CHAPTERS IN THE EPIDEMIOLOGY OF CHILD AND ADOLESCENT MENTAL HEALTH: RISK FACTORS, PREVENTION, TREATMENT AND OUTCOMES

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ABSTRACT

Mental illnesses are a substantial burden in Canada and worldwide. Early life conditions and experiences make individuals more susceptible to developing diseases. The primary goal of this thesis is to understand mental health issues in children and adolescents and to provide a basis for prevention planning and policy. The four core studies in this thesis utilize a variety of epidemiological methods and data sources.

The first study, a systematic review and meta-analysis of longitudinal studies, found that early childhood maltreatment is a strong risk factor for the latter onset of depression and anxiety disorders. Proportion attributable fractions (PAFs) indicated a very large reduction in depression and anxiety could result from reducing childhood maltreatment.

The second study explored epigenetic changes (DNA methylation) linked to depression. This systematic review found inconsistent results for candidate genes (e.g. BDNF, SLC6A4, NR3C1, OXTR, and others) and genome-wide studies. There was high heterogeneity in terms of experimental and statistical methodologies among the studies. Future studies should apply standardized experimental and laboratory methodologies and adopt longitudinal designs to trace changes overtime.

The third study using clinical administrative data examined whether current child and adolescent mental health services effectively improved clients' psychosocial functioning. Treatment was found to be effective though the initial severity of the problem affected outcomes. While shortening the length of treatment might improve resource use efficiency, it would be detrimental to some clients. Personalized treatment is required to meet clients' specific needs.

Finally, the potential iatrogenic effects (Bipolar Disorder (BPAD)) of pharmacological treatment (stimulant) of children and adolescents for ADHD is examined using a cohort study design and provincial administrative data. After adjusting for psychiatric comorbidity, it was found that stimulant use by itself does not lead to the development of BPAD, but rather the severity of the initial disease and comorbidity are predictors of future BPAD.

The clear message of this research is that early reduction in risk factor exposure in utero and in childhood and adolescence and the early treatment of mental health problems has a very positive

effect in reducing the onset and further development of psychiatric diseases and mental health problems.

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LIST OF ABBREVIATIONS

5caC	5-carboxylcytosin
5fC	5-formylcytosin
5-hmc	5-hydroxymethylcytosine
5-mc	5-methylcytosine
ACE	Adverse childhood experience
ACE	Angiotensin converting enzyme
ADHD	Attention Deficit Hyperactivity Disorder
AIC	Akaike information criterion
AMIS	Administrative and Management Information System
Anti-5mc	Anti-5'methylcytosine
APOE	Apolipoprotein E
BIC	Bayesian information criterion
BPA	Bisphenol A
BPAD	Bipolar Affective Disorder
CAFAS	Child and Adolescent Functional Assessment Scale
CDC	Centers for Disease Control and Prevention
CFA	Confirmatory factor analysis
CFI	Comparative Fit Index
CI	Confidence interval
CPS	Child Protective Services
CYMHAS	Child and Youth Mental Health and Addictions Services
DALY	Disability-adjusted life year
DEPDC7	DEP domain containing 7
EBM	Evidence-based medicine
Elovl5	Elongation of very long chain elongase 5
Fads1	Fatty acid desaturase 1
Fads2	Fatty acid desaturase 2
FKBP5	FK506 binding protein 5
GLUT1	Glucose transporter 1
GLUT4	Glucose transporter type 4

GRIN2A	Glutamate ionotropic receptor NMDA type subunit 2A
HP1BP3	Heterochromatin protein 1, binding protein 3
IGF2	Insulin-like Growth Factor 2
MAOA	Monoamine oxidase A
MDD	Major Depressive Disorder
MeDIP-seq	Methylated DNA immunoprecipitation combined with ultra-deep sequencing
MS-HRM	Methylation-specific high resolution melting
MS-PCR	Methylation-specific polymerase chain reaction
OR	Odds ratio
PAF	Population Attributable Fraction
PBI	Pervasiveness of behavioral impairment
PTSD	Post-traumatic stress disorder
RCT	Randomized controlled trials
RHA	Regional Health Authority
RMSEA	Room mean square error of approximation
RP-HPLC	Reversed-phase high performance liquid chromatography
SHR	Saskatoon Health Region
TET	Ten-eleven translocation
TLC	Thin-layer chromatography
TLI	Tucker Lewis Index
TPH2	Tryptophan hydroxylase 2
TTC9B	Tetratricopeptide repeat domain 9B
WDR26	WD repeat domain 26
WHO	World Health Organization
WRTC	Western Regional Training Centre
YLL	Years of Life Lost
ZBTB20	Zinc finger and BTB domain containing 20

CHAPTER 1 - INTRODUCTION

1.1 Burden of Mental Illness

Mental illnesses are defined as "alterations in thinking, mood or behavior (or some combination thereof) associated with significant distress and impaired functioning" (Government of Canada, 2006). Mental disorders have been a rising global burden for health systems from 1990 to 2010 (Murray, et al., 2012).

Mental illness is also a leading cause of disability in Canada (Mental Health Commission of Canada, 2014; Lim, Jacobs, Ohinmaa, Schopflocher, & Dewa, 2008). They account for nearly a quarter (23%) of Years of Life Lost (YLL) due to disability and 13% of YLL due to disability and premature mortality in Canada. It is estimated that 1 in 5 Canadians experiences a mental health or addiction problem every year (Smetanin, et al., 2011). By 40 years of age, 1 in 2 Canadians have, or have had, at least one mental illness (Smetanin, et al., 2011). Mental illness also causes a heavy economic burden. The costs in Canada were estimated at $51 billion per year, which included health care costs, lost productivity, and reductions in health-related quality of life (Lim, Jacobs, Ohinmaa, Schopflocher, & Dewa, 2008; Smetanin, et al., 2011).

People with mental illness and addictions are more likely to suffer from comorbidity of other mental or chronic health conditions. For example, people with early onset of depression and anxiety disorders are more likely to develop other chronic diseases in adult life, such as diabetes, heart disease, asthma, and chronic back pain (Scott, et al., 2011). Conversely, depression and anxiety disorders may be a concomitant consequence of the burden of chronic diseases or conditions, such as long-term medical conditions (Patten, 2001) and coronary heart disease (Frasure-Smith & Lesperance, 2005).

1.2 A Public Health Perspective on Mental Illness

In order to reduce the burden of mental and behavioural disorders, the World Health Organization (WHO, 2001) suggested that a public health approach would be the most appropriate method to respond to the multifaceted etiology, widespread stigma and discrimination, and significant treatment gap across the world. There are a number of actions that can be achieved, such as formulating policies, assuring universal access to mental health services (including health promotion and prevention), ensuring adequate care and protection of human rights, promoting healthy lifestyles and reducing risk factors, as well as enhancing research into the causes of mental disorders, the development of effective treatment, and the evaluation of mental health systems, etc. (WHO, 2001).

The core of public health is the prevention of disease particularly primary and secondary prevention. In order to prevent and intervene in the development of mental illness, knowledge of its nature – risk factors, and course and outcome – is needed. While we don't need a complete picture of a disease to effectively intervene to effectively intervene to alter the course of the disease, we do need knowledge of significant modifiable risk factors so we can intervene and alter the occurrence and trajectory of disease.

Prevention should be assessable to all, acceptable to the general population and be therapeutically and cost effective. One of the principles of a public health perspective is to focus on the health of an entire population or population at risk. It aims to provide the maximum benefit for the largest number of people and reduce health inequities. Prevention programs based on the public health approach are designed to expose a broad segment of a population to prevention measures and to reduce and prevent mental disorders at a population-level (WHO, 2017). The Public Health Agency of Canada (2013) suggested that the ultimate benefits of a population health approach should "extend beyond improved population health outcomes to include a sustainable and integrated health system, increased national growth and productivity, and strengthened social cohesion and citizen engagement".

Prevention should also not be harmful or iatrogenic. Iatrogenesis refers to injury or illness that result from the actions or activities of healthcare professionals or promoting products or services as beneficial to health, that potentially have untoward effects of people affected (Caplan & Caplan, 2001; Medical Dictionary, 2009; A Dictionary of Sociology, 1998). Some examples of

iatrogenesis include risk associated with medical interventions (e.g. adverse effects of prescription drugs, over-use of drugs, drug interaction), medical error, wrong prescription, negligence, nosocomial infections, and faulty procedures, techniques, information, methods, or equipment (Wikipedia, 2017).

The psychiatric treatment of some conditions and populations have been considered as carrying significant risks for iatrogenesis, such as substance abuse and antisocial youths (Moos, 2005; Weiss, et al., 2005). It was reported in a systematic review on substance use prevention programs that negative effects was found in 17 evaluation studies with 43 negative outcomes. Drug prevention programs led to increases in consumption of alcohol use, cigarette use, marijuana use, and multiple drug use (Werch & Owen, 2002). Additionally, poorly researched social marketing for the prevention of drug abuse may not only be ineffective, but it may also result in negative consequences (Sumnall & Bellis, 2007). For example, it was found that the higher exposure to the anti-drug advertisements in the US was associated with misperceptions of higher prevalence of drug use among young people which are strong predictors of intention to drug use (Donaldson, Graham, & Hansen, 1994; Rimal & Real, 2005). Finally, intervention programs for youth conduct problems, delinquency, and substance abuse applying group-delivery formats, such as group counseling, residential treatment, and school-based intervention programs, have been identified as producing iatrogenic effects. Evidence-based treatments were recommended to prevent and reduce the iatrogenic effects. Integrating research with clinical practice, including impressions, needs, and moderators of intervention outcomes, should also be required to guide prevention and treatment decisions (Rhule, 2005).

1.3 Why Study Children and Adolescents?

1.3.1 Epidemiology of Mental Illness in Children and Adolescents

It is reported that 70% of mental health problems have their onset during childhood or adolescence (Government of Canada, 2006). Young people aged 15 to 24 are more likely to experience mental illness and/or substance abuse than any other age group (Pearson, Janz, & Ali, 2015). In addition, the usage of health services for mental illness among children and adolescents increased from 1996/97 to 2009/10, which may be due to a real increase in the number of cases, but may also reflect an increased awareness, detection, and treatment of mental illness among children (Public Health Agency of Canada, 2015).

The development and diagnosis of mental health issues in children and adolescents are somewhat different from those in adults: behaviors that seem not to be a mental disorder at young age may develop a serious mental problem at older age; children's behavior and wellness are vulnerable to be affected by their familial environment; and they also lack the cognitive and linguistic sophistication to accurately describe their feelings and symptoms (Smetanin, et al., 2011).

1.3.2 Life Course Perspective

The life course perspective, as known as life course approach or life course theory, examines how an individual's early events influence their future decisions and events, such as marriage and divorce (White & Klein, 2007), engagement in crime, or disease incidence (Kuh & Ben-Shlomo, 1997). Life course epidemiology links adult health and disease risk to physical or social exposures during gestation, childhood, adolescence, earlier in adult life, or across generations. Early life conditions and experiences, such as poverty, adverse experience, and poor early growth, may make individuals more susceptible to developing adult risk factors and/or chronic diseases. Therefore, the life course strategies for prevention of chronic conditions suggest to intervene as early as possible before damage and disability set in (Factor-Litvak & Susser, 2004).

A population health approach directs investments to those areas that have the greatest potential to influence population health status positively. A population health approach is grounded in the notion that the earlier in the causal stream action is taken, the greater the potential for population health gains (Public Health Agency of Canada, 2013).

1.3.2.1 Early origins of mental disorders

A developmental model of the origins of disease, called fetal origins hypothesis, has been widely accepted and supported by various epidemiological and epi-genetic studies. The hypothesis proposes that the developmental health and wellbeing outcomes for an individual from infancy to adulthood are significantly impacted by the conditions in gestation period. For example, the association between low birthweight and coronary heart disease has been confirmed in longitudinal studies of men and women around the world (Barker, 2007).

Another example shows the importance of in-utero influences and the role of early attachment and emotional care. Maternal anxiety during pregnancy has been linked to problems of infant temperament, behavior, and cognitive development; emotional and behavioral problems in children and adolescents; and structural brain changes (Newman, et al., 2016). Newman et al. (2016) also indicated that epigenetic modifications, such as DNA methylation, can be one of the mechanisms underlying the in-utero effects on fetal development, and the association between childhood experience and quality of care and their regulation of psychological well-being. It was reported that epigenetic signatures may mediate the associations between childhood adversity and long-term alterations in an individual's stress response and immune system trajectories (Bick, et al., 2012). Prenatal "unhealthy diet" was also found to be associated with higher IGF2 methylation at birth which predicted ADHD symptoms (Rihlaarsdam, et al., 2016).

1.3.3 Potential for Early Intervention and Public Mental Health Measures

By understanding mental illnesses and conditions in children and adolescents, it is hoped that the potential of mental illness prevention can be maximized by intervening as early as possible, even during gestation; resilience from early-life adverse experiences can be promoted to reduce their impact in adulthood; and ultimately, the prevalence of mental disorders among both children and adults can be decreased, and the burden of health systems can be alleviated.

1.4 Context of This Research

The primary goal of this thesis study is to contribute to a further understanding of mental health issues in children and adolescents by applying various epidemiological methods, and to provide a possible basis for future health prevention planning and policy decision-making.

This research consists of four major studies targeting primary and secondary levels of prevention (see Table 1-1). Two studies regarding as primary prevention identify the risk factors of mental disorders and promote prevention on the development of mental disorders. One (Chapter 3) is a systematic review on the association between childhood maltreatment (as a common psychosocial environmental risk factor for mental illness) and later-onset depression and anxiety disorders. Meta-analysis was used to generate a pooled statistical indicator of risk. Population attributable fractions were calculated to understand to what extent that depression and anxiety incidence can be attributable to child maltreatment. The other risk factor study targeting primary prevention examines the association between DNA methylation modifications (as an

important biological risk factor affecting gene expression) and depression (Chapter 4). This is a systematic review of epigenetic effects using qualitative methods to summarize and compare the results across different laboratory factors and methodologies, such as tissues, platform/methods, sample size, etc.

The two other studies are conducted from a secondary prevention perspective controlling disease progression and recurrence. Chapter 5 examines the effectiveness of current outpatient therapy for children and adolescents with mental health issues in the Saskatoon Health Region, and identifies the factors that associated with favorable treatment outcomes. The second secondary prevention study examines the association between the use of stimulant medications as treatment for Attention Deficit Hyperactivity Disorder (ADHD), a major childhood and adolescent psychiatric disorder, and potential adverse outcomes (e.g. bipolar disorder) using a longitudinal administrative health data from the Province of Saskatchewan (Chapter 6). Studies on potential iatrogenic effects of treatment can be valuable for the prevention of comorbidity and other adverse outcomes due to early intervention and treatment.

Finally, in Chapter 7 the major findings of this thesis are summarized, and strengths and weaknesses enumerated, as well implications for mental health policy and intervention in children and adolescents' mental illness/health are identified.

Table 1-1 Summary of major studies in this research

Title of study	**Target area**	**Method & analysis**	**Level of prevention** (Katz & Ali, 2009)
Maltreatment in childhood substantially increases the risk of adult depression and anxiety in prospective cohort studies: systematic review, meta-analysis, and population attributable fractions (Chapter 3)	Psychosocial & environmental risk factor; Prevention	Systematic review & Meta-analysis; Population Attributable Fraction (PAF)	Primary - methods to avoid occurrence of disease either through eliminating disease agents or increasing resistance to disease
DNA methylation and major depressive disorder: a systematic review (Chapter 4)	Biological risk factor	Systematic review - Qualitative method	
Predictors of functional improvement in children and adolescents treated at child and youth mental health and addictions services in the Saskatoon Health Region (Chapter 5)	Outpatient treatment outcome	Descriptive analysis; Logistic regression; Sign test; Confirmative Factor Analysis	Secondary - methods to detect and address an existing disease prior to the appearance of symptoms.
Stimulant use and adverse events among children and youth (Chapter 6)	Medical treatment; Iatrogenic effects	Descriptive analysis; Penalized Maximum Likelihood Estimation (the Firth Method)	

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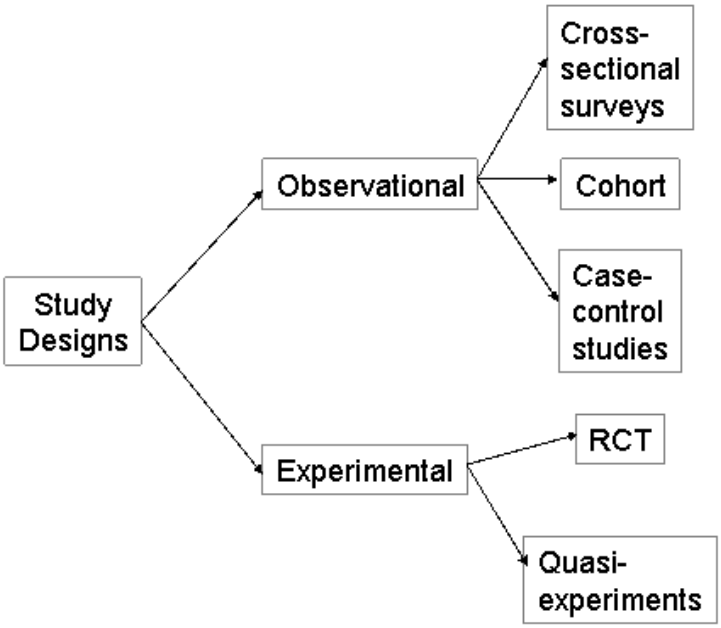
CHAPTER 2 – METHODS AND PROCEDURES

2.1 Study designs and level of evidence

Different epidemiological study designs provide information with different quality (Study Designs, n.d.). Observational studies, including cross-sectional, cohort, and case-control studies, observe and systematically collect information without changing the subjects being observed (no intervention. On the contrary, experimental studies, including randomized controlled trials (RCT) and quasi-experimental designs, intervene to change something (e.g., give some patients treatment) and observe what happens (Study Designs, n.d.). Figure 2-1 shows the summary and the advantages and disadvantages of each design.

Figure 2-1 Summary of designs, showing advantages and disadvantages of each [1]



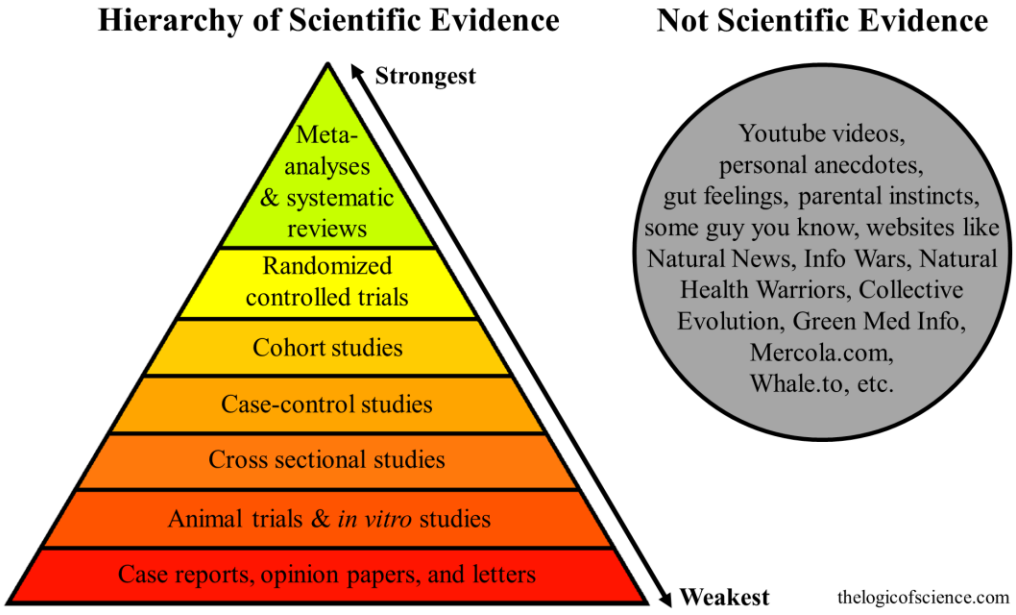
Advantages	Disadvantages
Quick; can cover whole population, giving representative information whether or not people are seeking care	Based mainly on self-report (biases?); diagnostic information usually inaccurate; can't establish causal sequence
Prospective, so can establish causal sequence; can estimate incidence	Time-consuming; costly; attrition of cohort?
Relatively cheap way of focusing on causal factors	Requires recall of past events (inaccurate?); controls not equivalent to cases
Controls for all main forms of bias; good for both etiological and evaluative research	Ethical concerns in etiological applications; Often uses selected populations: issue of generalizability?
May be more practical than RCT: can use "natural experiments"	Allocation bias often significant (exp'tal and control groups not equivalent)

[1] https://www.med.uottawa.ca/sim/data/Study_Designs_e.htm

Different study designs also provide different levels of evidence which are integral to evidence-based medicine (EBM). Figure 2-2 shows the hierarchy of evidence ranking from systematic reviews and meta-analyses as the strongest evidence to case reports and expert opinions as the weakest (The Logic of Science, 2016).

