk of psychopathology. Approximately one in four individuals carrying this deletion will develop schizophrenia.[17] Children with 22q11.2 deletion syndrome also experience elevated rates of a range of psychopathology, including attention-deficit/hyperactivity disorder (ADHD), anxiety, autism spectrum disorder (ASD), and oppositional defiant disorder.[18] Although these variants are associated with substantially elevated risk of SMI, they are uncommon (each occurring in less than 1% of the population) and thus cannot account for all cases of SMI.

1.2.4 Genome-wide association studies and polygenic scores

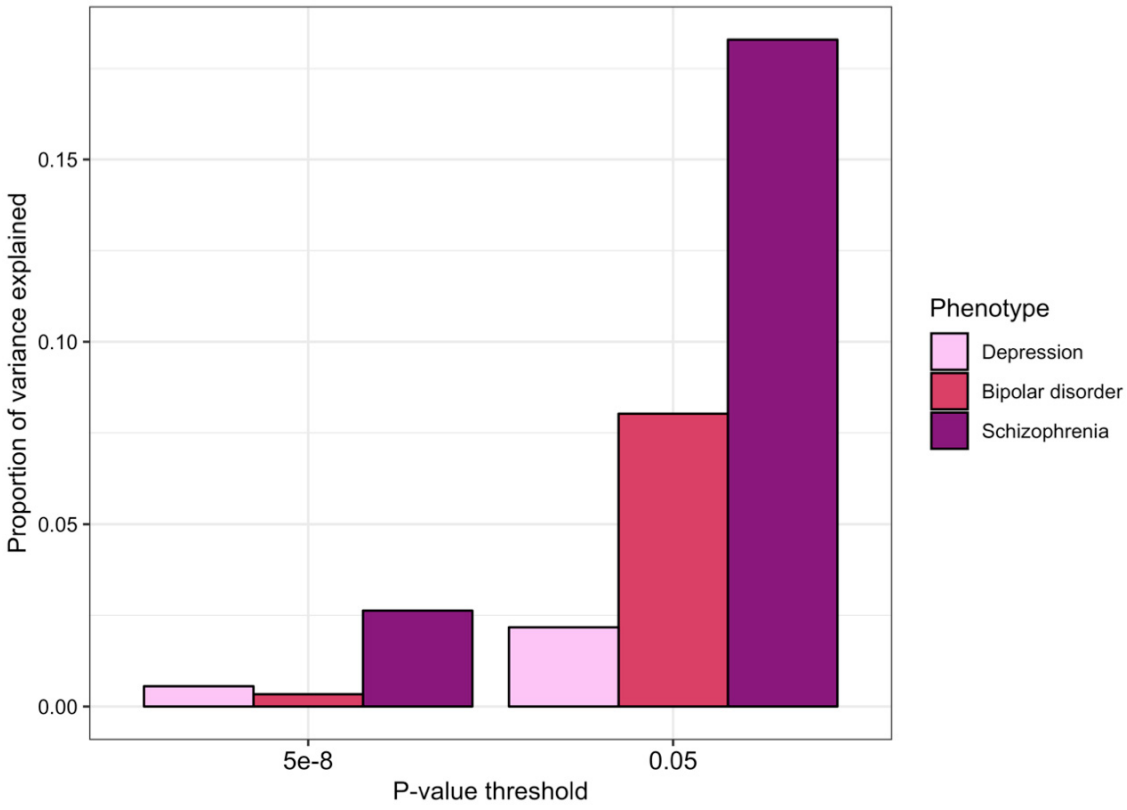
Technological advances have enabled large-scale genomic studies and consortia have formed to bring together the sample sizes necessary for adequately powered genome-wide analyses.[19] The focus has shifted from candidate gene studies to the exploration of molecular genetic contributions to SMI on a genome-wide scale. Genome-wide association studies (GWAS) have identified hundreds of common variants associated with SMI (see Table 1.1).

Table 1.1 Genetic variants associated with severe mental illness.

	Depression[20]	**Bipolar disorder**[21]	**Schizophrenia**[22]
Number of cases in largest sample	135,458	20,352	36,989
Number of controls in largest sample	344,901	31,358	113,075
Number of independent genome-wide significant loci	44	30	108
Odds ratio of most strongly associated genetic variant	1.05	1.15	1.21
Proportion of variance explained by top polygenic score	2%	8%	18%

One consistent finding from genome-wide association studies is that no single common genetic variant causes SMI. However, numerous common variants which are individually weakly related to SMI can be combined into polygenic scores (PGS), which strongly predict SMI.[23] It is likely that substantially more common variants contribute to genetic liability for mental illness, because the predictive ability of PGS for psychopathology improve with the inclusion of weakly associated variants, in addition to variants significantly associated with the disorder of interest (see Figure 1.1).[20–22,24] PGS may provide a potential means to quantify genetic risk of developing SMI among individuals without a family history of mental illness or for whom their family history is not known. It has been shown that PGS for schizophrenia and bipolar disorder can distinguish individuals affected with SMI from their unaffected siblings and from health community controls.[25] Additionally, PGS allow us to investigate how genetic liability for psychopathology manifests before illness onset and among individuals who may not develop a mental illness. In a general population sample, genetic risk for schizophrenia was associated with anxiety, negative symptoms, and worse neurodevelopmental outcomes (*e.g.*, worse language fluency) in childhood and adolescence, but surprisingly, not with adolescent psychotic symptoms.[26,27] Additionally, a polygenic score indexing genetic liability to ADHD was also associated with a range of phenotypes, including neurodevelopmental traits,[28] externalizing symptoms,[29] early onset depression,[30] lower cognitive performance,[31] worse educational outcomes,[32] substance use,[33] and higher body mass index (BMI).[33]

Figure 1.1 The polygenic risk for severe mental illness. The proportion of variance explained by polygenic scores derived from the most recent GWAS meta-analyses of major depressive disorder[20], bipolar disorder[21], and schizophrenia[22] with p-value thresholds at 5e-8 (genome-wide significance) and 0.05. The proportion of variance explained by the PGS for each disorder steeply increases with the inclusion of more, non-significantly associated variants. This suggests that SMI is highly polygenic and many more genetic loci than those that meet the genome-wide significance threshold likely contribute to risk.



1.2.5 Genetic overlap across disorders

Most genetic variation is broadly associated with a range of psychopathology.[34] Familial risk for SMI is not entirely disorder-specific; for example the offspring of individuals with psychotic disorders are also at increased risk for mood disorders and *vice versa*.[5] This finding is corroborated with molecular genetic[35–37] and neuropathological[38] findings, which show that the genetic variation and transcriptional dysregulation associated with illness are largely shared across forms of mental illness. Approximately two thirds of genetic associations are common to schizophrenia, bipolar disorder, and major depressive disorder.[35] Additionally, there are associations between genetic risk for SMI and genetic liability to a range of other phenotypes including common mental disorders (*e.g.*, ADHD, anxiety disorders), educational attainment, smoking status, BMI, and migraine.[36,37]

1.3 Environmental factors in the etiology of severe mental illness

In addition to confirming the genetic nature of mental illness, twin studies have shown that environmental exposures are important contributors to SMI risk. Concordance rates for monozygotic twins are not perfect, suggesting that environmental exposures contribute to disease risk.[9] Environmental risk factors can be clustered based on the stage in development when exposure is most likely to influence risk. Complex factors, such as socioeconomic status (SES), tend to remain constant throughout development and have wide-reaching implications on health across the life course.[39] Other factors, including maternal infection, influence risk of SMI if individuals are exposed during a 'sensitive' period in development.[40,41] Most individuals, with and without SMI, are exposed to at least one environmental risk factor, which complicates the investigation into the roles of

individual environmental exposures on SMI etiology.[42,43] Many individuals appear to be resilient and do not develop SMI, even if they are exposed to multiple environmental risk factors.[44,45] Additionally, it has become clear that the same type of environmental exposure increases the risk of many different mental disorders (see Figure 1.2).

Figure 1.2 Environmental factors associated with severe mental illness. For many of these factors, the impact of the exposures is dependent on when in development the exposure occurs. The number of plus signs indicates the strength of evidence for an association with each disorder: '+' indicates evidence from a single study or multiple small/low quality studies; '+ +' indicates evidence from multiple smaller studies or a strong association in a high-quality study; '+ + +' indicates consistent evidence from multiple large-scale studies or a meta-analysis; '0' indicates evidence of no association; blank cells indicate no evidence for or against an association.

	Timing						
Exposure	**Prenatal**	**Perinatal**	**Childhood**	**Adolescence**	**Depression**	**Bipolar disorder**	**Schizophrenia**
Malnutrition	→				+ +	+ +	+ + +
Heavy metals	→				+		+ +
Maternal stress	→				+ +	+	+
Infections	→	→	→		+	+ +	+ + +
Poverty	→	→	→	→	+ + +	+	+ + +
Preterm birth		→			+ +	+ +	+ +
Season of birth		→			+	+ +	+ + +
Birth complications		→				0	+ +
Urbanicity		→	→	→	+ + +	+	+ + +
Maltreatment			→	→	+ + +	+ +	+ + +
Bullying			→	→	+ + +	+	+ +
Head injury			→	→	+ +	+	+ +
Stimulants			→	→	0	+ +	+ + +
Cannabis				→	+	+ +	+ + +
Tobacco				→	+		+ + +

1.3.1 Pre- and perinatal risk factors for severe mental illness

Pre- and perinatal exposures that influence immune function have been linked to the development of SMI. It has been suggested that immune activation and subsequent inflammation could mediate the effects of pre- and perinatal insults, such as stress or infection, on SMI risk by contributing to abnormal neurodevelopment.[46] Individuals exposed to inflammation *in utero* are at increased risk of developing SMI in adulthood, particularly psychotic illness.[47,48] Investigations into the association between prenatal inflammation and mood disorders are limited, and current studies do not support a role of prenatal inflammation as a risk factor for mood disorders.[49,50] Results from animal studies suggest that the potential unfavorable effects of prenatal immune activation may extend across multiple generations. For example, some pathological neurobehavioural traits (*e.g.*, reduced sociability) resulting from *in utero* exposure to immune activation were observable for up to three generations in a mouse model.[51] This suggests that epigenetic mechanisms or learned behavioural transmission may influence these traits.

1.3.2 Childhood risk factors for severe mental illness

Exposure to adversity in childhood, including physical, sexual, and emotional abuse, neglect, and exposure to violence have been strongly implicated in the development of SMI.[52–55] Additionally, it has been shown that experiencing childhood maltreatment may render individuals more vulnerable to the mental health consequences of exposure to stressful life events later in life.[56] More recently, involvement in bullying, both as a victim and as a bully, has been recognized as a contributor to both SMI[57,58] and subclinical psychotic symptoms during adolescence.[59,60] Childhood trauma is associated with

increased blood levels of inflammatory markers in adulthood,[61] which provides a possible mechanism through which childhood adversities could impact the development of SMI.

1.3.3 Adolescent risk factors for severe mental illness

Substance use during adolescence is associated with later onset of SMI. Abuse of psychostimulants is typically associated with acute psychosis, however individuals with a family history of mental illness who use stimulants recreationally appear to be more vulnerable to persistent psychopathology than individuals without a family history.[62–65] Interestingly, the link between stimulants and psychopathology extends to children with a family history of mental illness who are taking stimulants to treat ADHD.[66] The risk of experiencing subclinical psychotic symptoms is more than four times higher among children taking prescribed stimulant medication compared to those who have never taken stimulants.[66] Additionally, monotherapy with psychostimulants has been associated with increased rates of manic episodes among individuals with bipolar disorder.[67] Regardless of family history status, cannabis use has been strongly and consistently associated with psychopathology,[68–71] particularly psychotic illness. In the case of cannabis exposure and the onset of psychosis, many criteria of causality are met,[72] including a positive dose-response relationship between cannabis use and psychotic outcomes,[73] the temporal sequence of cannabis use preceding the onset of psychosis,[74,75] and consistent evidence for an association. There is also evidence that tobacco use is associated with increased risk of SMI[76–78] and worse outcomes (*i.e.*, suicidality and psychotic symptoms) among individuals with SMI.[79,80] The fact that cannabis and tobacco are often used by the same individuals complicates the investigation into their individual relationships and SMI.[81] This highlights

the necessity of gathering information on exposure to multiple factors when examining environmental contributors to SMI.

1.3.4 The physical environment and risk of severe mental illness

Broad characteristics of the physical environment during development have been associated with SMI. Upbringing in an urban setting is associated with increased risk of many forms of psychopathology, including both psychotic and mood disorders.[82] Risk increases with total time spent living in an urban center. Additionally, subclinical psychotic symptoms among children and adolescents are more frequent and more likely to progress to SMI among youth living in urban environments.[83,84] The exact components of the urban environment that contribute to SMI risk are not well-defined. Toxins, such as heavy metals, are not likely to play a substantial role.[85] However, exposure to air pollutants such as nitrogen oxides has been associated with increased risk of psychotic symptoms among youth living in urban centers.[86] In the case of childhood and adolescent psychopathology, neighborhood factors including low social cohesion and high crime rate partially explain the elevated risk for psychotic symptoms among children living in urban areas.[87]

1.3.5 Aggregating environmental risk factors into a single score

Although some environmental risk factors for SMI have been identified, it is difficult to separate the effects of individual factors because they often cluster within the same person. The body of evidence suggests that environmental causation of SMI results from a complex combination of social, physical, and chemical exposures occurring at different stages of life, and influencing risk for multiple mental disorders. Similar to the notion of many

genetic factors of small effect sizes contributing to the genetic risk for SMI, it has been suggested that environmental risk is also attributable to the cumulative contribution of many exposures.[43] To improve the prediction of SMI, it may be useful to jointly examine a multitude of environmental risk factors across the life course, which may be combined into scores.[88] Although exposure to certain environments increases the risk of SMI, only a minority of individuals exposed to these factors will become ill. Genetic differences may render some individuals more vulnerable or resilient to the impact of environmental exposures.

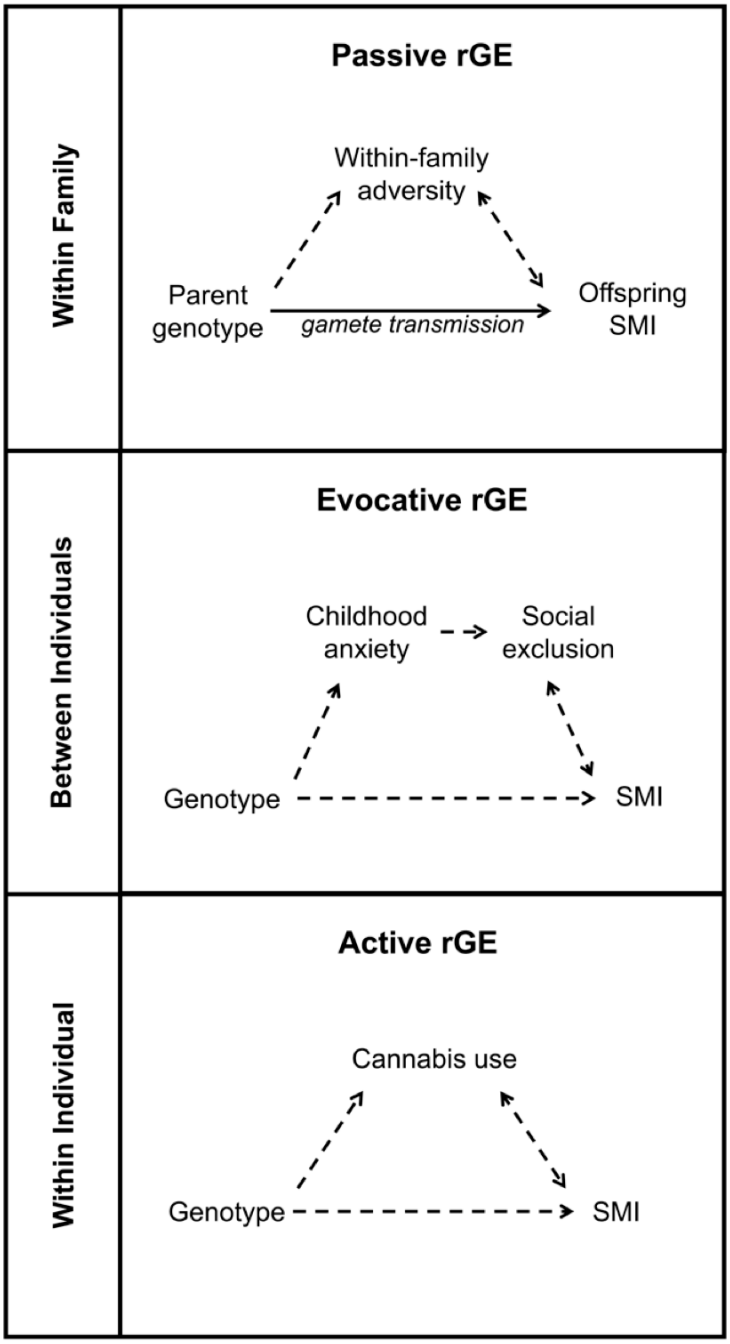
1.4 Gene-environment interplay in the etiology of severe mental illness

The term gene-environment interplay captures the combined contributory effects of both genetic and environmental factors to SMI. Gene-environment interplay encompasses gene-environment correlation (rGE) and gene-environment interaction (G × E).

1.4.1 Gene-environment correlation

Gene-environment correlation refers to the non-random relationship between genotype and exposure to specific environments. Three forms of rGE have been widely described: passive rGE, evocative rGE, and active rGE (see Figure 1.3).[89]

Figure 1.3 Examples of gene-environment correlations relevant to the etiology of severe mental illness.



1.4.2 Passive gene-environment correlation

Passive rGE refers to the association between genotype and rearing environment, both of which are influenced by an individual's parents' genes. For example, the association between childhood adversity and genetic risk for psychopathology represents a potential passive rGE. Children of parents with SMI are at increased risk for childhood maltreatment and individuals who experience adversity during childhood are more likely to develop SMI.[90] However, it is unclear whether the association between childhood maltreatment and psychopathology arises because abuse directly causes SMI or because genetic factors increase both the likelihood of experiencing abuse and the propensity to develop SMI. If the latter is true, the association between maltreatment and SMI represents a passive gene-environment correlation.

1.4.3 Evocative gene-environment correlation

Personal characteristics determine how individuals interact in social situations, and as a result, influence the responses they will elicit from others. Evocative rGE describes how differences in genotype could evoke different reactions. As an example, genetic liability to schizophrenia is associated with childhood anxiety.[26] Anxious children may evoke a different response from peers than their less anxious counterparts, resulting in an evocative rGE. Thus, anxiety-related behaviours may elicit adverse social reactions from peers, which could, in turn, influence risk of developing SMI.[44]

1.4.4 Active gene-environment correlation

Active rGE describes how genetic variation can contribute to differences in the likelihood of exposure to environments. For example, individuals at high genetic risk for schizophrenia are more likely to use cannabis.[91] Genotype, therefore, influences both the propensity to develop psychosis and the likelihood of being exposed to cannabis. Active rGE could account for a portion of the association between cannabis use and psychosis. However, genetic risk for schizophrenia only explains a small proportion (0.5%) of the variance in cannabis use.[91] Therefore, it is unlikely that rGE fully explains the association between cannabis use and psychosis. It does, however, bring attention to the need to consider rGE before making causal interpretations that assume relative independence of genetic and environmental factors.

1.4.5 Gene-environment interaction

Environmental exposures do not affect everyone equally. Some individuals remain healthy even when exposed to multiple known risk factors, whereas others will go on to develop SMI.[44,92] Genetic factors may render certain individuals more vulnerable to the impact of environmental exposures, resulting in gene-environment interactions. Two findings suggest that G × E play a substantial role in the development of SMI. First, much larger heritability estimates of SMI have been obtained from twin studies than for molecular genetic studies using unrelated individuals.[93] Second, despite the fact that epidemiological studies have robustly demonstrated that risk factors shared within families (*e.g.,* urbanicity, SES) are among the top contributors to SMI, twin studies suggest that shared environment plays little or no role.[9] These incompatible findings can be explained by the manner in

which heritability is calculated in twin studies: the interplay between genetic factors and environmental variables shared within a family are attributed to genetics and inflate heritability estimates while reducing the estimated contributions of shared environment.[94] Therefore, G × E offers a plausible explanation for these discrepant findings.

In addition to providing an explanation for the conflicting heritability estimates from twin studies, molecular genetic studies and epidemiology, identification of G × E could offer additional insight into both genetic and environmental contributions to SMI. First, G × E research can lead to the identification of novel genetic contributors to SMI that may not otherwise be identified in case-control studies.[95] Additionally, identification of G × E is valuable because unlike genetics, environment is malleable and can be modified selectively among those who are genetically sensitive. Therefore, identifying G × E could provide the opportunity for targeted interventions.

1.4.6 Gene-environment interaction by proxy

Family history of SMI can be used as a proxy for genetic risk. This method has been used to identify interactions between 'genetic liability' for SMI and environmental exposures. Individuals with a family history of SMI are particularly sensitive to the effects of multiple environmental risk factors including cannabis use,[96] urban upbringing,[97] and maternal infection *in utero*.[98] However, the applicability of findings using proxy measures of genetic contribution to illness is limited because the comprehensive family history of mental illness is not always known.

1.4.7 Gene-environment interaction involving molecular genetic variants

The search for gene-environment interactions involving specific molecular genetic variants began by testing interactions between environmental risk factors for SMI and variants within candidate genes. This approach relies on correctly selecting both the genetic variant and environmental exposure of interest based on prior knowledge. Surprisingly, this approach has led to the identification of interactions between variants in a handful of G × E. Replication of results, however, has been inconsistent. For example, homozygous carriers of the short allele within a length polymorphism in the promoter region of the serotonin transporter encoding gene *SLC6A4* have been shown to be at increased risk of depression following exposure to adversity.[99] This initial finding has seen a number of replications and non-replications.[100–102] A large meta-analysis found a strong relationship between the short allele at this locus and increased sensitivity to depression following childhood maltreatment.[100] However, a more recent meta-analysis did not find evidence for a strong association.[103,104] These conflicting results shed light on the fact that even large-scale meta-analyses do not agree on a single conclusion for or against a G × E in this longstanding debate. Broader searches screening hundreds of polymorphisms across functionally defined groups of genes have been carried out in search of a G × E. This methodology led to the identification of an interaction between cannabis use and a SNP in *AKT1*, a gene encoding a serine/threonine kinase involved in the transduction of signal following cannabinoid receptor activation.[105] Homozygous carriers of the C allele at rs2494732 in *AKT1* appear to be more vulnerable to the psychosis-inducing properties of cannabis. In a replication study, individuals with the C/C genotype at rs2494732 who used cannabis daily were found to be at 7-fold increased risk of developing psychotic illness

compared to T allele homozygotes.[106] This finding has been replicated in two independent samples since the original report, and may therefore represent a true G × E.[106,107]

Comprehensive genome-wide search strategies, referred to as genome–wide environment interaction studies (GWEIS), have been conducted to systematically search for G × E. GWEIS involves testing interactions between an environmental exposure of interest and hundreds of thousands of individual SNPs across the genome. This approach has led to the identification of G × E between environmental factors and genes that had not been previously associated with SMI.[95,108,109] A genome-wide approach to G × E research is likely to be superior to hypothesis-driven approaches, as many SMI-associated genetic loci that have been identified to date are found within genes that were not previously suspected to be implicated in psychopathology.[110] Due to a large number of loci being tested, GWEIS require very large samples to be adequately powered – even when examining interactions between common variants and common exposures. Although it is likely that gene-environment interactions play a significant role in the development of SMI, no specific G × E identified thus far explains a substantial proportion of cases.

1.5 The transdiagnostic nature of severe mental illness

All available evidence converges to show that genetic and environmental contributors to SMI are largely shared across disorders.[37,38,111] Therefore, it may be useful to conceptualize SMI as a transdiagnostic category composed of all major mood and psychotic disorders. This approach may allow for targeted transdiagnostic early interventions strategies aimed

to prevent the onset of SMI. However, it is first necessary to identify early, transdiagnostic indicators of risk for SMI.

1.6 Developmental psychopathology leading to severe mental illness

1.6.1 Antecedents to severe mental illness

Most cases of SMI are preceded and predicted by earlier and milder manifestations of psychopathology. These early risk indicators are conditions of the individual that are distressing to the individual without being severely impairing, predict SMI with substantial effect sizes, and precede its onset by several years.[112] They are not disorder-specific (each predicts multiple forms of SMI) and the presence of one or more antecedents does not guarantee progression to SMI. Based on previous literature, four key antecedents have been identified as potential predictive precursors to SMI: affective lability, anxiety, basic symptoms, and psychotic symptoms.[112] The antecedents that I will examine in this thesis are affective lability and basic symptoms.

1.6.2 Affective lability

Affective lability refers to the propensity to experience rapid changes in mood, occurring in an unpredictable and excessive manner.[113] Affective lability has been associated with SMI and may represent an early indicator of risk in childhood and adolescence. Bipolar disorder and affective lability are phenomenologically similar and the link between them is well established. Affective lability is increased among individuals with bipolar disorder[114] and their offspring.[115,116] Additionally, affective lability has been shown to predict the onset of bipolar disorder in prospective studies.[117] There is also support for a

relationship between affective lability and major depressive disorder. Affective lability has been shown to be elevated among individuals with depression.[118] In addition, irritability, a key component of affective lability,[115] has been shown to prospectively predict the onset of major depressive disorder.[119] Affective lability has also been shown to be elevated among individuals with schizophrenia and it prospectively predicts schizophrenia onset.[120,121] These findings suggest that affective lability may be an informative contributor to the prediction of SMI. However, it is not yet known whether affective lability during childhood and adolescence is specifically associated with disposition to bipolar disorder or whether it is more broadly associated with SMI.

1.6.3 Basic symptoms

Basic symptoms describe a group of subjectively perceived deficits and abnormalities across multiple domains, including thought, perception, and other essential mental processes. Basic symptoms may represent early manifestations of SMI, particularly psychotic illnesses. Positive symptoms of psychosis include hallucinations and/or delusions, which are perceived by the affected individual as real experiences. In contrast to positive psychotic symptoms, basic symptoms are immediately recognized by the individual as abnormal disturbances to their typical thoughts, senses, and feelings.[122] Basic symptoms strongly predict the onset of schizophrenia 5-10 years later[123,124] and they can be assessed in children as young as 8 years old [125]. Additionally, individuals who have a first degree biological relative living with psychotic illness experience more basic symptoms than controls.[126,127] Basic symptoms have also been linked to other forms of mental illness, including affective disorders[128,129] and are associated with lower global

functioning among individuals with a range psychiatric disorders[130] However, basic symptoms have not yet been examined as a potential marker of familial risk for SMI.

1.7 Early interventions

1.7.1 Early interventions to-date

Early interventions to-date have focused on treatment-seeking individuals in the early stages of illness. While these interventions have shown some benefit,[131] it is not typically maintained over follow-up.[132,133] Additionally, it has been suggested that earlier interventions provide the greatest benefit.[134] Gene-environment interactions provide a unique opportunity for early intervention because optional environmental exposures associated with risk of SMI (*e.g.*, cannabis use) can be selectively avoided among those who are genetically sensitive.

1.7.2 The use of genetic information in early interventions

The provision of genetic information may prompt individuals at risk to adopt risk-reducing behaviours, if the information is delivered appropriately. While some studies have shown a modest benefit effect of genetic information provision on behavioural modification,[135,136] other studies have shown little or no efficacy.[137,138] However, all of these studies involve the unidirectional transmission of information from researcher to participant. These trials are thus founded on the assumption that behavioural decisions are solely the result of available information and cognitive ability. This assumption fails to account for the fact that individual-level biases influence how people make decisions.[139] In contrast, genetic counselling is a psychotherapeutically-oriented approach and focused on helping the

individual understand all implications of genetic contributions to disease.[140] Interventions based on this bidirectional and highly personalized framework have the potential to generate a shared understanding of illness etiology and help participants identify protective mental health strategies for the future.

1.8 Research Objectives

In the present work, I will address three primary research objectives:

1) Examine the relationship between family history of SMI and specific developmental antecedents of SMI occurring during childhood and adolescence.
2) Examine the relationship between genetic liability to psychopathology and exposure to adversity.
3) Describe a novel genetic counseling-based intervention aimed at reducing exposure to cannabis among youth at high familial risk of SMI.

CHAPTER 2
METHODS

2.1 Cohort description

All of the data presented in my thesis were drawn from the Families Overcoming Risks and Building Opportunities for Well-being (FORBOW) study.[112] FORBOW is a longitudinal accelerated cohort study enriched for offspring of parents with SMI. Approximately three out of four youth participants in FORBOW have at least one biological parent living with a major mood or psychotic disorder. FORBOW participants and their parents attend annual assessments, which include a semi-structured psychiatric diagnostic interview, interview measures to assess adversity and drug use, cognitive testing, and questionnaires (see Table 2.1). Offspring are assessed by research staff blind to parent psychopathology. FORBOW parents are also interviewed by parent-specific research staff to assess parent psychopathology, family history of mental illness, and family socioeconomic status. Additionally, saliva samples are collected from all consenting offspring and parents. The mean age of FORBOW participants is 13 years (range 5-27 years). The median number of assessments completed per participant is 3 (range 1-7). Enrolment in FORBOW is ongoing, and we currently have 438 offspring from 242 families across Nova Scotia, Canada participating in our study.

The study protocol was approved by the Research Ethics Board of the Nova Scotia Health Authority. We obtained informed consent from participants who had the capacity to provide it. For participants who did not have the capacity to make an informed decision, a parent or guardian provided written informed consent and the participant provided assent.

Table 2.1. Assessment schedule for FORBOW cohort interview measures, cognitive assessments, and questionnaires relevant to my thesis.

	Assessment Year							
Measure	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Age**
Cognition								
WASI-II	✓				✓		✓	6+
WPPSI-III	✓							5
Interview								
KSADS-PL	✓	✓	✓	✓	✓	✓	✓	6+
SPI-CY	✓	✓	✓	✓	✓	✓	✓	6+
Bullying	✓	✓	✓	✓	✓	✓	✓	6+
SES	✓	✓	✓	✓	✓	✓	✓	6+
CEQ	✓	✓	✓	✓	✓	✓	✓	11+
SCID-5		✓	✓	✓	✓	✓	✓	18+
JVQ-P		✓		✓		✓		6-10
JVQ-C		✓		✓		✓		11-16
CECA		✓		✓		✓		17+
Questionnaire								
TOF	✓	✓	✓	✓	✓	✓	✓	6+
DUSI	✓	✓	✓	✓	✓	✓	✓	11+
CALS-P	✓	✓	✓	✓	✓	✓	✓	6-16
CALS-C	✓	✓	✓	✓	✓	✓	✓	9-17
CBCL-P	✓	✓	✓	✓	✓	✓	✓	6-16
CBCL-C		✓		✓	✓	✓	✓	11-14

WASI-II = Wechsler Abbreviated Scale of Intelligence – Second Edition; WPPSI-III = Wechsler Preschool and Primary Scale of Intelligence – Third Edition; KSADS-PL = Kiddie Schedule for Affective Disorders and Schizophrenia – Present and Lifetime version; SPI-CY = Schizophrenia Proneness Instrument – Child and Youth version; CEQ = Cannabis Experience Questionnaire; SCID-5 = Structured Clinical Interview for DSM-5; JVQ-P = Juvenile Victimization Questionnaire Parent Report; JVQ-C = Juvenile Victimization Questionnaire Child Report; CECA = Childhood Experiences of Care and Abuse; TOF = Test Observation Form; DUSI = Drug Use Screening Inventory; CALS-P = Children's Affective Lability Scale – Parent report; CALS-C = Children's Affective Lability Scale – Child report; CBCL-P = Child Behaviour Checklist Parent report; CBCL-C = Child Behaviour Checklist Child report.

2.1.1 Genetic counseling trial-within-cohort

FORBOW was designed to test early interventions aimed at preventing SMI.[112] A genetic counseling-based intervention is embedded within FORBOW (see Chapter 6). By embedding a randomized controlled trial within a longitudinal cohort, we are able to test the intervention effectiveness using trial-within-cohort (TwiC) design. This methodology allows for externally valid testing of long-term effectiveness and avoids disappointment bias that is often associated with allocation to a control group.[141] In TwiC, there are two stages of informed consent, which separates consent for cohort participation from consent for intervention participation.[142] For the genetic counseling intervention (see Chapter 6), we will randomly select one in every two eligible youth the be offered a single session of genetic counseling during which they will receive personalized genetic information regarding their risk of developing SMI, depending on whether or not they choose to use cannabis. The other eligible participants who are not allocated to receive an offer of intervention will not be informed of the intervention but will continue to receive annual FORBOW assessments.

2.2 Parent assessment

2.2.1 General psychopathology

Diagnoses of mental disorders according to the Diagnostic and Statistical Manual IV (DSM-IV) and DSM-5 were established by semi-structured interview using the *Schedule for Affective Disorders and Schizophrenia* (SADS-IV)[143] or the *Structured Clinical Interview for DSM-5* (SCID-5).[144] Parent assessors were blind to offspring diagnoses.

Parent diagnoses were confirmed in consensus meetings with a psychiatrist blind to offspring psychopathology.

2.2.2 Family history information

Detailed information on family history of schizophrenia, any psychosis, bipolar disorder, and depression are obtained for all first-degree relatives of each parent by interview using the Family Interview Guide for Genetic Studies (FIGS).[145]

2.2.3 Socioeconomic status

We obtained the highest level of education obtained by each biological parent, the family's annual household income, and whether or not the family owns their primary residence via interview with parents.

2.3 Offspring assessment

2.3.1 General psychopathology

Offspring were assessed for all Axis I disorders by semi-structured interview using the *Kiddie Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version* (K-SADS-PL; in offspring younger than 18 years)[146] or the *Structured Clinical Interview for DSM-5* (SCID; in offspring 18+ years old).[144] Both the parent and the participant completed the KSADS interview and the relative weight of each reporter's input was determined based on the participant's age and developmental stage. Offspring assessors were blind to parent psychopathology. Diagnoses were confirmed in consensus meetings with a psychiatrist blind to parent diagnoses.

2.3.1.1 Affective lability

Affective lability was assessed in children and youth using the self- and parent-report versions of the Children's Affective Lability Scale (CALS) questionnaire.[113] Participants with the capacity to do so completed the self-report version of the CALS. We also obtained the parent-report version of the CALS. To allow for comparison between the self- and parent-report measures, I standardized the total scores from each questionnaire by the mean and standard deviation of the control offspring scores. For assessments in which both the self- and parent-report measures were available, I used the mean of the two standardized scores as the dependent variable in analyses. For assessments in which we only obtained either the parent-reported CALS or self-reported CALS, the dependent variable was the standardized parent-reported or self-reported score, respectively.

2.3.1.2 Basic symptoms

We assessed basic symptoms via interview with participants using the *Schizophrenia Proneness Instrument – Child and Youth Version* (SPI-CY).[125] The SPI-CY was designed to be administered to children and youth aged 8 years and older. The SPI-CY contains two psychosis-risk basic symptom profiles: Cognitive-Perceptive (COPER) and Cognitive Disturbances (COGDIS). COGDIS items have been shown to strongly predict psychotic illness and are part of the clinical high-risk criteria that have been recommended for the early detection of psychosis.[147] I calculated a SPI-CY risk score as the total number of COPER or COGDIS items scored 3 (several times in a month or weekly) to 6 (daily), divided by the total number of items with a valid frequency rating. I calculated a COGDIS score, which incorporates the 9 items included in the COGDIS criteria, using the same

process. For analyses, I standardized the SPI-CY risk score and COGDIS score by the means and standard deviations of the control offspring scores.

2.3.1.3 Externalizing psychopathology

I calculated a dimensional index of externalizing symptoms by combining assessor-rated, parent-rated, and self-report questionnaires with consensus-confirmed diagnoses of externalizing disorders. I included the parent-rated and self-report versions of the Child Behaviour Checklist (CBCL) aggressive behaviour and delinquent behaviour syndrome scales.[148] I included the assessor-rated Test Observation Form (TOF) oppositional and attention problems syndrome scales, which were completed by the child assessor who administered the cognitive assessment. I scored each syndrome subscale from the CBCL and the TOF as the sum of the score for all items divided by the number of valid items. Consensus-confirmed diagnoses of oppositional defiant disorder, conduct disorder, any disruptive disorder, and ADHD were rated as present (scored 1) or absent (scored 0). I standardized the values for the TOF and CBCL syndrome scales and for consensus-confirmed externalizing disorders. I calculated the externalizing symptoms dimensional score as the mean of all available indicators of externalizing psychopathology.

2.3.2 General cognitive ability

Among offspring aged 6 years and older, we assessed general cognitive ability with the Wechsler Abbreviated Scale of Intelligence – Second Edition (WASI-II) [149]. There are four subtests on the WASI-II: block design, vocabulary, matrix reasoning, and similarities. These are combined to provide a reliable measure of full-scale IQ. Among 5 year old

offspring, we assessed general cognitive ability with the Wechsler Preschool and Primary Scale of Intelligence – Third Edition (WPPSI-III). We used the following subtests to obtain a measure of full-scale IQ: block design, information, matrix reasoning, vocabulary, word reasoning, and coding. These tests were administered by assessors trained in the administration of cognitive tests.

2.3.3 Victimization

Exposure to childhood maltreatment, defined as emotional abuse, physical abuse, sexual abuse, neglect, and/or exposure to violence at home, was prospectively assessed by interviewing parents (in offspring 10 years or younger) and youth participants (in offspring 11 years and older) using the Juvenile Victimization Questionnaire (JVQ).[150] This information was also retrospectively obtained from youth participants aged 17 years and older using the Childhood Experiences of Care and Abuse (CECA).[151] Peer victimization was assessed by asking parents (in offspring aged 10 years and younger) and youth participants (in offspring aged 11 years and older) if the participant had ever experienced bullying, with follow-up questions to gauge the frequency and severity of the victimization (adapted from the JVQ[150]).

2.3.4 Substance use

Use of cannabis and other recreational drugs (*e.g.,* nicotine, alcohol, stimulants) was collected annually among offspring aged 11 years and older with the validated Drug Use Screening Inventory (DUSI),[152] complemented with the Cannabis Experience Questionnaire (CEQ).[153] The CEQ provides additional questions to specifically assess

cannabis use (*i.e.*, type and frequency of cannabis use) and subjective experiences during cannabis intoxication and after cannabis use.

2.3.5 Adversity score

To calculate the adversity score, each indicator of victimization and SES was made binary. Indicators of maltreatment increased the adversity score if they were rated as "Yes" on the JVQ or CECA. Peer victimization increased the adversity score if bullying was rated as "Present" or "Severe". Mother's and father's education increased the adversity score if the respective parent did not complete education beyond high school. Home ownership increased the adversity score if the family did not own their home. Annual household income increased the adversity score if the annual household income was less than $60,000.

We calculated the adversity score as the mean of 10 possible binary indicators: 1) biological mother's education, 2) biological father's education, 3) home ownership status, 4) annual household income, 5) emotional abuse, 6) physical abuse, 7) sexual abuse, 8) neglect, 9) exposure to violence at home, and 10) bullying. The score was calculated by dividing the total count of adversities that the individual experienced by the total number of indicators with available information.

2.4 Biological samples

2.4.1 Collection, DNA extraction, and quantification

Saliva samples were collected using the Oragene Kit (DNA Genotek Inc, Kanata, ON). Saliva samples were catalogued and stored at room temperature until DNA extraction.

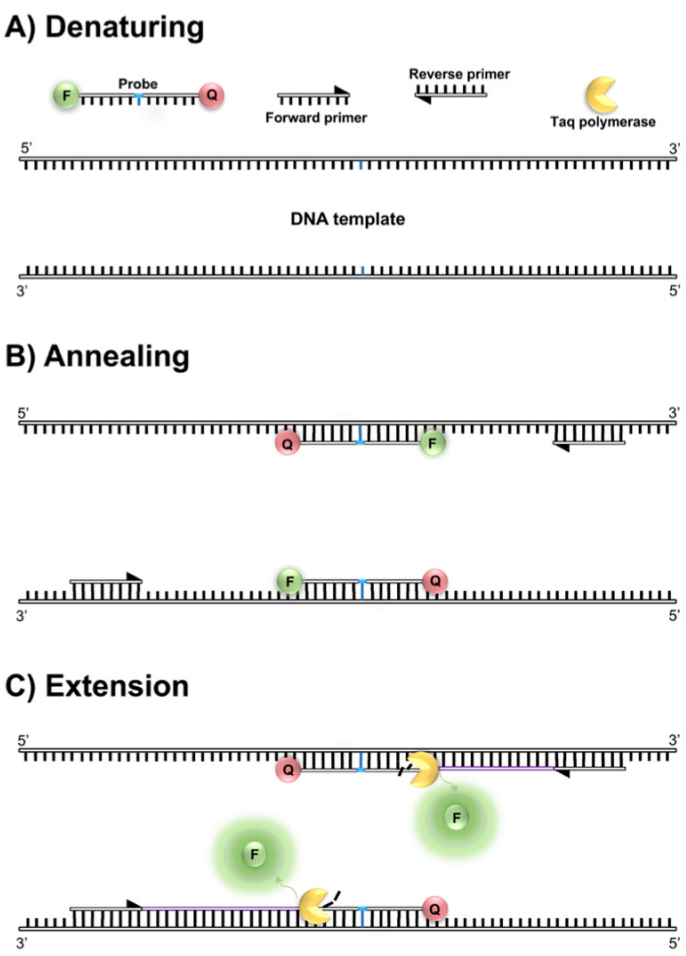
DNA extraction was performed as per the Oragene kit instructions. Briefly, samples were first mixed and incubated overnight at 50 °C to ensure that DNA was released and that nucleases were denatured. Next, prepIT®•L2P reagent was added to the mixed sample to precipitate impurities. Samples were then incubated on ice and spun to separate the impurity-containing pellet from the DNA-containing supernatant. Finally, the DNA was precipitated with ethanol and spun to isolate the DNA pellet. The full DNA extraction protocol is shown in Appendix B. DNA was quantified using the Nanodrop spectrophotometer. We excluded samples with a concentration less than 50ng/μL and a 260/280 ratio outside of the range of 1.7 to 2.0 from downstream applications. We assessed DNA degradation by running a random subset of 8 samples per rack of 60 samples on a 1% ethidium bromide gel with 50ng of DNA per well. We excluded samples that appeared to be degraded upon visualization of the gel from downstream applications.

2.4.2 Targeted genotyping

We genotyped a SNP within the *AKT1* gene (rs2494732) using the TaqMan assay. TaqMan is a polymerase chain reaction (PCR)-based genotyping assay that is ideal for genotyping a small number of SNPs in a large sample of individuals (see Figure 2.1).[154] TaqMan requires both forward and reverse primers to amplify the region surrounding the SNP of interest. Allele-specific probes hybridize to the polymorphic site. These probes have a fluorophore linked to their 5' end and a quencher linked to their 3' end. During the PCR amplification step, the probe is degraded by Taq polymerase as it extends the DNA from the primers. This step results in separation of the allele-specific fluorophore from its quencher, resulting in fluorescence. The level of fluorescence from each allele-specific

probe was detected for each sample to determine the genotype at this locus for each individual.[154] We obtained greater than 99% concordance between genotypes at rs2494732 obtained via TaqMan and genome-wide genotyping. The full TaqMan genotyping protocol is shown in Appendix B.

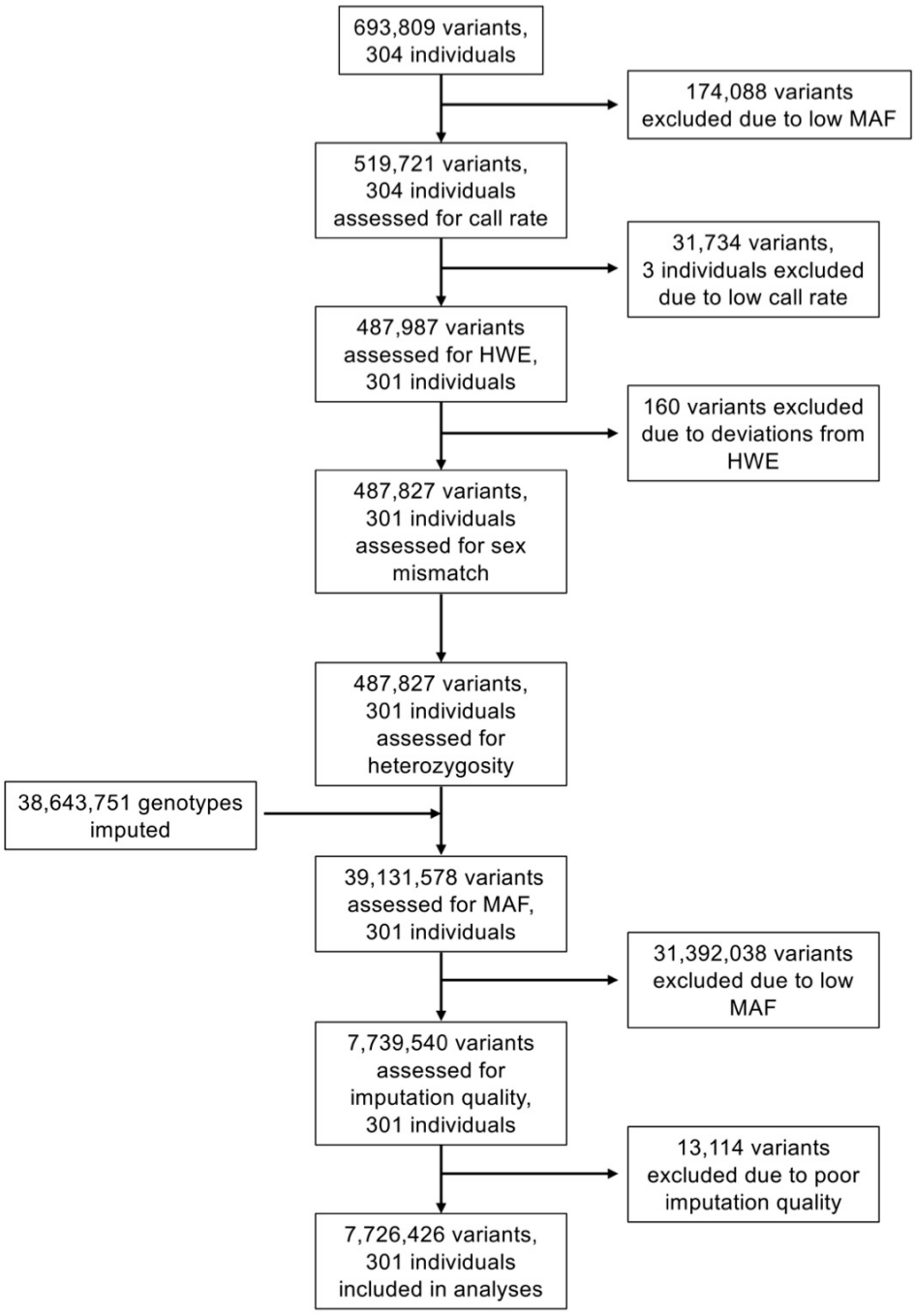
Figure 2.1. TaqMan genotyping. During the denaturing phase of the reaction, the template DNA strands separate. During the annealing phase, the probe with the fluorophore (represented by a green circle with the letter 'F') and the quencher (represented by a red circle with the letter 'Q') and the primers bind to the template DNA. In the extension phase, the Taq polymerase extends from the primer and cleaves the probe, separating the fluorophore from the quencher and resulting in detectable fluorescence. The two possible alleles at this locus are differentiated by different colored fluorophores.



2.4.3 Genome-wide genotyping, quality control, and imputation

We genotyped 693,809 single nucleotide polymorphisms (SNPs) using the Illumina Global Screening Array from DNA extracted from saliva. We completed pre-imputation quality control on genome-wide data by excluding variants and participants according to the following criteria (see Figure 2.2): 1) variants with minor allele frequency (MAF) less than 1%; 2) variants with missing call rate greater than 5%; 3) participants with genotyping rate less than 95%; 4) variants with significant deviations from Hardy-Weinberg equilibrium (HWE; $p < 10 \times 10^{-10}$);[155] 5) participants with discrepancies between self-reported sex and genetic sex; and 6) participants with abnormally high heterozygosity (> 4 SD above sample mean).[156] All quality control steps were implemented using PLINK2[157] or using scripts provided by Coleman *et al*.[158] Data were imputed using Minimac3 via the Michigan Imputation Server (https://imputationserver.sph.umich.edu/index.html). Phasing was completed using Eagle v2.3[159] and we used the Haplotype Reference Consortium as the reference panel.[160] Post-imputation quality control consisted of pruning variants with minor allele frequency less than 1% and with poor imputation quality ($R^2 < 0.30$). The protocol I created for quality control and imputation is shown in Appendix B.

Figure 2.2. Flow diagram of genome-wide genotyping quality control and imputation with variant and individual inclusion and exclusion information.



MAF = Minor allele frequency, HWE = Hardy-Weinberg equilibrium.

2.4.4 Ethnicity and population stratification

Genetic analyses can be affected by population stratification, leading to spurious associations between genetic factors and a given outcome. This is especially likely if individuals from different genetic backgrounds also differ on their risk for the outcome of interest.[161] I used PLINK2 to conduct principal components analysis.[157] I controlled for population structure by using principal components as covariates in all genetic analyses.

2.4.5 Polygenic scores

I constructed polygenic scores for ADHD, intelligence, and schizophrenia using PRSice2.[23] I pruned genotypes using clumping to obtain an independent set of SNPs in approximate linkage equilibrium with an $r^2 < 0.1$ within any 500 kb window. The predictive value of polygenic scores for traits with complex inheritance improve with the inclusion of weakly associated variants that do not meet the threshold for genome-wide significance.[162] Therefore, I constructed polygenic scores for each phenotype with *p*-value thresholds 0.50 for ADHD and 0.05 for schizophrenia and intelligence, because these thresholds maximally capture the phenotypic variance in each of these phenotypes.[163–165] To construct the polygenic scores, I weighted the contribution of each allele by the effect size of its association with each phenotype in the reference sample GWAS. The protocol containing instructions for constructing polygenic scores is shown in Appendix B.

2.5 Statistical analysis

I tested the effects of parent illness on offspring affective lability and basic symptom scores using mixed-effects linear regression, controlling for age, sex, and time in the study. I

accounted for the non-independence of repeated measures within individuals and observations from related individuals within the same family by including family and individual identifiers as random effects in the models. I tested the effect of (1) IQ, (2) externalizing symptoms, (3) ADHD polygenic score, (4) intelligence polygenic score, and (5) schizophrenia polygenic score on adversity score using mixed-effects linear regression models. I accounted for the non-independence of observations from related individuals by including the identifier as a random effect in the models. I adjusted to account for age, sex, time in the study, and the top ten ancestry informative genetic principal components. I used the standardized adjusted adversity score as the dependent variable in all regression models. I constructed five linear regression models, each with one of the five predictors listed above as the independent variable. I also constructed a full model, containing all five predictors as independent variables and with the standardized adjusted adversity score as the dependent variable. I quantified the accuracy of prediction from each model using variance explained (R^2). I also used partial variance explained to determine the variance in adversity that is uniquely explained by each of the five independent variables (IQ, externalizing symptom score, schizophrenia PGS, intelligence PGS, ADHD PGS). All analyses were implemented in R Studio (R versions 3.4 to 3.5.1).[166]

CHAPTER 3
AFFECTIVE LABILITY IN OFFSPRING OF PARENTS WITH MAJOR DEPRESSIVE DISORDER, BIPOLAR DISORDER AND SCHIZOPHRENIA

Contribution Statement

I drafted the manuscript used as the basis for this chapter, with guidance and editing from Dr. Rudolf Uher and the other co-authors. Data were collected by the FORBOW assessment team, which I am a part of. I completed the data analysis.

3.1 Abstract

Background: Affective lability, defined as the propensity to experience excessive and unpredictable changes in mood, has been proposed as a potential transdiagnostic predictor of major mood and psychotic disorder. A parental diagnosis of bipolar disorder has been associated with increased affective lability in offspring. However, the association between affective lability and family history of other mood and psychotic disorders has not been examined.

Methods: We measured affective lability using the self- and parent-reported Children's Affective Lability Scale in a cohort of 320 youth aged 6-17 years, including 137 offspring of a parent with major depressive disorder, 68 offspring of a parent with bipolar disorder, 24 offspring of a parent with schizophrenia, and 91 offspring of control parents. We tested differences in affective lability between groups using mixed-effects linear regression.

Results: Offspring of a parent with major depressive disorder (beta = 0.46, 95% CI 0.17 to 0.76, $p = 0.002$) or bipolar disorder (beta = 0.47, 95% CI 0.12 to 0.81, $p = 0.008$) had significantly higher affective lability scores than control offspring. Affective lability did not differ significantly between offspring of a parent with schizophrenia and offspring of control parents.

Conclusions: Our results suggest that elevated affective lability during childhood is a marker of familial risk for mood disorders.

3.2 Introduction

Severe mental illness (SMI), including mood and psychotic disorders, has wide-reaching implications on health across the life course. As a result, there have been calls to prioritize the early treatment and prevention of these disorders.[1,167] Recent advances show that major mental illnesses share many of the same genetic and environmental risk factors.[35,38,111,162,168–170] It may therefore be important to identify common, early manifestations shared across these disorders. Since SMI typically onsets in late adolescence and early adulthood, early risk identification strategies must focus on childhood and adolescence. The best known risk factor for SMI is a positive family history of mental illness.[4] However, family history-based risk identification is not sufficient because most individuals with a family history do not become ill themselves. Therefore, it is useful to know early manifestations of SMI that are associated with family history. This may enable the early identification of SMI risk and targeted prevention. In the present study, we examine whether affective lability is an early marker of familial risk for SMI.

SMI is typically preceded by childhood-onset disorders (*e.g.*, anxiety disorders or attention-deficit/hyperactivity disorder[171]) or other manifestations of psychopathology. Affective lability, also referred to as mood instability,[172] emotional dysregulation,[173] or cyclothymic temperament,[174] may represent an early manifestation of SMI risk. Affective lability describes the propensity to experience rapid, unpredictable, and excessive changes in mood.[113] It has been explored as a feature of SMI and as a potential early indicator of risk in childhood and adolescence. Bipolar disorder and affective lability have similar features and the link between them is well established. Affective lability is increased

among individuals with bipolar disorder[114] and their young offspring.[115,116] Additionally, affective lability has been shown to predict the onset of bipolar disorder in prospective studies of youth at familial risk.[117] There is moderate support for a relationship between affective lability and major depressive disorder. Affective lability has been shown to be elevated among individuals with depression.[118] In addition, irritability, a key component of affective lability,[115] has been shown to prospectively predict the onset of major depressive disorder among youth.[119] The evidence for an association between affective lability and schizophrenia is less conclusive. However, affective lability has been shown to be elevated among individuals with schizophrenia and it prospectively predicts schizophrenia onset.[120,121] Taken together, these findings suggest that affective lability may be an informative contributor to the prediction of SMI risk. However, it is not yet known whether affective lability during childhood and adolescence is specifically associated with disposition to bipolar disorder or whether it is more broadly associated with risk of multiple forms of SMI.

Currently, the best known predictor of SMI is a positive family history of mental illness. Individuals with a parent affected by SMI are more than twice as likely to become ill compared to offspring of control parents.[5] However, familial risk is not entirely disorder-specific and the offspring of individuals with psychotic disorders are also at increased risk for mood disorders and *vice versa*.[5] This is corroborated with molecular genetic[35,111,169] and neuropathology findings,[38] which show that the genetic variation and transcriptional dysregulation associated with illness are largely shared across forms of SMI. This provides further support for the need to identify psychopathological predictors of SMI that are

common across major mood and psychotic disorders. It is possible to examine early manifestations of SMI risk before they are shaped by illness by assessing them in offspring of parents with SMI. Using this methodology, we are able to distinguish possible predictors of illness from the effects of SMI. The link between affective lability and risk for major depressive disorder, bipolar disorder, and schizophrenia has yet to be examined in a single study.

The aim of the present study was to compare dimensional measures of affective lability among young offspring of parents with major depressive disorder, bipolar disorder, schizophrenia, and offspring of control parents. We assessed parental psychopathology and offspring affective lability in a cohort of children and youth enriched for offspring of parents with SMI.[112] We measured affective lability using validated parent- and self-report questionnaires.[113] We explored the relationship between parental diagnosis and affective lability in offspring. We hypothesized that offspring of parents with SMI will have more affective lability than control parents.

3.3 Methods

3.3.1 Sample description

The present study includes information from 808 assessments of 320 participants aged 6-17 years from 190 families, recruited as part of the Families Overcoming Risks and Building Opportunities for Well-being (FORBOW) study.[112] Affective lability was assessed annually, with the baseline assessment occurring at an average age of 10.06 years. Each participant completed an average of 3 assessments at 12-month intervals. Repeated affective lability measures from every available assessment were included in analyses. We included offspring of parents with major depressive disorder, bipolar disorder, schizophrenia spectrum disorders, and offspring of control parents. Offspring of parents with SMI were recruited through their parents' contact with mental health services in Nova Scotia, Canada. Offspring were included in FORBOW regardless of whether or not they had psychopathology. We excluded 1 individual due to severe intellectual disability (IQ < 70). Age matched offspring of control parents were recruited through local school boards. To ensure that control offspring were approximately matched with offspring of affected parents on socioeconomic status, we selectively recruited control offspring from the same schools and neighborhoods of the offspring of affected parents.

In the present study, we excluded observations from individuals collected after the onset of a final SMI diagnosis. Therefore, we excluded observations from offspring obtained after a diagnosis of bipolar disorder (N = 2 observations of 1 individual) or schizophrenia (N = 2 observations of 1 individual).

The study protocol was approved by the Research Ethics Board of the Nova Scotia Health Authority. We obtained informed consent from participants who had the capacity to provide it. For participants who did not have the capacity to make an informed decision, a parent or guardian provided written informed consent and the participant provided assent.

3.3.2 Parent assessment

Diagnoses of mental disorders according to the Diagnostic and Statistical Manual IV (DSM-IV) and DSM-5 were established using the *Schedule for Affective Disorders and Schizophrenia* (SADS-IV) and the *Structured Clinical Interview for DSM-5 Disorders* (SCID-5). Diagnoses were confirmed in consensus meetings with a psychiatrist blind to offspring psychopathology.

3.3.3 Offspring assessment

3.3.3.1 General psychopathology

Offspring were assessed for all Axis I disorders by semi-structured interview using the *Kiddie Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version* (K-SADS-PL).[146] Both the parent and the participant complete the KSADS interview and the relative weight of each reporter's input is determined based on the participant's age and developmental stage. Offspring assessors were blind to parent psychopathology. Diagnoses were confirmed in consensus meetings with a psychiatrist blind to parent diagnoses.

3.3.3.2 General cognitive ability

We assessed general cognitive ability with the Wechsler Abbreviated Scale of Intelligence – Second Edition (WASI-II).[149] The WASI-II was administered by research staff and graduate students with neuropsychological training. There are four subtests on the WASI-II: block design, vocabulary, matrix reasoning, and similarities. These four subtests are combined to provide a valid and reliable measure of full-scale IQ.