This thesis uses three basic study designs and several epidemiological techniques: population cohort design using clinical data (Chapter 5) and administrative data (Chapter 6), systematic reviews, both quantitative and qualitative, (Chapter 3 and 4), and population attributable fractions (Chapter 3) which is used to estimate the projected effect that reducing the prevalence of exposure would have on the incidence and prevalence of disease.

Figure 2-2 Hierarchy of evidence [2]



[2] thelogicofscience.com

2.2 Data Sources

Data used in this thesis research include both existing datasets, such as large-scale health administrative data from the Province of Saskatchewan (Chapter 6) and clinical data from Saskatoon Health Region (Chapter 5), and self-collecting data for review studies (Chapter 3 and 4).

For systematic reviews (Chapter 3 and 4), data has been collected via computerized search and manual search. Computerized search was conducted in various databases, such as PubMed, EMbase, Medline, and Cochrane Library etc., using search strategies. Inclusion and Exclusion criteria were applied to identify the eligibility of articles. Gray literature and reference lists in eligible articles were also be screened to include the most comprehensive articles.

2.3 Statistical Analysis

Descriptive analyses (Chapter 5 and 6) were applied to understand demographic and clinical characteristics of study populations, such as age, gender, living area, presenting mental health problem, referral source, prescription medication, etc.

Multivariate statistics were also employed. For example, multivariate logistic regression models were used for dichotomous outcomes (Chapter 5); Penalized Maximum Likelihood Estimation (the Firth Method) which was designed for analyzing rare events with logistic regression was also used (Chapter 6). Other analyses utilized include confirmatory factor analysis (CFA) which is applied to verify the relationship between observed factors and underlying constructs, and sign test for paired data which is used to compare median differences when the observations are not normally distributed (Chapter 5).

With regard to systematic reviews, both quantitative (meta-analysis, Chapter 3) and qualitative methods (Chapter 4) were applied respectively in the two studies. Meta-analysis was used to combine the results from two or more separate studies to answer a common question. It provides more power than separate studies, summarizes numerous and inconsistent findings, and investigate consistency of effect across different samples (Higgins & Green, 2011). Qualitative methods were used in Chapter 4 of the systematic review on the relationship between DNA methylation and depression due to the high heterogeneity existed among the studies included, in which case quantitative methods, such as meta-analysis, are not recommended by the Cochrane Guidelines.

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CHAPTER 3 – MALTREATMENT IN CHILDHOOD SUBSTANTIALLY INCREASES THE RISK OF ADULT DEPRESSION AND ANXIETY IN PROSPECTIVE COHORT STUDIES: SYSTEMATIC REVIEW, META-ANALYSIS, AND POPULATION ATTRIBUTABLE FRACTIONS

A version of this chapter has been published as: “Li, M., D'Arcy, C., & Meng, X. (2016). Maltreatment in childhood substantially increases the risk of adult depression and anxiety in prospective cohort studies: systematic review, meta-analysis, and population attributable fractions. *Psychological Medicine, 46*(4), 717-730. doi:10.1017/S0033291715002743”. My contributions to this study included contribution to study design, data collection, quality assessment, data synthesis and analysis, and manuscript writing and editing. This chapter also includes PAF estimates for Canada that were excluded in the published study.

3.1 Abstract

Literature supports a strong relationship between childhood maltreatment and mental illness but most studies reviewed are cross-sectional and/or use recall to assess maltreatment thus being prone to temporality and recall bias. Research on the potential prospective impact of maltreatment reduction on the incidence of psychiatric disorders is scarce. Electronic databases and grey literature from 1990 to 2014 were searched for English language cohort studies with criteria for depression and/or anxiety and non-recall measurement of childhood maltreatment. Systematic review with meta-analysis synthesized the results. Study quality, heterogeneity, and publication bias were examined. Initial screening of titles and abstracts resulted in 199 papers being reviewed. Eight high quality articles met eligibility criteria. Population attributable fractions (PAFs) estimated potential preventive impact. The pooled OR between any type of maltreatment and depression was 2.03 (95% CI 1.37-3.01) and 2.70 (95% CI 2.10-3.47) for anxiety. For specific types of maltreatment and depression or anxiety disorders, the ORs were: physical abuse OR=2.00 (95% CI 1.25-3.19), sexual abuse OR=2.66 (95% CI 1.88-3.75), and neglect OR=1.74, (95% CI 1.35-2.23). PAFs suggest that over one-half of global and one-third of Canadian depression and anxiety cases are potentially attributable to self-reported childhood maltreatment. A 10-25% reduction in maltreatment could potentially prevent 31.4 - 80.3 million depression and anxiety cases worldwide and 124,000 - 325,000 in Canada. This review provides robust evidence of childhood maltreatment increasing the risk for depression and anxiety, and reinforces the need for effective programs and policies to reduce its occurrence.

Key words: child abuse, depression, anxiety disorders, projected effect, population attributable fraction.

3.2 Introduction

Childhood maltreatment is a major public health and social welfare problem. Internationally, it is considered a serious public health, human rights, legal and social issue (Butchart *et al*. 2006). International studies estimate that 1 in 5 women and 1 in 13 of men have been sexually abused during childhood, while 25% of all adults report being physically abused (WHO, 2014).

Childhood maltreatment also results in psychological and neurobiological sequelae, which may contribute to the emergence of psychopathology (McCrory *et al*. 2010). It may be related to many neurobiological mechanisms: 1) stress systems (Ciccetti & Toth, 2005); 2) structural brain differences (Herringa *et al*. 2013), e.g. hippocampus, amygdala, corpus callosum and other white matter tracts, and prefrontal cortex; 3) functional brain differences, e.g. hyperactivity of amygdala in response to negative facial affect; and 4) genetics and epigenetics of resilience and vulnerability. Maltreatment in childhood has also been found to threaten the optimal development of affective processing abilities, attachment relationships, self-system processes, peer relationships, and adaptation to school (Ciccetti & Toth, 2005).

Depression and anxiety disorders are the major causes of morbidity worldwide. According to the report on the global burden of disease 2010 (Whiteford *et al*. 2013), depressive disorders contributed most of the burden of mental illness and substance use disorders (42.5%) followed by anxiety disorders (15.3%). Depressive disorders also accounted for 40.5% of disability-adjusted life years (DALYs) caused by mental illness and substance use disorders, with anxiety disorders accounting for 14.6%.

Previously studies of child maltreatment have shown its significant impact on psychological and health outcomes. Child abuse, including physical abuse, sexual abuse, and exposure to intimate partner violence, has been associated with a large number of psychiatric disorders, including depression, bipolar disorder, generalized anxiety disorder, alcohol and drug abuse, suicidal ideation and attempts, etc. (Afifi *et al*. 2014). Children from abusive families are significantly more likely to report depressive symptoms than those from non-abusive homes (Toth *et al*. 1992).

A number of reviews have also consistently shown the negative immediate and long-term psychological effects of the childhood maltreatment. Maniglio (2010, 2012) in systematic

reviews of reviews found that child sexual abuse was a significant risk factor for both depression and anxiety disorders. In addition, adults abused as children exhibited more posttraumatic stress symptoms, cognitive distortion, emotional distress (including depression and anxiety disorders), eating disorders, sleep disorders, substance abuse, and avoidance (Briere & Elliott, 1994; Chen *et al*. 2010; Nanni *et al*. 2012).

Although previous reviews have shown a significant direct relation between childhood maltreatment and depression and anxiety, they either reviewed cross-sectional studies or studies that did not have external documentation on child abuse history. Abuse exposure has generally been measured via recall methods. Such recall is prone to bias and false memory (Robins *et al*. 1985; Taylor & Brown, 1988; Coughlin, 1990; Maughan *et al*. 1995; Neumann *et al.* 1996; Hardt & Rutter, 2004). A substantial proportion of individuals known to have suffered abuse/maltreatment do not report such abuse when interviewed in adult life (Hardt & Rutter, 2004). Taylor and Brown (1988) indicated that mental health is associated with a filtering out of negative memories and/or re-representing them in non-threatening terms. People with good functioning in adult life are apt to forget early parental negativity whereas there is a tendency for people with poor functioning to retrospectively exaggerate negativity that was not reported contemporaneously during childhood (Robins *et al*. 1985; Maughan *et al*. 1995). In addition, cross-sectional studies cannot identify the temporal relationship between risk factors and outcomes. Questions have also been raised concerning the use of rating scales as opposed to diagnostic instruments to measure mental illness outcomes.

Little study can be found regarding the potential impact of reducing childhood abuse in decreasing the incidence of psychiatric disorders in a population. Population attributable fractions (PAFs) are used to indicate the proportional reduction in a population of a disease (incidence or mortality) that would occur if exposure to a risk factor were reduced to an alternative ideal exposure level (Rockhill *et al*. 1998). PAFs have been commonly recognized as effective tools to measure the potential effects of risk factors on psychiatric disorders (Sareen *et al*. 2008; Bolton & Robinson, 2010; Barnes & Yaffe, 2011; Meng & D'Arcy, 2013, 2014). Northridge (1995) believed that PAFs could help policymakers in judging priorities for public health action, intervention planning and decision-making.

This study aims to: 1) systematically review the evidence for the association between childhood maltreatment and depression and anxiety using longitudinal cohort studies and studies with external documented measures of child maltreatment and diagnostic measures of depression and/or anxiety; and 2) provide firm estimates of by how much the incidence of depression and anxiety in a population would be reduced if childhood maltreatment was reduced.

3.3 Method

The process and reporting of results systematic review and meta-analysis were guided by the PRISMA guidelines, 2009 revision (Moher *et al*. 2009), and the Meta-analysis of Observational Studies in Epidemiology recommendations (Stroup *et al*. 2000).

3.3.1 Inclusion and Exclusion Criteria

Prior to their inclusion in this review all articles were evaluated taking into account their internal validity and the following inclusion and exclusion criteria: 1) be published in English within the last 25 years (1990-2014); 2) be a cohort study; 3) did not use subject recall to assess maltreatment in childhood 4) gave clear information on the assessment of childhood abuse or adverse childhood experience (ACE) (e.g. types of abuse, years of age being abused, assessment and ascertainment tools, etc.); 5) use clear diagnosis criteria for depression or anxiety in adulthood, specifically DSM and its updates (American Psychiatric Association, 2013), ICD-10 (World Health Organization, 1992) or other generally accepted diagnostic criteria; 6) provide statistical indicators (e.g. relative risk) or original data to estimate the relationship between child abuse and depression/anxiety. Most importantly studies that measured exposure to childhood maltreatment merely via recall methods or referrals without official documentary support (e.g. police records, records from social services, child protective services, and criminal court) were *excluded* because subject recall methods are prone to bias and false memory criticisms.

3.3.2 Search Strategy

We conducted computerized searches in the PubMed, PsychINFO, EMBASE, Medline, and Cochrane Library databases for the 25-year period from January 1990 to December 2014 for published articles. Search strategy is in Appendix 1. In addition, we manually searched other resources for other relevant studies. The reference lists of selected articles, review articles on relevant topic, and the gray literatures were screened.

3.3.3 Data Collection and Quality Assessment

The full-text article was retrieved for all studies that initially appeared to meet the inclusion criteria for further examination. The two review authors (ML and XM) independently assessed the articles for eligibility. Any disagreements among reviewers were resolved through discussion. Data on author, publication year, journal, sample size, methods, indicators, outcomes, comorbidities, adjustments, study design and results were extracted independently by the two authors. The Newcastle Ottawa Scale criteria were used to characterize study quality (Wells *et al*. 2012). Assessment of study quality is essential for a proper understanding of non-randomized studies. One author of the selected articles was contacted for further information. One eligible study was excluded because of replication of same sample population and outcomes of interest.

3.3.4 Data Synthesis

The reviewed articles were grouped for five analyses: (1) *any maltreatment and depression,* to examine the relationship between any specified and unspecified child maltreatment and depression; (2) *any maltreatment and anxiety*; (3) *physical abuse* and either depression or anxiety disorders; (4) *sexual abuse* and either depression or anxiety disorders; and (5) *neglect* and either depression or anxiety disorders. We report on each category of analysis separately. Studies were involved in multiple separate analyses as their available data dictated.

3.3.5 Statistical Analyses

3.3.5.1 Meta-analysis

The analyses separately generated pooled estimates of the effects of child maltreatment in general and specific types of abuse on depression and/or anxiety. We also evaluated heterogeneity with DerSimonian and Laird I^2 statistics for each category to determine the proportion of heterogeneity that is not due to chance (Higgins *et al*. 2003). Funnel plots and Egger's tests were used to inspect for publication bias (Egger *et al*. 1997). Compared to funnel plot, the Egger's test provides a more objective way to estimate the reliability of the results. If these tests show non-significant heterogeneity, we used fixed effects model, whereas a more conservative random effects model was used if we saw the possibility of heterogeneity. Sensitivity analysis assessed the influence of each individual study on overall estimates by recalculating ORs with each study being removed one at a time. The quality of each study was

rated according to the Newcastle-Ottawa Scale, and meta-regression analyses were used to examine the impact of study quality on results. STATA, version 12, statistical software was used for the analyses.

3.3.5.2 Calculation of projected effects (PAFs)

The definition of PAF is the proportional reduction in average disease risk that would be achieved by elimination of exposure of interest (Rockhill *et al*. 1998). It indicates that the proportion of people with a disease in a population is potentially attributable to a given risk factor by assuming that there is a causal relationship (Benichou 2001). The PAF takes into account the strength of the association between the risk factor and the outcome as well as the prevalence of the risk factor. It was calculated by the following formula based on previous literature (Barnes & Yaffe, 2011; Rockhill *et al*. 1998; Sareen *et al*. 2008):

$$\text{PAF} = \frac{p(OR-1)}{p(OR-1)+1}$$

Where *p* is the population prevalence of the exposure, and OR is the pooled odds ratio of outcomes given different categories of child maltreatment. In the present study, worldwide and Canadian PAFs were calculated for specific types of abuse. Present worldwide prevalence estimates were obtained by the most recent review of a series of meta-analysis (Stoltenborgh *et al.* 2015). Both self-reported and informant prevalence estimates were used to generate self-reported and informant PAFs, respectively. Informant estimates are from studies collecting data from police records, social services, child protective services (CPS), child welfare workers, or teachers. Canadian prevalence estimates were obtained by searching journal databases (e.g. PubMed, etc.), World Health Organization, and Statistic Canada's websites. The most recent estimates were used.

Finally, we estimated the total number of depression and/or anxiety cases attributable to different categories of child maltreatment by multiplying PAF estimates and the present number of cases across the world and in Canada. We also calculated the number of cases that could potentially have been prevented if the prevalence of exposure to childhood maltreatment were 10% or 25% lower than present levels. We also calculated confidence ranges for PAF estimates, number of attributable cases, and number of cases potentially prevented by using the 95% CIs from the pooled OR estimates.

3.4 Results

3.4.1 Mata-Analysis

3.4.1.1 Selection of articles

Figure 3-1 shows the process of study selection. The initial search produced 5,340 titles, from which 392 abstracts were reviewed. After reviewing abstracts 199 articles were retrieved for full evaluation. Full-text articles were reviewed. The eight articles that met our inclusion and exclusion criteria are fully referenced in Appendix 2.

Table 3-1 presents the detailed data on characteristics of the reviewed articles. Articles included in the analysis were assessed for quality using the Newcastle-Ottawa Quality Assessment Criteria as well as for the external ascertainment of child to exposure as opposed to reliance on self-report measures. All the included studies rated highly in terms of quality (See Appendix 3).

The quality of the data is evident in the fact that none of the study characteristics examined had any impact on observed odds ratios in any of the analyses reported here. Nor was there any publication bias observed.

3.4.1.2 Relationship between any maltreatment and depression

Five articles (Brown *et al*. 1999; Widom *et al*. 2007; Danese *et al*. 2009; Scott *et al*. 2012; Cutuli *et al*. 2013) were included in the analysis to examine the relationship between any child maltreatment and depression. Figure 3-2a presents the individual study, pooled estimates, and funnel plots that were used to visually assess the presence of publication bias. A random effects model was used.

The pooled OR overall for depression for individuals with any type of child maltreatment compared to those without maltreatment history was 2.03 (95% CI 1.37 - 3.01, $\chi^2 = 10.94$, $I^2 = 63.4\%$, $p = 0.027$), indicating those with a child abuse history were 2.03 times more likely to have depression than those without such history.