3.3.3.3 Affective lability

Affective lability was assessed in children and youth using the self- and parent-report versions of the Children's Affective Lability Scale (CALS).[113] Participants with the capacity to do so completed the self-report version of the CALS. We also obtained the parent-report version of the CALS. To allow for comparison between the self- and parent-report measures, we standardized the total scores from each questionnaire by the mean and standard deviation of the control offspring scores. For assessments in which both the self- and parent-report measures were available (n = 489), we used the mean of the two standardized scores as the dependent variable, referred to as 'overall affective lability', in our primary analyses. For assessments in which we only obtained either the parent-reported CALS (n = 272) or self-reported CALS (n = 47), the dependent variable was the standardized parent-reported or self-reported score, respectively.

3.3.3.4 Antecedent affective lability

It may be desirable to use a dichotomous indicator of affective lability in applications that require yes or no decisions. Therefore, we defined the ‘presence’ of affective lability

(referred to as 'antecedent affective lability') as a score of one standard deviation or more above the mean of a large normative population sample.[115] To improve detection in the presence of underreporting, when both the self- and parent-report measures were available we considered the higher score. We report the rates of offspring who met our pre-defined antecedent affective lability threshold[112] at first assessment or at any assessment.

3.3.5 Statistical analysis

To test the effect of parent's primary diagnosis (no diagnosis, major depressive disorder, bipolar disorder, or schizophrenia) on offspring affective lability score, we implemented mixed-effects linear regression using the lme4 package in RStudio (R version 3.4).[175] We fitted a linear mixed regression model by maximum likelihood with fixed effects of age, biological sex and time in the study as covariates. We accounted for the non-independence of observations from related individuals and from repeated measures from the same individual by including family and individual identifiers as random effects in the model.

In sensitivity analyses, we tested the effect of parent diagnosis on the self- and parent-reported affective lability separately. We fitted two models, each with parent diagnosis as the independent variable and with the same fixed (age, biological sex and time) and random (family and individual identifiers) effects. The dependent variable for each of these models was: (1) total score on parent-report CALS and (2) total score on self-report CALS. We also tested the effect of parent's primary diagnosis on offspring overall affective lability score using only observations obtained prior to a diagnosis of major depressive disorder

using the same methodology described above. Finally, we tested the effect of affected parent's sex on offspring overall affective lability scores (see Appendix C).

3.4 Results

3.4.1 Demographic and clinical characteristics

The sample included 137 offspring of a parent with major depressive disorder, 68 offspring of a parent with bipolar disorder, 24 offspring of a parent with schizophrenia and 91 offspring of control parents. The characteristics of the sample are shown in Table 3.1.

Table 3.1. Demographic and clinical characteristics of the sample. Differences between groups were tested using univariate ANOVA for continuous variables or χ^2 tests for categorical variables. "*" denotes statistically significant group differences ($p < 0.05$).

	Parent Diagnosis			
	No Diagnosis *(n = 91)*	**Depression** *(n = 137)*	**Bipolar Disorder** *(n = 68)*	**Schizophrenia** *(n = 24)*
Families, *n*	58	79	44	17
Age, *mean (SD)*	10.49 (2.80)	10.95 (2.88)	11.66 (3.08)	8.90 (2.49)
Number of follow-ups, *mean (SD)**	2.18 (1.17)	2.28 (1.24)	2.81 (1.47)	2.90 (1.49)
Females, *n (%)*	44 (48.4)	72 (52.5)	35 (51.5)	13 (54.2)
IQ at baseline, *mean (SD)**	107 (13)	104 (13)	100 (12)	100 (14)
Offspring lifetime diagnoses, *n (%)*				
*Any anxiety disorder**	26 (28.6)	50 (36.5)	37 (54.4)	2 (8.3)
*Attention-deficit/hyperactivity disorder**	14 (15.4)	3 (27.7)	24 (35.3)	4 (16.7)
*Major depressive disorder**	4 (4.4)	11 (8.0)	13 (19.1)	0 (0)

3.4.2 Affective lability scores

The mean standardized and raw affective lability scores from each measure (CALS parent-report, CALS self-report) for each parent diagnostic group are listed in Table 3.2. The parent- and self-reported affective lability scores were moderately positively correlated (Pearson's r = 0.41).

Table 3.2 Affective lability scores and frequency of antecedent affective lability for each parent diagnostic group.

	Parent Diagnosis			
	No Diagnosis *(n = 91)*	**Depression** *(n = 137)*	**Bipolar Disorder** *(n = 68)*	**Schizophrenia** *(n = 24)*
Standardized AL score, *mean (SD)*				
Overall	-0.02 (0.9)	0.49 (1.2)	0.41 (1.3)	0.10 (1.0)
CALS-P	0.00 (1.0)	0.45 (1.3)	0.28 (1.2)	0.12 (1.0)
CALS-C	0.00 (1.0)	0.52 (1.3)	0.50 (1.5)	0.13 (1.13)
Raw AL score, *mean (SD)*				
CALS-P	7.41 (8.6)	11.28 (10.8)	9.80 (10.3)	8.43 (9.0)
CALS-C	8.75 (8.4)	13.15 (11.0)	12.93 (12.4)	9.82 (9.5)
Antecedent AL baseline, *n (%)*	27 (29.7)	54 (39.4)	27 (39.7)	5 (20.8)
Antecedent AL ever, *n (%)*	34 (37.4)	71 (51.8)	40 (58.8)	6 (25.0)

AL = Affective lability; CALS-P = Children's Affective Lability Scale parent report; CALS-C = Children's Affective Lability Scale self-report.

3.4.3 Overall affective lability among offspring of parents with SMI

Across the 808 assessments of 320 children and youth with self- and/or parent-reported affective lability, offspring of a parent with SMI had significantly elevated overall affective lability scores compared to control offspring (ß = 0.43, 95% CI 0.16 to 0.70, p = 0.002). Offspring of a parent with major depressive disorder (ß = 0.46, 95% CI 0.17 to 0.76, p = 0.002) or bipolar disorder (ß = 0.47, 95% CI 0.12 to 0.81, p = 0.008) had significantly higher scores than offspring of control parents (see Figure 3.1). Offspring of a parent with schizophrenia had slightly higher scores than control offspring, but this difference was not statistically significant (ß = 0.15, 95% CI -0.34 to 0.63, p = 0.555).

When we exclude observations from offspring obtained after a diagnosis of major depressive disorder (N = 74 observations of 37 individuals), offspring of parents with major depressive disorder have significantly higher affective lability scores than controls (ß = 0.42, 95% CI 0.13 to 0.71, p = 0.005). Offspring of parents with bipolar disorder have numerically elevated affective lability scores compared to controls, but this difference is not statistically significant (ß = 0.28, 95% CI -0.06 to 0.63, p = 0.110).

3.4.4 Parent-reported affective lability among offspring of parents with SMI

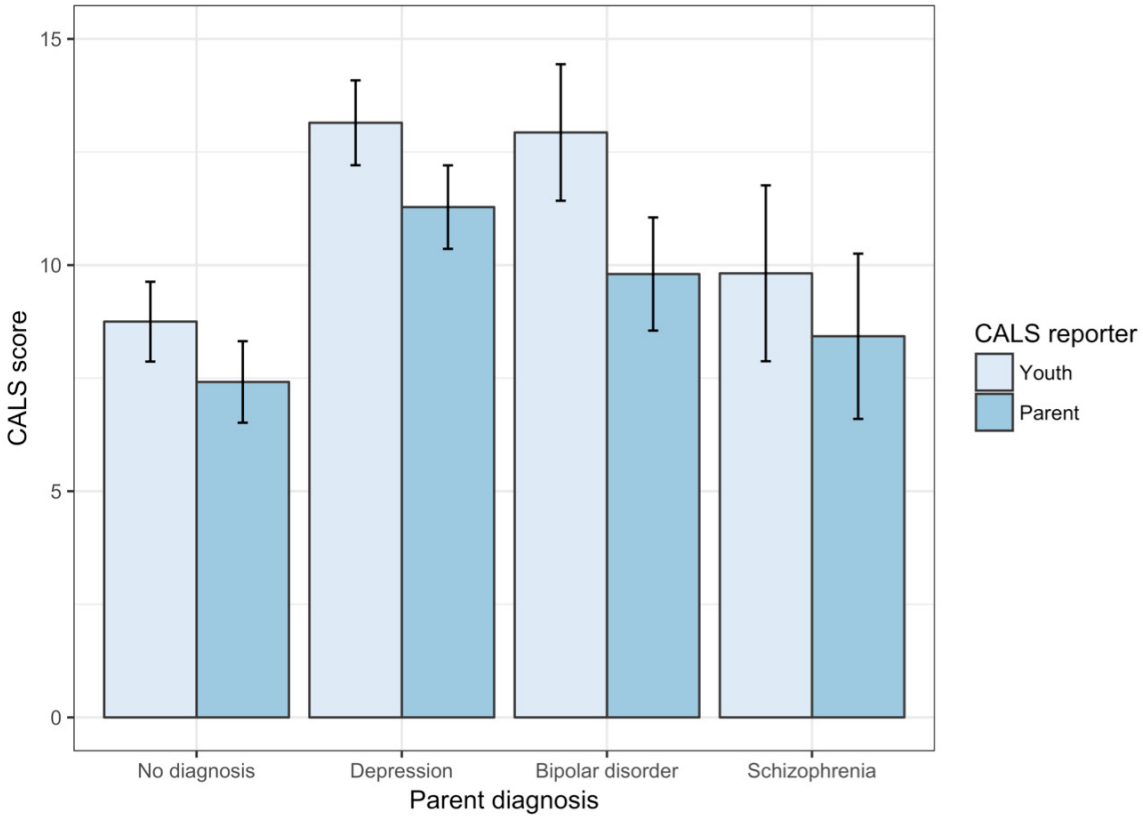
Across the 761 assessments of 313 children and youth with parent-reported affective lability, offspring of a parent with SMI had significantly elevated affective lability compared to control offspring (ß = 0.37, 95% CI 0.09 to 0.65, p = 0.009). Offspring of a parent with major depressive disorder had significantly higher scores than offspring of control parents (ß = 0.44, 95% CI 0.13 to 0.75, p = 0.005; see Figure 3.1). Offspring of a

parent with bipolar disorder had numerically higher scores than offspring of control parents, but the difference did not reach statistical significance (ß = 0.35, 95% CI -0.01 to 0.70, $p = 0.058$). Offspring of a parent with schizophrenia had slightly higher scores than control offspring, but this difference was not statistically significant (ß = 0.11, 95% CI -0.40 to 0.61, $p = 0.678$).

3.4.5 Self-reported affective lability among offspring of parents with SMI

Across the 536 assessments of 246 children and youth with self-reported affective lability, offspring of a parent with SMI had significantly elevated affective lability compared to control offspring (ß = 0.43, 95% CI 0.09 to 0.78, p = 0.014). Offspring of a parent with major depressive disorder (ß = 0.41, 95% CI 0.04 to 0.79, $p = 0.032$) or bipolar disorder (ß = 0.50, 95% CI 0.06 to 0.94, $p = 0.026$) had significantly higher scores than offspring of control parents (see Figure 3.1). Offspring of a parent with schizophrenia had slightly higher scores than control offspring, but this difference was not statistically significant (ß = 0.36, 95% CI -0.33 to 1.05, $p = 0.308$).

Figure 3.1 Mean parent- and youth-reported raw CALS scores for each parent diagnostic group. Error bars represent standard error of the mean.



3.4.6 Antecedent affective lability

We defined antecedent affective lability as a score 1 or more standard deviations above the mean of a large normative population sample on either the self- or parent-report measures. The rates of participants meeting this criterion from each parent diagnostic group are shown in Table 3.2. A substantially larger proportion of offspring of a parent with major depressive disorder (51.8%) or bipolar disorder (58.8%) had antecedent affective lability than did offspring of control parents (37.4%) and offspring of a parent with schizophrenia (25.0%).

3.5 Discussion

We compared affective lability among young offspring of a parent with major depressive disorder, bipolar disorder, schizophrenia, and offspring of control parents. We sought to test whether elevated affective lability in early life is specifically associated with parental bipolar disorder, or whether it is more generally associated with multiple forms of SMI. We found that dimensional affective lability was elevated among offspring of a parent with either major depressive disorder or bipolar disorder. We did not find a significant difference in affective lability between offspring of a parent with schizophrenia and offspring of control parents.

Our study was motivated by the need to examine the association between parental diagnoses of a major mood or psychotic disorders and affective lability in offspring with consistent assessment and analysis across parent diagnostic groups. It has been previously shown that affective lability is elevated among the offspring of a parent with bipolar

disorder and that affective lability predicts the onset of bipolar disorder later in life.[115,117] We confirmed that affective lability is elevated among offspring of a parent with bipolar disorder. We also found similarly elevated affective lability among the offspring of a parent with major depressive disorder. This finding was not entirely unexpected because irritability, a key component of affective lability,[115] strongly predicts the onset of major depressive disorder.[119] Additionally, this is consistent with our previous finding that disruptive mood dysregulation disorder, a condition characterized by temper outbursts and irritability,[176–178] was associated with a family history of major depressive disorder.[179] We did not find a difference in affective lability between offspring of a parent with schizophrenia and controls. Although this latter finding comes with a large degree of uncertainty due to a smaller sample of offspring of parents with schizophrenia, it is consistent with a recent finding that affective lability was elevated among patients with schizophrenia but not among their unaffected siblings.[120]

The results of the present study have potential implications for future research. The finding that affective lability is strongly associated with a parental diagnosis of major depressive disorder is new and informative. In conjunction with recent research suggesting that the familial and genetic risk for SMI may be shared across disorders,[5,35,38,111] our results suggest that affective lability may be broadly associated with a family history of mood disorders and may be an antecedent of mood disorders. The usefulness of affective lability as a predictor of multiple forms of mood disorders warrants exploration in longitudinal studies of high-risk offspring and in the general population. Since affective lability can be

effectively measured using a questionnaire,[113] its assessment can easily be adopted by larger studies of high-risk offspring.

The present study benefits from inclusion of offspring of parents with several types of SMI, resulting in a concentration of familial risk of SMI and thus a higher rate of psychopathology compared to the general population. Additionally, it is beneficial that we have included a broad age range of high-risk and control offspring, allowing us to map transdiagnostic commonality and specificity of affective lability across childhood and adolescence among youth at high familial risk for multiple forms of SMI. However, our results should be interpreted in the context of several limitations. The main limitation is the restricted statistical power due to smaller numbers of offspring of parents with bipolar disorder and with schizophrenia. The moderate size of the group of offspring of parents with bipolar disorder may have limited our ability to examine group differences separately for self-reported and parent-reported affective lability. However, the consistency with previous findings strongly suggests that increased affective lability is a robust feature of individuals at familial risk for bipolar disorder.[115] The small sample of offspring of parents with schizophrenia is a more significant limitation. The smaller enrolment of offspring of parents with schizophrenia may be partly due to the fact that individuals with schizophrenia have fewer children.[180] Since our analyses of offspring of a parent with schizophrenia had limited statistical power, any results regarding familial liability to schizophrenia should be viewed as preliminary. It would be useful for ongoing investigations of offspring of parents with schizophrenia to incorporate the assessment of affective lability to allow for further examination of the link between affective lability and family history of psychosis.

3.6 Conclusion

In conclusion, we found that affective lability is elevated among offspring of a parent with major depressive disorder or bipolar disorder compared to offspring of control parents. Our findings suggest that elevated affective lability may be broadly associated with familial risk for mood disorders, rather than being specifically associated with a family history of bipolar disorder. Future studies may explore the value of affective lability as a transdiagnostic predictor of risk of mood disorders.

CHAPTER 4
BASIC SYMPTOMS IN OFFSPRING OF PARENTS WITH SEVERE MENTAL ILLNESS

Contribution Statement

I drafted the manuscript used as the basis for this chapter, with guidance and editing from Dr. Rudolf Uher and the other co-authors. Data were collected by the FORBOW assessment team, which I am a part of. I completed the data analysis.

4.1 Abstract

Background: Basic symptoms, defined as subjectively perceived disturbances in thought, perception, and other essential mental processes, have been established as a predictor of psychotic disorders. However, the relationship between basic symptoms and family history of a transdiagnostic range of severe mental illness, including major depressive disorder, bipolar disorder and schizophrenia, has not been examined.

Aims: We sought to test whether non-severe mood disorders and severe mood and psychotic disorders (severe mental illness) in parents is associated with increased basic symptoms in their biological offspring.

Method: We measured basic symptoms using the Schizophrenia Proneness Instrument – Child and Youth Version in 332 youth aged 8-26 years, including 93 offspring of control parents, 92 offspring of a parent with non-severe mood disorders, and 147 offspring of a parent with severe mental illness. We tested the relationships between parent mental illness and offspring basic symptoms in mixed-effects linear regression models.

Results: Offspring of a parent with severe mental illness ($B = 0.69$, 95% CI 0.22 to 1.16, $p = 0.004$) or illness with psychotic features ($B = 0.68$, 95% CI 0.09 to 1.27, $p = 0.023$) had significantly higher basic symptom scores than control offspring. Offspring of a parent with non-severe mood disorders reported intermediate levels of basic symptoms, that did not significantly differ from control offspring.

Conclusions: Basic symptoms during childhood are a marker of familial risk of psychopathology that is related to severity and is not specific to psychotic illness.

4.2 Introduction

Severe mental illness (SMI) refers to mental disorders that cause functional impairment which substantially interferes with one or more major life activities, including mostly major mood or psychotic disorders. SMI often follows a chronic or recurrent course and available treatments have limited efficacy.[181–184] Improving upon our ability to predict SMI may be useful to inform targeted early interventions to prevent its onset.[147] Recent research has shown that there is substantial overlap in the genetic and environmental contributors to various forms of SMI.[35,38,111,162,185] Consequently, it may be useful to identify overlapping, transdiagnostic predictors of SMI that can be detected early enough to allow for preventive interventions. Self-experienced disruptions in thought, perception, and other essential mental processes, referred to as basic symptoms, are potential early indicators of SMI risk.[186]

Basic symptoms may represent an early manifestation of SMI, particularly psychotic illness.[124,147,186] Positive symptoms of psychosis include hallucinations and/or delusions, which are perceived by the affected individual as real experiences. In contrast to positive psychotic symptoms, basic symptoms are immediately recognized by the individual as abnormal disturbances to their typical thoughts, senses, and feelings.[122] These symptoms are often present years before the onset of illness and can be assessed in children as young as 8 years old.[125] Basic symptoms have been examined in detail as a potential precursor to psychotic illness. Basic symptoms strongly predict the onset of psychotic illness.[147] Basic symptoms have also been linked to other forms of mental illness, including affective disorders[128,129] and are associated with lower global functioning among individuals with a

range psychiatric disorders.[130] However, the utility of basic symptoms as an indicator of risk for a broader range mental disorders remains to be examined.

The best known predictor of SMI is a family history of illness.[4] Risk of illness is proportional to the degree of biological relatedness to the affected individual.[4] However, familial risk of mental illness is not disorder-specific. Individuals with a family history of schizophrenia are also at risk of mood disorders, and *vice versa.*[5] This finding is supported by molecular data which show that a substantial proportion of genetic variants and gene expression abnormalities associated with mental illness are shared across psychiatric disorders.[35,38,168,169] Taken together, these findings suggest that it may be useful to identify measurable experiences and behaviours that predict SMI and are shared across disorders. By examining early manifestations of risk among offspring of parents with SMI, we are able to distinguish possible causes or predictors of illness from the effects of SMI and its treatment. Individuals who have a first degree biological relative living with schizophrenia experience more basic symptoms than controls.[126,127] However, basic symptoms have not yet been examined among youth at high familial risk for other forms of mental illness.

Here we examine the relationship between basic symptoms and family history of a spectrum of non-severe mood disorders and severe mental illness, which includes both mood and psychotic disorders. We assessed basic symptoms in a sample of youth enriched for offspring of parents with major depressive disorder, bipolar disorder and schizophrenia, including both non-severe mood disorders and SMI. We aimed to test whether offspring

basic symptoms are associated with parent mental illness, its severity, psychotic features, or specific psychiatric diagnosis.

4.3 Methods

4.3.1 Sample description

The present study includes information from 909 assessments of 332 participants aged 8-26 years from 201 families, enrolled in the Families Overcoming Risks and Building Opportunities for Well-being (FORBOW) study.[187] Assessors blind to information on parents assessed basic symptoms annually, with the baseline assessment occurring at an average age of 11.84 years (range 8-24 years). Each participant completed a median of 3 assessments (range 1-6) at 12-month intervals. Repeated basic symptom measures from all assessments were included in analyses. We included offspring of parents with major depressive disorder, bipolar disorder, psychosis spectrum disorders, and offspring of control parents. Offspring of parents with SMI were recruited through their parents' contact with mental health services in Nova Scotia, Canada. Offspring were included regardless of whether or not they had psychopathology. Age matched offspring of control parents were recruited through local school boards. To ensure that control offspring were approximately matched with offspring of affected parents on socioeconomic status, we selectively recruited control offspring from the same schools and neighborhoods of the offspring of affected parents. We excluded offspring with a lifetime diagnosis of schizophrenia (n = 2 observations), schizoaffective disorder (n = 2 observations), or bipolar disorder (n = 8 observations).

We assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The study protocol was approved by the Research Ethics Board of the Nova Scotia Health Authority (file number 100266). We obtained written informed consent from participants who had the capacity to provide it. For participants who did not have the capacity to make an informed decision, a parent or guardian provided written informed consent and the participant provided assent.

4.3.2 Parent assessment

Diagnoses of mental disorders and psychotic symptoms according to the Diagnostic and Statistical Manual IV (DSM-IV) and DSM-5 were established using the *Schedule for Affective Disorders and Schizophrenia* (SADS-IV)[143] or the *Structured Clinical Interview for DSM-5 Disorders* (SCID-5).[144] Diagnoses were confirmed in consensus meetings with a psychiatrist blind to offspring psychopathology.

We defined severe mental illness (SMI) as a diagnosis of major depressive disorder, bipolar disorder, or a psychosis spectrum disorder accompanied by two or more of the following five severity criteria: (1) recurrent, (2) chronic, (3) presence of psychotic symptoms, (4) life threatening suicide attempt(s), or (5) required hospital admission.[187] We defined non-severe mood disorders (NSMD) as a diagnosis of any Axis I mood disorder that did not meet 2 or more severity criteria. In situations where one biological parent had NSMD and one biological parent had SMI, the offspring were placed in the SMI group.

4.3.3 Offspring assessment

4.3.3.1 General Psychopathology

Offspring were assessed for all Axis I disorders at 12-month intervals using the *Kiddie Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version* (K-SADS-PL; in offspring younger than 18 years)[146] or the *Structured Clinical Interview for DSM-5* (SCID; in offspring 18+ years old).[144] A single assessor completed both the diagnostic interview and the basic symptoms interview. Offspring assessors were blind to parent psychopathology. Diagnoses were confirmed in consensus meetings with a psychiatrist blind to parent diagnoses.

4.3.3.2 Basic symptoms

We assessed basic symptoms using the *Schizophrenia Proneness Instrument – Child and Youth Version* (SPI-CY).[125] The SPI-CY was designed to be administered to children and youth and it has been used among children aged 8 years and older with good inter-rater reliability.[125,188] The SPI-CY contains 2 psychosis-risk basic symptom profiles: Cognitive-Perceptive (COPER) and Cognitive Disturbances (COGDIS). COGDIS items have been shown to strongly predict psychotic illness and are part of the clinical high-risk criteria that have been recommended for the early detection of psychosis.[147] Descriptions of the items in both high-risk profiles are provided in Table 1. We calculated the SPI-CY risk score as the total number of COPER or COGDIS items scored 3 (several times in a month or weekly) to 6 (daily), divided by the total number of items with a valid frequency rating. We calculated a COGDIS score, which incorporates the 9 items included in the COGDIS

criteria, using the same process. For analyses, we standardized the SPI-CY risk score and COGDIS score by the means and standard deviations of the control offspring scores.

Table 4.1. Basic symptoms high-risk items. Items included only in the COPER high-risk profile are shown in light blue, items included only in the COGDIS high-risk profile are shown in dark blue, and items included in both COPER and COGDIS are shown in medium blue. To fulfill COPER criteria, an individual must experience 1 of the first 10 items at least several times in a month within the 3 months prior to assessment and the first occurrence must have been at least 12 months prior to the assessment. To fulfill COGDIS criteria, an individual must experience 2 of the last 9 items, each at least several times in a month within the 3 months prior to assessment.[125] Items B7, D7, and D15 can be consistently assessed in individuals aged 13 years and older.

Item Name (Item Number)	Description	Example Prompt
Decreased ability to discriminate between ideas and perception, fantasy and true memories (B1)	Difficulty locating the source of a memory resulting in an inability to distinguish between fantasy and true memories.	Do you become confused about whether you actually did something in the past or whether you just imagined it?
Unstable ideas of reference (B2)	Experiences of self-reference that are almost immediately rectified upon further consideration.	Do you ever think that the actions or comments of others are about you – but yet you are certain they are not?
Visual perception disturbances (B3, O1, O3)	Aspects of vision are misperceived but the individual is aware of their true appearance.	Do the outlines of objects sometimes appear broken, curved, or wavy?
Acoustic perception disturbances (B4.2, B5)	Non-verbal auditory pseudo-hallucinations, changes in the quality of sounds, or abnormally long-lasting residual sounds.	Do you sometimes have sudden short-lived difficulty with your hearing – like sounds being muffled or less loud?
Derealization (B7)	A change in how one relates emotionally to the environment: 1) the environment appears unreal or altered, or 2) an increased emotional affinity for the environment.	Do you sometimes experience your surroundings as changed or strange? As if the world around you is not real?
Thought interference (D9)	Irrelevant thoughts are intruding on and disturbing the train of thought.	If you want to concentrate on something, is your concentration suddenly interrupted by unimportant, irrelevant thoughts?
Thought pressure (D10)	Thoughts or images randomly enter the mind and disappear again in quick succession, without the individual being able to suppress or guide them.	Do you sometimes have the feeling that you are not able to control your thoughts anymore?
Disturbance of receptive speech (D11)	Disturbance in the understanding of words that are either read or heard.	Do you sometimes have difficulty understanding conversations that you know you should be able to follow?
Thought perseveration (D14)	The annoying rehearsal of unimportant, emotionally neutral thoughts related to trivial events of the recent past.	Do you sometimes find yourself thinking about past events that have no special meaning, even though you want to think about something else or go to sleep?
Thought blockages (D15)	A sudden interruption in the flow of thoughts, of the mind suddenly going blank, or the fading of thoughts.	Do your thoughts sometimes disappear suddenly, as if they were cut short?
Disturbances of abstract thinking (D7)	Deficits in the ability to understand abstract, figurative, or symbolic phrases beyond their literal meaning.	Do you have difficulty understanding the meaning of metaphors or abstract things like a saying or an idiom?
Inability to divide attention (D8)	Difficulty in dealing with demands that involve more than 1 sensory modality at a time.	Can you do 2 things at once as easily as you could before?
Disturbances of expressive speech (D12)	Subjective difficulty in finding the right words when trying to express oneself.	When you want to say something, do you struggle to find the right words?
Captivation of attention by details of the visual field (O2)	An ordinary visual stimulus stands out in a striking manner so that it appears almost isolated from the rest of the environment.	Is your attention sometimes caught by a detail in your surroundings, so that you need to look at it without wanting to?

4.3.3.3 Antecedent basic symptoms

It may be desirable to use a dichotomous indicator of basic symptoms in applications that require yes or no decisions. Therefore, we defined antecedent basic symptoms as the presence of COPER and/or COGDIS criteria.[187] We report the rates of offspring who met our pre-defined antecedent basic symptoms threshold at their first assessment and at any assessment.

4.3.4 Statistical analysis

We tested the effect of parent mental illness on offspring basic symptoms in mixed-effects linear regression models using the lme4 package,[175] implemented in R Studio (R version 3.4.3).[189] We accounted for the non-independence of observations from related individuals and from repeated measures within the same individual by including family and individual identifiers as random effects in the models. We included fixed effects of age, biological sex, and time in the study as covariates. To test the effect of parent's primary illness severity (control, NSMD, SMI) on offspring basic symptoms, we fitted a linear mixed regression model with standardized offspring SPI-CY risk score as the dependent variable and parent illness severity as the independent variable. We tested the effect of parent psychosis (control, non-psychotic illness, psychotic illness) on offspring basic symptoms by fitting a linear mixed regression model with standardized offspring SPI-CY risk score as the dependent variable and parent psychosis as the independent variable. We tested the effect of parent's primary diagnosis (control, major depressive disorder, bipolar disorder, psychosis spectrum disorder) on offspring basic symptoms by fitting a linear mixed regression model with standardized offspring SPI-CY risk score as the dependent variable

and parent diagnosis as the independent variable (see Appendix C, Supplementary Tables 4.9 and 4.10, Supplementary Figure 4.2). We also tested the effects of parent illness severity, parent psychotic symptoms, and parent diagnosis on offspring COGDIS scores separately, using the same methodology as described above. Effect sizes are summarized with standardized beta coefficients and their 95% confidence intervals.

4.3.5 Sensitivity analyses

We opted not to exclude offspring with major depressive disorder from the primary analyses because depression was common and excluding it would reduce the representativeness of the sample. However, to ensure that our results were not unduly influenced by offspring depressive disorders, we performed sensitivity analysis by excluding observations in which offspring had experienced a major depressive episode within the 12 months prior to the assessment. Additionally, the prevalence and clinical significance of basic symptoms has been shown to vary with age.[190] Since we included participants across a broad range of ages, we stratified analyses by age and tested the effect of parent illness severity (control, NSMD, SMI) on offspring basic symptoms among participants aged 11 years and under and 12 years and older separately (see Appendix C).