Figure 3-1 PRISMA flow diagram – Childhood maltreatment and later depression and/or anxiety

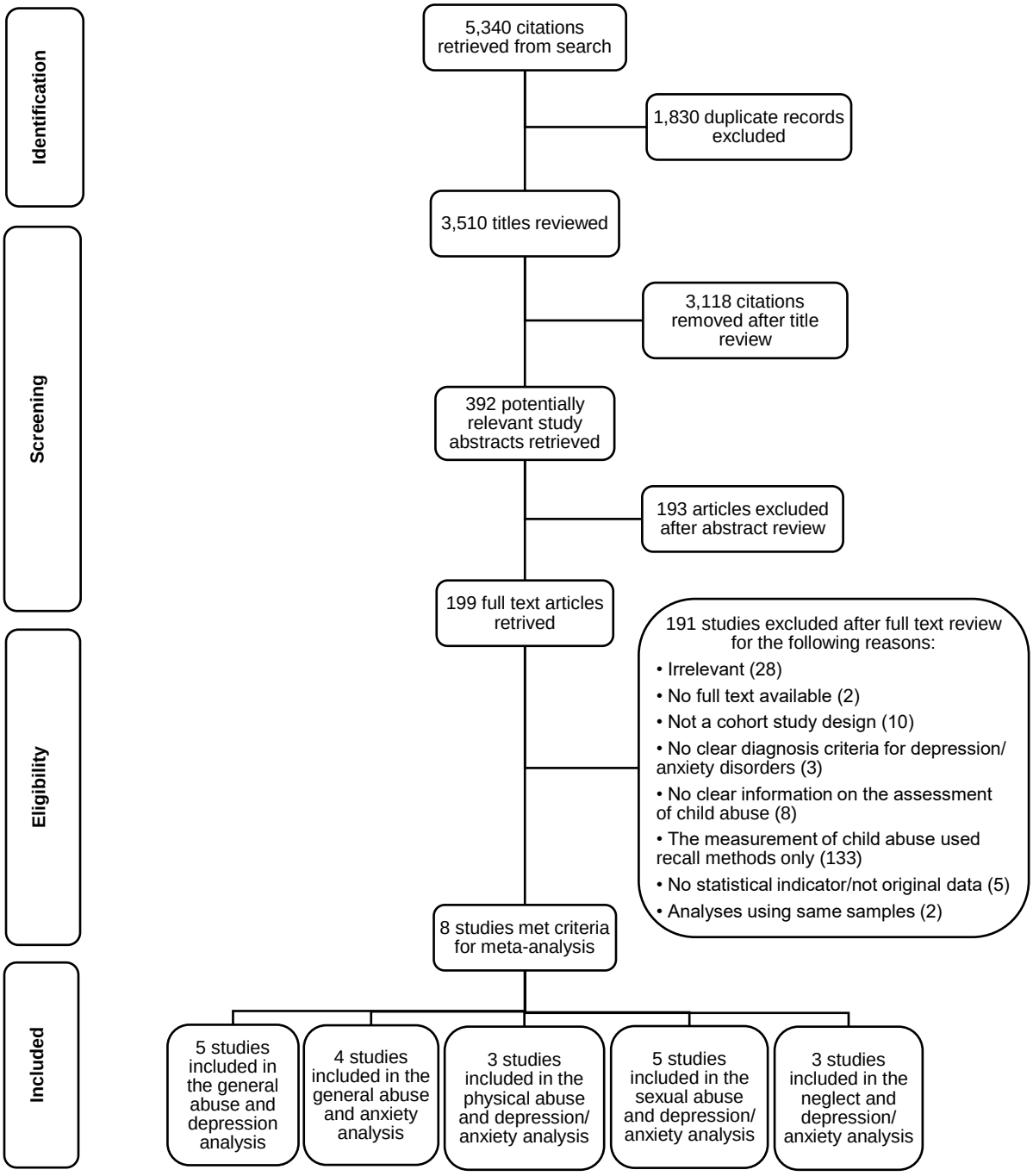


Table 3-1 *Summary of the Studies Attributes*

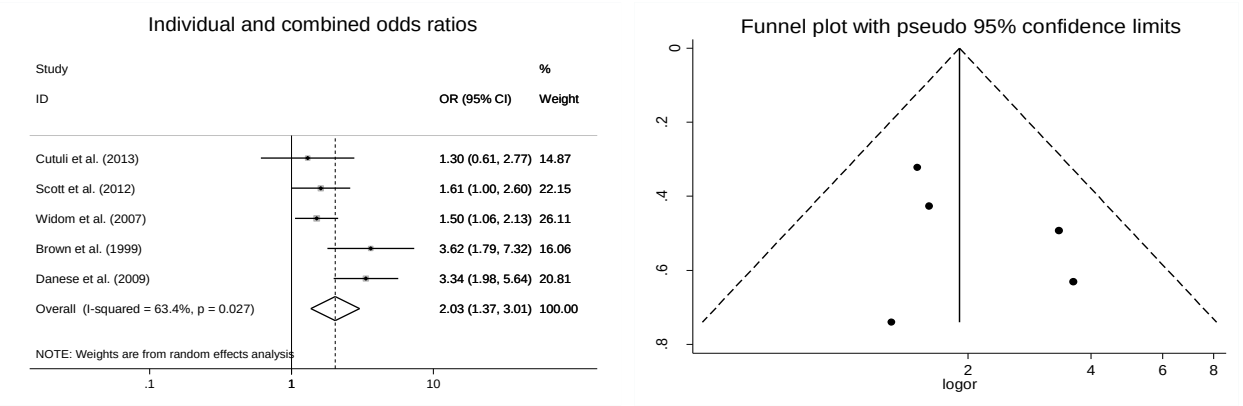
First Author	Year	Setting	Sample Source	Sample Size	Type of Exposure	Age of Exposure (Year)	Ascertainment of Exposure	Health Outcome	Age of Outcome Assessed (Year)	Assessment of Health Outcome
Cutuli et al.	2013	USA	Children of primiparous women	157	Maltreatment in general	Birth to 17.5	Observation, caregiver interviews, reviews of child protection & medical records	Depression	18 – 28	ASCID
Cutajar et al.	2010	Australia	Victoria residents	5,365	Sexual abuse	Birth to 16	Official records from VIFM	Anxiety & PTSD	18 and above	Diagnosed using DSM, then transferred Codes using WHO-ICD
Widom	1999	USA	General population	1,196	Child abuse in general, physical abuse, sexual abuse, & neglect	Birth to 11	Official records from the county juvenile or adult criminal court	PPTSD	18 and above	NDIS
Scott et al.	2012	New Zealand	National population	1,413	Maltreatment in general	Birth to 17	Official records from CYF	Major depressive disorder & anxiety	16 – 27	WCIDI
Widom et al.	2007	USA	General population	1,196	Child abuse in general, physical abuse, sexual abuse, neglect	Birth to 11	Official records from the county juvenile or adult criminal court	Major depressive disorder	18 & above	N DIS-III-Revised
Spataro et al.	2004	Australia	Victoria residents	3,141,357	Child sexual abuse	Birth to 16	Official records from VIFM	Anxiety	18 & above	Registered cases on the Victorian Psychiatric Case Register
Brown et al.	1999	USA	General population	776	Child maltreatment in general, neglect, physical abuse, sexual abuse	Non-specify ("Youth")	Official records from NYSCR & retrospective self-report	Depressive disorder	Non-specify ("Young adult")	N DISC
Danese et al.	2009	New Zealand	Members of the Dunedin Multi-disciplinary Health and Development Study	1,037	Childhood maltreatment in general	Birth to 10	Retrospectively self-report & assessment from cumulative index	Major depressive disorder	32	DDSM-IV

VIFM, Victorian Institute of Forensic Medicine; CYF, Child, Youth and Family Agency; NYSCR, New York State Central Registry for Child Abuse and neglect; PTSD, Post-traumatic stress disorder; SCID, Structured Clinical Interview for DSM disorders; DSM, Diagnostic and Statistical Manual of Mental Disorders; WHO-ICD, WHO International Diagnostic Interview; DIS, National Institute of Mental Health Diagnostic Interview Schedule; CIDI, WHO Composite International Diagnostic Interview; DISC, National Institute of Mental Health Diagnostic Interview Schedule for Children

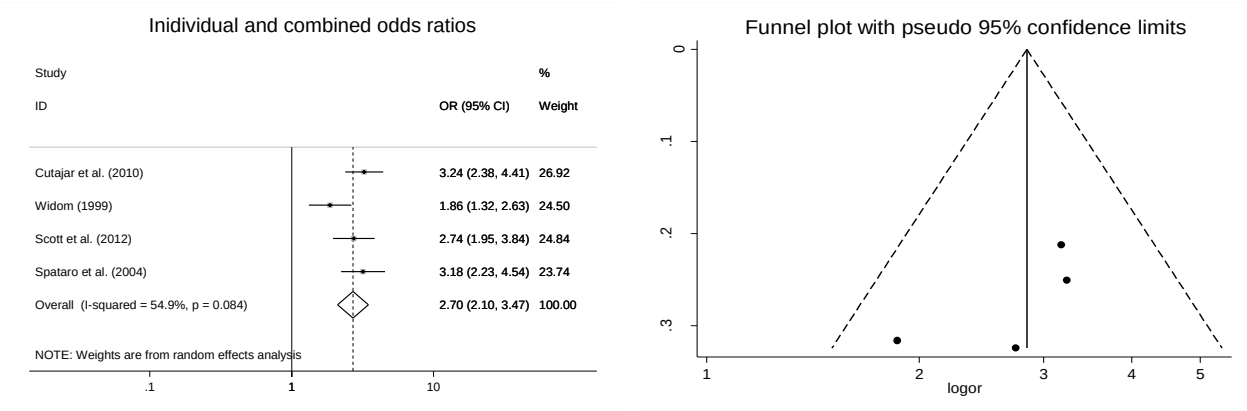
Figure 3-2 Odds ratios between childhood maltreatment and depression and/or anxiety and funnel plots.

In the funnel plots, the X-axis shows the logarithmic scale of odds ratio estimate for each study and Y-axis is standard error of the logarithmic function of the odds ratio. The dashed line represents the 95% confidence interval and the point estimate of logarithmic transition of odds ratio illustrates as the solid line. OR = odds ratio.

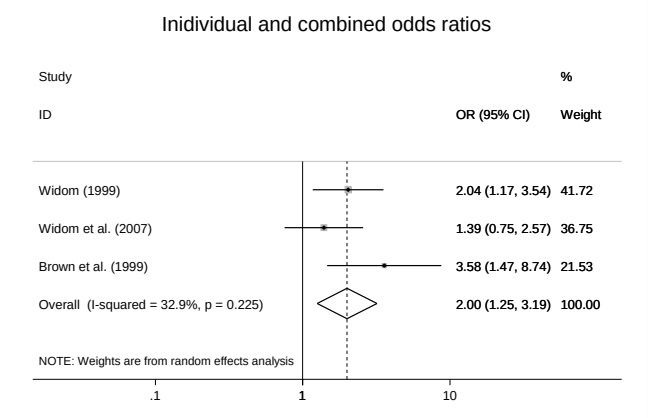
a. Relationship between any maltreatment and depression

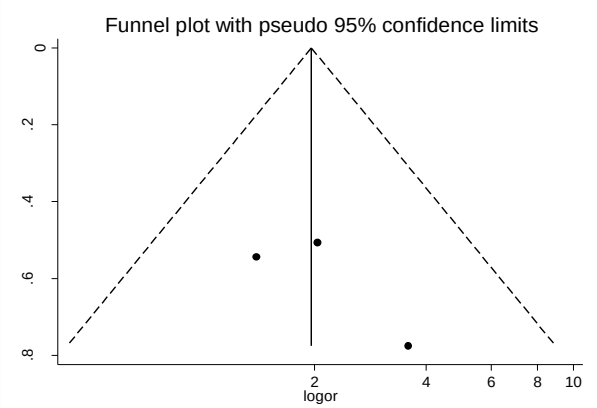


b. Relationship between any maltreatment and anxiety

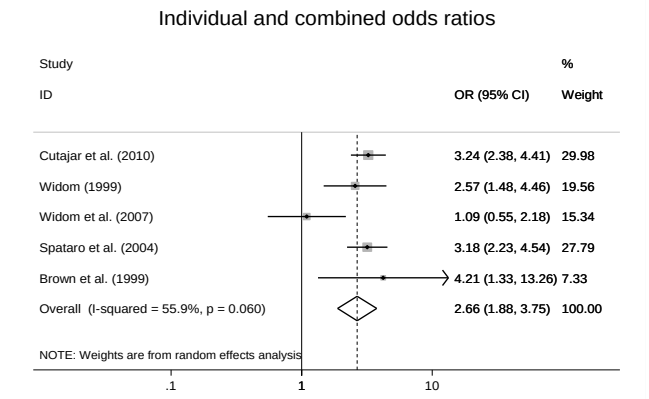


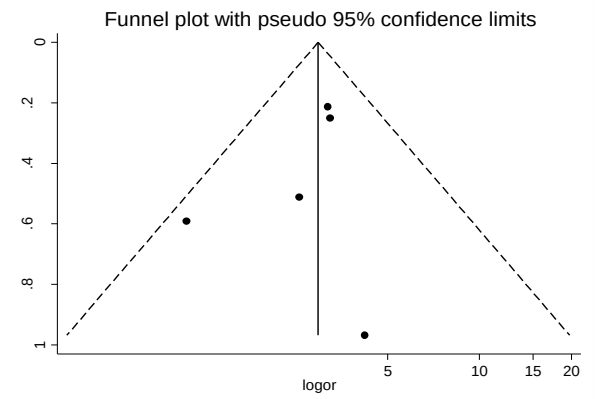
c. Relationship between physical abuse and depression and/or anxiety



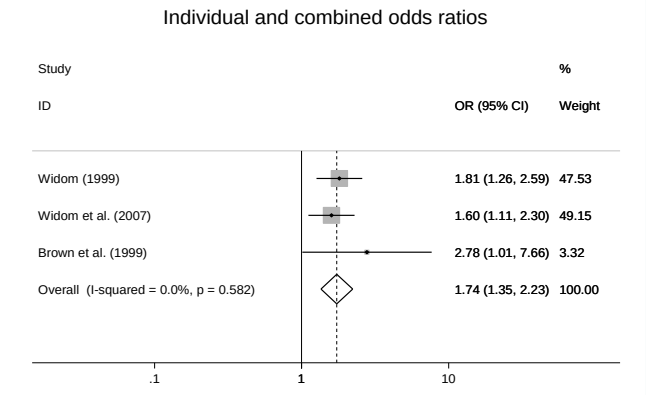


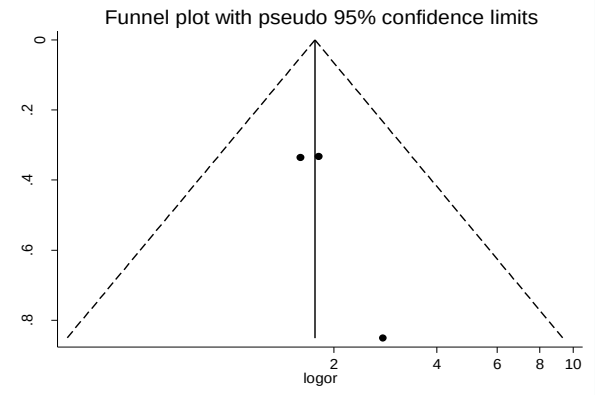
d. Relationship between sexual abuse and depression and/or anxiety





e. Relationship between neglect and depression and/or anxiety





As shown in the funnel plot in Figure 3-2a, all the studies were within the domain, which represents 95% confidence interval limits around the estimate. No asymmetry was shown in the funnel plot. There was no evidence of publication bias in this meta-analysis (Egger's test, p = 0.46).

Sensitivity analysis was also used to assess the influence of each study on overall estimates by omitting one study at a time. The analysis yielded with/without childhood maltreatment ORs ranging from 1.69 (95% CI 1.09 -2.64) to 2.24 (95% CI 1.33 - 3.79). The combined OR was 1.91 (95% CI 1.27 - 2.85), which clearly indicates the experience of childhood maltreatment was a risk factor for depression.

3.4.1.3 Relationship between general maltreatment and anxiety

Four articles (Widom, 1999; Spataro *et al*. 2004; Cutajar *et al*. 2010; Scott *et al*. 2012) were used to examine the relationship between any type of child maltreatment and anxiety disorders. Figure 3-2b shows the individual study and pooled estimates, and funnel plots. A random effects model was used.

The pooled OR overall for anxiety for individuals who experienced any type of child maltreatment compared to those who did not was 2.70 (95% CI 2.10-3.47, χ^2 = 6.65, I^2 = 54.9%, p = 0.084), indicating that those who experienced child abuse were 2.70 times more likely to have anxiety disorders in adulthood than those who did not experience.

The funnel plot in Figure 3-2b showed that all the studies were within the domain of 95% confidence interval limits around the estimate. No asymmetry was found in the funnel plot. No evidence of publication bias was found in this meta-analysis (Egger's test, p = 0.26).

Sensitivity analyses found that with/without childhood maltreatment ORs ranging from 2.65 (95% CI 1.91 - 3.68) to 3.11 (95% CI 2.34 - 4.13). The combined OR was 2.84 (95% CI 2.19 - 3.68), clearly indicating that childhood maltreatment experience was a risk factor for anxiety disorders.

3.4.1.4 Relationship between physical abuse and depression or anxiety

Three articles (Brown *et al.* 1999; Widom, 1999; Widom *et al.* 2007) were used to examine the relationship between physical abuse and either depression or anxiety disorders.

Figure 3-2c presents the individual study, pooled estimates, and funnel plots. A random effects model was used. Analysis using a fixed effects model did not affect the results.

The pooled OR for depression and/or anxiety for individuals who were physically abused in childhood compared to those who were not was 2.00 (95% CI 1.25 -3.19, χ^2 = 2.98, I^2 = 32.9%, p = 0.225), indicating that children who were physically abused were 2 times more likely to develop depression or anxiety in adulthood than those who were not.

The funnel plot in Figure 3-2c indicated that all the studies were within the domain of 95% confidence interval limits around the estimate. No asymmetry was found in the funnel plot. No evidence of publication bias found in this meta-analysis (Egger's test, p = 0.449).

Sensitivity analysis produced with/without physical abuse ORs ranging from 1.71 (95% CI 0.83 - 3.53) to 2.41 (95% CI 1.05 - 5.54). The combined OR was 1.96 (95% CI 1.02 - 3.78), indicating that childhood experience of physical abuse was an important risk factor for depression and anxiety disorders.

3.4.1.5 Relationship between sexual abuse and depression or anxiety

Five articles (Brown *et al*. 1999; Widom, 1999; Spataro *et al*. 2004; Widom *et al.* 2007; Cutajar *et al.* 2010) were included in the analysis of the relationship between sexual abuse and depression and anxiety disorders. Figure 3-2d presents the individual study and pooled estimates, and funnel plots. A random effects model was used.

The pooled OR overall for depression and anxiety for individuals sexually abused in childhood compared to those who were not was 2.66 (95% CI 1.88 - 3.75, χ^2 = 9.06, I^2 = 55.9%, p = 0.06), indicating that children experienced sexually abuse were 2.66 times more likely to develop depression or anxiety in adulthood than those without such experience.

As shown in Figure 3-2d, the funnel plot indicated that all the studies were within the domain of 95% confidence interval limits around the estimate. No asymmetry was found in the funnel plot. No evidence of publication bias found in this meta-analysis (Egger's test, p = 0.417).

Sensitivity analysis yielded with/without sexual abuse ORs ranging from 2.75 (95% CI 1.81- 4.16) to 3.16 (95% CI 2.35- 4.27). The combined OR was 2.96 (95% CI 2.22 - 3.95), showing that childhood experience of sexual abuse was a strong risk factor for depression and anxiety disorders.

3.4.1.6 Relationship between neglect and depression or anxiety

Three articles (Brown *et al.* 1999; Widom, 1999; Widom *et al.* 2007) contributed to the analysis of the relationship between neglect and depression and anxiety disorders. Figure 3-2e presents the individual study and pooled estimates, and funnel plots. A fixed effects model was used.

The pooled OR overall for depression and anxiety for individuals experienced neglect compared to those did not experience neglect was 1.75 (95% CI 1.37 - 2.24, $\chi^2 = 1.08$, $I^2 = 0.0\%$, $p = 0.58$), indicating that children who experienced neglect were 1.75 times more likely to develop depression or anxiety in adulthood than those who were not neglected.

As shown in Figure 3-2e, the funnel plot indicated that all the studies were within the domain of 95% confidence interval limits around the estimate. No asymmetry was found in the funnel plot. There was no evidence of publication bias found in this meta-analysis (Egger's test, $p = 0.284$).

Sensitivity analysis for with/without neglect ORs ranged from 1.70 (95% CI 1.07 - 2.70) to 1.91 (95% CI 1.04 - 3.51). The combined OR was 1.76 (95% CI 1.13 - 2.75), pointing to childhood experience of neglect as a significant risk factor for depression and anxiety disorders.

3.4.2 Projected Effects (PAFs)

3.4.2.1 World wide

3.4.2.1.1 Self-reported prevalence of child maltreatment and PAFs

In 2014, the worldwide self-reported prevalence of physical abuse is 22.6% (Stoltenborgh *et al.* 2015). Worldwide depression and anxiety disorders are estimated to affect 350 million (WHO, 2012) and 273 million adults (Vos *et al.* 2012), respectively. The PAF estimate used here for the effect of physical abuse on the incidence of depression and anxiety disorders was 18.4%, which indicates that nearly 115 millions of depression and anxiety cases are potentially attributable to childhood physical abuse (Table 3-2). If the global prevalence of physical abuse was reduced by 10%, we estimated that there would be 9.5 million fewer depression and anxiety cases worldwide, whereas a 25% reduction could reduce prevalence by 24.6 million cases (Figure 3-3). The numbers of cases attributable to any specific type of abuse and any specific

mental disorder maybe an overestimate due to the existence of co-morbidity among mental disorders and the potential for an individual to suffer multiple types of abuse.