4.4 Results

4.4.1 Sample characteristics and basic symptom scores

The sample included 93 offspring of control parents, 92 offspring of a parent with non-severe mood disorders, and 147 offspring of a parent with severe mental illness. The characteristics of the sample and the rates of antecedent basic symptoms across parent groups are shown in Table 4.2.

Table 4.2. Demographic and clinical characteristics of the sample. Differences between groups were tested using univariate ANOVA for continuous variables or χ^2 tests for categorical variables. "*" denotes statistically significant group differences.

	Parent Group		
	Control *(n = 93)*	**NSMD** *(n = 92)*	**SMI** *(n = 147)*
Parent major depressive disorder, *n*	0	85	62
Parent bipolar disorder, *n*	0	7	68
Parent schizophrenia, *n*	0	0	17
Parent illness psychotic features, *n*	0	1	58
Families, *n*	60	59	88
Age, *mean (SD)**	12.11 (3.1)	13.65 (4.2)	13.78 (4.0)
Number of follow-ups, *mean (SD)**	2.43 (1.3)	2.66 (1.4)	3.02 (1.6)
Females, *n (%)**	42 (45.2)	44 (47.8)	83 (56.5)
Antecedent BS at baseline, *n (%)*	12 (12.90)	16 (17.39)	35 (23.81)
Antecedent BS ever, *n (%)**	18 (19.35)	26 (28.26)	54 (36.73)

4.4.2 Differences in SPI-CY risk scores by parent illness severity

Across the 909 assessments of 332 children and youth with valid SPI-CY risk scores, basic symptoms were significantly elevated among the offspring of parents with SMI compared to controls (ß = 0.69, 95% CI 0.22 to 1.16, p = 0.004; see Figures 4.1 and 4.2). Basic symptom scores were numerically elevated among offspring of parents with NSMD, but this difference was not statistically significant (ß = 0.22, 95% CI -0.30 to 0.73, p = 0.415). When we excluded observations at which offspring experienced a major depressive episode within 12 months prior to the assessment, basic symptoms remained significantly elevated among the offspring of parents with SMI (ß = 0.49, 95% CI 0.10 to 0.87, p = 0.014). Full regression results are shown in Appendix C, Supplementary Tables 4.1 and 4.2. In age-stratified analyses, these findings remained consistent in both the younger (8-11 year olds) and older (12 years and older) subsets (see Appendix C, Supplementary Tables 4.11 and 4.13, Supplementary Figures 4.3 and 4.4).

4.4.3 Differences in COGDIS score by parent illness severity

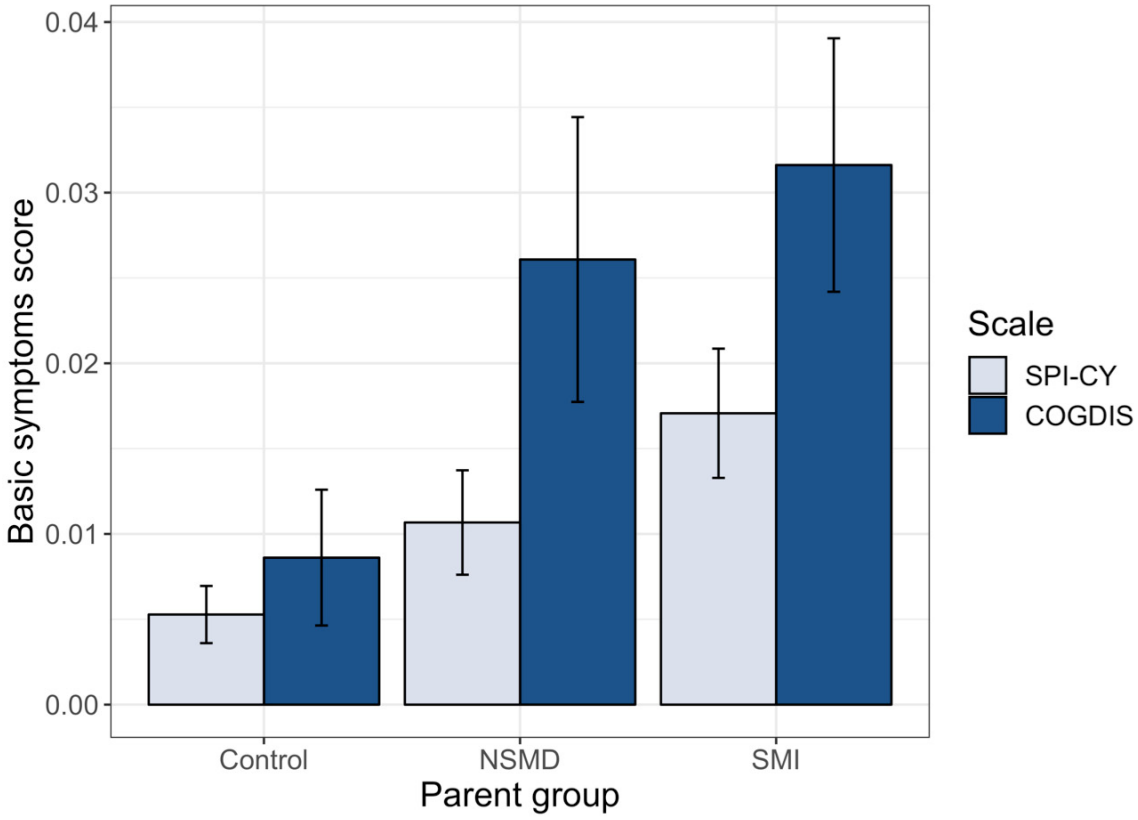
Across the 905 assessments of 331 children and youth with valid COGDIS scores, basic symptoms were significantly elevated among the offspring of parents with SMI compared to controls (ß = 0.53, 95% CI 0.13 to 0.93, p = 0.009; see Figures 4.1 and 4.2). When we excluded observations at which offspring experienced a major depressive episode within 12 months prior to the assessment, basic symptoms remained significantly elevated among the offspring of parents with SMI (ß = 0.39, 95% CI 0.04 to 0.73, p = 0.028). Full regression results are shown in Appendix C, Supplementary Tables 4.3 and 4.4. In age-stratified analyses, COGDIS scores were numerically increased among offspring of parents with

SMI, however these differences were only statistically significant in the younger (8-11 year olds) subset (see Appendix C, Supplementary Tables 4.12 and 4.14, Supplementary Figures 4.3 and 4.4).

4.4.4 Differences in basic symptom scores by parent psychosis

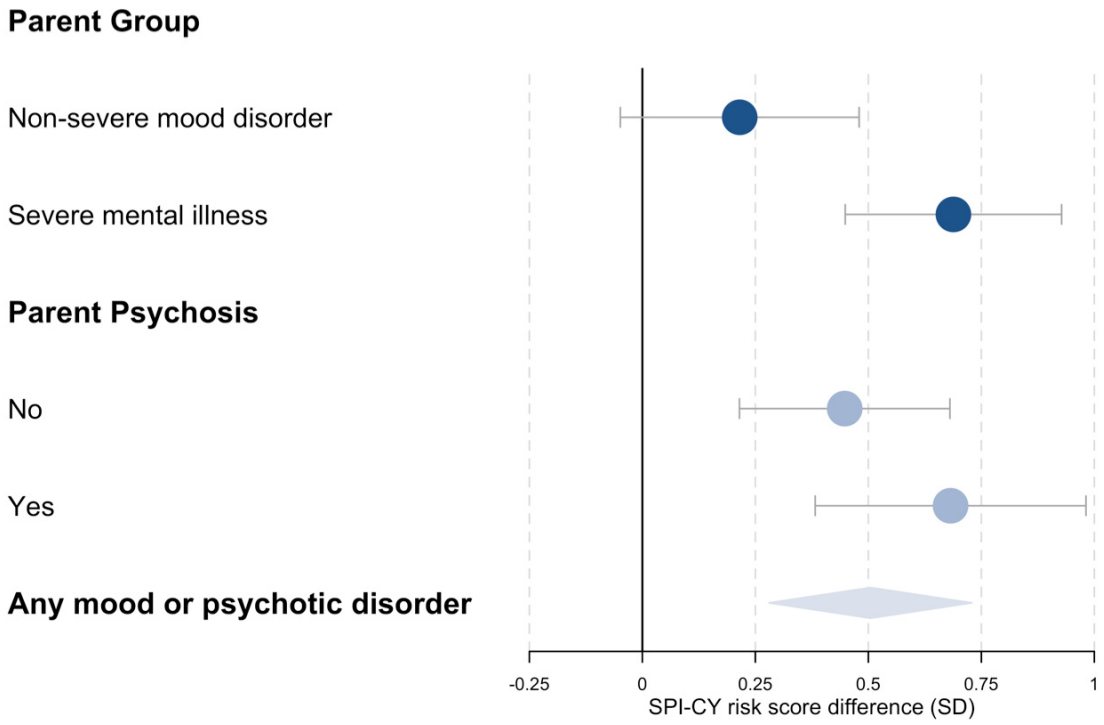
Across the 909 assessments of 332 children and youth with valid SPI-CY risk scores, basic symptoms were significantly elevated among offspring of a parent with psychotic mental illness compared to controls (ß = 0.68, 95% CI 0.09 to 1.27, $p = 0.023$; see Appendix C, Supplementary Figure 4.1). Offspring of a parent with non-psychotic mental illness had numerically higher SPI-CY risk scores than controls, but this difference was not statistically significant (ß = 0.45, 95% CI -0.01 to 0.90, $p = 0.055$, see Appendix C, Supplementary Figure 4.1). When we excluded observations at which offspring experienced a major depressive episode within 12 months prior to the assessment, both the offspring of parents with psychotic mental illness (ß = 0.44, 95% CI -0.05 to 0.92, $p = 0.078$) and with non-psychotic mental illness (ß = 0.35, 95% CI -0.02 to 0.72, $p = 0.067$) had numerically higher SPI-CY risk scores than controls, but the difference was not statistically significant. Similarly, COGDIS scores were significantly elevated among the offspring of parents with psychotic mental illness (ß = 0.55, 95% CI 0.05 to 1.04, $p = 0.030$) and with non-psychotic mental illness (ß = 0.41, 95% CI -0.02 to 0.80, $p = 0.037$). Full regression results are shown in Appendix C, Supplementary Tables 4.5-4.8.

Figure 4.1. Mean SPI-CY risk score and COGDIS score, stratified by parent illness severity group. Error bars represent standard error of the mean.



Control = No history of mood or psychotic disorder; NSMD = Non-severe mental illness; SMI = Severe mental illness.

Figure 4.2. Effect of parent illness on offspring basic symptoms. Circles represent the effect size of the standard deviation increase in SPI-CY risk score compared to controls for each parent group. Whiskers represent the standard error.



4.4.5 Antecedent basic symptoms

We defined antecedent basic symptoms as the presence of COPER and/or COGDIS high-risk criteria. The rate of youth meeting these high-risk criteria increased with increasing parent severity: 12.9% control offspring, 17.4% of offspring of parents with NSMD and 23.8% of offspring of parents with SMI had antecedent basic symptoms at baseline (Table 4.2).

4.5 Discussion

We sought to test whether basic symptoms during childhood and adolescence are elevated among offspring of parents with a spectrum of non-severe mood disorders (NSMD) and severe mental illness (SMI), which included mood and psychotic disorders. We found that basic symptoms were most elevated among offspring of parents with severe mental illness, intermediate in offspring of parents with non-severe mood disorders, and lowest in offspring of control parents.

Our study was motivated by a need to identify early transdiagnostic indicators of risk for SMI among youth. Previous studies have established that basic symptoms predict psychosis, and can be present years before its onset.[123,147] However, basic symptoms have not been previously examined among offspring of parents with a broad range of major mood and psychotic disorders. Consistent with prior studies which show offspring of parents with SMI are at risk of developing SMI themselves,[5] we found that basic symptoms are elevated among the offspring of parents with SMI compared to controls. We also confirmed that basic symptom scores are elevated among first-degree relatives of

individuals with psychosis.[127] Our results are consistent with prior findings showing that offspring of parents with SMI are at increased risk for multiple forms of SMI, in addition to the disorder present in the parent.[5] Additionally, it has been suggested that psychosis may represent a transdiagnostic indicator of illness severity.[191,192] This is supported by studies showing that the presence of psychotic symptoms in non-psychotic disorders has been associated with more severe illness and worse treatment outcomes.[193–196] Our results suggest that basic symptoms represent a transdiagnostic marker of risk for severe mental illness that is not specific to psychotic illness.

The present study benefits from the inclusion of offspring of parents with mental illness, resulting in a concentration of familial risk of psychopathology. As a result, our sample has a higher rate of basic symptoms than in the general population. We also benefit from a longitudinal design, with repeated assessments allowing to capture basic symptoms over the period of several years. However, our results should be interpreted in the context of our study limitations. The main limitation is the smaller number of offspring of parents with a schizophrenia spectrum disorder. The majority parents in our sample who experience psychosis have bipolar disorder or major depressive disorder with psychotic features. The smaller enrolment of offspring of parents with schizophrenia may be due in part to the fact that individuals with schizophrenia tend to have fewer children.[180] However, enrolment in our cohort is ongoing and will include more offspring of parents with schizophrenia spectrum disorders in the future. Additionally, since basic symptoms are more prevalent and may be more clinically relevant among older adolescents,[188,190] our study was limited by the inclusion of younger adolescents and children. However, in our age-stratified

sensitivity analyses, we found that basic symptom scores were independently associated with parent mental illness among 8-11 year old and among 12-27 year old offspring.

The results of our study have potential implications for future research. The finding that basic symptoms are elevated among young offspring of parents with SMI can help target interventions to youth at high risk of SMI, long before the onset of illness. Interventions aimed at preventing psychosis among individuals experiencing prodromal symptoms have been criticized, in part because "good" outcomes may be synonymous with onsets of other, non-psychotic illnesses among intervention recipients.[192] It has been shown that earlier interventions produce better outcomes.[134,197] Our results suggest that basic symptoms may represent a useful transdiagnostic risk indicator. Basic symptoms could be used, in combination with other factors, to identify high-risk youth who may benefit from targeted interventions before the onset of major mental illnesses. Our results warrant further investigation in other familial high-risk cohorts. Additionally, the basic symptom assessment tool[125] could be adopted by cohorts currently using interview measures of psychopathology.

4.6 Conclusion

In conclusion, we found that basic symptoms are elevated among offspring of parents with severe mental illness, in addition to offspring of parents with psychosis. Our results suggest that basic symptoms during childhood are a marker of familial risk for psychopathology that is related to severity and is not specific to psychotic illness. Future studies could explore the value of basic symptoms as a transdiagnostic predictor of mental illness.

CHAPTER 5
NEURODEVELOPMENTAL AND GENETIC DETERMINANTS OF EXPOSURE TO ADVERSITY AMONG YOUTH AT RISK FOR MENTAL ILLNESS

Contribution Statement

I drafted the manuscript used as the basis for this chapter, with guidance and editing from Dr. Rudolf Uher and the other co-authors. Phenotypic data and saliva samples were collected by the FORBOW assessment team, which I am a part of. Dr. Amina Bagher and I completed the benchwork. I completed the genomic quality control and data analysis.

5.1 Abstract

Background: Attention-deficit/hyperactivity disorder (ADHD) and lower cognitive ability have been linked with increased likelihood of exposure to adversity. We hypothesized that these associations may be partly due to genetic factors.

Methods: We calculated polygenic scores for ADHD and intelligence and assessed psychopathology and general cognitive ability in a sample of 301 youth aged 5-27 years enriched for offspring of parents with mood and psychotic disorders. We calculated an adversity score as a mean of 10 indicators, including socioeconomic disadvantage, childhood maltreatment, and bullying. We tested the effects of polygenic scores, externalizing symptoms, and IQ on adversity scores using mixed-effects linear regression.

Results: Externalizing symptoms and general cognitive ability showed expected positive and negative relationships with adversity, respectively. Polygenic scores for intelligence were unrelated to adversity, but polygenic scores for ADHD was associated with adversity (ß = 0.25, 95% CI 0.14 to 0.35, $p < 0.0001$). ADHD polygenic scores uniquely explained 5% of variance in adversity score. The relationship between polygenic scores for ADHD and adversity was independently significant among individuals with (ß = 0.51, 95% CI 0.26 to 0.76, $p < 0.0001$) and without (ß = 0.16, 95% CI 0.04 to 0.29, $p = 0.009$) ADHD.

Conclusions: A genetic score indexing liability to ADHD was associated with exposure to adversity in early life. Previously observed associations between externalizing symptoms, lower cognitive ability and adversity may be partially attributed to genetic liability to ADHD.

5.2 Introduction

Many individuals experience adversity in childhood, including poverty, neglect, abuse or bullying.[198] These early exposures are implicated in later development of psychopathology,[199,200] and are associated with more severe and treatment-resistant forms of mental illness.[53,54] It has commonly been assumed that exposure to adversity causes mental disorders and limits development of cognitive ability.[201] However, externalizing symptoms[202] and lower cognitive ability[203] may also render individuals more likely to experience adversity. Genetic factors may help us understand the direction of causality because they are stable over the lifespan. In the present study, we used polygenic scores to examine the relationships between intelligence, attention-deficit/hyperactivity disorder (ADHD) and adversity.

Lower cognitive ability has been linked to adversity. Individuals who report a history of childhood maltreatment have lower cognitive abilities on average.[204] However, it has been shown that the lower cognitive performance among victimized individuals pre-dates the occurrence of their victimization.[203] This suggests that lower cognitive ability may be a risk factor for adversity. Similarly, individuals with ADHD exhibit lower performance on cognitive tests on average than individuals without ADHD.[205]

ADHD is a heritable neurodevelopmental disorder characterized by persistent inattention and/or hyperactivity and impulsivity that causes distress or impairment for the affected individual.[206] ADHD onsets early in life, and frequently persist into adulthood.[207] ADHD is associated with a range of adverse outcomes, including lower socioeconomic status

(SES),[208] poor physical and mental health,[171,208] and death due to accidents.[209] Importantly, the presence of ADHD in childhood prospectively predicts abuse and neglect later in life.[202] Taken together, the available evidence suggests that ADHD and lower cognitive ability render an individual more likely to experience adversity. However, it is not known to what extent the previously observed associations can be explained by genetic factors.

Genetic factors influence both intelligence and risk of psychopathology. Genetic variants that are individually weakly related to a given phenotype can be combined into polygenic scores (PGS) that are more consistently predictive.[23] Genetic studies have found that genetic predisposition to higher intelligence may have protective effects against ADHD.[164] Additionally, genetic studies of ADHD have found that a PGS for ADHD is associated with both externalizing symptoms[29] and lower cognitive performance.[31] However, no previous study has examined the relationship between genetic liability to ADHD and intelligence and adversity.

In the present study, we examined the associations between PGS for ADHD and PGS for intelligence and exposure to adversity. To test the specificity of our results to genetic predisposition to ADHD or intelligence, we also examined the PGS for schizophrenia. We selected the PGS for schizophrenia because it reliably predicts psychopathology[165] and because it indexes genetic risk for a disorder that typically onsets in late adolescence or in adulthood, and thus it is distinct from ADHD which onsets in childhood. We tested these associations in a cohort of youth enriched for offspring of parents with major mood and psychotic disorders. We defined adversity as a score composed of multiple indicators,

including socioeconomic disadvantage, childhood maltreatment, and peer victimization. We also explored associations between externalizing symptoms, cognitive ability, and adversity. We hypothesized that genetic scores for ADHD and intelligence would be associated with exposure to adversity.

5.3 Methods

5.3.1 Sample Description

Participants ranged in age from 5 to 27 years (mean = 13.5, SD = 4.4) and were enrolled in the Families Overcoming Risks and Building Opportunities for Well-being (FORBOW) study.[187] FORBOW is enriched for offspring of parents with major depressive disorder, bipolar disorder, and schizophrenia spectrum disorders. Offspring were assessed by research staff blind to information on parent psychopathology. FORBOW participants are assessed at 12-month intervals, and participants in the present study have completed a median of 4 annual assessments (range 1-7). We included the most recent observation of general cognitive ability and externalizing symptoms from all participants. We included lifetime diagnoses of ADHD, conduct disorder and oppositional defiant disorder and lifetime history of adversity. Offspring of parents with mental illness were recruited through their parents' contact with mental health services in Nova Scotia, Canada. Additional offspring matched on age and socioeconomic status were recruited through local school boards and community organizations. Offspring were included regardless of whether or not they had psychopathology.

The study protocol was approved by the Research Ethics Board of the Nova Scotia Health Authority. We obtained informed consent from participants who had the capacity to provide it. For participants who did not have the capacity to make an informed decision, a parent or guardian provided written informed consent and the participant provided assent.
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5.3.2 Participant Assessment

Each youth participant was assessed by three research staff. One assessor completed the youth cognitive assessment, one assessor interviewed the parent(s) and the youth participant to assess youth psychopathology, and a third assessor interviewed the parents to assess parent psychopathology and socioeconomic factors. Youth assessors were blind to information on parent psychopathology and parent assessors were blind to information on youth psychopathology.

5.3.2.1 Adversity

When examining factors contributing to or resulting from adversity, it is difficult to separate individual adversities because they often co-occur within the same individual. Therefore, it may be useful to jointly examine multiple environmental risk factors.[88] We assessed multiple indicators of adversity encompassing socioeconomic disadvantage, childhood maltreatment, and bullying. We selected these categories because they are

external to the individual, they are assessed in our sample, and they are strongly associated with onsets of major mood and psychotic disorders.[39,53,57,199,200]

5.3.2.1.1 Socioeconomic disadvantage

In interviews with biological parents, we assessed the highest level of education obtained by each biological parent, the family's annual household income, and whether or not the family owned their primary residence. Mother's and father's education increased the adversity score if the respective parent did not complete education beyond high school. Home ownership increased the adversity score if the family did not own their home. Annual household income increased the adversity score if it was less than $60,000.

5.3.2.1.2 Childhood maltreatment

Exposure to childhood maltreatment, defined as emotional abuse, physical abuse, sexual abuse, neglect, and/or exposure to violence at home, was assessed by interviewing parents (in offspring aged 10 years and younger) and youth participants (in offspring aged 11 years and older) using the Juvenile Victimization Questionnaire (JVQ).[150] This information was also retrospectively obtained from youth participants aged 17 and older using the Childhood Experiences of Care and Abuse.[151] To calculate an adversity score, each indicator of childhood maltreatment was made binary. Indicators of maltreatment increased the adversity score if they were rated as "Yes" on the JVQ or CECA.

5.3.2.1.3 *Peer victimization*

Peer victimization was assessed by asking parents (in offspring aged 10 years and younger) and youth participants (in offspring aged 11 years and older) if the participant had ever experienced bullying, with follow-up questions to gauge the frequency and severity (adapted from the JVQ). To calculate an adversity score, each indicator of peer victimization was made binary. Peer victimization increased the adversity score if bullying was rated as "Present" or "Severe".

5.3.2.1.4 Adversity scores

We calculated three adversity scores: an overall adversity score, a socioeconomic adversity score and a victimization score. The total adversity score was calculated as the mean of 10 binary indicators: 1) biological mother's education, 2) biological father's education, 3) home ownership status, 4) annual household income, 5) emotional abuse, 6) physical abuse, 7) sexual abuse, 8) neglect, 9) exposure to violence at home, and 10) bullying. The socioeconomic adversity score was calculated as the mean of the first 4 indicators. The victimization adversity score was calculated as the mean of the last 6 indicators. Each score was calculated by dividing the total count of adversities that the individual experienced by the number of indicators with available information.

5.3.2.2 General psychopathology

Offspring were assessed for mental disorders using the *Kiddie Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version* (K-SADS-PL; in offspring younger than 18 years)[146] or the *Structured Clinical Interview for DSM-5* (SCID; in

offspring 18+ years old).[144] Offspring assessors were blind to parent psychopathology. Diagnoses were confirmed in consensus meetings with a psychiatrist blind to parent diagnoses. In the present study, we included consensus-confirmed diagnoses of ADHD, oppositional defiant disorder, and conduct disorder.

5.3.2.3 Externalizing psychopathology

We calculated a dimensional index of externalizing symptoms by combining assessor-rated, parent-rated, and self-report questionnaires with consensus-confirmed diagnoses. We included the parent-rated and self-report versions of the Child Behaviour Checklist (CBCL) aggressive behaviour and delinquent behaviour syndrome scales.[148] We included the assessor-rated Test Observation Form (TOF) oppositional and attention problems syndrome scales, which were completed by the cognitive assessors.[210] We scored each syndrome subscale from the CBCL and the TOF as the sum of the score for all items divided by the number of valid items. Consensus-confirmed diagnoses of oppositional defiant disorder, conduct disorder and ADHD were rated as present (scored 1) or absent (scored 0). We calculated the externalizing symptoms dimensional score as the mean of standardized available indicators.

5.3.2.4 Full-scale intelligence quotient (IQ)

Among offspring aged 6 years and older, we assessed general cognitive ability with the Wechsler Abbreviated Scale of Intelligence – Second Edition (WASI-II).[149] There are four subtests on the WASI-II: block design, vocabulary, matrix reasoning, and similarities. These are combined to provide a reliable measure of full-scale IQ. Among 5 year old

offspring, we assessed general cognitive ability with the Wechsler Preschool and Primary Scale of Intelligence – Third Edition (WPPSI-III). We used the following subtests to obtain a measure of full-scale IQ: block design, information, matrix reasoning, vocabulary, word reasoning, and coding. These tests were administered by assessors trained in the administration of cognitive tests.

5.3.3 Genotyping, quality control, and imputation

We genotyped 693,809 single nucleotide polymorphisms (SNPs) using the Illumina Global Screening Array from DNA extracted from saliva collected via the Oragene kit (DNA Genotek Inc, Kanata, ON). We completed pre-imputation quality control on genome-wide data by excluding variants and participants according to the following criteria: 1) variants with minor allele frequency less than 1%; 2) variants with missing rate greater than 5%; 3) participants with genotyping rate less than 95%; 4) variants with significant deviations from Hardy-Weinberg equilibrium ($p < 10 \times 10^{-10}$);[155] 5) participants with discrepancies between self-reported sex and genetic sex; and 6) participants with abnormally high heterozygosity (> 4 SD above sample mean).[156] Data were imputed using Minimac3 via the Michigan Imputation Server (https://imputationserver.sph.umich.edu/index.html). Post-imputation quality control consisted of pruning variants with minor allele frequency less than 1% and with poor imputation quality ($R^2 < 0.30$).

5.3.4 Polygenic scores

5.3.4.1 Reference samples for polygenic score derivation

We constructed the intelligence PGS using the results of a meta-analysis of genome-wide association study (GWAS) data for intelligence.[164] We constructed the ADHD PGS based on the results of a meta-analysis of case-control GWAS data for diagnosed cases of ADHD.[163] We constructed a PGS for schizophrenia based on the results of a meta-analysis of case-control GWAS data for diagnosed cases of schizophrenia.[165] We selected the schizophrenia PGS as a test of specificity of our results to intelligence PGS or ADHD PGS because this is one of the most widely used and well-documented psychiatric polygenic scores.

5.3.4.2 Polygenic score calculation

We constructed polygenic scores for ADHD, intelligence, and schizophrenia using PRSice2.[23] We pruned genotypes using clumping to obtain an independent set of SNPs in approximate linkage equilibrium with an $r^2 < 0.1$ within any 500 kb window. The predictive value of polygenic scores for traits with complex inheritance improve with the inclusion of weakly associated variants that do not meet the threshold for genome-wide significance.[162] Therefore, we constructed polygenic scores for each phenotype with *p*-value thresholds 0.50 for ADHD and 0.05 for schizophrenia and intelligence, because these thresholds maximally capture the phenotypic variance in each of these phenotypes.[163–165] To construct the PGS, we weighted the contribution of each allele by the effect size of its association with each phenotype in the reference sample GWAS.

5.3.5 Statistical analysis

To test our primary hypotheses that genetic predisposition to ADHD and intelligence influence risk of adversity, we tested the effect of ADHD PGS and intelligence PGS on adversity score using mixed-effects linear regression models via the lme4 package, implemented in R Studio (R version 3.5.1). We also tested the effects of IQ, externalizing symptoms and schizophrenia PGS on adversity using the same method. We accounted for the non-independence of observations from related individuals by including the family identifier as a random effect. We adjusted for age, sex, time in the study, and the top ten ancestry informative genetic principal components. We constructed five regression models, each with one of the five predictors listed above as the independent variable. We also fitted a full model, containing all five predictors as independent variables. We quantified the accuracy of prediction from each model using variance explained (R^2). We used partial variance explained to determine the variance in adversity that could be uniquely explained by each of the five independent variables (IQ, externalizing symptom score, schizophrenia PGS, intelligence PGS, ADHD PGS).

5.3.5.1 Sensitivity analyses

To ensure that the effects of ADHD PGS were not driven by individuals with ADHD, we tested the effects of ADHD PGS on adversity separately among individuals with and without ADHD. All primary analyses were repeated using PGS derived with different *p*-value thresholds for variant inclusion (see Appendix C). We also repeated our primary analyses among the subset of participants aged 17 years or younger, among the subset of participants who have a biological parent with a major mental illness, and among the subset

of individuals of European descent (see Appendix C). We implemented mediation analysis to examine the mechanisms underlying the associations between adversity and ADHD PGS, externalizing symptoms, and IQ (see Appendix C).