The self-reported prevalence of sexual abuse is 12.7% based on the Stoltenberg *et al.* (2011 & 2015) meta-analysis. Approximately 17.4% (over 108 million) of depression and anxiety cases in the world are potentially attributable to sexual abuse in childhood. If the prevalence of sexual abuse was reduced by 10%, about 9.1 million cases could potentially be prevented; a 25% reduction in sexual abuse prevalence could potentially prevent about 23.4 million cases worldwide.

It was estimated that 16.3% and 18.4% of worldwide population respectively have been exposed to physical and emotional neglect (Stoltenborgh *et al.* 2015). Our meta-analysis suggests about 10.8% (67 million) and 12.0% (75 million) of depression and anxiety cases respectively are potentially attributable to physical and emotional neglect. A 10% of reduction in the prevalence of physical neglect could potentially lower the number of cases of depression and anxiety by 6.1 million globally; this number would increase to 15.4 million if the prevalence physical neglect were reduced by 25%. Similarly, around 6.7 and 16.9 millions of cases of depression and anxiety, respectively could be prevented by a 10% and a 25% reduction in the prevalence of emotional neglect.

Adding up specific types of maltreatment, over half (58.59%) of depression and anxiety cases worldwide were potentially attributable to childhood maltreatment. A 10% reduction in child maltreatment could potentially prevent 31.36 million depression and anxiety cases, and a 25% reduction could potentially prevent 80.28 million cases.

Table 3-2 Depression and anxiety disorders cases attributable to specific types of childhood maltreatment worldwide

	Pooled OR (95% CI)	Self-report			Informant		
		Population Prevalence of maltreatment	PAF (confidence range)	Number of cases attributable – millions (Confidence range)	Population Prevalence of maltreatment	PAF (confidence range)	Number of cases attributable – millions (Confidence range)
Physical abuse *vs.* Anxiety and/or depression	2.00 (1.25, 3.19)	22.60%	18.43% (5.35%, 33.11%)	114.84 (33.32, 206.26)	0.30%	0.30% (0.07%, 0.65%)	1.86 (0.47, 4.07)
Sexual abuse *vs.* Anxiety and/or depression	2.66 (1.88, 3.75)	12.70%	17.41% (10.05%, 25.88%)	108.47 (62.63, 161.26)	0.40%	0.66% (0.35%, 1.09%)	4.11 (2.19, 6.78)
Physical neglect *vs.* Anxiety and/or depression	1.74 (1.35, 2.23)	16.30%	10.76% (5.40%, 16.70%)	67.06 (33.62, 104.05)	--	--	--
Emotional neglect *vs.* Anxiety and/or depression	1.74 (1.35, 2.23)	18.40%	11.98% (6.05%, 18.46%)	74.66 (37.69, 114.98)	--	--	--

OR, odds ratio; PAF, Population Attributable Fraction.

Figure 3-3 Potential depression and anxiety cases that could be prevented through child maltreatment reduction worldwide

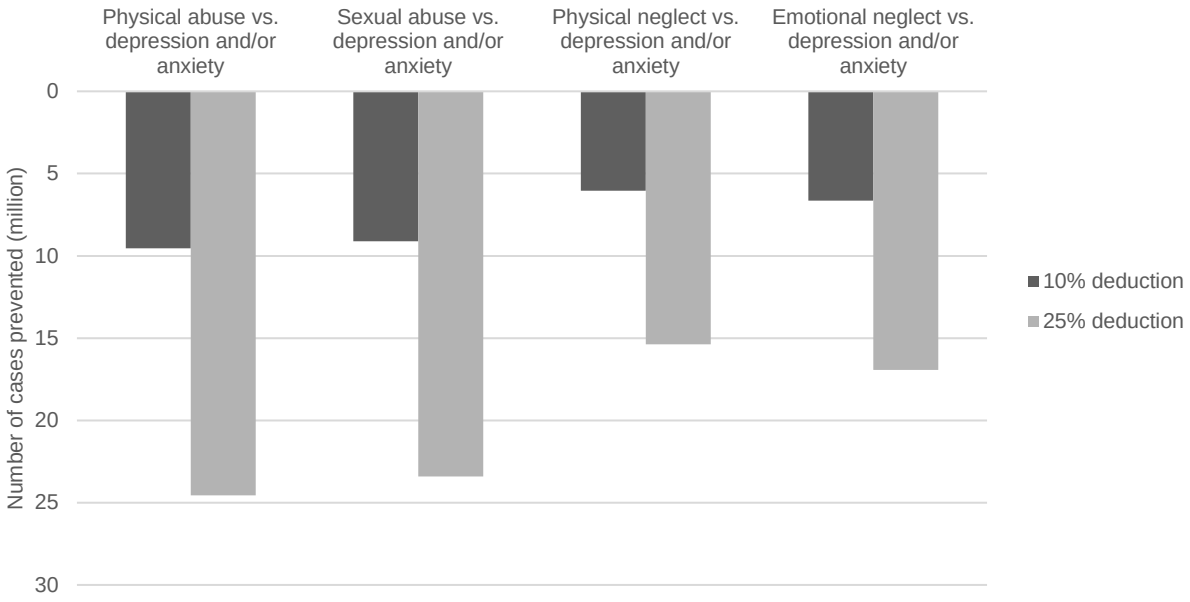
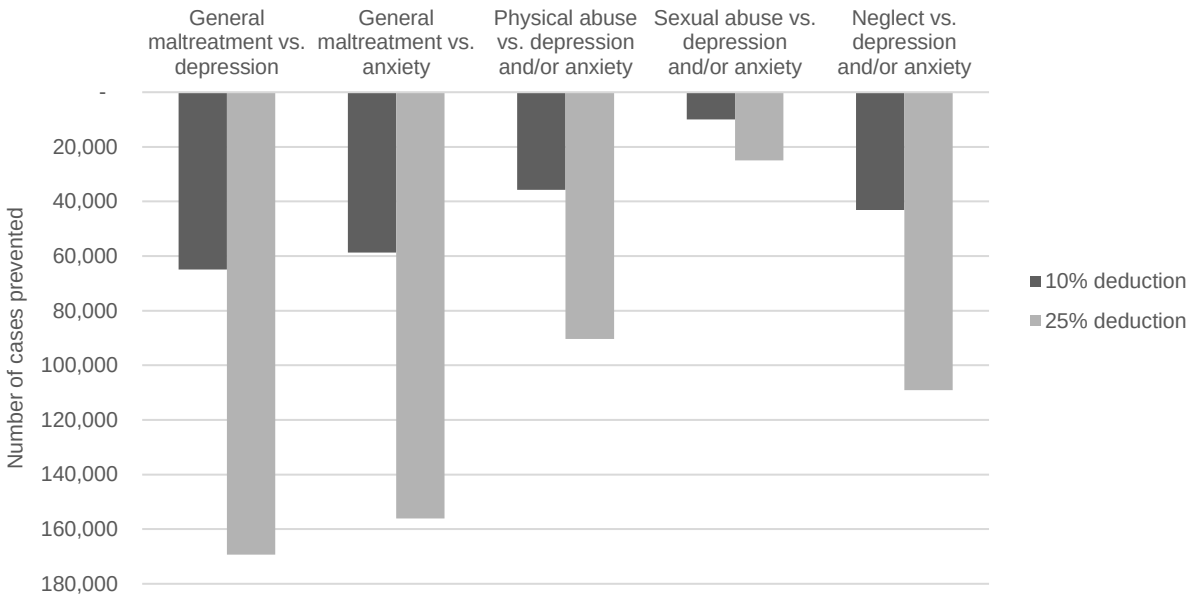


Figure 3-4 Potential depression and anxiety cases that could be prevented through child maltreatment reduction in Canada



3.4.2.1.2 Informant prevalence of child maltreatment and PAFs

The informant prevalence of physical abuse was 0.3% (Stoltenborgh *et al.* 2015). It is estimated that 0.3% (nearly 1.9 million) of depression or anxiety cases are potentially attributable to childhood physical abuse. A 10% to 25% reduction of the prevalence could potentially prevent 190,000 to 460,000 of depression and anxiety cases.

It reported that 0.4% of the worldwide population have been exposed to sexual abuse based on informant studies (Stoltenborgh *et al.* 2015). The PAF estimate is 0.66%, which indicates that 4.1 millions of depression and anxiety cases are potentially attributable to the exposure of sexual abuse in childhood. If the prevalence of sexual abuse was reduced by 10%, about 410,000 cases could potentially be prevented; a 25% reduction could potentially prevent about 1,020,000 cases.

3.4.2.2 Canada

As of 2008, the Canadian prevalence of child maltreatment in general was estimated at 36% (Public Health Agency of Canada, 2010). There are 3.2 million people aged 15 years and above in Canada suffering from depression in 2012 (Statistics Canada, 2013). The PAF estimate for child maltreatment leading to depression was about 27.1%, corresponding to nearly 870,000 depression cases potentially attributable to childhood maltreatment (Table 3-3). If the prevalence of child abuse was 10% lower than at present, we estimated that there would be 60,000 fewer depression cases across Canada, whereas a 25% reduction could result in 170,000 fewer cases (Figure 3-4).

It was reported that 2.4 million people aged 15 years and above in Canada were affected by anxiety disorders in 2012 (Statistics Canada, 2013). Approximately 38.0% (910,000) of anxiety disorders in Canada are potentially attributable to some form of child abuse. If the prevalence of child abuse was reduced by 10%, about 60,000 anxiety disorder cases could potentially be prevented, while a 25% deduction in prevalence could potentially prevent about 160,000 cases.

The prevalence of physical abuse in Canada is estimated at 7.3% (Public Health Agency of Canada, 2010). We calculated that 6.8% (380,000) of depression and anxiety cases are potentially attributable to physical abuse. A 10% of reduction in the prevalence of physical abuse

could result in 40,000 fewer cases and a 25% of reduction could lead to a further decrease of 50,000 cases.

The prevalence of childhood sexual abuse in Canada was estimated at 1.11% as of 2008 (Public Health Agency of Canada, 2010). The PAF estimate indicated that around 1.8% (100,000) of depression and anxiety cases are potentially attributable to sexual abuse in childhood. Ten thousand cases could be potentially prevented by reducing the prevalence of sexual abuse by 10%; and the number of prevented cases could be more than doubled if the prevalence reduced by 25%.

It was estimated that approximately 12.3% of people in Canada experienced neglect in childhood (Public Health Agency of Canada, 2010). PAF estimates indicate about 8.3% (470,000) of depression and anxiety cases are potentially attributable to neglect. If the prevalence of neglect reduced by 10%, about 40,000 cases could potentially be prevented, while a 25% deduction of prevalence could potentially prevent about 110,000 cases.

Together, 31.79% of depression and anxiety cases in Canada are potentially attributable to child maltreatment in general. If the prevalence of child maltreatment reduced by 10%, 123,656 depression and anxiety cases could potentially be prevented, while a 25% reduction of prevalence could potentially prevent 325,442 cases in Canada.

Table 3-3 Depression and anxiety disorders cases attributable to specific types of childhood maltreatment in Canada

	Population Prevalence of maltreatment	Pooled OR (95% CI)	PAF (confidence range)	Number of cases attributable – millions (Confidence range)
Any Maltreatment *vs.* Depression	36%	2.03 (1.37, 3.01)	27.05% (11.75%, 41.98%)	0.87 (0.38, 1.34)
Any Maltreatment *vs.* Anxiety	36%	2.70 (2.10, 3.47)	37.97% (28.37%, 47.07%)	0.91 (0.68, 1.13)
Physical abuse *vs.* Anxiety and/or depression	7.30%	2.00 (1.25, 3.19)	6.80% (1.79%, 13.78%)	0.38 (0.10, 0.77)
Sexual abuse *vs.* Anxiety and/or depression	1.11%	2.66 (1.88, 3.75)	1.81% (0.97%, 2.96%)	0.10 (0.05, 0.17)
Neglect *vs.* Anxiety and/or depression	12.27%	1.74 (1.35, 2.23)	8.32% (4.12%, 13.11%)	0.47 (0.23, 0.73)

OR, odds ratio; PAF, Population Attributable Fraction.

3.5 Discussion

This meta-analyses consistently showed significant relationships between various types of maltreatment and depression and/or anxiety outcomes. The pooled OR between any type of maltreatment and depression was 2.03 (95% CI 1.37-3.01) and 2.70 (95% CI 2.10-3.47) for anxiety. For specific types of maltreatment and depression or anxiety disorders, the ORs were: physical abuse OR=2.00 (95% CI 1.25-3.19); sexual abuse OR=2.66 (95% CI 1.88-3.75); and neglect OR=1.75 (95% CI 1.37-2.24).

Consistent with previous reviews, our results show childhood maltreatment is a risk factor for depression and anxiety disorders. Several meta-analyses, using less rigorous criteria for the measurement of maltreatment exposure, evaluating the short- and long-term effects of various types of childhood maltreatment on mental health support our finding that all types of child maltreatment are associated with an elevated risk of developing psychological disorders, including depression and anxiety disorders (Maniglio, 2009; Paolucci *et al*. 2010; Nanni *et al*. 2012; Norman *et al*. 2012).

To the best of our knowledge, this is the first paper to provide quantitative estimates on the projected reduction of mental disorders cases that could result from a reduction in child maltreatment. The PAFs estimate that over half of depression and anxiety cases worldwide and approximately 32% of cases in Canada are potentially attributable to self-reported childhood maltreatment. A 10% to 25% reduction in child maltreatment could potentially prevent 31.36 to 80.28 million depression and anxiety cases worldwide and 123,656 to 325,442 cases in Canada. Approximately 9 million cases are attributable to informant child physical or sexual abuse. A 10% to 25% of reduction in the informant prevalence of child abuse could potentially prevent 0.4 to 1 million cases.

Both self-reported and informant worldwide prevalence have strengths and limitations. An obvious drawback of self-reported is the reliance on retrospective memory, which is often seen as unreliable and could be biased; whereas informant reports are often reflecting the most severe cases of maltreatment. Informant measures could better assess the continuity and circumstances of maltreatment experiences, such as neglect or emotional abuse; while self-reported measures work better for some types of maltreatment, such as sexual abuse, which may be more invisible to informants. Additionally, the prevalence from informant studies is

considered as an underestimate since they are substantially based on reports by professionals to Child Protection Services and cover shorter periods of childhood (usually a one-year period), compared to self-report studies. However, a conclusion as to whether self-report prevalence rates are over- or underestimates is less clear-cut. It could overestimate when emotional abuse or neglect is measured without taking into account the chronicity of the maltreated behaviors; it could also underestimate when abuse is measured at a single time point (Fergusson *et al*. 2000; Stoltenborgh *et al.* 2015).

PAFs estimates provide quantitative measures of the impact that could be achieved by reducing the prevalence of child maltreatment on depression and anxiety. Our study strongly suggests that decreasing the amount of maltreatment in childhood should be the target for mental illness prevention and mental health promotion. This is not only because adverse experiences in childhood significantly increase the risk of adult depression and anxiety, but also its own threats to children's psychological and neurobiological sequelae. Interventions and services for maltreatment should also promote resilience to further improve the mental health of general populations.

3.5.1 Strength and Limitations of the Current Study

The strengths of this study come from the pooled the findings from longitudinal cohort studies with the external proof of documented child maltreatment, thus avoiding the issue of recall bias, effort after meaning and potential false memories. The studies reviewed here used strong mental health measures, are relatively recent, and were of good quality. PAF estimates show how the incidence of depression and anxiety could be decreased by reducing childhood abuse.

There are several limitations. Firstly, the small numbers of articles reviewed is an obvious limitation. It is unfortunate that more studies did not meet our stringent inclusion/exclusion criteria. Secondly, the studies reviewed are not representative of large sections of world's population with the study samples coming from the USA (4), Australia (2), and New Zealand (2). Studies from developing countries are lacking. PAFs measures used the global prevalence of child abuse (in general and individual type), which is more generalizable. Therefore, PAFs measures may be influenced by the inconsistent measures between global prevalence of maltreatment and associations between maltreatment and depression and anxiety.

Thirdly, heterogeneity was found to be high in the studies reviewed indicating substantial variation in the degree of association between child maltreatment and mental health outcomes reported in the various studies. This reinforces the need for standardization in the measurement of child maltreatment and its various types. It also may indicate that there are significant moderators that influence the maltreatment and mental health relationship. There is a need for better tracking of potential moderators in future studies of the effects of childhood abuse. Fourthly, this review only included studies without subject recall bias in maltreatment assessment. The nature of these abuse reports often deals with more severe cases. Because the prevalence of child abuse was not provided for severity levels, we used the crude prevalence to calculate PAF. The estimates of PAFs may be influenced by the severity levels of child abuse. Finally, most selected studies did not report whether outcome of interest was present at baseline, except one study. No randomized clinical trials can be performed for the relationship between maltreatment and depression and anxiety. This systematic review is an observational study to explore the association between child abuse and adulthood depression and anxiety. No causality could be inferred, as there is a lack of data on baseline depression and anxiety.

Using externally documented measure of maltreatment thus avoiding potential recall biases, this systematic review provides robust evidence about the effects of childhood maltreatment on the subsequent incidence of depression and anxiety in adulthood. The calculated PAFs showed the large reduction in the incidence of depression and anxiety that could result from reducing the prevalence of child maltreatment. This analysis reinforces the need for legal, health and social services programs and policies aimed at reducing the prevalence of childhood maltreatment.

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Appendix 1 Search Strategy

To get maximum number of relevant citations, we used the following search strings: ‘child’ AND (depress* OR anxiet* OR phobi* OR panic OR PTSD OR post-trauma* OR posttrauma* OR OCD OR obsessive* OR agraphobi*) AND (abus* OR maltreat* OR neglect OR abandon* OR illtreat* OR ill-treat* OR mal-treat* OR advers* OR trauma* OR ACE*)’ as the keywords for study retrieval.

Appendix 2 Data References

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Appendix 3 Assessment of Studies Quality Characteristics

First Author	Year	Represen-tativeness[1]	Selection of control[2]	Ascertainment of exposure to child abuse[3]	Assessment of exposure[4]	Assessment of outcome[5]	Temporality[6]	Adequacy of follow-up of cohorts or response rate[7]	Was follow-up long enough[8]	Appropriate analysis[9]	Appropriate confounding control[10]	TOTAL
Cutuli et al.	2013	0	1	1	1	1	0	1	1	1	0	7
Cutajar et al.	2010	1	1	0	2	1	1	1	1	1	0	9
Widom	1999	1	1	1	2	1	0	1	1	1	1	10
Scott et al.	2012	1	1	0	2	1	0	1	1	1	0	8
Widom et al.	2007	1	1	0	2	1	0	1	1	1	1	9
Spataro et al.	2004	1	1	0	2	1	0	1	1	1	1	9
Brown et al.	1999	1	1	0	1	1	0	1	1	1	1	8
Danese et al.	2009	1	1	0	1	1	0	1	1	1	1	8

[1]Representativeness of the population: population-based representative = 1; Not representative, selected group, volunteers, or no description = 0.

[2]Selection of the non-exposed cohort/control: drawn from the same population =1; drawn from a different source or no description =0.

[3]Assertainment of exposure to child abuse: data on child abuse collected prospectively, or collected retrospectively although the official reports were generated in real-time = 1; data on child abuse collected retrospectively = 0.

[4]Assessment of exposure: all cases from secure official record (court-substantiated abuse) = 2; cases partially from secure official record = 1; self-reported or structured interview or self-administered questionnaire or no description = 0.

[5]Assessment of outcome: use of structured clinical interview for DSM-III/IV (DIS, DISC, CIDI) = 1; questions from published health surveys/screening instruments, own system, symptoms described, no system, not specified, or self-reported = 0.

[6]Demonstration that outcome of interest was not present at start of study: yes = 1; no = 0.