5.4 Results

5.4.1 Demographic and clinical characteristics

Following genetic quality control, the final sample included 301 participants aged 5-27 years from 180 families. Three-quarters (74.4%) of participants experienced some form of adversity. Table 1 presents the demographic and descriptive characteristics of the participants, stratified by adversity score. As expected, the intelligence PGS was significantly positively associated with full-scale IQ, the ADHD PGS was significantly positively associated with externalizing symptoms, and the schizophrenia PGS was significantly positively associated with family history of schizophrenia (see Appendix C, Figure 5.1).

Table 5.1. Demographic and clinical characteristics of the sample.

	Adversity score		
	None *(N = 77)*	**Low-Moderate** *(N = 117)*	**High** *(N = 107)*
Adversity score, *mean (SD)*	0 (0)	0.15 (0.05)	0.45 (0.15)
Age in years, *mean (SD)*	11.4 (3.0)	12.8 (4.3)	15.7 (4.5)
Females, *N (%)*	38 (49)	62 (53)	61 (57)
IQ, *mean (SD)*	110 (12)	106 (12)	102 (14)
Parent diagnoses			
Parent depression, *N (%)*	24 (31)	51 (44)	52 (49)
Parent bipolar disorder, *N (%)*	16 (21)	26 (22)	20 (19)
Parent schizophrenia, *N (%)*	3 (4)	7 (6)	13 (12)
Offspring psychopathology			
Lifetime ADHD diagnosis, *N (%)*	17 (22)	31 (26)	37 (35)
Lifetime ODD diagnosis, *N (%)*	4 (5)	10 (9)	17 (16)
Lifetime CD diagnosis, *N (%)*	0 (0)	0 (0)	8 (7)
Externalizing symptom score, *mean (SD)*	-0.12 (0.46)	-0.05 (0.53)	0.14 (0.78)

ADHD = Attention-deficit/hyperactivity disorder; ODD = Oppositional defiant disorder; CD = Conduct disorder

5.4.3 The association between IQ and exposure to adversity

Lower cognitive performance was associated with higher likelihood of being exposed to adversity. After accounting for age, sex, time in the study, and genetic principal components, IQ was significantly negatively associated with overall adversity (ß = -0.16, 95% CI -0.27 to -0.05, p = 0.004) and socioeconomic adversity (ß = -0.13, 95% CI -0.22 to -0.04, p = 0.003), see Figure 1. IQ was also negatively associated with victimization, but this was not statistically significant (ß = 0.11, 95% CI -0.23 to 0.003, p = 0.052). IQ explained 7% of variance in adversity scores (Figure 2). After further accounting for externalizing symptom scores and PGS for schizophrenia, intelligence, and ADHD, IQ uniquely explained 3% of variance in exposure to adversity.

5.4.4 The association between externalizing symptoms and exposure to adversity

Externalizing symptoms were associated with higher likelihood of being exposed to adversity. After accounting for age, sex, time in the study, and genetic principal components, externalizing symptom scores were significantly positively associated with overall adversity (ß = 0.20, 95% CI 0.09 to 0.30, p < 0.001), socioeconomic adversity (ß = 0.15, 95% CI 0.06 to 0.23, p < 0.001), and victimization (ß = 0.22, 95% CI 0.10 to 0.33, p < 0.001), see Figure 1. Externalizing symptoms explained 6% of variance in adversity scores (Figure 2). After further accounting for IQ and PGS for schizophrenia, intelligence, and ADHD, externalizing symptoms uniquely explained 2% of variance in exposure to adversity.

5.4.5 The impact of genetic predisposition to intelligence on exposure to adversity

In contrast to IQ, we did not find any relationship between genetic predisposition to intelligence and adversity. After accounting for age, sex, time in the study, and genetic principal components, genetic predisposition to intelligence was not associated with overall adversity (ß = -0.04, 95% CI -0.17 to 0.08, p = 0.514), socioeconomic adversity (ß = -0.04, 95% CI -0.15 to 0.07, p = 0.508), or victimization (ß = -0.03, 95% CI -0.15 to 0.09, p = 0.628), see Figure 1. The results were consistent across PGS with differing *p*-value thresholds for variant inclusion (see Appendix C, Figure 5.2).

5.4.6 The impact of genetic predisposition to ADHD on exposure to adversity

Higher genetic liability to ADHD was associated with greater likelihood of being exposed to a range of adversities. After accounting for age, sex, time in the study, and genetic principal components, genetic liability to ADHD was significantly associated with overall adversity (ß = 0.25, 95% CI 0.14 to 0.35, p < 0.0001), socioeconomic adversity (ß = 0.11, 95% CI 0.01 to 0.20, p = 0.023) and victimization (ß = 0.25, 95% CI 0.14 to 0.37, p < 0.0001) (Figure 3). The polygenic score for ADHD explained 8% of variance in overall adversity scores. After accounting for IQ, externalizing symptoms and PGS for schizophrenia and intelligence, ADHD PGS uniquely explained 5% of variance in exposure to adversity.

ADHD PGS was significantly independently associated with adversity among 48 individuals with a diagnosis of ADHD (ß = 0.51, 95% CI 0.26 to 0.76, p < 0.0001) and 253 individuals without this diagnosis (ß = 0.16, 95% CI 0.04 to 0.29, p = 0.009). The results

were consistent across PGS with differing p-value thresholds for variant inclusion (see Appendix C, Figure 5.2).

5.4.7 The impact of genetic predisposition to schizophrenia on exposure to adversity

Genetic liability to schizophrenia was not associated with exposure to adversity. After accounting for age, sex, time in the study, and genetic principal components, genetic predisposition to schizophrenia was not associated with overall adversity (ß = -0.02, 95% CI -0.16 to 0.12, p = 0.805), socioeconomic adversity (ß = -0.04, 95% CI -0.17 to 0.09, p = 0.574), or victimization (ß = 0.04, 95% CI -0.09 to 0.17, p = 0.543). The results were consistent across PGS with differing *p*-value thresholds for variant inclusion (see Appendix C, Figure 5.2).

Figure 5.1. The effects of PGS for intelligence, ADHD and schizophrenia, IQ, and externalizing symptoms on adversity. Analyses controlled for age, sex, time in the study, 10 ancestry informative principal components. For each PGS, we selected the p-value threshold for variant inclusion that maximally captured the phenotypic variance in the original studies (P_T = 0.50 for ADHD PGS and 0.05 for intelligence and schizophrenia PGSs). Error bars represent standard error.

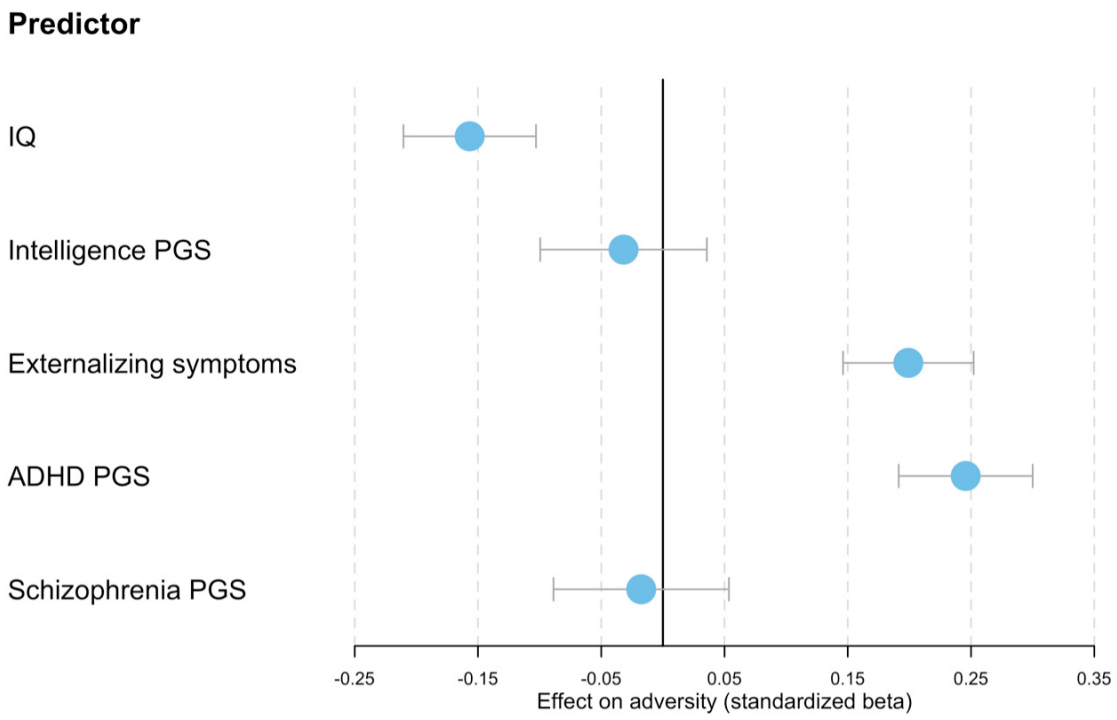


Figure 5.2. Variance in adversity scores explained by PGS for intelligence, ADHD and schizophrenia, IQ, and externalizing symptoms. All models controlled for age, sex, time in the study, and 10 ancestry informative principal components. The full bar represents the variance explained by each predictor. The blue portion of the bar represents the unique variance explained by each predictor once all other predictors have been accounted for.

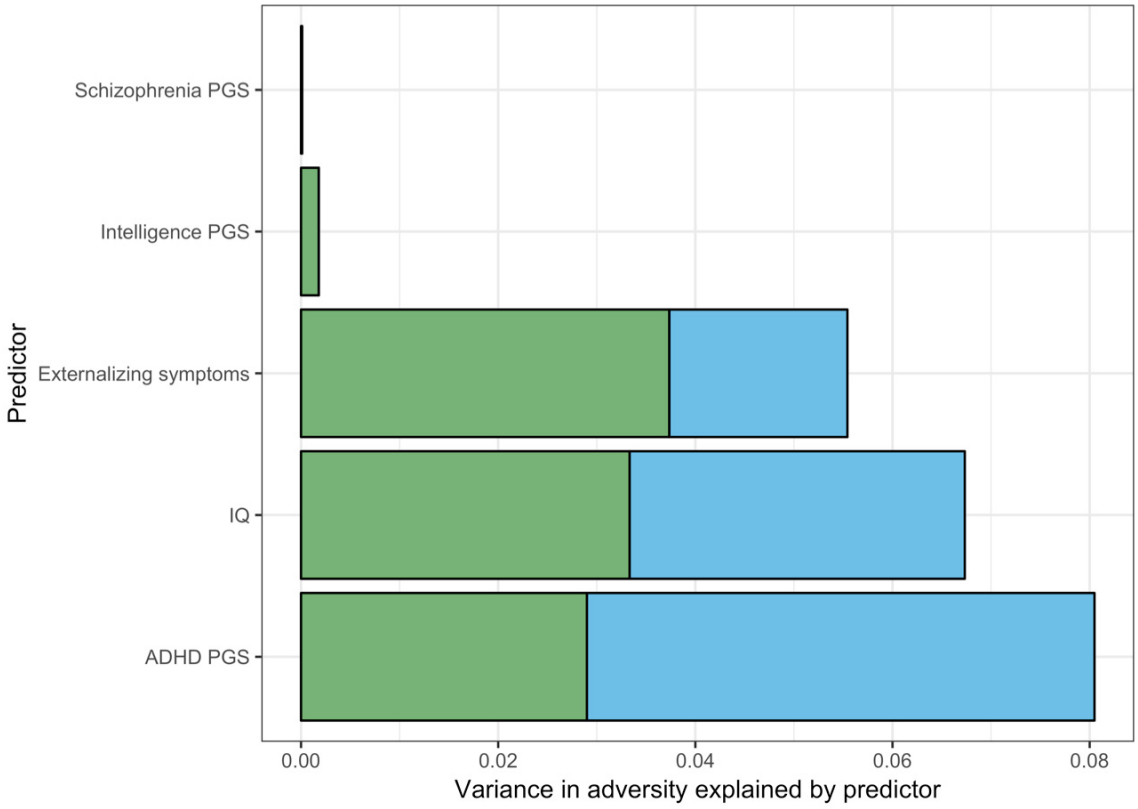
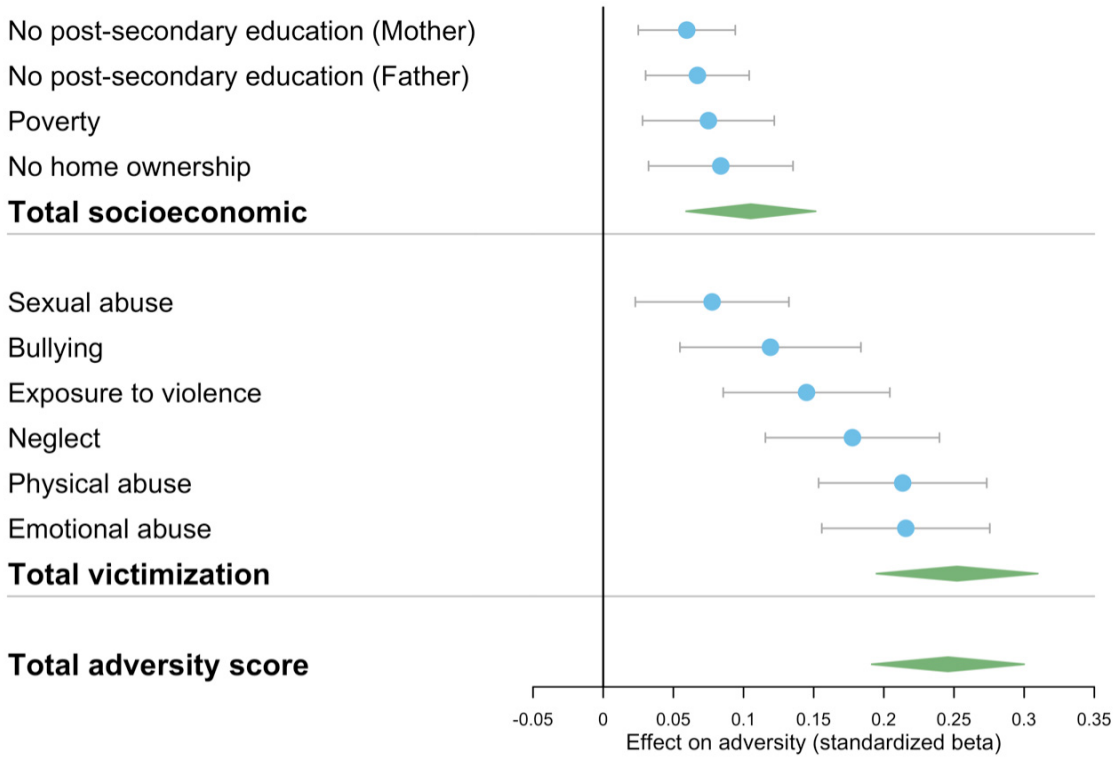


Figure 5.3. The effect of ADHD PGS on each indicator of adversity, on socioeconomic adversity, and on victimization. The offspring ADHD PGS was significantly associated with socioeconomic adversity score (ß = 0.11, 95% CI 0.01 to 0.20, p = 0.023) and victimization score (ß = 0.25, 95% CI 0.14 to 0.37, p < 0.001). The offspring ADHD PGS was also significantly associated with physical abuse (ß = 0.21, 95% CI 0.10 to 0.33, p < 0.001), emotional abuse (ß = 0.22, 95% CI 0.10 to 0.33, p < 0.001), exposure to violence (ß = 0.14, 95% CI 0.03 to 0.26, p = 0.015), and neglect (ß = 0.18, 95% CI 0.06 to 0.30, p = 0.004). Error bars represent standard error.



5.5 Discussion

The present study identified a gene-environment correlation between genetic liability to ADHD and a range of adverse experiences in childhood. Specifically, we found that individuals with higher genetic risk for ADHD were more likely to experience adversity during childhood. Polygenic scores for ADHD strongly and uniquely contributed to risk of experiencing adversity, even after accounting for general cognitive ability and externalizing symptoms. This relationship was independent of a diagnosis of ADHD. In contrast, genetic predisposition to intelligence or schizophrenia were not significantly associated with adversity.

The present study was motivated by the need to identify potential mechanisms underlying the relationships between lower cognitive ability, ADHD and adversity. We hypothesized that genetic factors associated with intelligence and ADHD would influence risk of experiencing adversity, including childhood maltreatment, socioeconomic disadvantage, and peer victimization. We found that genetic liability to ADHD was associated with adversity over and above general cognitive ability and externalizing psychopathology. This is consistent with a recent study that found that higher genetic liability to ADHD is associated with increased risk of exposure to bullying.[211] Our results suggest that genetic liability to ADHD contributes to risk of adversity partially through its influence on externalizing symptoms, but also independently contributes directly to risk. In contrast, genetic predisposition to intelligence did not influence risk of experiencing adversity. These results suggest that the effect of polygenic score on risk of adversity is specific to genetic liability to ADHD.

Our findings represent an example of a gene-environment correlation, which occurs when genetic and environmental factors contributing to psychopathology are associated.[89,162] Based on our results, we can speculate about the mechanisms through which genetic liability to ADHD may contribute to exposure to adversity. Parents provide both genetic material and the rearing environment for their offspring. Parental genetic factors are therefore often associated with components of the rearing environment. Higher maternal genetic liability to ADHD has been associated with lower offspring educational attainment.[32] Higher parental polygenic scores for ADHD may contribute to both increased risk of socioeconomic disadvantage among offspring and higher offspring polygenic scores through passive gene-environment correlation. This is supported by our finding that higher genetic liability to ADHD was associated with socioeconomic adversity. Thus, the relationship between ADHD PGS and adversity is partially driven by passive gene-environment correlation. Additionally, genetic factors can impact behaviour and thus can influence the social responses we elicit from others. Genetic liability to ADHD has been associated with greater symptoms of hyperactivity, impulsivity, and inattention in the general population.[28] These characteristics may elicit unfavorable responses from caretakers and peers, resulting in increased risk of experiencing victimization through evocative gene-environment correlation. Our finding that genetic liability to ADHD was associated with victimization suggests that the relationship between adversity and ADHD PGS can be partially attributed to evocative gene-environment correlation.

The present study benefits from the inclusion of offspring of parents with major mood and psychotic disorders. Approximately three out of every four participants in the present study have a biological parent with major depressive disorder, bipolar disorder, or schizophrenia spectrum disorder. Thus, our sample is enriched for genetic liability to psychopathology and has a higher rate of mental disorders compared to the general population. Due to the concentration of genetic and psychosocial risk for mental illness, our results will be readily generalizable to clinical and high-risk populations. However, extension of these findings to unselected populations may need to be probed in future studies. We assessed psychopathology and adversity using validated in-person interviews conducted by research staff blind to parent psychopathology. This allowed us to conduct PGS analyses among individuals with and without diagnoses of ADHD. Assessments across multiple ages and from multiple raters allowed for rich phenotyping within our sample. Additionally, our study has a very high retention rate (94%), and is not limited by the nonrandom attrition that has been previously reported in genetic studies.[212] However, our study is not without limitations. The main limitation is that the ADHD polygenic score is restricted to common additive effects and only accounts for 5.5% of the variance in ADHD.[163] However, this proportion of variance explained is in keeping with other large-scale genomic studies of mental illness, including major depressive disorder and bipolar disorder.[20,21] Additionally, the statistical power is limited by the sample size. However, due to the strength and consistency of our results, it is highly unlikely that they are due to chance alone.

The present study has implications for future research. The finding that genetic liability to ADHD is associated with exposure to adversity provides a potential explanation for the previously reported relationship between externalizing psychopathology and victimization. Future research could examine mechanisms underlying the relationship between genetic liability to ADHD and adversity, including the role of parental polygenic scores for ADHD and offspring exposure to adversity. Additionally, our results have implications for risk identification. It is known that youth with ADHD are at increased risk of experiencing adversity.[202] However, our results suggest that individuals with high genetic liability to ADHD who do not manifest the disorder are also at increased risk.

5.6 Conclusion

In conclusion, we found that genetic liability to ADHD is associated with exposure to adversity among youth at familial risk for mood and psychotic disorders. Future studies could explore the mechanisms underlying the relationship between genetic liability to ADHD and adversity. This information may help inform the development of appropriate interventions to maximize the psychological well-being of children and youth with high genetic liability to ADHD.

CHAPTER 6
GENETIC COUNSELLING FOR THE PREVENTION OF MENTAL HEALTH CONSEQUENCES OF CANNABIS USE: A RANDOMIZED CONTROLLED TRIAL

Copyright Statement

This chapter is based on a manuscript that will be submitted as: Alyson Zwicker, Marissa A. LeBlanc, Barbara Pavlova, Martin Alda, Eileen M. Denovan-Wright, Jehannine Austin, and Rudolf Uher. Genetic counselling for the prevention of mental health consequences of cannabis use: A randomized controlled trial.

Contribution Statement

I drafted the manuscript that was used for the basis of this chapter. The original intervention idea was conceived by Dr. Rudolf Uher and Jehannine Austin. I developed the idea to a grant proposal and intervention protocol. I was awarded funding to complete this intervention.

6.1 Abstract

Background: Cannabis use is an established risk factor for severe mental illness. However, cannabis does not affect everyone equally. Genetic information may help identify individuals who are vulnerable to the detrimental effects of cannabis on mental health. A common genetic variant within the *AKT1* gene selectively increases risk of psychosis, only in the presence of cannabis use. Therapeutically-oriented genetic counselling may reduce cannabis exposure among genetically sensitive individuals.

Methods: Using a trial-within-cohort design, we will test the efficacy of a genetic counselling-based intervention aimed at reducing cannabis exposure, titled Interdisciplinary approach to Maximize Adolescent potential: Genetic counselling Intervention to reduce Negative Environmental effects (IMAGINE). This will be implemented in a cohort of youth enriched for familial risk for major mood and psychotic disorders located in Nova Scotia, Canada. One in every two eligible youth aged 12-21 years will be randomly selected to be offered a single genetic counselling session with a board-certified genetic counsellor. Youth will also be invited to attend a follow-up session with research staff approximately 1 month following the intervention. The primary outcome will be cannabis use (both self-report and urine screen) at 1-month follow up and subsequent annual assessments as part of the parent cohort. Secondary outcomes include intervention acceptability and psychopathology.

Discussion: This study will be the first translational application of a gene-environment interaction to improve mental health and test an intervention with potential public health benefits.

6.2 Background

Severe mental illness (SMI) refers to mental disorders that substantially interfere with one or more major life activities. These include major mood or psychotic disorders such as major depressive disorder, bipolar disorder, or schizophrenia spectrum disorders. SMI often follows a chronic or recurrent course and available treatments have limited efficacy.[182–184,213] It is therefore desirable to implement targeted early interventions to prevent its onset. Recent research has shown that there is substantial overlap in the genetic and environmental contributors to various forms of SMI.[35,38,111,162] Many risk factors for SMI are either not modifiable (*e.g.*, genetics) or difficult to alter (*e.g.*, low socioeconomic status). In contrast, exposure to cannabis is one specific environmental risk factor that could be avoided among cannabis-sensitive individuals.

One in three Canadians aged 15-24 have used cannabis in the past year, and youth describe perceived harmlessness as a top reason for experimenting with cannabis.[214–216] These findings are worrisome because cannabis is estimated to be responsible for 14-24% of schizophrenia cases.[217,218] Cannabis has also been linked to suicidality, earlier onset and poor outcomes of depression, bipolar disorder and psychosis.[71,219,220] The recent trend earlier age of cannabis use[214,216] and increase in the amount of psychoactive $^{\Delta 9}$-tetrahydrocannabinol (THC) in cannabis[221] are also problematic because cannabis use in adolescence may disrupt brain maturation and may be more damaging than use in adulthood.[222] Additionally, some individuals are more vulnerable to the detrimental effects of cannabis on mental health.

It may possible to identify individuals who are more vulnerable to the harmful effects of cannabis. A genetic polymorphism, rs2494732 in the *AKT1* gene, influences the risk of developing psychosis, only among individuals who are exposed to cannabis. Approximately one third of the population carries the C/C genotype, and these individuals are 7-fold more likely to develop psychotic illness after regular cannabis use than T/T allele homozygotes.[223] Heterozygous C/T individuals have comparable risk to T/T homozygotes. This finding is robust and has been replicated in three large, independent samples.[105,223,224] *AKT1* encodes a serine-threonine kinase that is involved in many signal transduction pathways. Importantly, it is involved in signal transduction following cannabinoid receptor 1 activation.[225] The impact of this polymorphism on protein function or expression has not been defined in brain, although levels of AKT1 have been shown to be decreased in peripheral blood of individuals with schizophrenia.[226] Regardless of mechanism, rs2494732 genotype is a robust indicator of SMI risk following cannabis use.

This may be the first gene-environment interaction related to SMI risk that has an effect size large enough to be meaningful on an individual level.[93] Although the majority of psychiatric genetic counselling does not currently involve the provision of molecular genetic information, this information is becoming increasingly available to the general population. It is therefore essential that we establish methodology to deliver psychiatric genetic counselling that will be acceptable and empowering to young people, with the potential to encourage positive behavioural change. Since individuals actively participate in the decision to use cannabis, knowledge of personal risk of SMI based on genotype has the potential to influence their decisions. Importantly, in the case of cannabis, modification

of behavior for even a limited period could be beneficial if it coincides with the key developmental stage when individuals are most vulnerable to the negative effects of cannabis exposure.

To date, early interventions have focused on treatment-seeking individuals in the prodromal stage of illness.[227] These interventions have demonstrated benefits,[228] but the prodromal stage of illness is already associated with impairment, and overall functional outcomes often remain poor.[229,230] Models suggest that the earlier interventions are delivered, the greater benefit they bring.[134] Genetic information offers the potential to provide targeted, early interventions before the onset of symptoms.

Providing genetic risk information may promote risk-reducing behaviors if the information is delivered appropriately. While some studies have found that genetic information can induce behavioural modifications,[231] other studies have shown little or no effect.[137,138,232] However, all of these studies involve the unidirectional transmission of information from researcher to participant. These trials are thus founded on the assumption that behavioural decisions are the result of available information and cognitive ability. This assumption fails to account for the fact that other factors, including individual-level biases, agency in the learning process, and framing influence how people make decisions.[139] In contrast, genetic counselling is a psychotherapeutically-oriented approach and focused on helping the individual understand all implications of genetic contributions to disease.[140] In the context of psychiatric illnesses, genetic counselling involves personalized discussion of both genetic and environmental contributing factors. Genetic variations are framed as conferring

a vulnerability to illness, rather than to the illness itself. Additionally, genetic factors are discussed in terms of their potential to increase susceptibility to illness in the context of detrimental environments.[233] Interventions based on this bidirectional and highly personalized framework have the potential to generate a shared understanding of illness etiology and help participants identify protective mental health strategies for the future.

Genetic information has the potential to provide a personalized health message that counteracts the perceived harmlessness of cannabis use. This is particularly relevant now that recreational cannabis use is legal in Canada. Within a cohort enriched for offspring of parents with SMI located in Nova Scotia, Canada, we aim to determine if strategic delivery of personalized genetic information will decrease exposure to cannabis. The *AKT1* genotype is ideal for this purpose, because it is only related to negative outcomes in the presence of cannabis use and, therefore, knowing one's *AKT1* genotype does not carry an inherent ominous risk message. This paper will describe the design of a genetic counselling-based intervention that aims to reduce cannabis exposure among vulnerable youth, titled Interdisciplinary approach to Maximize Adolescent potential: Genetic counselling Intervention to reduce Negative Environmental effects (IMAGINE).

6.3 Aims and hypotheses

6.3.1 Aims

We aim to test if personalized risk information based on a replicated gene-cannabis interaction reduces cannabis use in a cohort of youth enriched for familial risk for mental illness. Additionally, we will evaluate the acceptability of IMAGINE as the proportion of participants who accept the offer of intervention and the proportion of intervention recipients who opt to receive their personalized *AKT1* genotype.

6.3.2 Hypotheses

We will test the following hypotheses:

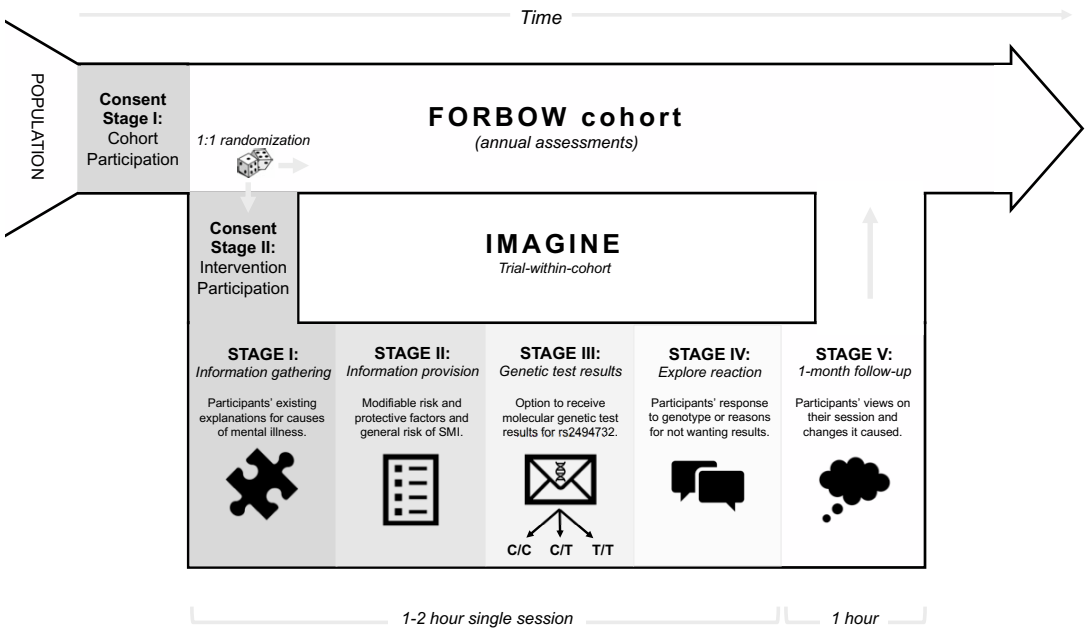
1) A genetic counselling intervention with personalized feedback of *AKT1* genotype will reduce cannabis use among vulnerable youth.
2) The effect of the intervention will be moderated by *AKT1* genotype, with greater reduction of cannabis use among the sensitive CC homozygotes.