[7]Adequacy of follow-up of cohorts or response rate: completeness good (>= 80%), with description of those lost to follow-up = 1; completeness poor (< 80%) or no statement = 0.

[8]Was follow-up long enough for outcomes to occur: yes = 1; no = 0.

[9]Appropriate statistical analysis: yes = 1; no = 0.

[10]Appropriate methods to control confounding: yes (multivariable adjusted OR including SES, education, or family dysfunction in models) = 1; no (univariate analysis or controls for age/sex only) = 0.

CIDI, Composite International Diagnostic Interview; DIS, Diagnostic Interview Schedule; DISC, Diagnostic Interview Schedule for Children; SES, socioeconomic status.

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CHAPTER 4 – WHAT DO DNA METHYLATION STUDIES TELL US ABOUT DEPRESSION: A SYSTEMATIC REVIEW

A revised version of this chapter will be submitted for publication. The target journal initially is Molecular Psychiatry.

4.1 Abstract

Background. While there has been a few reviews conducted to explore the association between DNA methylation modification and the etiology of depression, there has been no comprehensive review of *epigenetic* studies of depression critically exploring experimental methodologies and verification of laboratory testing factors that may significantly affect the accuracy and validity of results. This systematic review corrects for this knowledge deficit.

Methods. Electronic databases and grey literatures up to June 2016 were searched for English-language studies with clear criteria for diagnosis of depression. Fifty seven articles met our eligibility criteria and included in this review along with a summary of study characteristics. We grouped the findings into etiological and treatment studies according to the following genomic attributes: (1) *BDNF*; (2) *SLC6A4*; (3) *NR3C1*; (4) *OXTR*; (5) *other candidate genes*; (6) *genome-wide*; and, (7) *treatment response*.

Results. Majority of the studies were recently published and from developed countries. Whole blood and saliva samples were the most common tissues used in study analyses. Bisulfite conversion, along with pyrosequencing, were widely used to test DNA methylation level across all studies. High heterogeneity existed among the studies in terms of experimental and statistical methodologies and study designs. Given such heterogeneity it is recommended that a systematic review without meta-analysis be undertaken. Inconsistent findings were identified in each study subgroup. The majority of the studies on BDNF (10/11) and nearly half of studies on SLC6A4 (5/11) showed that an increased DNA methylation was associated with depression. Significant (with both hyper- and hypo-methylation) and insignificant relationships were found in all other subgroups.

Conclusion. This review generally supported that DNA methylation changes is associated with depression. It is suggested that more longitudinal studies using standardized experimental and

laboratory methodologies are needed in future epigenetic studies to enable more systematical comparisons and quantitative synthesis.

4.2 Introduction

A number of systematic reviews on susceptible genes and gene by environment interactions provide a comprehensive list of putative genetic and environmental risk factors for major depressive disorder (MDD) (Levinson, 2006; Lohoff, 2010; Shyn & Hamilton, 2010; Saveanu & Nemeroff, 2012; Cohen-Woods, Craig, & McGuffin, 2013; Dunn, et al., 2015). However, there has been little compilation of our knowledge of DNA methylation and depression. Furthermore, there has been no comprehensive review of epigenetic studies in depression critically exploring experimental methodologies and verification of laboratory testing in humans, which may significantly affect the accuracy and validity of results.

To fill this information gap, and provide a critical update on recent findings of DNA methylation in depression, we aimed to: 1) systematically synthesize major findings on DNA methylation and depression; 2) compare similarities and differences across different studies, including experimental and laboratory factors and statistical analyses, which might partially explain some inconsistencies in results; and, 3) comment on the challenges and opportunities for future studies.

4.3 Background

4.3.1 The Ubiquity of Depression

Major depressive disorder (MDD) is one of the most prevalent mental disorders. The Global Burden of Disease 2012 systematic review of 291 diseases and injuries in 21 world regions from 1990 to 2010 concluded that MDD accounted for 2.5% of global DALYs and its ranking increased from 15th to 11th (Murray, et al., 2012). MDD is not only commonly known for its impact on health and wellbeing, but also has an economic impact on absenteeism, loss of productivity, unemployment, and health care expenditure.

Studies have consistently found that genetic and psychosocial environment substantially contribute to the risk of depression (Saveanu & Nemeroff, 2012; Silberg, et al., 1999; Rice,

Harold, & Thapar, 2001). However, replications of these research findings have been hampered by phenotypic and genetic heterogeneities, thus even occurs in large-scale genome wide association studies (Lewis, et al., 2010; Shi, et al., 2011; Akula, et al., 2010; Muglia, et al., 2010). It is now generally accepted that the pathogenesis of MDD not only includes genetic, psycho-socio environmental factors and their interactions, but also involves epigenetic modifications, especially those altered by DNA methylations. DNA methylation has been identified in a number of studies as an etiological and diagnostic biomarker for many mental disorders (Dempster, et al., 2011; Fuchikami, et al., 2011; Walker, et al., 2016; Kaminsky, et al., 2012). Both genetic and environmental factors can affect the extent of DNA methylation. DNA methylation also integrates the impact of both genetic and environmental factors on the potential downstream functional outcomes on a phenotype (Lienert, et al., 2011; Schadt, 2009).

4.3.2 Gene Expression and Epigenetics

A number of gene have been putatively linked to major depression. A gene is a string of DNA encoding information and hiding in a cell's nucleus. Gene expression refers to the process of synthesizing the information in a gene to produce functional gene products which can be proteins or non-proteins, such as transfer RNA (tRNA) or small nuclear RNA (snRNA). Gene expression consists from several steps, including transcription, RNA splicing, translation, and post-translational modification. Genes are expressed by being transcribed into messenger RNA (mRNA), and then be translated into protein via tRNA (Wikipedia, 2016).

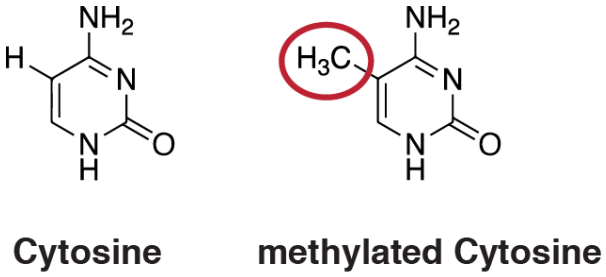
The regulation of gene expression is crucial to an organism's development. It ensures the genetic information in DNA is properly interpreted and allows the genotype to give rise to organism's phenotype. Genes can interact with and respond to organism's environment. External environmental factors or endocrine signals (Nguyen, Nioi, & Pickett, 2009) may cause modification of regulatory proteins (Paul, 2008) and intracellular signals (Los, Maddika, Erb, & Schulze-Osthoff, 2009), thus further affecting regulation of gene expression.

Epigenetics refers to the external changes in a chromosome, which affects transcription and gene expression, and alters the heritable phenotype. Epigenetic modifications of gene expression include alterations in DNA methylation – the addition of a methyl group which prevents certain genes from being expressed, and histone modifications (Dalton, Kolshus, & McLoughlin, 2014; Rettner, 2013; Ennis, 2014). Histones are proteins that DNA wraps around. Modifications that squeeze DNA tightly making the DNA unable to be "read" by the cell; on the contrary, relaxed histones can make the DNA accessible to be "read" (Rettner, 2013). Epigenetic modifications can be potentially caused by many outside stimulus from chemicals to lifestyle factors, such as Bisphenol A (BPA), exercise, and child abuse and other forms of early trauma (Ennis, 2014). DNA methylation is the most studied epigenetic modification, and can change the activity of a DNA segment turning genes "on" or "off" without change in the DNA's sequence (Dalton, Kolshus, & McLoughlin, 2014).

4.3.2.1 DNA methylation

DNA methylation is the reversible and heritable attachment of a methyl group to a nucleotide. The most common form of DNA methylation occurs at the 5´carbon of cytosine in CpG dinucleotides, creating 5-methylcytosine. CpG dinucleotides are often located in CpG islands (clusters of CpG sites) within the promoter region or first exon of genes, or upstream from genes within CpG island shores (DNA regions within 2 Kb of CpG islands) or shelves (within 2 Kb of shores) (Jones, 2012). Two of DNA's four bases, cytosine and adenine, are found to be able to be methylated. Figure 4-1 shows an example of cytosine methylation which is widespread in both eukaryotes and prokaryotes (Wikipedia, 2017). DNA methylation is a key epigenetic mechanism in developmental regulation of gene expression, and plays an important role in transcriptional regulation of genes and miRNAs (Lopez-Serra & Esteller, 2012), control of alternative promoter usage (Laurent, et al., 2010), and alternative splicing (Laurent, et al., 2010).

Figure 4-1 Cytosine methylation



4.3.3 The Mediating Role of DNA Methylation Modification in the Relationship between Early Life Experience and Later On Psychiatric Disorders

Significant associations between early life exposure and psychiatric disorders have been consistently reported by various epidemiological and epigenetic studies (Moffitt & Tank, 2013; Danese, et al., 2008; Vythilingam, et al., 2002; Kessler, et al., 2010). Early life conditions and experiences, such as poverty, adverse experience, and poor early growth, can make individuals more prone to developing physical and psychiatric diseases. A developmental model of the pathogenesis of disease - fetal origins hypothesis, proposes that the developmental health and wellbeing outcomes for an individual from infancy to adulthood are significantly impacted by the conditions during gestation, as in the association between low birth weight and coronary heart disease that has been confirmed in longitudinal studies of men and women around the world (Barker, 2007).

Various studies have demonstrated that early life exposures are also associated with DNA methylation modifications, such as the history of childhood abuse (McGowan, et al., 2009), exposure to intimate partner violence during pregnancy (Radtke, et al., 2011), prenatal maternal depressive symptoms (Oberlander, et al., 2008), poor maternal care (Unternaehrer, et al., 2015), and early life socioeconomic status (Lam, et al., 2012). Early life, as an especially sensitive period, critically affects the structure and function of the genome, and this effect is not limited to the brain or susceptible genes, but is more genome- and system-wide (Szyf & Bick, 2013).

Animal findings have provided the evidence to support that early life stress is linked to persistent modifications of DNA methylation in the central nervous systems, by changing gene expressions throughout the life span and passing the changes to offspring (Roth, Lubin, Funk, & Sweatt, 2009; Murgatroyd, et al., 2009).

In addition, epigenetic mechanisms have been found to contribute to the establishment and maintenance of regular gene expressions (Xu, et al., 2010). Modifications of DNA methylation are associated with either gene silencing for *hyper*methylation or the inducement of gene transcription for *hypo*methylation, both of which are assumed to increase vulnerability of psychiatric disorders (Kosztolany, 2011; Ptak & Petronis, 2010). Both inherited and acquired epigenetic dysregulations may play a role in the etiology of MDD and other psychiatric disorders (D'Addario, et al., 2013; Oh, et al., 2015; Kahl, et al., 2016). A prospective cohort study indicated that lower DNA methylation levels of seven candidate genes assessed at birth were associated with more attention deficit hyperactivity disorder (ADHD) symptoms in children at the year of six (van Mil, et al., 2014).

Furthermore, epigenetic alterations could mediate the relationship between early life exposures and psychiatric disorders. Prenatal “unhealthy diet” was associated with higher ADHD symptoms, indirectly via hypermethylation of insulin-like growth factor 2 (IGF2), which plays an essential role in growth and development before birth (Rihlaarsdam, et al., 2016). Similarly, epigenetic signatures probably mediate associations between early adverse events and long-term alterations in human stress and immune systems response (Bick, et al., 2012). Newman et al. (2016) in their review summarizing the early origins of psychiatric disorders suggest that epigenetic modifications, such as DNA methylation, could be one of the mechanisms underlying the in-utero effects on fetal development, and the association between early childhood experiences and quality of parental care provided and emotional regulation.

4.3.4 The Development of DNA Methylation Arrays

Harrison and Parle-McDermott reviewed the major developments in the methodologies used for DNA methylation analysis over the past 30 years (Harrison & Parle-McDermott, 2011). The earliest techniques were based on the separation of methylated and unmethylated cytosines using reversed-phase high performance liquid chromatography (RP-HPLC), which was further improved throughout the 1980s (Kuo, McCune, Gehrke, Midgett, & Ehrlich, 1980; Gomes & Chang, 1983; Patel & Gopinathan, 1987) and thin-layer chromatography (TLC). In the following years, molecular techniques were then applied to indirectly examine DNA methylation levels at both a genome-wide and gene-specific context, such as immunoprecipitation via anti-5′methylcytosine (anti-5mC) antibody (Oakeley, A, & Jost, 1997), and methylation-sensitive restriction enzymes, which cut DNA via recognition of digested fragments (Santos, Hendrich, Reik, & Dean, 2002). The advent of sodium bisulfite treatment of DNA, a deamination reaction converts cytosine to uracil when unmethylated but remains cytosine when methylated. Various approaches can detect conversions, such as bisulfite sequencing (Frommer, et al., 1992), methylation-specific polymerase chain reaction (MS-PCR) (Herman, Graff, Moyhanen, Nelkin, & Baylin, 1996), and methylation-specific high resolution melting (MS-HRM) (Wojdacz & Dobrovic, 2007). In recent years, these three techniques, immunoprecipitation, methyl-sensitive RE, and bisulphite treatment, have become principal methods for DNA methylation differentiation and have been applied to DNA microarrays/beadchips and next-generation sequencing platforms (Harrison & Parle-McDermott, 2011). The microarrays separate and analyze methylated and unmethylated DNA fragments; whereas next generation sequencing is a newly developed parallel sequencing, which allows the sequencing of DNA and RNA more quickly and cheaply compared with the previously used Sanger sequencing. Although all these recent techniques have their strengths and weaknesses, new methodologies and analytical tools will be developed in the near future to lower the cost of genome-wide sequencing and improved

consistency of laboratory testing. These evolutions promote epigenetic studies and bring us closer to exploring a complete human epigenetic profile (Harrison & Parle-McDermott, 2011).

4.3.5 What Have Been Found by Previous Reviews on This Topic?

To our knowledge, there are five reviews including only one systematic review so far on the general topic of DNA methylation and depression (Lockwood, Su, & Youssef, 2015; Uddin, Sipahi, Li, & Koenen, 2013; Dalton, Kolshus, & McLoughlin, 2014; Bakusic, Schaufeli, Claes, & Godderis, 2017; Chen, Meng, Pei, Zheng, & Leng, 2017). Generally they suggest that altered DNA methylations may be associated with the etiology of depression. Lockwood, Su, and Youssef in their narrative review of epigenetic findings in both animal models and human studies concluded epigenetics could play an important role in depression and suicide in humans (Lockwood, Su, & Youssef, 2015). Again, Uddin et al. (2013) using a same approach studied the role of sex in DNA methylation and post-traumatic stress disorder (PTSD) and MDD, and suggested that sex differences in DNA methylation among genes known to influence brain development may explain the sexually dimorphic risk for developing PTSD and MDD. Another narrative review found the inverse association between adverse environmental factors, i.e. early life stress, and the epigenetic modification of gene expression (Dalton, Kolshus, & McLoughlin, 2014). A recent review examined the association between DNA methylation of seven candidate genes and depression and found that BDNF and NR3C1 gene methylation levels may be related to depression, whereas the relationship between SLC6A4 and depression was inconsistent (Chen, Meng, Pei, Zheng, & Leng, 2017).

In contrast to a sufficient number of systematic reviews on susceptible genes and gene by environment interactions, that provide a list of putative genetic and environmental risk factors for MDD (Levinson, 2006; Lohoff, 2010; Shyn & Hamilton, 2010; Saveanu & Nemeroff, 2012; Cohen-Woods, Craig, & McGuffin, 2013; Dunn, et al., 2015), there has been a limited progress in systematically reviewing DNA methylation in depression. There has been no comprehensive

review(s) of epigenetic studies in depression critically exploring the role of experimental methodologies and verification of laboratory testing factors, which may significantly affect the accuracy and validity of results. One recent systematic review assessed both animal and human studies and identified the correlation between burnout/depression and global and candidate-gene DNA methylation (Bakusic, Schaufeli, Claes, & Godderis, 2017). However, the review in question did not examine the influence of experimental and statistical methodologies and analyses on the findings.

This systematic review aims to: 1) systematically synthesize major findings on DNA methylation and depression; 2) compare similarities and differences across different studies, including experimental and laboratory factors and statistical analyses, which might partially explain some inconsistencies of results; and, 3) note the challenges and opportunities for future studies.

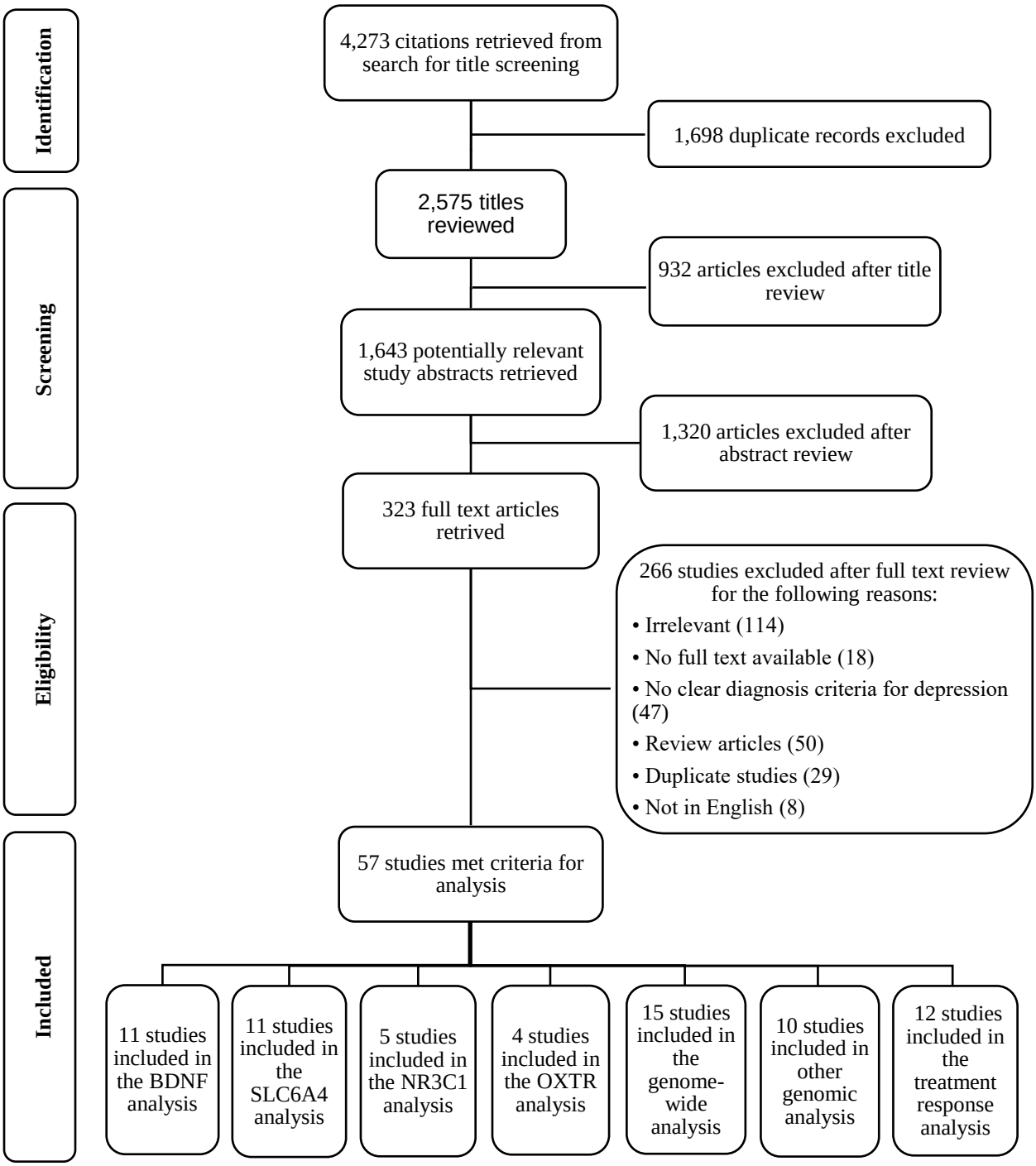
4.4 Methods

The processing and reporting of results of this systematic review were guided by the PRISMA 2009 guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009).