6.4 Methods

6.4.1 Overview

IMAGINE is nested within an ongoing longitudinal cohort enriched for offspring of parents with severe mental illness, the Families Overcoming Risks and Building Opportunities for Well-being (FORBOW) study (see Figure 6.1).[187] FORBOW participants attend annual assessments, which includes a battery of comprehensive clinical and cognitive testing.[187] FORBOW participants who meet the study inclusion criteria (see below) will be contacted and offered the opportunity to participate in IMAGINE. Participants who accept the offer will receive a single session intervention with a board-certified genetic counsellor. During this session, they will have the opportunity to receive their personalized *AKT1* genotype at rs2494732, which reflects their sensitivity to the detrimental effects of cannabis on mental health. Participants will also be invited to attend a 1-month follow-up interview, which includes a urine screen for cannabinoids and an interview regarding how IMAGINE changed their behaviour and perception of cannabis use, their intervention experience, and their opinions on the use of genetic information in this context.

Figure 6.1. Intervention design. IMAGINE is nested within a longitudinal cohort enriched for offspring of parents with mood and psychotic disorders.



6.4.2 Design

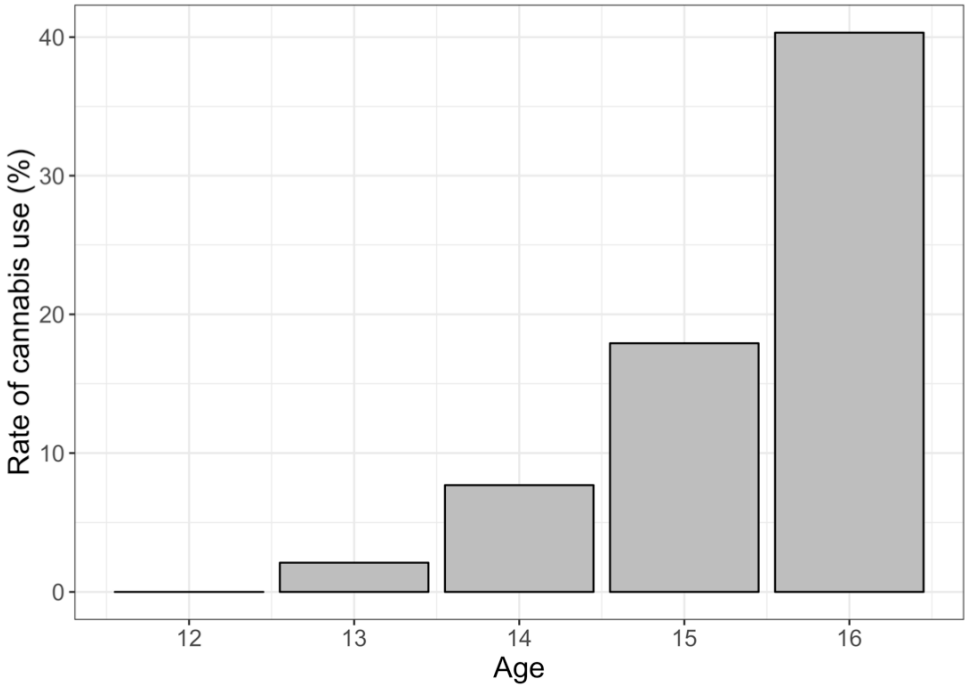
We will test IMAGINE effectiveness in an innovative trial-within-cohort (TwiC) design, which allows externally valid testing of long-term effectiveness and avoids 'disappointment bias' secondary to allocation to a control group.[141] In TwiC, two-stage informed consent separates consent to cohort participation from consent to receive an intervention.[234] Within the TwiC design, we will randomly select one in every two eligible youth to be offered a single session of genetic counselling during which they will be provided with personalized genetic information and their risk of developing mental illness based on genetic test results, family history to the best of the participant's knowledge, and whether or not they choose to use (or continue to use) cannabis. The other participants will not be offered any intervention so that IMAGINE is compared with the current standard (no intervention). These participants, however, will continue to be invited for annual assessments within the FORBOW study. We will follow the participants through adolescence and young adulthood. The primary outcomes will be abstinence from cannabis reported by participants in confidential interviews and confirmed by measurement of the $^{\Delta 9}$-THC and its major metabolites in urine samples. Secondary outcomes include intervention acceptability, participant empowerment, attitudes and perceptions regarding the session and the use of genetic information, and psychopathology.

6.4.3 Recruitment and Participants

The study will be carried out in Nova Scotia, the province with highest use of cannabis in Canada.[214] The FORBOW cohort includes offspring of parents with major depressive disorder, bipolar disorder and schizophrenia, in addition to offspring of parents without

SMI. The average age of FORBOW participants is 12 years (range 6-27). We have retained 94% of participants in follow-up over up to six years. Our pilot data show a steep increase in the use of cannabis from 0% at age 12 years to over 40% at age 16 years (see Figure 6.2).

Figure 6.2. Rate of cannabis use by age in the FORBOW cohort.



6.4.4 Inclusion and exclusion criteria

FORBOW participants will be randomized to receive an offer of intervention if they meet the following inclusion criteria: (1) 12 to 21 years old, (2) provided a genetic sample, and (3) had the capacity to provide informed consent at their most recent FORBOW assessment. Exclusion criteria are a diagnosis of bipolar disorder or a psychotic disorder at baseline, autism, and severe intellectual disability (IQ < 70).

6.4.5 DNA samples and genotyping

Participants provide saliva samples using the Oragene kit (DNA Genotek Inc, Kanata, ON) at FORBOW assessments. We extract genomic DNA from these saliva samples as per the kit instructions. We are genotyping rs2494732 prior to participant allocation using a validated PCR-based TaqMan method.[154]

6.4.6 Allocation

Once SNP genotyping is completed, participants who meet inclusion criteria will be randomly selected in 1:1 ratio to be offered the intervention or not. A dedicated research method unit staff member will independently carry out random selection using random number tables with variable block size, stratified by the rs2494732 genotype. Allocation will be concealed from study investigators until the decision on eligibility is finalized. Participants will be advised not to discuss the attendance of the intervention with anyone outside their family and will be reminded not to mention it to assessors prior to each follow-up assessment with FORBOW research staff.

6.4.7 Intervention

6.4.7.1 Content

Genetic counseling combines educational provision of information with a person-centered counselling approach, considering key determinants of behavioral change. IMAGINE will follow an established psychiatric genetic counselling protocol that has demonstrated acceptability and positive outcomes in empowerment and self-efficacy.[235,236] IMAGINE is specifically designed for an adolescent/young adult population.

6.4.7.2 Process – Intervention group

Participants will be given the option to attend the intervention alone or with their parent(s). The session begins with an information gathering stage, which involves uncovering the participants' existing explanation for cause of illness and detailed family history based on the participants' knowledge (see Figure 6.1). This is followed by information delivery, including numeric information about the general risk of mental illness based on information other than the participant's own genetic test. In a third stage, the counsellor opens the participant's envelope during the session and reveals the inner envelope, marked with "genetic test results inside." At this point, the participant will have the opportunity to consider again whether they would like to receive the information. All participants will provide informed consent to receive genetic information prior to the commencement of the intervention, but this procedure will serve as an additional layer of security. It will also allow the participants the opportunity to change their mind regarding their willingness to receive genetic information following the initial discussion with the genetic counselor. Those who opt to receive their genetic test results will then be provided with more specific

tailored information. The absolute risk of developing mental illness is higher in the presence of cannabis for all participants, but the difference between absolute risk with and without cannabis varies greatly according to genotype. Those with the C/C genotype (expected: 33%) will be counselled that if they use cannabis, they have a substantially higher risk of developing SMI than someone without that variant (58% in the presence of regular cannabis use versus 7% in the absence of cannabis). Those with the C/T genotype (expected: 49%) and the T/T genotype (expected: 18%) will be counselled that their risk of developing mental illness is slightly higher if they use cannabis (7% in the absence of cannabis versus 20% in the presence of regular cannabis use), see Figure 6.3. As per standard genetic counselling practice and based on evidence about how people understand risk,[233,237] information will be framed in terms of absolute probabilities, and in terms of probability both to develop and not to develop SMI. All participants receive the information on avoidable risk factors for SMI, including cannabis, and all receive information that cannabis should not be considered 'safe'. Participants' reactions to the information will be explored. The counsellor will anticipate, uncover and effectively address the participant's psychological reaction to the test result, working with the participant towards mitigating fear associated with having a history of use, while consolidating a resolve to minimize exposure in the future.

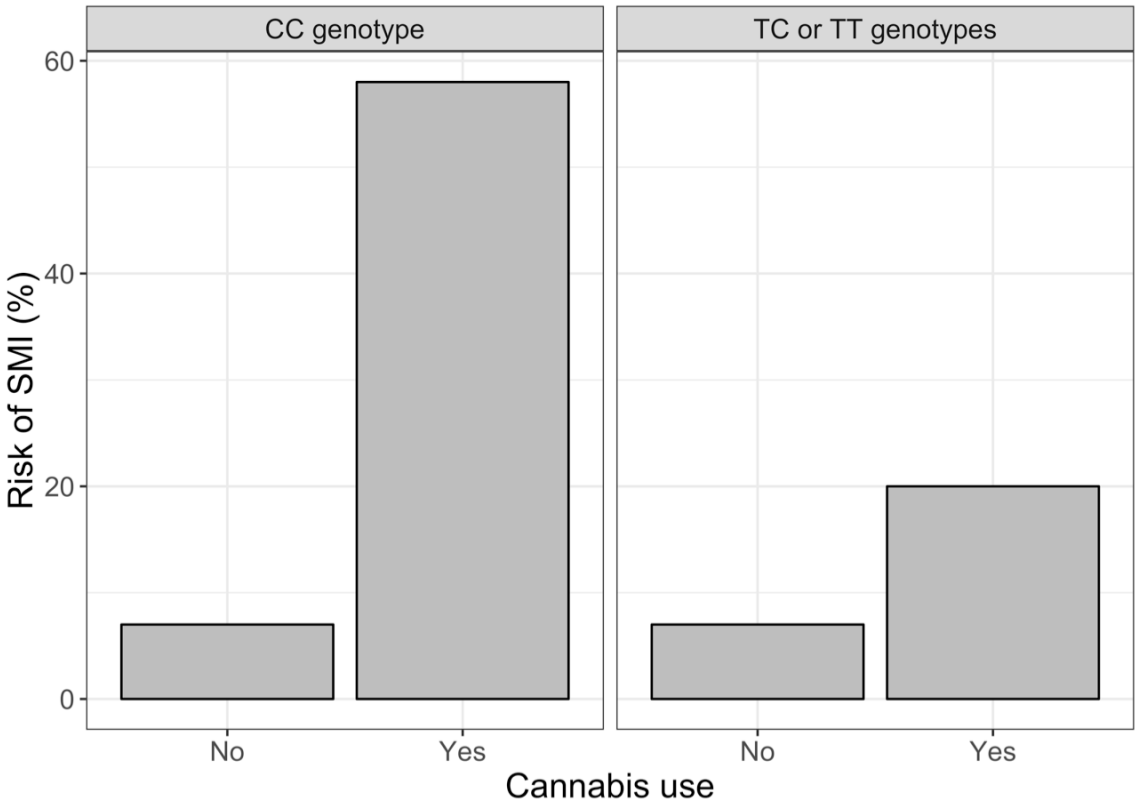
6.4.7.3 Process – Comparison group

There is no additional consent required to participate in the comparison group. Eligible individuals in FORBOW who are not allocated to be offered the intervention will continue with annual follow-up appointments. At these appointments, we will continue to collect information on outcomes of interest (*i.e.*, cannabis use, psychopathology).

6.4.8 One-month follow-up

The participants will meet with an independent researcher 1 month after the intervention session. The follow-up session will include a review of what the participant remembered from the counselling session and invite questions that may have emerged. The researcher will inquire about the experience of the intervention, the participant's views on ethical issues surrounding feedback of genetic test results, and the impact of the session on self-efficacy, beliefs about causation of illness, stigma and empowerment. We will also assess cannabis use, both via self-report and by urine screen for cannabinoids.

Figure 6.3. Risk of SMI by genotype and cannabis exposure.



6.4.9 Outcomes

6.4.9.1 Cannabis use

Use of cannabis and other recreational drugs (nicotine, alcohol, stimulants, opiates, sedative hypnotics, hallucinogens) will be collected at 1-month follow-up and subsequent annual FORBOW assessments with the validated Drug Use Screening Inventory (DUSI),[152] complemented with the Cannabis Experience Questionnaire (CEQ).[153] The CEQ provides additional questions to specifically assess cannabis use (*i.e.*, type and frequency of cannabis use) and subjective experiences during cannabis intoxication and after cannabis use. In addition, exposure to cannabis and other drugs (amphetamine, cocaine, methamphetamine, opiates) will be measured objectively in urine samples. Urine drug screens will be used to corroborate self-reported drug use and quickly provides a positive or negative reading reflecting any use of cannabis in the previous approximately 30 days.

6.4.9.2 Acceptability

The acceptability of IMAGINE will be determined as the proportion of participants who attend the offered genetic counselling session.

6.4.9.3 Psychopathology

As part of the FORBOW parent study, we will assess psychiatric disorders using the Kiddie Schedule for Affective Disorders and Schizophrenia Present and Lifetime Version (K-SADS-PL),[146] for participants aged 12-18 years, and the Structured Clinical Interview for

DSM (SCID) for participants 18 years and older.[144] Diagnoses are confirmed in consensus meetings with psychiatrists who are blind to family history and intervention allocation.

6.4.9.4 Therapeutic Alliance

Therapeutic alliance will be assessed immediately post-intervention using the Working Alliance Inventory.[238] Both a client and genetic counsellor version will be completed by the participant and the genetic counsellor, respectively. This is a very brief 4-item questionnaire designed to assess the perceived bond between genetic counsellor and participant. This questionnaire will be administered to participants at the end of the intervention by a research assistant in the absence of the genetic counsellor to minimize desirability bias in completing this measure.

6.4.10 Sample size

A power calculation suggests that a sample of 104 individuals is required to detect the effect of an intervention that halves the risk of cannabis use with 80% power. To account for participant withdrawal, we propose to enroll 120 youth participants. This number of participants will allow us to determine whether the intervention is acceptable to an adolescent and young adult population. This will also allow us to obtain preliminary results on its effectiveness in reducing initiation of cannabis use and/or frequency of cannabis use in this population.

6.4.11 Statistical analysis

6.4.11.1 Intervention acceptability

IMAGINE will be deemed acceptable if 70% or more participants allocated to the intervention attend both the intervention and the 1-month follow-up interview.

6.4.11.2 Cannabis use

The short-term effectiveness of IMAGINE in reducing cannabis use will follow the intention-to-treat principle with allocation (rather than intervention receipt) as the primary independent variable.[239] We will use logistic regression to test the effect of intervention allocation on any cannabis use at 1-month follow-up and subsequent FORBOW assessments. To reduce the number of tests, we will count any reported cannabis use post-intervention or a positive urine test as positive cannabis use. We will control for cannabis use at baseline, age, and sex as covariates. Secondary analyses will examine self-reported cannabis use and urine test results separately and assess the agreement between the two measures.

6.4.11.3 One-month follow-up interview

In addition to the quantitative analysis, we will qualitatively analyze the transcripts of 1-month follow-up about intervention experience. Iterative thematic and phenomenological analysis will be used to identify salient topics in the experiences and attitudes of participants towards the use of genetic testing, genetic counselling in a psychiatric context, cannabis, and mental illness. The topic endorsement will be aligned with quantitative information on genotype and cannabis use.

6.5 Discussion

We describe a genetic counselling-based intervention aimed at reducing cannabis use among youth at familial risk for major mood and psychotic disorders. In contrast to other studies that have tested the effects of provision of genetic information on behaviour,[135,136] this study relies on a therapeutically-oriented genetic counselling protocol with bidirectional transmission of information between genetic counsellors and youth participants. IMAGINE is designed to uncover and address existing explanations for cause of illness, stigma, shame, and guilt that may be associated with a family history of mental illness. The study will also help youth develop their understanding of risk and protective factors for mental illness. In an era of increasing availability of genetic information, this study explores a potential method to improve the communication and understanding of genetic contributors to psychopathology to youth in an empowering and non-stigmatizing manner. We will test the acceptability of genetic counselling to young people who are not treatment-seeking and test the usefulness of genetic counselling to promote positive health-related behaviour among youth at familial risk for mental illness.

6.5.1 Ethical aspects

The individuals who will participate in the present study are not seeking treatment. Therefore, we have broadly consulted on offering interventions to non-treatment seeking youth and we have piloted this procedure with other psychological interventions that are embedded within our cohort.[187] To ensure that all participants in the present study are able to fully benefit from the intervention sessions, we are limiting inclusion to young people who are able to provide informed consent. The genetic counselling model that will be used

in the present study is suitable for non-treatment seeking individuals and it is designed to normalize mental illness and reduce the feelings of stigma, shame, and blame that are often associated with illness. The genetic counsellors will address the participants' feelings and perspectives related to stigma and focus on normalizing their experiences. Additionally, we have opted to restrict the use of family history information used in IMAGINE to the information that is provided by the youth, rather than including all family history information that was obtained as part of the parent study. This strategy minimizes the risk of the youth inadvertently learning details of family history of mood and psychotic disorders that were not previously known during the intervention.

6.6 Conclusion

In conclusion, we are conducting an intervention aimed at reducing cannabis exposure among genetically sensitive individuals. IMAGINE is particularly timely now due to the legalization of recreational cannabis use in Canada. This intervention will provide youth with the tools to understand causes and contributors to mental health and illness and make informed behavioural decisions related to their health. This study represents the first translational application of a gene-environment interaction to improve mental health and tests an intervention with potential public health benefits.

CHAPTER 7
GENERAL DISCUSSION

7.1 Objectives of the research

The overall objective of my thesis was to better understand specific early factors that contribute to risk of SMI. Family history is currently the strongest known predictor of SMI.[4] However, the majority of individuals who develop SMI do not have an affected parent. Additionally, two out of every three offspring of a parent with SMI will not become ill themselves.[5] The predictive value of family history information is also limited by smaller family sizes and the incomplete penetrance of mental illness.[240] Therefore, I sought to examine genetic and developmental psychopathology factors that could be used to complement family history information when predicting risk of SMI among children and youth.

7.2 Summary of the research

I sought to examine associations between family history of mental illness and phenotypes that are identifiable early in life. I chose to examine affective lability and basic symptoms because these phenotypes have been shown to predict the onset of SMI.[117,119,147] Specifically, affective lability has been previously associated with a family history of bipolar disorder[115] and that basic symptoms have been identified as a strong predictor of psychotic illness.[147] Additionally, they have been previously identified by our group as antecedents to severe mental illness that may be useful for targeting individuals to interventions.[112] I found that affective lability is associated with a family history of major mood disorders, suggesting that this phenotype is an indicator of familial liability to mood

disorders (see Chapter 3). I also found that basic symptoms are transdiagnostically associated with parental illness severity, suggesting that basic symptoms during childhood are a marker of familial risk of psychopathology that is related to severity and is not specific to psychotic illness (see Chapter 4). Taken together, these findings suggest that affective lability and basic symptoms may represent useful transdiagnostic indicators of SMI risk that could facilitate allocation of youth at familial risk of SMI to targeted preventative interventions.

Genetic factors influence risk of psychopathology but can also influence risk of being exposed to environmental factors that are strongly associated with psychopathology, such as adversity.[52,200] Previous research has demonstrated that externalizing psychopathology and lower cognitive ability are associated with increased risk of exposure to adversity. I sought to test whether polygenic scores indexing genetic liability to intelligence and ADHD also predicted exposure to adversity, encompassing maltreatment, socioeconomic disadvantage, and peer victimization. I found that genetic disposition to ADHD strongly predicted exposure to adversity (see Chapter 5). In contrast, there was no significant relationship between genetic disposition to intelligence and adversity, although lower intelligence itself is associated with exposure to adversity. These results suggest that the previously observed associations between externalizing symptoms, lower cognitive ability and adversity may be partially attributable to genetic liability to ADHD. Future research could examine potential mechanisms underlying the relationship between genetic liability to ADHD and adversity, including the role of parental polygenic scores for ADHD and offspring exposure to adversity.

The combination of genetic and environmental risk factors for psychopathology may improve our ability to predict who will become ill in the future, thus providing opportunity for intervention. We can use genetic information to identify individuals who are vulnerable to certain environments and reduce the likelihood of exposure. In Chapter 6 of my thesis, I described a genetic counselling-based intervention that uses genetic information to communicate risk of developing SMI, depending on whether or not individuals choose to use cannabis. The primary aim of the described study is to test if personalized risk information based on a replicated gene-cannabis interaction reduces cannabis use among youth enrolled in a cohort enriched for familial risk of mental illness. If found to be effective, this study will demonstrate the potential for genetic counselling to promote positive health-related behaviours among youth at risk for SMI.

7.3 Future directions

7.3.1 Using antecedents to target interventions

The findings presented in my thesis have implications for future research on early interventions in psychiatry. The information presented here will be used to improve our ability to target early preventative interventions to high-risk children and youth years before the onset of major mood or psychotic disorders. Affective lability and basic symptoms are currently being used in combination with other antecedents (anxiety, psychotic symptoms) to target a cognitive-behavioural therapy intervention to youth in the FORBOW cohort.[112] This intervention if currently being offered to 9 to 19 year olds who have at least one antecedent and who have not yet experienced a major mood or psychotic

episode. This ongoing research will determine whether treating antecedents early in life can reduce distress and prevent the onset of major mood or psychotic illness.

7.3.2 Using genetic information to improve prediction of severe mental illness

Genetic information offers the possibility to improve our ability to predict who is at risk of SMI. Family history is a strong predictor of SMI, but it is limited by small family sizes and incomplete penetrance of mental illness.[4,240] Additionally, family history information is often not known in full due to the stigma associated with mental illness. Molecular genetic information may be useful in complementing family history information, particularly in cases where family history information is sparse or absent. Future research could focus on testing whether polygenic scores for psychopathology can be used to improve prediction of SMI risk over family history information alone. If polygenic scores are found to improve prediction of SMI, then genetic information could also be used to target high-risk individuals to preventative early interventions.

7.4 Conclusions

In conclusion, I identified psychopathological and genetic factors that may be useful in complementing family history information to improve the prediction and prevention of SMI. I found that two specific developmental antecedents of SMI are transdiagnostically associated with a positive family history of illness. Additionally, I found that genetic liability to ADHD strongly predicts exposure to adversity during childhood and adolescence. I also describe a genetically-informed early intervention aimed at reducing cannabis use among youth who are sensitive to the detrimental effects of cannabis use on

mental health. These findings will contribute to a better understanding of early risk factors of SMI and will inform future early preventative interventions.

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APPENDIX B
PROTOCOLS

DNA Purification from Saliva

Before beginning, label two 2mL and one 500uL Eppendorf tubes per aliquot of sample.

1. Mix the sample in the DNA Genotek kit by inversion and gentle shaking for a few seconds.
2. Incubate the sample at 50°C in the air incubator overnight.
3. Add 40µL of PT-L2P to a clean 2mL tube. Cap the tube.
4. Transfer 1000µL of sample to the tube containing PT-L2P and mix by vortexing for 5 seconds.
5. Incubate on ice for 10 minutes.
6. Centrifuge at RT for 10 minutes at 15,000 x *g*.
7. While the samples are spinning, add 1000uL RT 100% ethanol to the fresh, labeled 2mL tubes. **Cap the tubes**.
8. **Carefully** transfer the majority of the supernatant from step 6 to the ethanol-containing tube. Discard the pellet. Mix **gently** by inversion 10 times.
9. Let the sample stand at RT for 10 minutes to allow DNA to fully precipitate.
10. Place the tube into the centrifuge with a known orientation. Centrifuge at RT for 2 minutes at 15,000 x *g*.
11. **Carefully** pipette off the supernatant and discard it. **If the DNA pellet is disturbed, repeat previous spin.**
12. Add 1000µL of 70% ethanol, mix **gently**. Let stand 2 minutes. Centrifuge at RT for 2 minutes at 15,000 x *g*.
13. Remove supernatant completely. Pulse spin if necessary, then remove residual. Let sit on the bench for at least 2 minutes to allow leftover residual ethanol to evaporate.
14. Add 150µL TE buffer and vortex until pellet is resuspended (minimum 10 seconds).
15. Incubate overnight at RT. Vortex prior to Nanodrop quantification.
16. Store in aliquots at -80°C.

TaqMan Genotyping

1. **Plan experiment and plate layout.**
    - Calculate total volumes of Master Mix and Assay Mix needed. Per sample requirements are:
        - 12.50 uL **2x** TaqMan Master Mix
        - 1.25 uL 20x Assay Working Stock
            - **NOTE:** Assay Stock is purchased at **40x** concentration so must be diluted
    - Plan plate layout using template. Attach to PCR hood using a magnet.

2. **Prepare samples for genotyping.**
    - Thaw 1 ng/uL samples completely
        - 1 ng/uL sample dilutions should be prepared ahead of time and stored separately
    - Vortex for 10 seconds.
    - Pulse spin.
    - Place on bench in tube rack at RT.

3. **Clean out PCR hood.**
    - Turn PCR hood on and remove all items from PCR hood
    - Wipe down PCR hood with DNA Away
    - Return each item to clean PCR hood after wiping it down with DNA Away

4. **Prepare reaction mix.**
    - Remove Assay Mix from -20 freezer and put on ice in small ice box.
        - Keep assay mix out of direct light by placing cover on ice box.
        - **NOTE:** If Assay Stock not already diluted to **20x** concentration do so now
    - Remove Master Mix from 4-degree fridge. Place in tube rack.
    - Add required volumes of Master Mix and Assay Mix to a fresh 1.5 mL tube.
    - Vortex briefly. Pulse spin.
    - Place reaction mix on ice, covered.
    - Return any remaining Assay Mix to freezer, return Master Mix to fridge.

5. **Bring required components to PCR hood.**
    - Diluted DNA samples (in tube rack), reaction mix (in covered ice box), necessary pipettes and tips, tip disposal container, 96-well plate, and plate film.

6. **Add sample followed by reaction mix to 96-well plate (25 uL reaction).**
    - Add 11.25uL diluted DNA sample.
    - Add 13.75uL reaction mix (keep covered until use).
    - **NOTE:** Always add negative control to plate last after loading all samples.

7. **Seal the plate using plate film. Place the plate in the ice box, covered.**

8. **Transport downstairs for PCR.**
    - Select “New Experiment”

- Select “From Template” and then select “forbow” and click create

PCR Reaction Settings:

Step	Predesigned and Custom TaqMan® SNP Genotyping Assays		
	Temp.	Length	Cycles
Polymerase activation	95°C	10 minutes	HOLD
Denaturation	95°C	15 seconds	40
Annealing/ extension	60°C	1 minute	

9. **Exporting Data**
    - Insert USB stick and select your completed experiment and hit “Synchronize”
    - Transfer USB to computer beside PCR machine and bring up LaRoche Software
    - Label the raw data with Sample ID numbers and mark empty wells and negative controls
    - Select analysis tab and choose EndPoint Analysis
    - Export End Point Fluorescence Table to USB drive and ensure before leaving that it will open in Microsoft Excel correctly

Genotyping Quality Control, Imputation, and Polygenic Risk Scoring

A) Pre-Imputation Quality Control

1. Update sample names.

```
$plink \
--bfile forbow18_aligned \
--update-ids $namesf18 \
--make-bed \
--out forbow18.updated_names
```

2. Exclude duplicates.

```
$plink \
--bfile forbow18.updated_names \
--remove $excludesf18 \
--make-bed \
--out forbow18.kept_names
```

3. Filter by minor allele frequency (MAF) - retain variants with MAF > 0.01.

```
$plink \
--bfile $root.updated_sex \
--maf 0.01 \
--make-bed \
--out $root.common
```

4. Iteratively filter for call rate - remove SNPs then samples in turn beneath specified thresholds (90% to 95% in 1% steps) - produces $root.filtered.

```
sh ./Iterative_Missingness.sh 90 95 1
```

5. Review missingness to ensure all missing SNPs and individuals have been dropped.

```
$plink \
--bfile $root.filtered \
--missing \
--out $root.filtered_missing

sort -k 5 -gr $root.filtered_missing.lmiss | head
```

- Check that no variants above missingness threshold remain in column 5.

```
sort -k 6 -gr $root.filtered_missing.imiss | head
```

- Check that no individuals above missingness threshold remain in column 6.

6. Assess SNPs for deviation from Hardy-Weinberg equilibrium (HWE).

```
$plink \
--bfile $root.filtered \
--hardy \
--out $root.hw_p_values
```

Departures from HWE are expected in a case-only sample (Wittke-Thompson et al 2005) - exclude variants with a low P-value threshold because of the high-risk nature of the sameple (P < 10e-10).

```
$plink \
--bfile $root.filtered \
--hwe 0.000000001 \
--make-bed \
--out $root.hw_dropped
```

7. Prune for linkage disequilibrium - window of 1500 variants with a shift of 150 variants between windows, and an r^2 cut-off of 0.2.

```
$plink \
--bfile $root.hw_dropped \
--indep-pairwise 1500 150 0.2 \
--out $root.LD_one
```

Extract prune-in SNPs.

```
$plink \
--bfile $root.hw_dropped \
--extract $root.LD_one.prune.in \
--make-bed \
--out $root.LD_two
```

Generate file lists of SNPs from high-LD regions and non-autosomal regions to exclude from the pruned file.