4.4.1 Search Strategy

Two methods were used to retrieve all studies with relevant topics. First, we conducted a search of the computerized bibliographic databases PubMed, Web of Science, EMBASE, Medline, and Cochrane Library. The search strategy is detailed in Supplementary Appendix 1. The literature search comprised articles up to June, 2016. Second, a snowball technique was then applied to identify further studies. We manually searched other resources for other relevant studies. The reference lists of selected articles, review articles on relevant topic, and the gray literatures were screened. Figure 4-2 presents the process of study selection.

Figure 4-2 PRISMA flow diagram – DNA methylation and depression



4.4.2 Inclusion and Exclusion Criteria

All suitable articles were evaluated with regards to their internal validity and four selection criteria as follows: 1) used a clear diagnosis criteria for depression, specifically DSM and its updates (APA, 2013), ICD-10 (WHO, 1992) or other generally accepted diagnostic criteria; 2) examined the association between DNA methylation and depression; and, 3) provided a statistical indicator (i.e. coefficient) or original data to estimate the relationship between DNA methylation and depression.

Articles were excluded if they 1) did not specify the "depressed" patients as patients suffering from major depression, major depressive disorder, unipolar depression, or other types of depression; 2) were not written in English.

4.4.3 Data Collection

1. *Selection of studies.* Two authors (M Li & X Li) independently screened all the retrieved articles. Inconsistencies in interpretation were resolved through group discussions (X Li, M Li & Meng). Endnote and RefWorks were used as bibliographic software.
2. *Data extraction and management.* Data on author(s), year of publication, sample size, study designs, study cohort, experimental methods, type of tissues, target genes/genome, DNA purification method (a method of DNA isolation from a sample to assess the purity of the extracted DNA), DNA methylation method, DNA methylation validation (verification of methylation patterns), genotyping, gene expression, experimental factors, statistical methods, and major findings were extracted independently. For those studies with multiple reports, a single record denoted one study with information extracted from multiple reports. Group discussions dealt with all the inconsistent interpretations.
3. *Dealing with missing data.* The reviewers endeavored to contact the original authors of the studies with missing information in order to gather complete and consistent study information. Open-ended questions were used to prevent misleading answers.

4. *Data synthesis.* Because the divergence of targeted genes/genomes, we grouped the summarized findings as follows: (1) *BDNF*; (2) *SLC6A4*; (3) *NR3C1*; (4) *OXTR*; (5) *other candidate genes*; (6) *genome-wide*; and, (7) *treatment response*. Some studies were involved in multiple separate analyses as their data permitted. Because the different experimental study designs and various statistical methods used, we first summarized the overall review findings and then discussed factors/characteristics could confound the review findings. Findings for each subgroup analysis are also provided.

4.5 Results

Some 4, 273 citations were initially retrieved from the title search. A total of 1, 643 abstracts were reviewed with 323 full text articles being retrieved for review. Finally, 57 articles met our eligibility criteria and are included in this review (see Figure 4-2). Appendix 2 lists all the selected articles by analysis group.

Supplementary Appendix 3 presents details on the study characteristics of the selected studies. We did notice a significant heterogeneity in terms of study characteristics across studies. Most studies were published between 2008 and 2016, especially in the past six years. The selected studies mainly focused on adult and senior age groups (49/57), covered a total number of 10,857 subjects worldwide (North America: 16/57, Asia: 16/57, Europe: 22/57, and Australia: 5/57). We also evaluated study quality covering from *design* (study design, sample size, and type of subject), *implementation* (biological sample, DNA methylation method, purification of DNA extraction, and validation of methylation), *analysis* (analytical method, batch effect, genotyping and gene expression), to *results interpretation* (major findings and their implications). The majority of the studies in this review were case-control or longitudinal studies with hospital or general population-based cohort. There was a wide variety in terms of sample size ranging from 11 to 1,024. Blood and saliva were most commonly used as biological samples analyzed by generally accepted DNA methylation methods, such as bisulfite conversion with pyrosequencing.

Both parametric and non-parametric statistics were used. Importantly, most of these studies did not analyze the influence of batch effect on their results (55/57), except two genome-wide studies.

This review was designed to apply evidence-based approaches to evaluate the findings of studies on the relationship between DNA methylation and depression. High heterogeneity existed among the studies reviewed, in such a case the Cochrane guidelines does not recommend using quantitative methods, such as meta-analysis. As a consequence, qualitative methods were used.

We present our review results divided into the major categories of etiological-genome-wide studies, etiological-candidate genes studies, and treatment response studies. Table 4-1 summarizes study characteristics.

Table 4-1 Summary of study characteristics

Genomic Category	BDNF	SLC6A4
Number of articles	11	11
Years	2011-2016	2008-2016
Country/continent	Asia (7); Europe (3); North America (1); not mentioned (1)	Asia (3); Europe (2); North America (4); Australia (2)
Age group	Adult to senior	Adult to senior (9); adolescent (2)
Sample size	4,184 (38-1024)	2,188 (43-954)
Diagnostic standard	DSM-IV (9); Hamilton Depression Rating Scale (1); GMS AGECAT & Geriatric Depression Scale (1)	DSM-IV (8); SCID (1); CIDI (1); Beck Depression Inventory (BDI-II) (2); AAGA-II (1); CIS-R (clinical interview schedule-revised) (1)
Study design	case-control (7); longitudinal study (4) followed-up for 1 to 2 years	case-control (7); longitudinal study (3) followed-up for 6 weeks to 1 years; twins study (1)
Cohort	Hospital-based (5); general population (4)	Cases were hosptal- or population-based; controls from general population or hospital (1)
Biological sample	blood (9); buccal tissue (1); saliva (1)	blood (9); saliva (1); buccal cell (1)
Purification of DNA extraction	Yes (7) - QIAamp DNA Blood Mini Kit (5); PUREGENE DNPurification Kit (1); Dneasy Blood & Tissue Kit (1); No (4)	Yes (5) - QIAamp DNA Blood Mini Kit (3); Dneasy Blood & Tissue Kit (1); cold protein precipitation (1); No (6)
DNA methylation method /kit	All used bisulfite conversion (11) - EpiTect Bisulfite Kit (4); EZ-96 DNA Methylation Kit (3). Methylation-specific quantitativePCR (1), PCR and sequencing (1), pyrosequencing (7) using PSQ 96M System(5) and PyroMark ID System with Pyro Gold Reagents Kit(2)	All used bisulfite conversion (11) - EpiTect kit (2); EZ DNA methylation kits (3). Pyrosequencing (6) using PyroMark Software (3); PSQ 96 System (2). PCR and sequencing (2). Analyzed using EpiTYPER analysis (3) and MassARRAY (3)
Methylation validation	Yes (1) - Bisulfite-modified universal methylated DNA was used as negative control (1); No (10)	Yes (4) - mean of methylation percentage (1); bisulfite conversion (1); pyrosequencing on duplicate samples (2); No validation (6)
Genotyping	Yes (7); No (4)	Yes (9); No (2)
Gene expression	All No (11)	Yes (4); No (7)
Analytical method	Pearson's correlation coefficient test (1); ANOVA (1); hierarchical clustering analyses (1); t-test (5); multivariate logistic regression (4); linear regression (2); Wilcoxon-MannWhitney test (1)	Regression analyses (6) - linear (2); logistic (2); both (1). MannWhitney U test (1). ANOVA (2). T-test (2).
Major finding	Higher level of BDNF DNA methylation were associated with depression (CpG 1,2,3,4,5,9) or poststroke depression (10); No significant difference (CpG 1,2,3,4) (1).	No significant association (6); SLC6A4 methylation level was independently associated with poststroke depression/ depressive symptoms (3); Higher methylation is associated with lifetime depression, compared with alcohol dependence (1) and depression patients compared with controls (1);

Genomic Category	NR3C1	OXTR
Number of articles	5	4
Years	2014-2016	2015-2016
Country/ continent	Asia (2); Europe (2); North America (1)	North America (3); Europe (1)
Age group	Adult to senior (5); adolescent (1)	Adult to senior
Sample size	1,292 (12-954)	1,025 (43-545)
Diagnostic standard	DSM-IV (2); CIDI (1); Patient Health Questionnaire (PHQ-9) consistent with DSM-IV (1); diagnosed by psychiatrist (1)	DSM-IV (2); SCID-I/II (1); Edinburgh Postnatal Depression Scale (EPDS) (1)
Study design	Case-control (4); longitudinal study (1)	Case-control (3); longitudinal study (1)
Cohort	Participants recruited from hospital- or population-based studies (4); hospital outpatients (1)	participants recruited from population-based studies/databases (3); hospital inpatients, controls from advertisement (1)
Biological sample	blood (4); post-mortem brain tissues (1)	blood (3); saliva (1)
Purification of DNA extraction	Yes (4) - EZ DNA Methylation-Gold kit, Prep Mini Spin Kit, QIAamp DNA Blood Mini Kit (2) & LifeSciences's Quickgene DNA Whole Blood Kit; No (1);	Yes (2) - QIAamp DNA Blood Mini Kit (2); No (2)
DNA methylation method /kit	All used bisulfite conversion (5) using EpiTect Bisulfite Kit (1) or EZ DNA Methylation Kit (1). Pyrosequencing using PyroMark kit (4). PCR (1). Analyses using EpiTYPER method (1)	All used bisulfite treatment (4) using EpiTect Bisulfite Kit (1) or EZ DNA methylation Gold kit (1). Pyrosequencing analysis using PyroMark system (3). PCR and sequencing using BigDye Terminator Cycle Sequencing Kit (1)
Methylation validation	Yes (2) - bisulfite treatment using EpiTect bisulfite kit or pyrosequencing using PyroMark; No validation (3)	No (4)
Genotyping	Yes (1); No (4)	Yes (4)
Gene expression	Yes (3); No (2)	Yes (1); No (3)
Analytical method	Analysis of covariance (ANCOVA) (1); T-test (2); linear and logistic regression (1); Mann-Whitney U-test (1)	T-test (1); linear mixed effect model (1); logistic regression (1); linear regression (1)
Major finding	Depression /depressive symptom was associated with higher methylation level (NR3C1_1, CpG 7 in female) (2); depression was related to lower methylation level (CpG 3,4, 5-13) (2); no significant findings (1)	Greater methylation levels were found in cases compared with controls (1); decreased methylation levels were found in depressed female patients (1) or serum estradiol levels in postpartum depression (1); no significant association with postpartum depression(1)

Genomic Category	Genome
Number of articles	15
Years	2011-2016
Country/ continent	Asia (2); Europe (4); North America (6); Australia (1); mixed UK & Australia & Canada (2)
Age group	Adult to senior (13); not mentioned (2)
Sample size	1,952 (12-454)
Diagnostic standard	DSM-III/IV (10); SCIDI (1); Patient Health Questionnaire (PHQ-9) (1); Beck Depression Inventory (BDI) & BDI-II (1); Hamilton Depression Rating Scale (HDRS) (1); Edinburgh Postnatal Depression Scale (1); not mentioned (1)
Study design	Case-control (11); longitudinal study (2); twin study (3); discovery/pilot-replication (5)
Cohort	Participants recruited from hospital- or population-based studies or databases (Twin Registry, Brain Bank) (12); not mentioned (3)
Biological sample	blood (11); post-mortem brain (3); postmortem frontal cortex (3); sperm (1)
Purification of DNA extraction	Yes (6) - Nucleon Genomic DNA Extraction Kit (2), salt extraction method (AU) (1), MasterPure DNA Purification kit (1), QIAamp DNA Blood Maxi Kit (2), Qiagen DNA mini kit (1); No (9)
DNA methylation method /kit	Bisulfite conversion (12) using EZ DNA methylation Kit (4), ZymoResearch bisulfite kit (1), EpiTect Bisulfite Kit (1). Bead array using Infinium Human Methylation Beadchips (10). CHARM assay platform (1). Enrichment for methylated regions using methylated DNA immunoprecipitation combined with ultra-deep sequencing (MeDIP-seq) (1). Pyrosequencing (2) using Gold Q96 Reagents (1) and Pyromark system (2). PCR and sequencing (1). ELISA-based for global DNA methylation profiling (1) - MethylFlash methylated DNA quantification kit (for 5-mc), MethylFlash hydroxymethylated DNA quantification kit (for 5-hmc).
Methylation validation	Yes (10) - next generation sequencing (1), pyrosequencing (4), Bisulfite conversion (2), replicates (2), high-resolution melting and bisulfite Sanger sequencing (1), ; No (5)
Genotyping	Yes (1); No (14)
Gene expression	Yes (7); No (8)
Analytical method	Mann-Whitney U-test (2); linear mixed effect model (2); linear regression (1); logistic regression (1); t-test (5); ANOVA (1); pairwise comparisons (1); linear modelling using Limma and methyAnalysis package (1); ranking analysis (1); functional annotation cluster analyses (1)
Major finding	Significant differential modifications were found in depression, but can be increase or decrease (15) - Lower DNA methylation in depressive patients than in controls (5); hypermethylation was found in depressive /postpartum depressive patients (5); increased methylation found in pilot, not in replication (1); significant difference in mean methylation found in females, not in males (1); both directions were found (1): some processes (e.g. brain development, tryptophan metabolism) showed patterns suggestive of increased methylation among individuals with depression whereas others (e.g. lipoprotein) showed patterns suggestive of decreased methylation among individuals with depression. No difference among severe MDD and remitted patient at 5-hmc and 5-mc levels (1).

Genomic Category	Others
Number of articles	10
Years	2012-2016
Country/continent	Asia (1); Europe (6); North America (3);
Age group	Adult to senior (7); adolescent (1); not mentioned (2)
Sample size	888 (34-174)
Diagnostic standard	DSM-IV (7); SCID-I/II (1); Composite International Diagnostic Interview (CIDI) (1); MDI (1); Brief Symptom Inventory (BSI)
Study design	Case-control (8); longitudinal study (1); twin's study (1)
Cohort	hospital inpatients /outpatients & controls from general population (3); population-based study (5); not mentioned (2)
Biological sample	blood (7); saliva (3)
Purification of DNA extraction	Yes (6) - QIAamp DNA Blood Mini Kit (2), Wizard Genomic DNA Purification kit (1), Invisorb Blood Giga Kit (1), Qiagen DNA mini kit (1), Puregene whole blood DNA-extraction kit (1); No (4)
DNA methylation method /kit	Bisulfite convertion (9) using EpiTect Bisulfite Kit (3), EZ DNA methylation Kit (2). Analyzed using EpiTYPER platform (3). Methylation-specific PCR (1). PCR and sequencing (5) using BigDye Terminator Cycle Sequencing Kit (1). Bead array using the Illumina Infinium HumanMethylation450 (450K) BeadChip (2). Pyrosequencing (1) using PyroMark Q96 MD (1). Not mentioned (1)
Methylation validation	Yes (2) - circle sequencing (1), pyrosequencing & replication (1); No (8)
Genotyping	Yes (4); No (6)
Gene expression	Yes (2); No (8)
Analytical method	Mixed linear models (1); linear regression (2); Pearson's correlation coefficient test (2); Fisher's exact test (1); t-test (2); ANCOVA (2); non-parametric analyses (1)
Major finding	Increased methylation was found in depression (GLUT1, CpG 1,5,12 of ACE, Elovl5, FKBP5) (4); lower level of methylation found in depression (Fads2, MAOA(2), DEPDC7) (4); No difference found (GLUT4, APOE) (2); The TPH2 promoter was methylated in 36.0% of MDD + suicide patients, as compared with in 13.0% of MDD patients (1); HP1BP3 and TTC9B predicted PPD, In a replication analysis, these biomarkers also functioned to segregate PPD status in women who developed depression during the antenatal period with 88% accuracy; however the prediction was in the opposite direction.

Genomic Category	Treatment response
Number of articles	11
Years	2014-2016
Country/ continent	Asia (3); North America (1); Europe (5); mixed UK & Australia (1); not mentioned (1)
Age group	Adult to senior (9); not mentioned (2)
Sample size	1,740 (11-554)
Diagnostic standard	DSM-IV (8); SCID-I/II (1); BDI-I/II (3); CSID-I (2); HAM-D-21 (2); GAF (2); Hamilton Depression Rating Scale-21 (1)
Study design	Case-control (4); longitudinal study (7)
Cohort	Hospital-based cohort (5); population-based database (1); controls from general population (1); not mentioned (3)
Biological sample	blood (11)
Purification of DNA extraction	Yes (8) - PUREGENE DNPurification Kit (1), Nucleon BACC Genomic DNA Extraction Kit (2), QIAamp DNA Blood Mini Kit (3), DNeasy Blood and Tissue Kits (1), FlexiGene DNA Kit (2); No (3)
DNA methylation method /kit	Bisulfite convertion (10) using EpiTect Bisulfite Kit (2), EZ DNA methylation kits (4). Analyzed using EpiTyper software (1). Pyrosequencing (3) using PyroMark system (2), PSQ 96M System (1). PCR and sequencing (5) using BigDye Terminator (4). Methylation-specific quantitativePCR following the MethyLight protocol using SYBR green (1). Methylated DNA immunoprecipitation combined with ultra-deep sequencing (MeDIP-seq) (1)
Methylation validation	Yes (3) - bisulfite conversion for negative controls (1), fully methylated and fully non-methylated DNA was used in all experiments (3); No (8)
Genotyping	Yes (6); No (5)
Gene expression	Yes (3); No (8)
Analytical method	Linear regression (4); linear mixed effect model (3); Pearson's correlation coefficient test (2); ANOVA (1); Wilcoxon signed-rank test (1)
Major finding	Greater methylation levels were associated with SSRIs at 2 CpG, anti-depressant therapy (2); hypomethylation of the 5-HTT & MAOA transcriptional control region might impair antidepressant treatment response in Caucasian patients with MDD (2); Remitters had a significantly lower mean BDNF promoter methylation rate than non-remitters (exon I) (1); No significant methylation (e.g. MAOA, BDNF) change related to antidepressant use (6); The pre-treatment methylation rate(CpG3) of SLC6A4 is associated with therapeutic responses to antidepressants in unmedicated patients with MD (1); lithium and valproate tended to decrease, even though not significantly, DNA methylation level at BDNF gene promoter, when compared to other classes of medications (e.g. antidepressants and atypical antipsychotics).

4.5.1 Etiological Studies

4.5.1.1 Whole genome-wide studies

There were 15 studies, published from 2013 to 2016, using whole-genome wide approaches to examine the relationship between DNA methylation and depression. Study sample sizes ranged from 12 to 454 subjects, and over half (53.3%, 8/15) of the studies contained relatively small sample sizes of <100. Subjects were recruited from existing hospital, or population-based studies or databases. The study designs of this category consisted of 11 case-control studies, 3 twins' studies, and 2 cohort studies, and 5 studies applied the discovery/pilot-replication method. Most of biological specimens were whole blood samples, followed by post-mortem brains, and sperm. Most studies included methylation validation procedure (66.7%, 10/15), but no DNA purification in their arrays (60.0%, 9/15). The majority (80.0%, 12/15) of these studies used bisulfite conversion. Bead arrays were widely applied using Infinium Human Methylation Beadchips (10/15), followed by pyrosequencing, and methylated DNA immunoprecipitation combined with ultra-deep sequencing (MeDIP-seq). Almost half of the studies (46.7%, 7/15) also did gene expression afterwards.

Although all these studies did find significant modifications in the level of DNA methylation among depression cases, both positive (hypermethylation) and negative (hypomethylation) correlations were noted. Inconsistent results were also identified. For instance, increased methylation that was previously shown in a pilot study was not present in its replication (Sabunciyan, et al., 2012); a significant decrease in mean methylation was observed among females, but not for males (Byrne, et al., 2013); lower methylation levels were found among severe MDD patients vs. healthy controls, but no difference between severe vs. remitted patients (Tseng, et al., 2014); one study found both *hyper*methylations in some processes (e.g. brain development and tryptophan metabolism), and *hypo*methylations in other tissues (e.g. lipoprotein) (Uddin, et al., 2011).

Although studies with both large and small sample sizes did not show significant differences in terms of study design and major findings, studies with large sample sizes were more likely to use DNA purification methods and examine gene expression than those with smaller samples. Thus results from studies with large sample sizes may be considered to be more reliable.

4.5.1.2 BDNF

There were 11 articles published from 2011 to 2016, studying the relationship between DNA methylation on *BDNF* gene and depression. Their sample sizes ranged from 38 to 1,024 subjects. Studies in this group used case-control (63.6%, 7/11) and short-term longitudinal cohorts (36.4%, 4/11) designs. Subjects were from both hospitals and general populations. The whole blood samples were the primary choice (81.8%, 9/11) for biological testing, followed by buccal cells and saliva samples. DNA methylation was mostly tested using bisulfite pyrosequencing (63.6%, 7/11), followed by methylation-specific quantitative PCR. Most studies checked DNA purification (63.6%, 7/11), but did not included methylation validation (90.9%, 10/11). Seven studies also did genotyping (63.6%, 7/11), but none conducted gene expression. Most of studies in this group had the relatively large sample sizes (>200, 6/11) and majority of studies with large sample size performed DNA purification (5/6).

Consistently, most studies (90.9%, 10/11) found that subjects with depression or post-stroke depression were more likely to have hypermethylation on multiple *BDNF* CpG sites. Only one study did not replicate this finding, but it had a small sample size and did not report on laboratory factors in their analyses (Choi, et al., 2015).

4.5.1.3 SLC6A4

There were 11 studies examined the relationship between DNA methylation on *SLC6A4* and depression. The sample sizes in this group ranged from 43 to 286 subjects, except for one study had 954 subjects. Seven of the studies were case-control studies, three were longitudinal

studies with less one-year follow-up, and one was a "twins" study. Subjects were from hospitals and population databases or from general population. Whole blood was the primary biological sample (9/11), followed by buccal cells and saliva. Less than half studies tested for both DNA purification and methylation validation. More than half of studies (54.5%, 6/11) applied pyrosequencing followed by EpiTYPER or MassARRAY analysis, for DNA methylation. Most of the studies (9/11) also tested genotyping, but only four (4/11) examined gene expression.

The three longitudinal studies which together represented 65.4% of the group subjects consistently found that *SLC6A4 hyper*methylation was significantly associated with depression and depressive symptoms (Kim, et al., 2013; Philibert, et al., 2008; van der Knaap, van Oort, Verhulst, Oldehinkel, & Riese, 2015). This finding was supported by a twins study (Zhao, Goldberg, Bremner, & Vaccarino, 2013) and a case-control study that had a most comprehensive consideration of laboratory factors and statistical analysis (Iga, et al., 2016). Five studies with relatively large sample sizes consistently found that *SLC6A4 hyper*methylation were linked to the risk of depression, depressive symptoms, or post-stroke depression, but other six small sample-sized studies did not replicate this finding.

4.5.1.4 NR3C1

In this group there was a great variation in terms of sample size, which ranged from 12 to 954 subjects. Most of studies were case-control, except for one longitudinal cohort study. Subjects were from hospital or the general population. Whole blood was the primary choice as the biological sample. However, one study used post-mortem brains. Most of studies tested for DNA purification and methylation validation. Pyrosequencing, followed by EpiTYPER analysis, were used to test the level of DNA methylation. Most studies did also tested gene expression and one did genotyping.

The major findings of the five studies were inconsistent with both *hypo*- and *hyper*-methylated CpG sites on *NR3C1* gene reported. However, studies with longitudinal study

designs, more reliable laboratory arrays and statistical analyses consistently showed that people with *NR3C1 hyper*methylation were more likely to report depression and/or depressive symptoms. (Nantharat, Wanitchanon, Amesbutr, Tammachote, & Praphanphoj, 2015; van der Knaap, van Oort, Verhulst, Oldehinkel, & Riese, 2015).

4.5.1.5 OXTR

Four articles met the criteria to explore the relationship between DNA methylation on *OXTR* gene and depression. Sample sizes ranged from 43 to 545 subjects. Most studies in this group of them were case-control designs (3/4), one was a longitudinal cohort study. Subjects were mainly recruited from the general population (91.7%), with the remaining being inpatients and controls recruited from advertisements (8.3%). Again, the whole blood was the primary tissue choice (75%), followed by saliva. DNA purification was tested by half of these studies. None tested for methylation validation. Bisulfite treatment and pyrosequencing were used for methylation arrays. All the studies did also test genotyping, while only one study examined gene expression.

Due to the defects in study design (i.e. small sample sizes), and lab factors (lack of DNA purification, or methylation validation), findings from these four studies are difficult to interpret. Two case-control studies had small sample sizes (N<100) found inconsistent results. The third case-control failed to apply valid lab arrays and found a non-significant association. The longitudinal cohort study with 353 subjects did not perform DNA purification or methylation validation.

4.5.1.6 Other candidate genes

Ten studies met the eligibility criteria for the relationship between DNA methylation on other candidate genes and depression. They included: *Glucose transporter 1 (GLUT1), Glucose transporter type 4 (GLUT4), Tryptophan hydroxylase 2 (TPH2), Angiotensin Converting Enzyme (ACE), Apolipoprotein E (APOE), Fatty acid desaturase 1 (Fads1), Fatty acid desaturase 2*

(Fads2), Elongation of very long chain fatty acid elongase 5 (Elovl5), Heterochromatin protein 1, binding protein 3 (HP1BP3), tetratricopeptide repeat domain 9B (TTC9B), FK506 binding protein 5 (FKBP5), monoamine oxidase A (MAOA), and DEP domain containing 7 (DEPDC7). This group of studies had a relatively small sample sizes ranging from 34 to 174 subjects. Case-control study designs were more frequently used, followed by longitudinal cohort design and twins' studies designs. Subjects were hospital inpatients or outpatients or from the general population, or from an existing population-based study. Two studies in this group did not mention the source of their samples. Most studies (7/10) used whole blood as the biological sample for arrays, the rest of the studies used saliva. Most of studies tested DNA purification, but only two examined methylation validation. Consistently, DNA methylation was measured via bisulfite conversion and pyrosequencing and then analyzed by EpiTYPER. A few studies did genotyping and/or gene expression. Notably, in one study the same outcomes were obtained from the initial study on MAOA and its replication study using an independent sample (Melas & Forsell, 2015; Melas, et al., 2013).

*Hyper*methylation was found in depressed cases in four studies (GLUT1, CpG 1,5,12 of ACE, Elovl5, FKBP5) (Kahl, et al., 2016; Zill, et al., 2012; Haghighi, et al., 2015; Hohne, et al., 2015); in contrast, *hypo*methylation was found in depression in four studies (Fads2, MAOA, DEPDC7) (Haghighi, et al., 2015; Melas, et al., 2013; Melas & Forsell, 2015; Cordova-Palomera, et al., 2015); and no significant difference in methylation levels was found between depressed patients and their healthy controls in two studies (*GLUT4, APOE*) (Kahl, et al., 2016; Chagnon, Potvin, Hudon, & Preville, 2015). In addition, one study found that patients who were both depression and suicidal had *hyper*methylation in the *TPH2* promoter region, as compared with in depression-only patients (Zhang, et al., 2015). Finally, one study found methylated *HP1BP3* and *TTC9B* predicted postpartum depression. In their replication analysis, these biomarkers were able to accurately segregate postpartum depression status in women, but the

prediction was in the opposite direction to that found in a pilot analysis (Kaminsky & Payne, 2014).

Due to the fact that many genes were studied in this group and most studies failed to apply strong study designs, or better laboratory and analyses factors in their execution, it is hard to weigh the value of their findings.

4.5.2 Treatment Studies

There were 11 articles published from 2013 to 2016, included in this analysis to explore the association between DNA methylation modification and treatment. The sample sizes in this category ranged widely from 11 to 554 subjects, with most of the studies having relatively small sample sizes (<100). Most of the studies' subjects were adults and seniors were from hospital-based cohorts, followed by population-based databases and general population. Study characteristics, such as study design, applying DNA purification, and genotyping and gene expression did not vary between small and large sample sized studies. The only tissue used in this group of studies was the whole blood. Most studies (72.7%, 8/11) tested for DNA purification, but not methylation validation. DNA methylation was mostly for tested by bisulfite conversion and pyrosequencing. Over half (6/11) of these studies also included genotyping and gene expression in their arrays.

More than half studies (6/11) did not identify significant methylation modifications related to antidepressants use, including the only study on *MAOA*, one of three studies on *SLC6A4*, three of five studies on *BDNF*, and one genome-wide study (Dell'Osso, et al., 2014; Davies, et al., 2014; Kang H.-J. , et al., 2013; Na, et al., 2016; Domschke, et al., 2015; Tadic, et al., 2014). One of five studies on *BDNF* and one of three studies on *SLC6A4* at 2 CpG sites hypermethylations were associated with antidepressant therapy (Booij, et al., 2015; Carlberg, et al., 2014). Whereas, the only study on *5-HTT* transcriptional control region indicated that its hypomethylation might impair antidepressant response in Caucasians patients with MDD, and

one of five studies on *BDNF* promoter region hypomethylations were linked to antidepressant treatment response in remitters compared with non-remitters (Kleimann, et al., 2015; Domschke, et al., 2014). Okada, et al. (2014) identified the positive correlation between pre-treatment DNA methylation on *SLC6A4 CpG3* and antidepressants in un-medicated patients (Okada, et al., 2014). In addition, one study tested the association between methylation modifications and classes of antidepressants, and demonstrated that lithium and valproate tended to decrease, though not significantly, DNA methylation level on *BDNF* promoter, compared with other classes of medications, such as antidepressants and atypical antipsychotics (Dell'Osso, et al., 2014). Only the small sample sized studies in this group did methylation validation. The only two negative correlations between methylation levels on *BDNF* and *5-HTT* gene and antidepressants were found by studies with relatively small samples (Kleimann, et al., 2015; Domschke, et al., 2014).

4.6 Discussion

To the best of our knowledge, this is the first review comprehensively exploring the role of DNA methylation in depression taking into account of both laboratory and analytic factors that could confound findings. A total of 57 articles were included in this review. The majority of the studies reviewed were recently published and were from developed countries. Whole blood and saliva samples were the most common tissues used in these analyses. Bisulfite conversion along with pyrosequencing, were widely used to test DNA methylation level. There was a high heterogeneity among the studies in terms of laboratory and statistical methodologies used and study designs. Larger sample size and laboratory verification (DNA purification and DNA methylation validation) are the major characteristics important for accurate results.

Due to the high level of heterogeneity in studies reviewed, qualitative analyses were used three subgroup analyses were done, including etiological-genome-wide studies, etiological-candidate genes studies, and treatment response studies.

4.6.1 Findings on Etiological-Whole-Genome Studies

We found that the level of DNA methylation were significantly different between depression patients and controls in the whole genome-wide association studies. Hypermethylations were observed in six studies on the following genes as zinc finger and BTB domain containing 20 (*ZBTB20*), Heterochromatin protein 1, binding protein 3 (*HP1BP3*), Tetratricopeptide repeat domain 9B (*TTC9B*), and Glutamate Ionotropic Receptor NMDA Type Subunit 2A (*GRIN2A*) (Davies, et al., 2014; Guintivano, Arad, Gould, Payne, & Kaminsky, 2014; Osborne, et al., 2016; Kaut, et al., 2015; Haghighi, et al., 2014; Walker, et al., 2016).

ZBTB20 exists hippocampal neurons and cerebellum granule cells (Mitchelmore, et al., 2002) and plays a role in many processes including neurogenesis, glucose homeostasis, and postnatal growth (ZBTB20 gene, 2017). It may also have an impact on the development and regionalization of the human hippocampus, which has been found to be related to depression by many studies (Sheline, Mittler, & Mintun, 2002; Bremner, et al., 2000; Sheline, Wang, Gado, Csernansky, & Vannier, 1996).

Both *HP1BP3* and *TTC9B* are linked to estrogen signaling. *HP1BP3* is highly expressed in brain and related to a number of physical and behavioral phenotypes for mice, such as dwarfism, impaired bone mass, impaired maternal behavior, and anxiety (Garfinkel, et al., 2015; Garfinkel, et al., 2016). Lower *HP1BP3* has been found to be associated with postpartum depression and Alzheimer's disease in humans (Guintivano, Arad, Gould, Payne, & Kaminsky, 2014; Neuner, et al., 2016). *TTC9B* has been identified to be related to gonadal hormones (Cao, Iyer, & Lin, 2006) and may be linked to hippocampal synaptic plasticity, which is critical for hippocampal long-term potentiation and depression (Gerges, et al., 2004). These markers in peripheral blood may indicate estrogen-mediated epigenetic changes in hippocampus and in turn potentially raise the vulnerable phenotypes based on their actions in brain (Guintivano, Arad, Gould, Payne, & Kaminsky, 2014).

The *GRIN2A* gene provides instructions for making a protein called glutamate receptor subunit epsilon-1- in human encoded GluN2A, which is one component (subunit) of a subset of NMDA receptors. They are involved in normal brain development, changes in the brain in response to experience (synaptic plasticity), learning, and memory (GRIN2A gene, 2017). Methylation modifications in *GRIN2A* may play a key role in determining the function of NMDA receptors. Generally, gene promoter region methylation could repress the gene expression, but the methylation on gene body can be positively correlated with expression activity (Hellman & Chess, 2007). This suggests that the hypermethylation of the GRIN2A gene body may result in the overexpression of NR2A, and thus promote vulnerability for MDD via up-regulating NMDA receptor-dependent glutamatergic signaling (Calabrese, et al., 2012).

*Hypo*methylations among depression patients were also observed on the following genes: WD repeat domain 26 (WDR26), 5-hydroxymethylcytosine (5-hmc), and 5-methylcytosine (5-mc) (Numata, et al., 2015; Kaut, et al., 2015; Cordova-Palomera, et al., 2015; Khulan, et al., 2014; Nagy, et al., 2015; Tseng, et al., 2014). Consistent with our findings on WDR 26, previous studies have found that the hypomethylation of WDR26 in depressed individuals may be related to lower gene expression levels (Pajer, et al., 2012). Additionally, the decreased blood transcription levels of WDR26 were associated with depression-related phenotypes (Pajer, et al., 2012; Karanges, et al., 2013; Wray, et al., 2012; Lee, et al., 2005).

5-Methylcytosine (5-mc) is a methylated form of the DNA base cytosine, which could be involved in the regulation of gene transcription. Its presence is important for the maintenance of the active chromatic state and for neurogenesis at non-promoter CpG islands (Wu, et al., 2010), and is associated with stable and long-term transcriptional silencing of promoters (Butler, 2009). 5-mc is also found as the critical mechanism mediating genomic imprinting. This process has been identified to be a key for normal development, and its abnormal imprinting can result in disorders such as Prader-Willi, Angelman, and Beckwith-Wiedemann syndrome (Butler, 2009).

5-Hydroxymethylcytosine (5-hmc) is a product of conversion of 5-mc. It is related to the regulation of gene expression and prompting DNA demethylation. The three Ten-eleven translocation (TET) enzymes oxidize each step in the demethylation of 5-mc. 5-mc is converted to 5hmC, then 5-formylcytosine (5fC), then 5-carboxylcytosine (5caC), each by TET1-3 (Ito, et al., 2011). Reduced level of TET1 and subsequently 5hmC cause impaired self-renewal of stem cells (Freudenberg, et al., 2012).

Notably, inconsistent results were identified within the same studies among different subgroups, for example, different sexes (Byrne, et al., 2013), processes (e.g. brain development, tryptophan metabolism, lipoprotein) (Uddin, et al., 2011), tissues (white blood cells, brain and sperm) (Oh, et al., 2015), or between pilot and replication studies (Sabunciyan, et al., 2012).

4.6.2 Findings on Etiological-Candidate Genes Studies

For candidate genes studies, we selected 11 studies on *BDNF* gene, with the majority (10/11) of them indicating that *BDNF* hypermethylation links with depression. Most of studies had the relatively large sample sizes and examined DNA purification. This is consistent with recent reviews on *BDNF* and depression (Chen, Meng, Pei, Zheng, & Leng, 2017; Bakusic, Schaufeli, Claes, & Godderis, 2017). Chen et al. (2017) indicated that more than half of the studies showed an increased BDNF methylation in depressed patients. Bakusic et al. (2017) concluded in their review that hypermethylation was consistently found in MDD subjects across three reviewed studies (Bakusic, Schaufeli, Claes, & Godderis, 2017). The *BDNF* gene provides instructions for making a protein found in the brain and spinal cord, and promotes the survival of nerve cells (neurons). It is actively involved in the growth, maturation, maintenance of these neurons, and regulation of synaptic plasticity, which is important for learning and memory (BDNF gene, 2017; Malcangio & Lessmann, 2003). It is reported that changes in the methylation level of the *BDNF* promoter is associated with its lower expression in prefrontal cortex (Zheleznyakova, Cao, & Schioth, 2016) and activity in the hippocampus in animal studies (Lee

& Kim, 2010). Similar decrease in BDNF levels were also found in serum and plasma in MDD patients, thus it is hypothesized that MDD is related to impaired neuronal plasticity (Lee & Kim, 2010).

Positive associations between *SLC6A4* methylation modifications and depression have also been identified in many studies in this review and previous reviews (Chen, Meng, Pei, Zheng, & Leng, 2017; Bakusic, Schaufeli, Claes, & Godderis, 2017). All longitudinal studies in this review and studies with more comprehensive considerations of lab and statistical work consistently found depression patients had *SLC6A4* hypermethylation compared to controls. *SLC6A4* gives instructions for making a protein in the brain involving in the regulation of serotonergic signaling by transporting 5-HT from synaptic spaces into presynaptic neurons (Tao-Cheng & Zhou, 1999) and the regulation of emotional behaviors (Meyer-Lindenberg, 2009). The alterations of *SLC6A4* play an important role in the brain development and function in human (Booij, Wang, Levesque, Tremblay, & Szyf, 2013). It has been hypothesized that DNA hypermethylation may result in the reduction of SLC6A4 expression and 5-HT reuptake, which in turn increase the vulnerability to affective disorder at critical stages of development (Gaspar, Cases, & Maroteaux, 2003; Olsson, et al., 2010).

The results of *NR3C1, OXTR,* and other target genes studies were controversial. Both hypo- and hyper- methylation levels were noted in depressive patients compared to controls. No significant associations between DNA methylation on these genes and depression were also reported by some studies. The similar findings were also found by previous reviews (Chen, Meng, Pei, Zheng, & Leng, 2017; Bakusic, Schaufeli, Claes, & Godderis, 2017). *NR3C1* is the receptor of cortisol and glucocorticoids bind. It regulates gene transcriptions and links to the development, metabolism, and immune response (Lu, et al., 2006; Rhen & Cidlowski, 2005). *OXTR* is a receptor of the hormone and neurotransmitter oxytocin (Gimpl & Fahrenholz, 2001; Zingg & Laporte, 2003). It presents in the central nervous system and plays an important role in modulating various behaviors, such as stress and anxiety, social memory and recognition, sexual

and aggressive behaviors, bonding/affiliation and maternal behavior (Caldwell & Young, 2006; Kiss & Mikkelsen, 2005; Veenema & Neumann, 2008). We found some of NR3CI and OXTR studies reviewed here had limitations in terms of type of study design, sample size, and range of laboratory work and statistical analyses. Due to the high heterogeneity across studies, this review could not provide more conclusive results on these genes in terms of relationships between DNA methylation modifications on these genes and depression.