For the list of high-LD regions used in analysis of samples of European ancestry, please refer to Price AL, Weale ME, Patterson N, et al. (2008), “Long-range LD can confound genome scans in admixed populations”, Am J Hum Genet 83: 132-5, Table 1.

```
awk –f ./highLDregions4bim_b37.awk $root.LD_two.bim > highLDexcludes

awk '($1 < 1) || ($1 > 22) {print $2}' $root.LD_two.bim >
autosomeexcludes

cat highLDexcludes autosomeexcludes > highLD_and_autosomal_excludes
```

Exclude high LD and non-autosomal regions.

```
$plink \
--bfile $root.LD_two \
--exclude highLD_and_autosomal_excludes \
--make-bed \
--out $root.LD_three
```

8. Check for sex mismatch using the X chromosome F statistic.

```
$plink \
--bfile $root.LD_two \
--check-sex 0.2 0.8 \
--out $root.sex_check_x
```

Exclude any definite problems.

```
$plink \
--bfile $root.hw_dropped \
--remove discordant_individuals.txt \
--make-bed \
--out $root.sexcheck_cleaned

$plink \
--bfile $root.LD_three \
--remove discordant_individuals.txt \
--make-bed \
--out $root.LD_four
```

9. Check genome-wide heterozygosity.

```
$plink \
--bfile $root.LD_four \
--ibc \
--out $root.het
```

10. Calculate pairwise identity-by-descent (IBD).

```
$plink \
--bfile $root.LD_four \
--genome \
--make-bed \
--out $root.IBD
```

Calculate IBD specifically for those with reported relations within the sample.

```
$plink \
--bfile $root.LD_four \
--genome \
--rel-check \
--make-bed \
--out $root.IBD_relcheck
```

B) Imputation Prep

1. Use the script provided at http://www.well.ox.ac.uk/~wrayner/tools/#Checking to check PLINK file against the Haplotype Reference Consortium reference panel.

Calculate MAF:

```
./plink --bfile $root.no_dups --freq --out $root.final
```

Run script:

```
perl HRC-1000G-check-bim.pl -b $root.no_dups.bim -f $root.final.frq
-r HRC.r1-1.GRCh37.wgs.mac5.sites.tab -h
```

Run output script to update PLINK files:

```
sh ./Run-plink.sh
```

2. Convert PLINK individual chromosome files to VCF files.

3. bgzip VCF files.

4. Upload to Michigan Imputation Server with the following settings:

```
Reference panel - HRC r1.1 2016
Phasing - Eagle v2.3
Population - mixed
```

C) Post-Imputation Quality Control

1. Duplicate gzVCF files output from the imputation server.

2. Assign unique variant identifiers for each chromosome file.

```
$bcftools norm -Ou -m-any chr1.dose.vcf.gz | $bcftools annotate -Oz
-x 'ID' -I +'%CHROM:%POS:%REF:%ALT' > unique_chr1.vcf.gz
```

-Ou: output to uncompressed BCF (speeds up computational time when moving between commands)
-m-any: split multi allelic sites into biallelic records for all variants
-N: do not normalize
-Oz: output to compressed VCF
-x 'ID': deletes the IDs
-I +'%CHROM:%POS:%REF:%ALT': assigns IDs

3. Convert VCF to PLINK binary file format and retain variants with MAF < 0.01 for each chromosome.

```
$plink --vcf unique_chr1.vcf.gz --keep-allele-order --maf 0.01 --
make-bed --out uchr1_refalt
```

4. Exclude SNPs with poor imputation quality for each chromosome.

```
$plink --bfile uchr1_refalt --qual-scores chr1_info.txt --qual-
threshold 0.3 --make-bed --out uchr1qc
```

5. Merge chromosomes into a single file.

```
$plink --merge-list $mergechr --make-bed --out uhrns18
```

6. Add sex to the phenotype file.

```
$plink \
--bfile $clean \
```

```
--update-sex $sex \
--make-bed \
--out $clean.sex
```

D) Polygenic Risk Scoring

Schizophrenia (PGC2):

```
Rscript $prsR --dir /Users/alysonzwicker/Documents/PRSice_mac/ \
--prsice $prs \
--base scz_pgc_base_clean.txt \
--stat or \
--pvalue p \
--chr chr \
--snp snp \
--A1 a1 \
--A2 a2 \
--se se \
--target $rs.valid \
--binary-target F \
--out scz_rs_2 \
--bar-levels 0.01,0.05,0.1,0.2,0.5,1 \
--thread max \
--fastscore T \
--no-regress
```

Bipolar (PGC2):

```
Rscript $prsR --dir /Users/alysonzwicker/Documents/PRSice_mac/ \
--prsice $prs \
--base daner_PGC_BIP32b_mds7a_0416a \
--stat OR \
--pvalue P \
--chr CHR \
--snp SNP \
--A1 A1 \
--A2 A2 \
--se SE \
--target $rs.valid \
--binary-target F \
--out bp_rs_2 \
--bar-levels 0.01,0.05,0.1,0.2,0.5,1 \
--thread max \
--fastscore T \
--no-regress
```

MDD (PGC2):

```
Rscript $prsR --dir /Users/alysonzwicker/Documents/PRSice_mac/ \
--prsice $prs \
--base MDD2018_ex23andMe \
--stat OR \
--pvalue P \
--chr CHR \
```

```
--snp SNP \
--A1 A1 \
--A2 A2 \
--se SE \
--target $rs.valid \
--binary-target F \
--out mdd_rs_2 \
--bar-levels 0.01,0.05,0.1,0.2,0.5,1 \
--thread max \
--fastscore T \
--no-regress
```

ADHD (PGC2):

```
Rscript $prsR --dir /Users/alysonzwicker/Documents/PRSice_mac/ \
--prsice $prs \
--base adhd_jul2017 \
--stat OR \
--pvalue P \
--chr CHR \
--snp SNP \
--A1 A1 \
--A2 A2 \
--se SE \
--target $rs.valid \
--binary-target F \
--out adhd_rs_2 \
--bar-levels 0.01,0.05,0.1,0.2,0.5,1 \
--thread max \
--fastscore T \
--no-regress
```

Intelligence:

```
Rscript $prsR --dir /Users/alysonzwicker/Documents/PRSice_mac/ \
--prsice $prs \
--base SavageJansen_2018_intelligence_metaanalysis.txt \
--stat stdBeta \
--beta \
--pvalue P \
--chr CHR \
--snp SNP \
--A1 A1 \
--A2 A2 \
--se SE \
--target $rs.valid \
--binary-target F \
--out intell_rs_2 \
--bar-levels 0.01,0.05,0.1,0.2,0.5,1 \
--thread max \
--fastscore T \
--no-regress
```

Anxiety factor score:

```
Rscript $prsR --dir /Users/alysonzwicker/Documents/PRSice_mac/ \
--prsice $prs \
--base anxiety.meta.full.fs.tbl \
--stat Effect \
--beta \
--pvalue P.value \
--chr CHR \
--snp SNPID \
--A1 Allele1 \
--A2 Allele2 \
--se StdErr \
--target $rs.valid \
--binary-target F \
--out anx_fs \
--bar-levels 0.01,0.05,0.1,0.2,0.5,1 \
--thread max \
--fastscore T \
--no-regress
```

Anxiety case-control:

```
Rscript $prsR --dir /Users/alysonzwicker/Documents/PRSice_mac/ \
--prsice $prs \
--base anxiety.meta.full.cc.tbl \
--stat Effect \
--pvalue P.value \
--chr CHR \
--snp SNPID \
--A1 Allele1 \
--A2 Allele2 \
--se StdErr \
--target $rs.valid \
--binary-target F \
--out anx_cc \
--bar-levels 0.01,0.05,0.1,0.2,0.5,1 \
--thread max \
--fastscore T \
--no-regress
```

E) Principal Components Analysis

1. LD prune - window of 1500 variants with a shift of 150 variants between windows, and an r^2 cut-off of 0.2.

```
$plink \
--bfile $rs.valid \
--indep-pairwise 1500 150 0.2 \
--out $rs.valid_one
```

Extract prune-in SNPs.

```
$plink \
--bfile $rs.valid \
--extract $rs.valid_one.prune.in \
--make-bed \
```

```
--out $rs.valid.LD_two
```

Generate file lists of SNPs from high-LD regions and non-autosomal regions to exclude from the pruned file.

```
awk -f ./highLDregions4bim_b37.awk $rs.valid.LD_two.bim >
highLDexcludes

awk '($1 < 1) || ($1 > 22) {print $2}' $rs.valid.LD_two.bim >
autosomeexcludes

cat highLDexcludes autosomeexcludes > highLD_and_autosomal_excludes
```

Exclude high LD and non-autosomal regions.

```
$plink \
--bfile $rs.valid.LD_two \
--exclude highLD_and_autosomal_excludes \
--make-bed \
--out $rs.valid.LD_three
```

2. Run PCA.

```
$plink \
--bfile $rs.valid.LD_three \
--pca 10 header
```

APPENDIX C
RESULTS SUPPLEMENT

Supplementary Materials: Affective lability in offspring of parents with major depressive disorder, bipolar disorder and schizophrenia

Full regression results from primary analysis

Supplementary Table 3.1. Overall dimensional affective lability across parent diagnostic groups.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent depression	0.46	**0.002**	0.17	0.76
Parent bipolar	0.47	**0.008**	0.12	0.81
Parent schizophrenia	0.15	0.555	-0.34	0.63
Time	-0.13	**< 0.001**	-0.18	-0.08
Age	0.02	0.249	-0.01	0.05
Sex	0.08	0.462	-0.13	0.30

Full regression results from sensitivity analyses

Supplementary Table 3.2. CALS parent-report across parent diagnostic groups.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent depression	0.44	**0.005**	0.13	0.75
Parent bipolar	0.35	0.058	-0.01	0.70
Parent schizophrenia	0.11	0.678	-0.40	0.61
Time	-0.12	**< 0.001**	-0.18	-0.07
Age	-0.001	0.969	-0.04	0.04
Sex	-0.01	0.949	-0.23	0.22

Supplementary Table 3.3. CALS self-report across parent diagnostic groups.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent depression	0.41	**0.032**	0.04	0.79
Parent bipolar	0.50	**0.026**	0.06	0.94
Parent schizophrenia	0.36	0.308	-0.33	1.05
Time	-0.13	**< 0.001**	-0.21	-0.06
Age	0.07	**0.017**	0.01	0.12
Sex	0.22	0.134	-0.07	0.51

The sex of the affected parent and offspring overall affective lability

We tested the effect of affected parent's sex on offspring affective lability. We constructed a mixed-effects linear regression model with both mother's and father's illness, controlling for age, sex, and time in the study. To account for the non-independence of observations from related individuals and repeated measures within individuals, we included family and individual identifiers as random effects in the model. The effect of mother's SMI on offspring affective lability was significant (beta = 0.34, 95% CI 0.08 to 0.60, p = 0.010), whereas the effect of father's SMI was not (beta = -0.02, 95% CI -0.36 to 0.33, p = 0.923), see Supplementary Table 4. This analysis includes 141 observations from individuals with affected fathers and 281 observations from individuals with affected mothers.

Supplementary Table 3.4. Full regression results from analyses of affected parent sex

Regression term	Beta	P-value	95% CI lower	95% CI upper
Mother affected	0.34	**0.010**	0.08	0.60
Father affected	-0.02	0.923	-0.36	0.33
Time	-0.13	**< 0.001**	-0.19	-0.08
Age	0.02	0.169	0.01	0.06
Sex	0.05	0.652	-0.17	0.27

Supplementary Materials: Basic symptoms in offspring of parents with severe mental illness

Full regression results from parent illness severity analyses

Supplementary Table 4.1. SPI-CY risk scores by parent illness severity.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent NSMD	0.22	0.415	-0.30	0.73
Parent SMI	0.69	**0.004**	0.22	1.16
Time in study	-0.16	**< 0.001**	-0.25	-0.07
Age	0.11	**< 0.001**	0.06	0.16
Sex	-0.004	0.980	-0.39	0.38

Supplementary Table 4.2. Sensitivity analysis of SPI-CY risk scores by parent illness severity. We excluded observations at which offspring experienced a major depressive episode within 12 months prior to the assessment.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent NSMD	0.19	0.380	-0.23	0.61
Parent SMI	0.49	**0.014**	0.10	0.87
Time in study	-0.14	**< 0.001**	-0.22	-0.06
Age	0.08	**< 0.001**	0.04	0.12
Sex	-0.12	0.476	-0.43	0.20

Supplementary Table 4.3. COGDIS scores by parent illness severity.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent NSMD	0.31	0.176	-0.14	0.75
Parent SMI	0.53	**0.009**	0.13	0.93
Time in study	-0.12	**0.005**	-0.21	-0.04
Age	0.10	**< 0.001**	0.06	0.14
Sex	0.03	0.837	-0.29	0.36

Supplementary Table 4.4. Sensitivity analysis of COGDIS scores by parent illness severity. We excluded observations at which offspring experienced a major depressive episode within 12 months prior to the assessment.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent NSMD	0.27	**0.163**	-0.11	0.65
Parent SMI	0.39	**0.028**	0.04	0.73
Time in study	-0.09	**0.027**	-0.17	-0.01
Age	0.07	**< 0.001**	0.04	0.11
Sex	0.04	0.791	-0.32	0.25

Full regression results from parent psychosis analyses

Supplementary Table 4.5. SPI-CY risk scores by parent psychosis.

Regression term	Beta	P-value	95% CI lower	95% CI upper
No parent psychosis	0.45	0.055	-0.01	0.90
Parent psychosis	0.68	**0.023**	0.09	1.27
Time in study	-0.16	**0.001**	-0.25	-0.06
Age	0.11	**< 0.001**	0.06	0.16
Sex	0.01	0.940	-0.37	0.40

Supplementary Table 4.6. Sensitivity analysis of SPI-CY risk scores by parent psychosis. We excluded observations at which offspring experienced a major depressive episode within 12 months prior to the assessment.

Regression term	Beta	P-value	95% CI lower	95% CI upper
No parent psychosis	0.35	0.067	-0.02	0.72
Parent psychosis	0.44	0.078	-0.05	0.92
Time in study	-0.13	**0.001**	-0.21	-0.05
Age	0.08	**< 0.001**	0.04	0.12
Sex	-0.10	0.550	-0.42	0.22

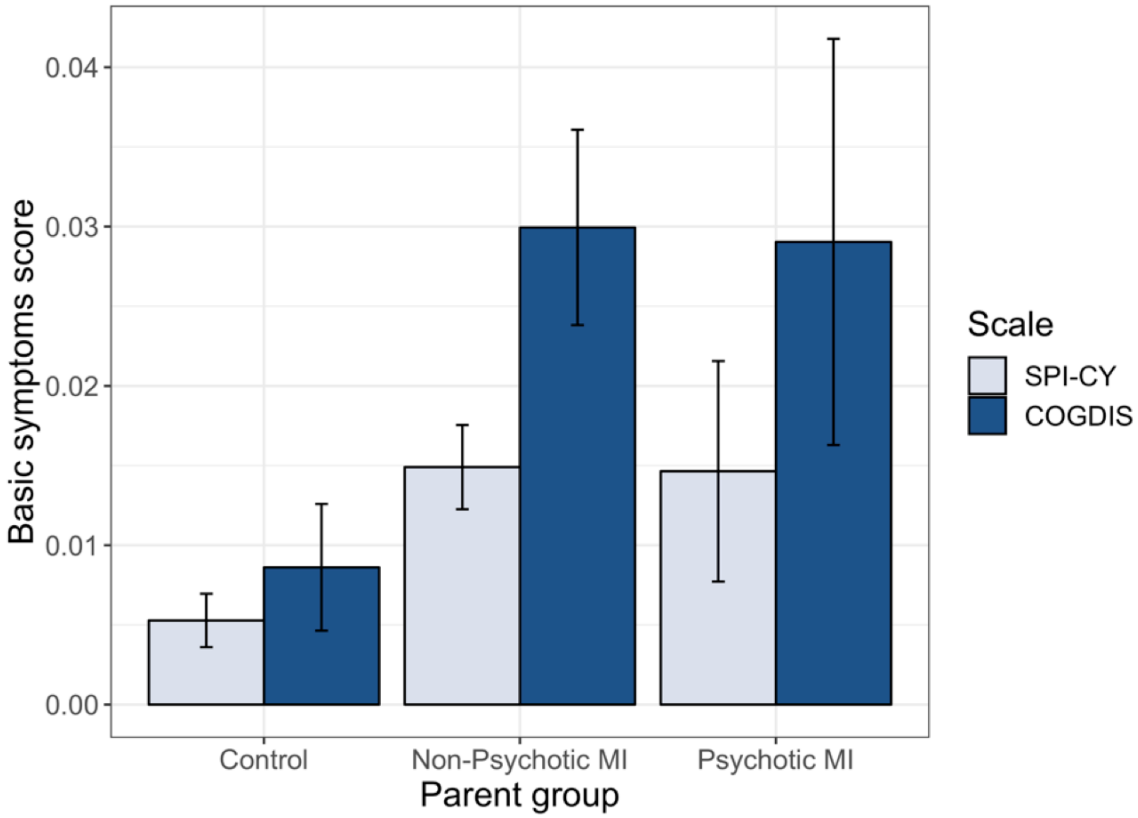
Supplementary Table 4.7. Differences in COGDIS scores by parent psychosis.

Regression term	Beta	P-value	95% CI lower	95% CI upper
No parent psychosis	0.41	**0.037**	0.02	0.80
Parent psychosis	0.55	**0.030**	0.05	1.04
Time in study	-0.12	**0.005**	-0.21	-0.04
Age	0.10	**< 0.001**	0.06	0.14
Sex	0.04	0.797	-0.28	0.37

Supplementary Table 4.8. Sensitivity analysis of COGDIS scores by parent psychosis. We excluded observations at which offspring experienced a major depressive episode within 12 months prior to the assessment.

Regression term	Beta	P-value	95% CI lower	95% CI upper
No parent psychosis	0.34	**0.043**	0.01	0.68
Parent psychosis	0.34	0.123	-0.09	0.77
Time in study	-0.09	**0.032**	-0.17	-0.01
Age	0.07	**< 0.001**	0.03	0.11
Sex	-0.03	0.844	-0.31	0.26

Supplementary Figure 4.1. Mean SPI-CY risk scores and COGDIS scores, stratified by parent psychosis. Error bars represent standard error of the mean.



Basic symptom scores by parent diagnosis

Differences in SPI-CY risk score by parent diagnosis

Across the 909 assessments of 332 youth with valid SPI-CY risk scores, offspring basic symptom scores were significantly elevated among offspring of parents with bipolar disorder compared to controls (B = 0.78, 95% CI 0.24 to 1.31, p = 0.005; see Supplementary Figure 2). SPI-CY risk scores were numerically elevated among offspring of parents with major depressive disorder (B = 0.40, 95% CI -0.08 to 0.87, p = 0.101) and schizophrenia (B = 0.21, 95% CI -0.68 to 1.09, p = 0.647), but these differences were not statistically significant.

Supplementary Table 4.9. Differences in SPI-CY risk scores by parent diagnosis.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent depression	0.40	0.101	-0.08	0.87
Parent bipolar	0.78	**0.005**	0.24	1.31
Parent schizophrenia	0.21	0.647	-0.68	1.09
Time in study	-0.15	**0.001**	-0.25	-0.06
Age	0.10	**< 0.001**	0.06	0.15
Sex	0.03	0.867	-0.35	0.41

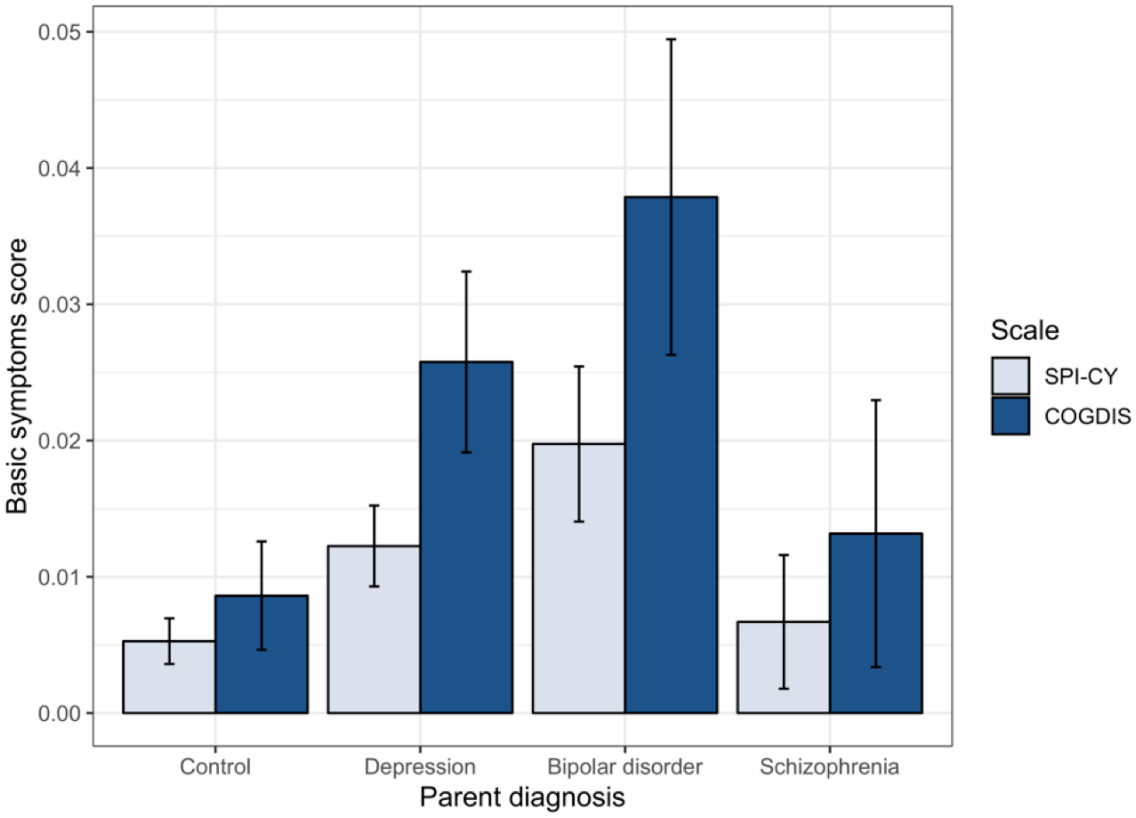
Differences in COGDIS score by diagnosis

Across the 905 assessments of 331 youth with valid COGDIS scores, COGDIS scores were significantly elevated among offspring of parents with bipolar disorder (B = 0.62, 95% CI 0.17 to 1.08, p = 0.007; see Supplementary Figure 2). COGDIS scores were numerically elevated among offspring of parents with major depressive disorder (B = 0.37, 95% CI -0.03 to 0.77, p = 0.072) and psychosis spectrum disorders (B = 0.26, 95% CI -0.50 to 1.01, p = 0.504), however these differences were not statistically significant.

Supplementary Table 4.10. Differences in COGDIS scores by parent diagnosis.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent depression	0.37	0.072	-0.03	0.77
Parent bipolar	0.62	**0.007**	0.17	1.08
Parent schizophrenia	0.26	0.504	-0.50	1.01
Time in study	-0.12	**0.006**	-0.21	-0.03
Age	0.09	**< 0.001**	0.05	0.13
Sex	0.05	0.747	-0.27	0.37

Supplementary Figure 4.2. Mean SPI-CY risk scores and COGDIS scores by parent diagnosis. Error bars represent standard error of the mean.



Age stratified analyses

SPI-CY risk score by parent illness severity in 8-11 year olds

Across the 395 assessments of 195 children and youth aged 8-11 years with valid SPI-CY risk scores, basic symptoms were significantly elevated among offspring of a parent with SMI (B = 0.45, 95% CI 0.10 to 0.79, p = 0.011; see Supplementary Figure 3). Full regression results are shown below.

Supplementary Table 4.11. Differences in SPI-CY risk scores by parent illness severity in offspring aged 8-11 years.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent NSMD	0.20	0.318	-0.19	0.60
Parent SMI	0.45	**0.011**	0.10	0.79
Time in study	-0.09	0.070	-0.18	0.01
Age	0.07	0.224	-0.05	0.19
Sex	-0.28	0.068	-0.57	0.02

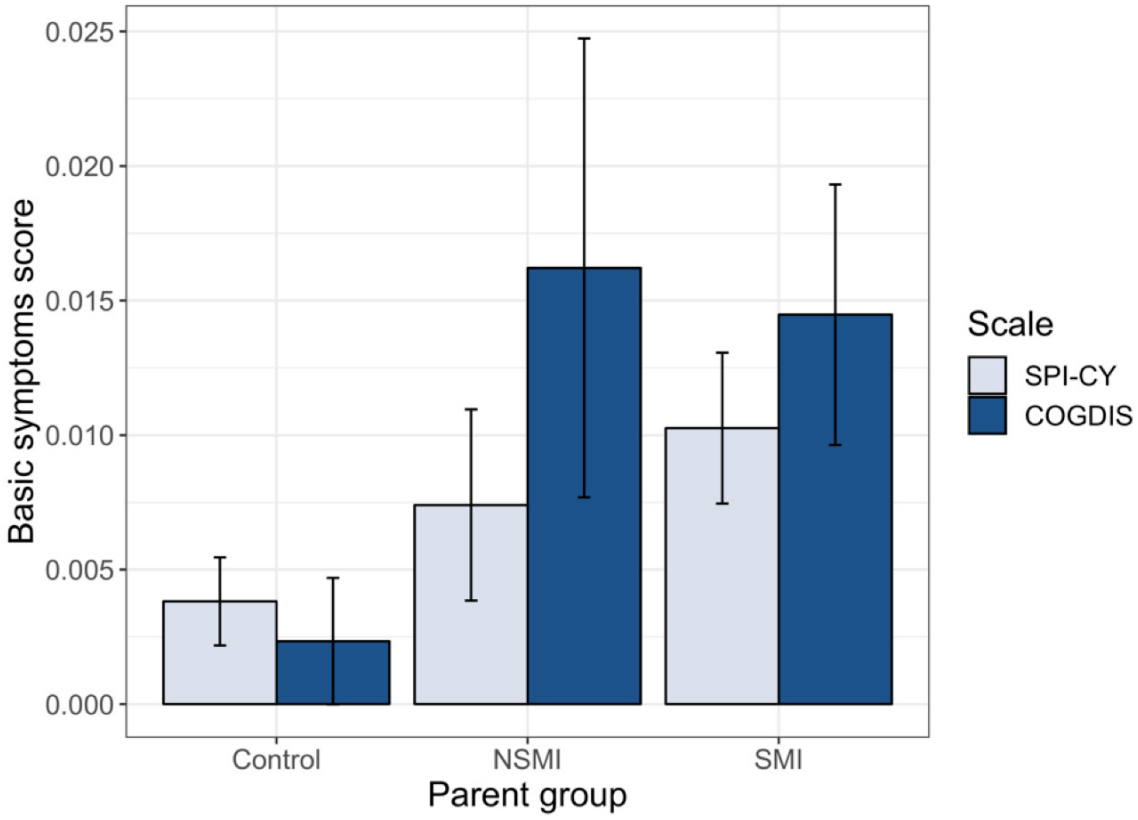
COGDIS score by parent illness severity in 8-11 year olds

Across the 391 assessments of 194 children and youth aged 8-11 years with valid COGDIS scores, basic symptoms were significantly elevated among offspring of parents with NSMD (B = 0.36, 95% CI 0.05 to 0.66, p = 0.023) and SMI (B = 0.34, 95% CI 0.08 to 0.60, p = 0.012; see Supplementary Figure 3). Full regression results are shown below.

Supplementary Table 4.12. Differences in COGDIS scores by parent illness severity in offspring aged 8-11 years.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent NSMD	0.36	**0.023**	0.05	0.66
Parent SMI	0.34	**0.012**	0.08	0.60
Time in study	-0.04	0.258	-0.12	0.03
Age	-0.03	0.615	-0.12	0.07
Sex	-0.09	0.435	-0.32	0.14

Supplementary Figure 4.3. Mean SPI-CY risk score and COGDIS score, stratified by parent illness severity group for participants aged 8-11 years. Error bars represent standard error of the mean.



SPI-CY risk score by parent illness severity in 12+ year olds

Across the 514 assessments of 196 children and youth aged 12-27 years with valid SPI-CY risk scores, basic symptoms were significantly elevated among offspring of a parent with SMI (B = 0.83, 95% CI 0.03 to 1.62, p = 0.043; see Supplementary Figure 4). Full regression results are shown below.

Supplementary Table 4.13. Differences in SPI-CY risk scores by parent illness severity in offspring aged 12-27 years.

Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent NSMD	0.18	0.693	-0.70	1.05
Parent SMI	0.83	**0.043**	0.03	1.62
Time in study	-0.20	**0.008**	-0.36	-0.05
Age	0.16	**0.001**	0.06	0.25
Sex	0.15	0.631	-0.47	0.77

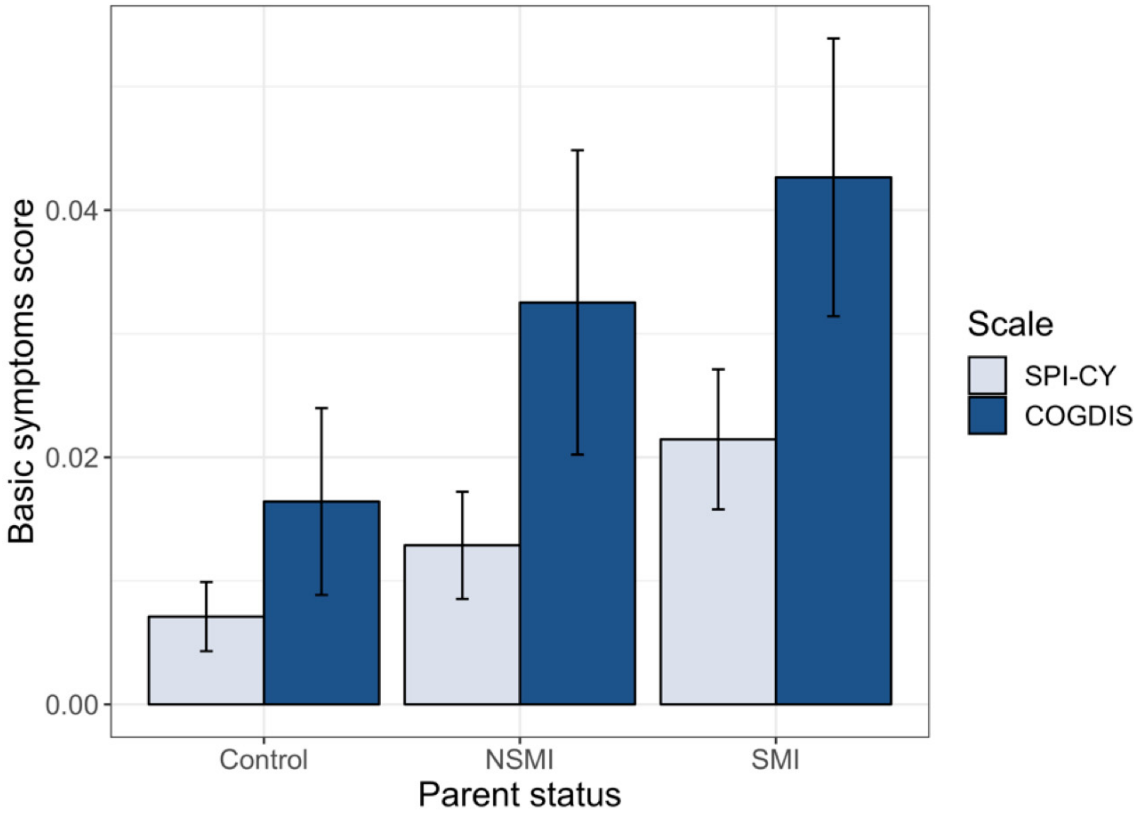
COGDIS score by parent illness severity in 12+ year olds

Across the 514 assessments of 196 children and youth aged 12-27 years with valid COGDIS scores, COGDIS scores numerically increased with increasing parent illness severity, basic symptoms were numerically elevated among offspring of a parent with NSMD or SMI, but these differences were not statistically significant (see Supplementary Figure 4). Full regression results are shown below.

Supplementary Table 4.14. Differences in COGDIS scores by parent illness severity in offspring aged 12-27 years.

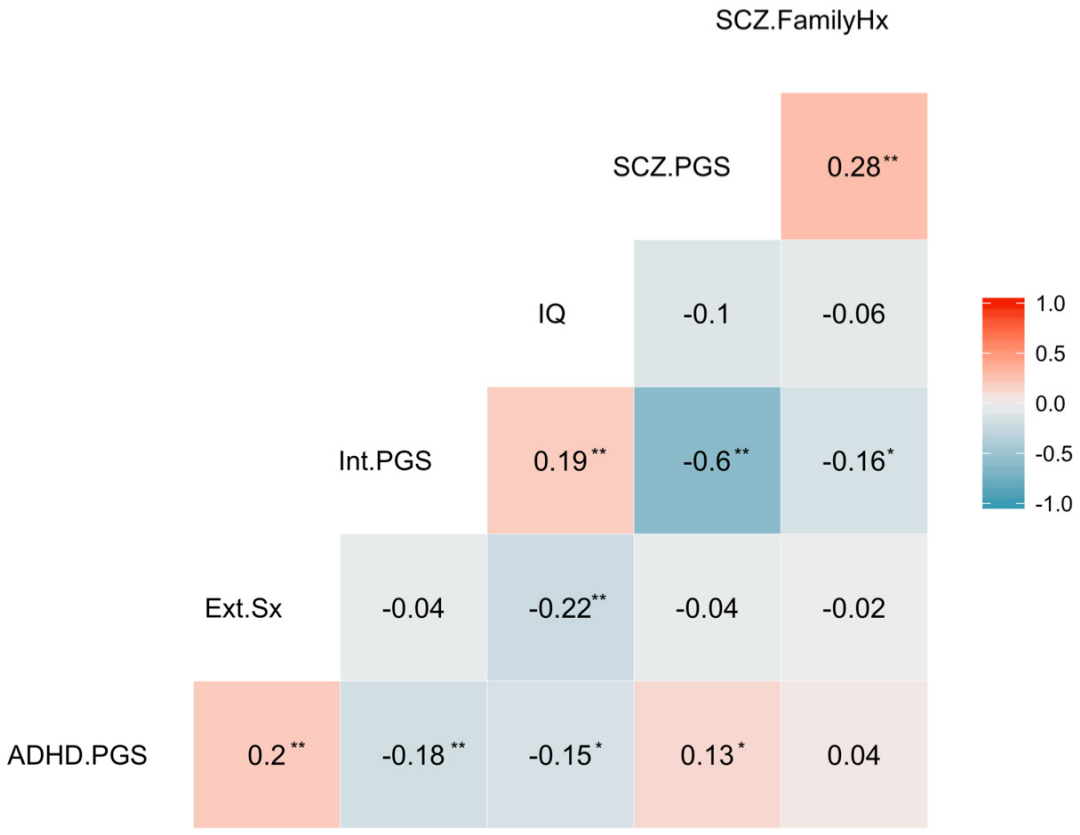
Regression term	Beta	P-value	95% CI lower	95% CI upper
Parent NSMD	0.23	0.547	-0.53	0.99
Parent SMI	0.65	0.068	-0.05	1.34
Time in study	-0.17	**0.015**	-0.31	-0.03
Age	0.12	**0.005**	0.04	0.20
Sex	0.09	0.744	-0.45	0.63

Supplementary Figure 4.4. Mean SPI-CY risk score and COGDIS score, stratified by parent illness severity group for participants aged 12-27 years. Error bars represent standard error of the mean.



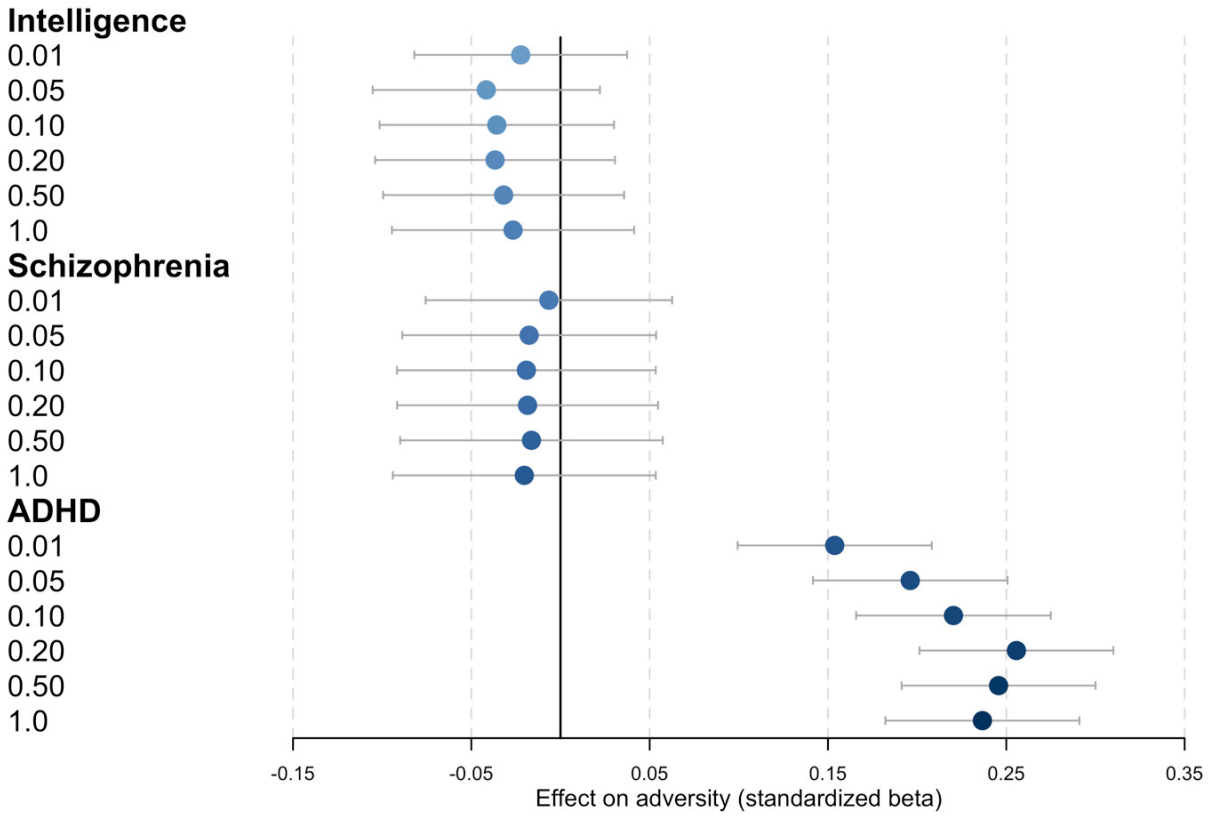
Supplementary Materials: Neurodevelopmental and genetic determinants of exposure to adversity among youth at risk for mental illness

Supplementary Figure 5.1. Correlation matrix showing relationships between our predictors. The numbers within the boxes represent Pearson product-moment correlation coefficients, '*' denotes p-value less than 0.05 and '**' denotes p-value less than the Bonferroni corrected p-value of 0.003 (accounting for 15 tests).

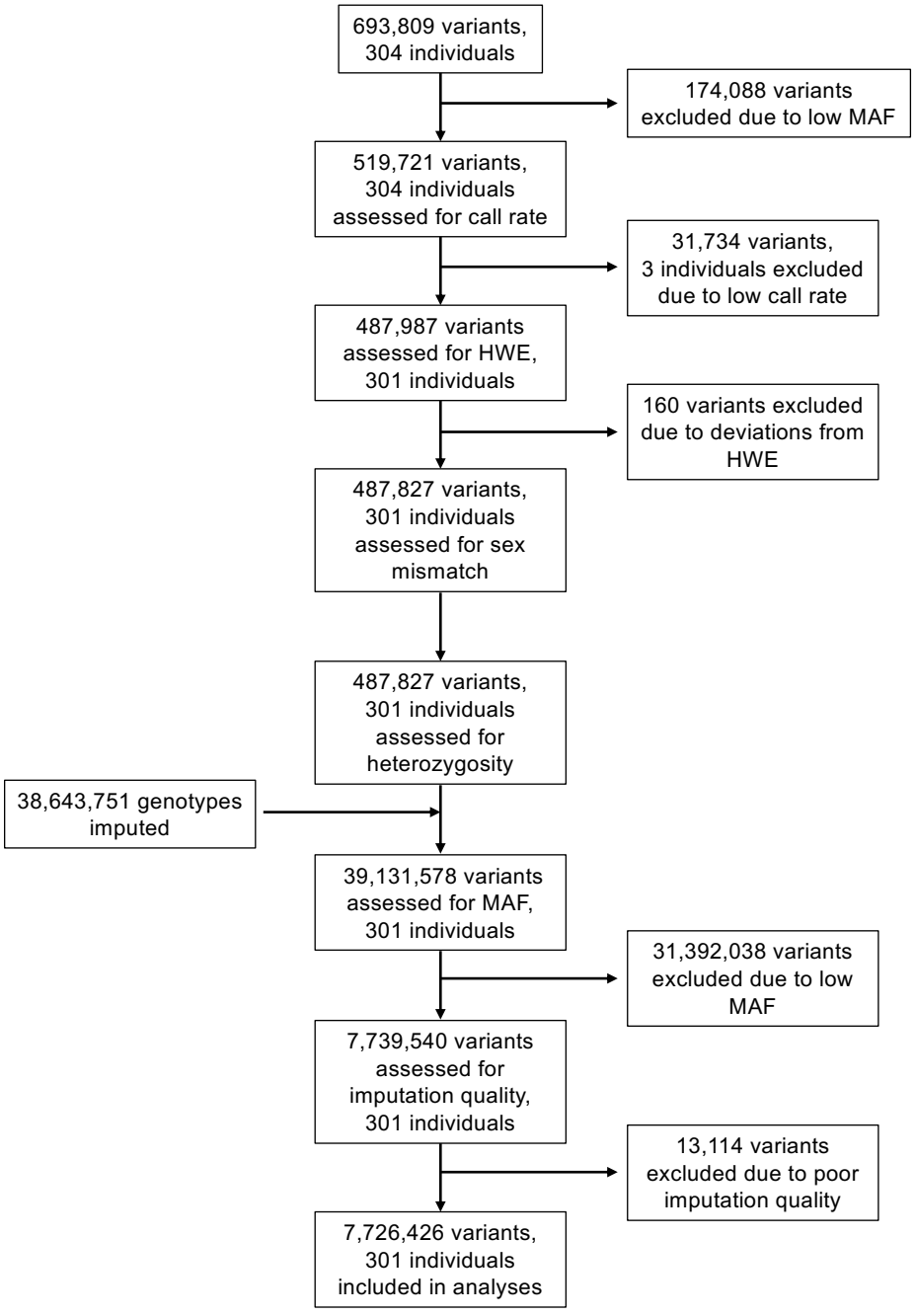


SCZ.FamilyHx = Family history of schizophrenia; SCZ.PGS = Schizophrenia polygenic score; Int.PGS = Intelligence polygenic score; Ext.Sx = Externalizing symptom score; ADHD.PGS = ADHD polygenic score.

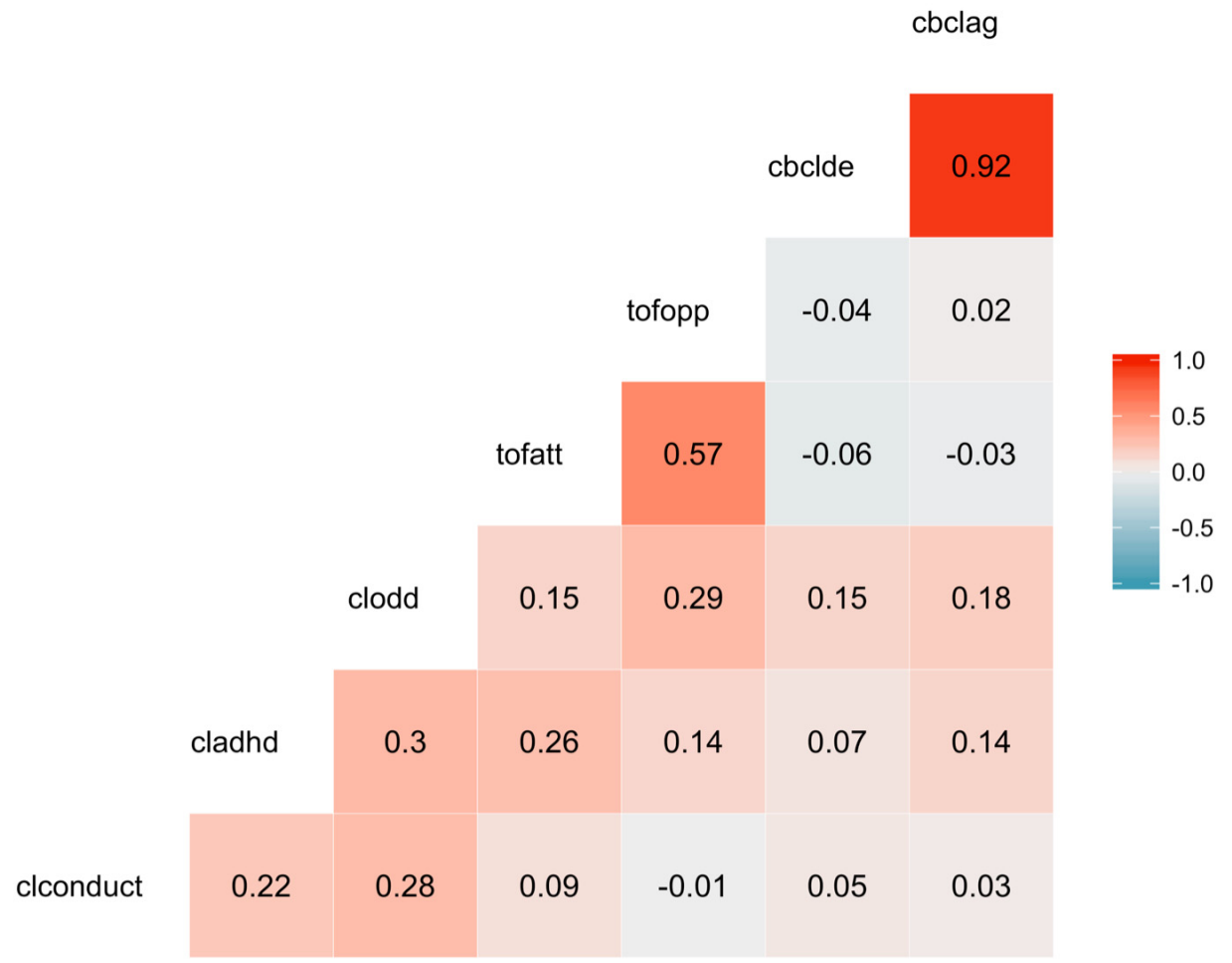
Supplementary Figure 5.2. The effect of polygenic scores for intelligence, schizophrenia and ADHD at multiple p-value thresholds for variant inclusion (ranging from 0.01 to 1 for each phenotype) on adversity. None of the polygenic scores for intelligence or schizophrenia were significantly associated with exposure to adversity. The polygenic score for ADHD was significantly associated with adversity at all *P* value thresholds except for $P_T = 0.01$, with $p < 0.003$ (Bonferroni significance threshold corrected for 18 tests).



Supplementary Figure 5.3. Flow diagram of genome-wide genotyping quality control and imputation with variant and individual inclusion and exclusion information. MAF = Minor allele frequency, HWE = Hardy-Weinberg equilibrium.



Supplementary Figure 5.4. The relationships between lifetime consensus-confirmed diagnoses of ADHD, oppositional defiant disorder, conduct disorder and the scores on TOF attention problems and oppositional scales and CBCL aggressive behaviour and delinquent behaviour scales. The TOF attention problems and oppositional scales contain 17 items each. The CBCL aggressive behaviour and delinquent behaviour scales contain 20 items and 16 items, respectively.



clconduct = consensus-confirmed lifetime conduct disorder diagnosis, cladhd = consensus-confirmed lifetime ADHD diagnosis, clodd = consensus-confirmed lifetime oppositional defiant disorder diagnosis, tofatt = TOF attention problems scale score, tofopp = TOF oppositional scale score, cbclde = CBCL delinquent behaviour scale, cbclag = CBCL aggressive behaviour scale.

Supplementary Table 5.1. The prevalence of adversity exposures.

Adversity	N (%)
No post-secondary education (Mother)	55 (18.3)
No post-secondary education (Father)	128 (42.5)
No home ownership	109 (36.2)
Poverty	88 (29.2)
Emotional abuse	34 (11.3)
Physical abuse	19 (6.3)
Sexual abuse	14 (4.7)
Neglect	42 (14.0)
Exposure to violence	65 (21.6)
Bullying	42 (14.0)

Supplementary Table 5.2. The number of SNPs included in each polygenic score at each p-value threshold. P_T = p-value threshold for SNP inclusion.

Polygenic score	Number of SNPs included
ADHD	
$P_T = 0.01$	10290
$P_T = 0.05$	32704
$P_T = 0.10$	53146
$P_T = 0.20$	84918
$P_T = 0.50$	148253
$P_T = 1.0$	198847
Intelligence	
$P_T = 0.01$	23940
$P_T = 0.05$	63327
$P_T = 0.10$	97008
$P_T = 0.20$	146810
$P_T = 0.50$	242275
$P_T = 1.0$	317875
Schizophrenia	
$P_T = 0.01$	19508
$P_T = 0.05$	54237
$P_T = 0.10$	85168
$P_T = 0.20$	132591
$P_T = 0.50$	227597
$P_T = 1.0$	306655

Supplementary Table 5.3. Point estimates for regression results presented in Figure 1 of the main text. The association between adversity score and IQ, intelligence PGS, externalizing symptoms, ADHD PGS, and schizophrenia PGS.

Predictor	Standardized beta	Standard error	p-value
IQ	-0.16	0.05	0.004
Intelligence PGS	-0.03	0.07	0.637
Externalizing symptoms	0.20	0.05	< 0.001
ADHD PGS	0.25	0.05	< 0.001
Schizophrenia PGS	-0.02	0.07	0.805

Supplementary Table 5.4. Point estimates for variance explained presented in Figure 2 of the main text.

Predictor	Specific variance explained (%)	Unique variance explained (%)
IQ	6.73	3.40
Intelligence PGS	0.18	0
Externalizing symptoms	5.54	1.80
ADHD PGS	8.05	5.15
Schizophrenia PGS	0.01	0

Supplementary Table 5.5. Point estimates for regression results presented in Figure 3 of the main text. The association between polygenic score for ADHD ($P_T = 0.50$) and adversities.

Predictor	Standardized beta	Standard error	p-value
No post-secondary education (Mother)	0.06	0.03	0.085
No post-secondary education (Father)	0.07	0.04	0.069
Poverty	0.07	0.05	0.110
No home ownership	0.08	0.05	0.104
Sexual abuse	0.08	0.05	0.156
Bullying	0.12	0.64	0.064
Exposure to violence	0.14	0.06	0.015
Neglect	0.18	0.06	0.004
Physical abuse	0.21	0.06	< 0.001
Emotional abuse	0.22	0.06	< 0.001
Total socioeconomic	0.11	0.05	0.023
Total victimization	0.25	0.06	< 0.001
Total adversity score	0.25	0.05	< 0.001

Supplementary Table 5.6. Regression results from multivariable analysis testing the relationship between all of the listed predictors and overall adversity score.

Predictor	Standardized beta	95% CI lower	95% CI upper	p-value
ADHD PGS	0.23	0.12	0.34	< 0.001
Intelligence PGS	-0.02	-0.16	0.11	0.741
Schizophrenia PGS	0.01	-0.15	0.16	0.937
Externalizing symptoms	0.14	0.03	0.25	0.013
IQ	-0.19	-0.30	-0.08	0.001

Chapter 5: Supplementary Methods

Family history assessment and calculation of family history scores

Detailed information on family history of schizophrenia, bipolar disorder, and depression up to second-degree relatives was obtained for all offspring participants based on interviews with their parents using the Family Interview for Genetic Studies (FIGS) (Maxwell, 1992). We calculated the family history score for schizophrenia as a count of the number of family members with a positive history of schizophrenia, divided by the number of family members with available information on mental health status. In both the numerator and denominator, a count of '1' was given for each first-degree family member and a count of '0.5' was given for each second-degree family member (Milne et al., 2008). This method takes into account both missing information and the density of psychiatric illness within a family.

Mediation analysis

To examine the mechanisms underlying the associations between adversity and ADHD PGS, externalizing symptoms, and IQ, we implemented mediation analysis using the 'mediation' package (Tingley, Yamamoto, Hirose, Keele, & Imai, 2014) in R version 3.5.1. We fitted mediator and outcome models using mixed-effects linear regression using the lme4 package (Bates, Mächler, Bolker, & Walker, 2015, p. 4). To account for the non-independence of observations from related individuals within families, we included the family identifier as a random effect in the models. We controlled for the effects of age, sex, time in the study, and the top 10 ancestry informative genetic principal components. To test whether externalizing symptoms mediate the relationship between ADHD PGS and adversity, we fitted a mediator model with externalizing symptoms explained by ADHD PGS. Next, we fitted an outcome model, with adversity explained by ADHD PGS and externalizing symptoms. To test whether IQ mediates the relationship between ADHD PGS and adversity, we fitted a mediator model with IQ explained by ADHD PGS. Next,

we fitted an outcome model, with adversity explained by ADHD PGS and IQ. We used the 'mediate' function to estimate the average causal mediation effects (ACME) and average direct effects (ADE) of externalizing symptoms and IQ. We calculated confidence intervals using the quasi-Bayesian Monte Carlo method with 1000 simulations.

Genotyping, quality control, and imputation

We genotyped 693,809 single nucleotide polymorphisms (SNPs) using the Illumina Global Screening Array from DNA extracted from saliva collected via the Oragene kit (DNA Genotek Inc, Kanata, ON), see Supplementary Figure 4. We completed pre-imputation quality control on genome-wide data by excluding variants and participants according to the following criteria: 1) variants with minor allele frequency less than 1%; 2) variants with missing rate greater than 5%; 3) participants with genotyping rate less than 95% (n = 3); 4) variants with significant deviations from Hardy-Weinberg equilibrium ($p < 10 \times 10^{-10}$); 5) participants with discrepancies between self-reported sex and genetic sex; and 6) participants with abnormally high heterozygosity (> 4 SD above sample mean) (Medina-Gomez et al., 2015). Data were imputed using Minimac3 via the Michigan Imputation Server (https://imputationserver.sph.umich.edu/index.html). Post-imputation quality control consisted of pruning variants with minor allele frequency less than 1% and with poor imputation quality ($R^2 < 0.30$).

Reference samples for polygenic score derivation

We constructed the intelligence PGS using the results of a meta-analysis of genome-wide association studies (GWAS) of intelligence (Savage et al., 2018). We constructed the ADHD PGS based on the results of a meta-analysis of GWAS of ADHD (Demontis et al., 2019). We

constructed a PGS for schizophrenia based on the results of a meta-analysis of GWAS of schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). We selected the schizophrenia PGS to test specificity of our results to intelligence PGS and ADHD PGS because this is one of the best validated psychiatric polygenic scores.

Chapter 5: Supplementary Results

Sensitivity analyses excluding offspring of control parents

When we exclude offspring of control parents (n = 84), our primary results were unchanged. Externalizing symptoms were positively associated with adversity (ß =0.22, 95% CI 0.10 to 0.35, p < 0.001). IQ was significantly negatively associated with adversity (ß =-0.18, 95% CI -0.32 to -0.05, p = 0.005). Polygenic score for ADHD was significantly positively associated with adversity (ß =0.24, 95% CI 0.10 to 0.37, p < 0.001). Polygenic scores for schizophrenia (ß =-0.07, 95% CI -0.22 to 0.08, p = 0.352) and intelligence (ß =-0.04, 95% CI -0.19 to 0.10, p = 0.560) were not significantly associated with adversity.

Sensitivity analyses excluding adult participants (aged 18 years or older)

When we exclude offspring aged 18 years and older (n = 51), our primary results were unchanged. Externalizing symptoms were positively associated with adversity (ß =0.18, 95% CI 0.07 to 0.29, p = 0.001). IQ was significantly negatively associated with adversity (ß =-0.17, 95% CI -0.28 to -0.05, p = 0.004). Polygenic score for ADHD was significantly positively associated with adversity (ß =0.23, 95% CI 0.11 to 0.34, p < 0.001). Polygenic scores for schizophrenia (ß =-0.02, 95% CI -0.18 to 0.13, p = 0.791) and intelligence (ß =-0.02, 95% CI -0.15 to 0.12, p = 0.786) were not significantly associated with adversity.

Sensitivity analyses excluding individuals with non-European ancestry

When we exclude individuals who reported having non-European or mixed ancestry, our primary results were unchanged. Externalizing symptoms were positively associated with adversity (ß =0.20, 95% CI 0.09 to 0.31, p < 0.001). IQ was significantly negatively

associated with adversity (ß =-0.14, 95% CI -0.26 to -0.03, p = 0.010). Polygenic score for ADHD was significantly positively associated with adversity (ß =0.27, 95% CI 0.16 to 0.39, p < 0.0001). Polygenic scores for schizophrenia (ß =0.02, 95% CI -0.20 to 0.24, p = 0.826) and intelligence (ß =-0.13, 95% CI -0.28 to 0.01, p = 0.0749) were not significantly associated with adversity.

Externalizing symptoms as a mediator of the relationship between ADHD PGS and adversity

When we examined externalizing symptoms as a mediator of the relationship between ADHD PGS and adversity, we found that both the estimated average causal mediation effects of externalizing symptoms (ß =0.04, 95% CI 0.01 to 0.07, p < 0.001) and the average direct effects of ADHD PGS on adversity (ß =0.21, 95% CI 0.11 to 0.32, p < 0.001) were both statistically significantly different from zero. These results suggest that ADHD PGS contributes to risk of adversity, both by directly increasing risk of adversity and through its influence on externalizing symptoms.

IQ as a mediator of the relationship between ADHD PGS and adversity

When we examined IQ as a mediator of the relationship between ADHD PGS and adversity, we found that the average direct effects of ADHD PGS on adversity were statistically significantly different from zero (ß =0.23, 95% CI 0.12 to 0.34, p < 0.001), but the average causal mediation effects of IQ were not (ß =0.02, 95% CI -0.003 to 0.04, p = 0.098. These results suggest that ADHD PGS contributes directly to risk of adversity, but that IQ does not mediate this relationship.