4.6.3 Findings on Treatment Studies

Inconsistent results were also identified in treatment studies on anti-depressant therapy in this review. Consistently, another recent review on DNA methylation and clinical response to anti-depressant medication in MDD patients was unable to find consistent support for such a relationship (Lisoway, Zai, Tiwari, & Kennedy, 2017). Both increased and decreased DNA methylation levels on *SLC6A4* and *BDNF* genes were associated with the use of anti-depressant medications, whereas *MAOA* methylation modification was not linked to anti-depressant response. The relationship between antidepressant treatment and DNA methylation of certain genes has been reported, i.e. *BDNF* DNA methylation modification was associated with the decreased gene expression, which can lead to MDD (Duman, 2002). The use of antidepressants can restore the decreased *BDNF* up to the normal level and alleviate depressive symptoms (Duman, 2002; Lee & Kim, 2010). Inconsistencies across all these findings may be explained by different ethnicities, duration of treatments, and pharmacogenetic heterogeneities (Kang H. , et al., 2013; Domschke, et al., 2014). It was suggested to investigate anti-depressant response across different treatment modalities since the level of DNA methylation may be altered during the treatment (Roberts, et al., 2014).

4.6.4 Strengths and Limitations

This review was the first study to apply the evidence-based approach to summarize an overall profile of the relationship between DNA methylation and depression. We critically

reviewed various study characteristics that can significantly impact on this association, including study design, study population, targeted gene/genome, methylation arrays, type of tissue, DNA purification, methylation validation, appropriate statistical methods, and the consideration of downstream analyses, e.g. genotyping and gene expression. We found that DNA methylation level was associated with depression, including both hypo- and hyper-methylations. Many genes are involved in the epigenetic changes of depression. In addition, study characteristics are also critical in this exploration. We suggest that results from more carefully crafted studies were more reliable and more likely to be replicated.

However, there are several limitations of this review to be noted. First, this review aimed to provide an overview of the cutting edge findings on the relationship between DNA methylation and depression. Therefore, all eligible studies with a wide range of genomic coverage, i.e. targeted genes or whole-genome and different types of study designs were included. No pooled results were made to simply estimate this relationship, as many factors were involved. Second, although we used a systematic approach and subgroup analysis to retrieve all relevant studies and analyze more homogeneous studies, heterogeneity still exist in this review. Different types of tissues, study designs, depressive phenotypes (including MDD, depressive symptoms, postpartum depression, and post-stroke depression), comparison groups (depressive patients vs. healthy controls, severe patients vs. remitted patients), analytic methods, and sample sizes could be sources of heterogeneity and may lead to inconsistent results. Finally, most of the studies in this review were cross-sectional. Given that DNA methylation level is dynamic and potentially reversible, and can be affected by a number of environmental factors (such as chemical exposure, drug use, stress, aging, gender, diet, and lifestyle), cross-sectional DNA methylation measures may not be able to reveal the true relationship between this epigenetic modification and depression.

4.7 Conclusion

Even though this review identified a high heterogeneity across studies on the relationship between DNA methylation and MDD, we did find in this overview a rising tide in the recognition of DNA methylation modification in depression, and generally that DNA methylation changes are associated with the disease. But there are some inconsistencies across studies because of the wide range of study characteristics, which directly influence the results. Most of studies have applied the widely acceptable lab techniques and statistical analyses for this relationship, which makes the pooled results more likely to reach a consistent findings. Future epigenetic studies should also adopt longitudinal study designs to trace the change of methylation levels at different phases of disease, for example pre- and post-treatment stages. To allow for a systematic comparison of studies there should be an agreed upon consistent set of standards involving a minimum set for items for the execution and reporting of methylation studies similar to what is required for the reporting of clinical trials and systematic reviews and meta-analysis (Schulz, Altman, Moher, & The CONSORT Group, 2010; Moher, Liberati, Tetzlaff, & Altman, 2009). This would advance the field and provide a firm base for evidence on the relationship between DNA methylation and major depressive disorder.

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Appendix 1 Search Strategy

PubMed

((((((((depressive disorder [MeSH Terms]) OR major depressive disorder [Text Word]) OR major depression [Text Word]) OR unipolar depression [Text Word]) OR depression [Text Word]) OR depressed [Text Word]) OR depressive [Text Word])) AND ((((DNA methylation [MeSH Terms]) OR methylation [Text Word]) OR epigenetic*[Text Word])) filters: Humans

MEDLINE

(Mesh (depressive disorder) OR (major depressive disorder) OR (major depression) OR (unipolar depression) OR depression OR depressed OR depressive) AND (mesh (DNA methylation) OR methylation OR epigenetic*)

Web of Sciences

#1 TS="DNA methylation" OR TS=methylation OR TS=epigenetic*
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Timespan=All years
#2 TS="depressive disorder" OR TS="major depressive disorder" OR TS="major depression" OR TS="unipolar depression" OR TS=depression OR TS=depressed OR TS=depressive
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Timespan=All years
#3 #2 AND #1

EMBASE

1 ("DNA methylation" or "methylation" or epigenetic*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
2 limit 1 to human
3 1 and 2
4 ("depressive disorder" or "major depressive disorder" or "major depression" or "unipolar depression" or depression or depressed or depressive).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
5 limit 4 to human
6 4 and 5
7 3 and 6

Cochrane Library

#1 MeSH descriptor: [Depressive Disorder] explode all trees
#2 "major depressive disorder" or "major depression" or "unipolar depression" or "depressed" or "depression" (Word variations have been searched)
#3 #2 or #1 or "depressive" (Word variations have been searched)

#4 MeSH descriptor: [DNA Methylation] explode all trees
#5 methylation or epigenetic* (Word variations have been searched)
#6 #4 or #5
#7 #3 and #6

Appendix 2 Data References

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Appendix 3 Characteristics Table

ID	1	2	3
First Author	Numata	Bayles	Booij
Year	2015	2013	2015
Country	Japan	Australia	Canada
Age	cases: 44.2±15.2; controls: 42.4±12.3	cases: 39 ± 2; controls: 42 ± 2	cases: 40.3 ± 9.5; controls: 35.3 ± 12.8
Sample Size	63 (39 discovery and 24 replication)	106	69
Cases	32 (20 discovery and 12 replication)	36 (18 males/18 females)	33 (23 females/ 10 males)
Diagnostic standard	DSM-IV	DSM-IV	DSM-IV, SCID interview confirmed, BDI-II
Controls	31 (19 discovery and 12 replication)	70 (47 males/23 females)	36 (21 females/ 15 males)
Study design	discovery-replication cohort (case-control)	case-control	case-control
Cohorts	Hospital-based cases and matched controls from Japanese	media advertised recruitment	mental health service of hospital
Biological Sample	Peripheral blood	blood (leukocytes)	peripheral cells (whole blood DNA)
Purification of DNA extraction	Not mentioned	not mentioned	Not mentioned
DNA methylation methods/ Kits	Bisulfite conversion EZ DNA methylation Kit (ZYMO research), Infinium Human Methylation 450 Beadchips	Bisulfite conversion, PCR and sequencing; EpiTYPER methylation analysis	Bisulfite conversion, pyrosequencing; PyroMark Q24 Software
Candidate genes vs. genome	Genome (485,764 CpG inlands)	Two regions in Promoter methylation of SLC6A4	SLC6A4 promoter, targeted CpG sites 5–15
Methylation Validation	Next generation sequencing (R2=0.81)	two methodologies	not mentioned
Genotyping	No	No	Yes
Gene expression	Yes	No	Yes
Analytical method of methylation difference	Mann-Whitney U test	two-way ANOVA	linear regression
Major findings	363 CpG sites demonstrated lower DNA methylation in MDD patients than in controls. 18 MDD-associated DNA methylation markers to discriminate cases from controls	No significant differences between MDE cases and controls in terms of the pattern of methylation of the SLC6A2 promoter. Antidepressant treatment did not change the result.	MDD diagnosis was not significantly associated with DNA methylation. Patients with SSRIs had greater methylation levels at 2 CpG.
Category	Genome	SLC6A4	SLC6A4; treatment response

ID	4	5
First Author	Carlberg	Dell'Osso
Year	2014	2014
Country	Austria	Italy
Age	cases: 46.03 ± 1.07; controls: 31.8 ± 0.55	Age matched
Sample Size	554 (207 MDD, 59 BD, 278 control)	87
Cases	207	43
Diagnostic standard	DSM-IV	DSM IV
Controls	278	Age-matched, 44
Study design	case-control	case-control
Cohorts	not mentioned	Not mentioned
Biological Sample	peripheral blood mononuclear cells	peripheral blood mononuclear cells (PBMCs)
Purification of DNA extraction	PUREGENE DNPurification Kit	Not mentioned
DNA methylation methods/ Kits	Bisulfite convertion, EZ-96 DNA Methylation Kit. Used methylation-specific quantitativePCR	bisulfite convertion, PCR and sequencing
Candidate genes vs. genome	BDNF exon I promoter	BDNF exon I promoter, 17CpG sites.
Methylation Validation	not mentioned	Bisulfite-modified universal unmethylated DNA as negative control
Genotyping	Yes	No
Gene expression	No	No
Analytical method of methylation difference	Pearson's correlation coefficient test	ANOVA followed by Bonferroni's post-hoc test
Major findings	BDNF exon I promoter significantly increased in MDD. Anti-depressant therapy associated with increase methylation.	Higher level of BDNF DNA methylation: MDD statistical significance compared with BD-I; Overall lithium and valproate tended to decrease, even though not significantly, DNA methylation level at BDNF gene promoter. However, mood stabilizers did not seem to affect DNA methylation.
Category	BDNF; treatment response	BDNF; treatment response

ID	6	7	8
First Author	Davies	Fuchikami	Frodl
Year	2014	2011	2015
Country	UK & Australia	Japan	Ireland
Age	Age matched	cases: 45.6 ± 12.5; controls: 42.3 ± 9.6	cases: 41.6 ± 10.8; controls: 35.6 ± 13.0
Sample Size	454 - A. 50 twin pairs: A. 50 MZ twins [27MZT pairs(UK) + 23 pairs(AU)]; B. replication: 354 age-matched [118 MDD, 236 control (female)]	38	60
Cases	B. replication: 118 MDD	20 (8 males/ 12 females)	25
Diagnostic standard	DSM IV	DSM IV	DSM IV
Controls	B. replication: 236 control	18 (10 males/ 8 females)	35
Study design	A. twin study; B case-control	case-control	case-control
Cohorts	UK: TwinsUK Registry. AU: Australian Twin Registry.	control recruited by advertisement	Patient: hospital based. Control from local community
Biological Sample	Whole blood samples	peripheral blood	peripheral blood
Purification of DNA extraction	Nucleon Genomic DNA Extraction Kit BACC3 (UK) ; salt extraction method (AU)	DNeasy Blood &Tissue Kits	Not mentioned
DNA methylation methods/ Kits	Methylated DNA immunoprecipitation combined with ultra-deep sequencing (MeDIP-seq) (enrichment for methylated regions)	Bisulfite convertion using EZ DNA methylation kit	Bisulfite convertion, pyrosequencing, PyroMark Q24
Candidate genes vs. genome	Genome-wide MeDIP-Sequencing. 4 DMRs for replication.	BDNF gene , 2 CpG islands(I and IV)	SLC6A4 promoter CpG5-15
Methylation Validation	Not mentioned	Not mentioned	The mean of methylation percentage from sites 5–15
Genotyping	No	No	No
Gene expression	Yes	No	No
Analytical method of methylation difference	linear mixed effect model	Hierarchical clustering analyses	Regression analysis
Major findings	Both AU&UK did not identify DMR of genome-wide significance. MDD is hypermethylation on coding region ZBTB20. Meta-analysis: 17 DMRs of genome-wide significance ZBTB20, AGTPBP1, TBC1D8 and CLSTN1 for replication. Case-control: increased methylation. Methylation changes do not relate to anti-depression use.	Significant methylation difference in CpG I, not in IV.	Diagnosis not significantly associated with methylation.
Category	Genome; treatment response	BDNF	SLC6A4

ID	9	10
First Author	Iga	Januar
Year	2016	2015
Country	Japan	France
Age	cases: 45.0 ± 13.1; controls: 42.2 ± 12.1	cases: 72.0 ± 4.5; controls: 71.4 ± 4.5
Sample Size	57	1024
Cases	28 (8 males/ 20 females)	773
Diagnostic standard	DSM IV	DSM IV, Late-life depression - CES-D⩾16 or current MDD.
Controls	29 (8 males/ 21 females)	251
Study design	case-control	case-control
Cohorts	both health and control form hospital	A longitudinal study of general population
Biological Sample	leukocytes	Buccal tissue
Purification of DNA extraction	QIAamp DNA Blood Maxi Kit	Not mentioned
DNA methylation methods/ Kits	Bisulfite convertion, pyrosequencing, EpiTect Plus DNA Bisulfite Kit	Bisulphite convertion, EZ-96 DNA Methylation-Lightning MagPrep
Candidate genes vs. genome	5HTT promoter region, 9 CpGs	BDNF PROMOTER 1 AND IV
Methylation Validation	The methylation percentage at each CpG region was quantified in duplicate using PyroMark Q24	not mentioned
Genotyping	Yes	Yes
Gene expression	Yes	No
Analytical method of methylation difference	Unpaired t-test	Linear regression
Major findings	Mean methylation level was significantly increase in patients compared with controls. No significant difference in single CpG site.	Depression at baseline and chronic late-life is associated with higher BDNF methylation, CpG3,4,5.
Category	SLC6A4	BDNF

ID	11	12
First Author	Kahl	Kang
Year	2016	2015
Country	German	Korea
Age	cases: 41.8 ± 11.1; controls: 43.2 ± 13.1	50.8 ± 9.7
Sample Size	70	309 at baseline, 244 followed-up
Cases	52 (37 of which finished treatment)	Baseline: 74 diagnosed with depression; Follow-up: 44 diagnosed
Diagnostic standard	DSM IV	DSM IV
Controls	18	Not applicable
Study design	case-control	Longitudinal study, followed at 1 year
Cohorts	Case were inpatients with MDD treated; Controls from university announcements	Hospital based, all women with breast cancer undergoing breast surgery
Biological Sample	Genomic DNA, frozen EDTA-blood	leukocyte DNA
Purification of DNA extraction	QIAamp DNA Blood Mini Kit	QIAamp DNA Blood Mini Kit
DNA methylation methods/ Kits	Bisulfite conversion, PCR and sequencing. Sodium-bisulfite using the EpiTect Bisulfite Kit); Sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit.	Bisulfite conversion using EpiTech Bisulfite Kit, Pyrosequencing using the PSQ 96M System
Candidate genes vs. genome	Core promoter regions of GLUT1 and GLUT4.	BDNF CpG1-9 (-612 -- -463)
Methylation Validation	not mentioned	not mentioned
Genotyping	No	Yes
Gene expression	No	No
Analytical method of methylation difference	Mixed linear models	T-test and multivariate logistic regression models
Major findings	Increased methylation of GLUT1 in MDD. Not difference found in GLUT4.	Higher methylation percentage at CpG9 with depression, both 1 week and 1 year, after breast cancer.
Category	Others	BDNF

ID	13	14	15
First Author	Kang	Kang	Kaut
Year	2015	2013	2015
Country	South Korea	Korea	Netherlands
Age	72.8 ± 5.9	54.9 ± 14.9	cases: not mentioned; controls: 78.8 ± 14.2
Sample Size	631 without depression at baseline (521 of which were followed-up)	108 MDD patients	12
Cases	86/521 were identified depression at follow-up	Not applicable	6
Diagnostic standard	GMS AGECAT; severity - Geriatric Depression Scale (GDS)	DSM IV	DSM III
Controls	Not applicable	Not applicable	6
Study design	Longitudinal, followed-up for 2 years.	Longitudinal, Baseline, 12-week treatment with antidepressants.	Pilot-replcation, 5 genes selected for replication.
Cohorts	A community-based prospective survey of late-life psychiatric morbidity	Hospital based	Netherlands Brain Bank
Biological Sample	Venous blood, leukocyte	Leukocytes	Post-mortem brain, HIP, PFC tissue
Purification of DNA extraction	QIAamp DNA Blood Mini Kit	QIAamp DNA Blood Mini Kit	Not mentioned
DNA methylation methods/ Kits	Bisulfite conversion using EpiTech Bisulfite Kit, Pyrosequencing using the PSQ 96M System	Bisulfite conversion using EpiTech Bisulfite Kit, Pyrosequencing using the PSQ 96M System	Bisulfite conversion with a ZymoResearch bisulfite kit and Iminium Human Methylation 450 K bead arrays
Candidate genes vs. genome	BDNF	SLC6A4. −479 and −350, 7 CpG sites.	Epigenome-wide. Selected genes for replication.
Methylation Validation	not mentioned	not mentioned	Pyrosequencing, Pyromark Q24 Kit
Genotyping	Yes	No	No
Gene expression	No	No	No
Analytical method of methylation difference	T-test and multivariate logistic regression models	Association between methylation status and treatment outcome: Pearson's correlation tests	Mann-Whitney U test
Major findings	Higher BDNF methylation was associated with depression and severe depressive symptoms.	SLC6A4 methylation status as a marker for childhood adversities among MMD; but was not associated with treatment outcomes.	11 genes in hippocampus and 20 genes in prefrontal cortex revealed differential methylation. In replication, GRIN2A was found hypermethylated in both tissues and single CpG level.
Category	BDNF	Treatment response	Genome

ID	16	17
First Author	Kim	Kim
Year	2013	2013
Country	South Korea	South Korea
Age	64.5 ± 9.5	64.5 ± 9.6
Sample Size	286 stroke patients at baseline, 222 of which were followed-up for 1 year.	286 stroke patients at baseline (222 of which were followed-up for 1 year)
Cases	Poststroke depression (PSD), 80 with depression at baseline	Poststroke depression (PSD); baseline: 80 any PSD, 32 major PSD. Follow-up: 53 any, 21 major.
Diagnostic standard	DSM IV (depression: major/ minor depressive disorder)	DSM IV (depression: major/ minor depressive disorder)
Controls	Not applicable	Not applicable
Study design	Longitudinal, followed-up for 1 year after stroke.	Longitudinal, followed-up for 1 year after stroke
Cohorts	Post-stroke cohort, hospitalized	Post-stroke cohort, hospitalized
Biological Sample	Venous blood, leukocytes	Venous blood, leukocytes
Purification of DNA extraction	QIAamp DNA Blood Mini Kit	QIAamp DNA Blood Mini Kit
DNA methylation methods/ Kits	Bisulfite conversion using EpiTech Bisulfite Kit, Pyrosequencing using the PSQ 96M System	Bisulfite conversion using EpiTech Bisulfite Kit, Pyrosequencing using the PSQ 96M System
Candidate genes vs. genome	BDNF promoter region. Between -694 and -577, 7 CpG cites.	SLC6A4 promoter region between -479 and -350, including 7 CpG sites.
Methylation Validation	not mentioned	not mentioned
Genotyping	Yes	Yes
Gene expression	No	No
Analytical method of methylation difference	Multivariate logistic regression model	Multivariate logistic regression model
Major findings	Prevalent, persistent, and incident PSD have higher BDNF methylation status. CpG site 6 significantly associated with incident PSD. CpG 1,2,4 higher methylation.	Higher SLC6A4 methylation status was independently associated with major PSD at both baseline and follow-up.
Category	BDNF	SLC6A4

ID	18
First Author	Kim
Year	2015
Country	South Korea
Age	18-85
Sample Size	969 Acute Coronary Syndrome (711 of which were followed-up). At baseline, 378 depressive disorder (255 of which randomised to a 24-week trial)
Cases	Trail: 127 received escitalopram
Diagnostic standard	DSM IV (depression : major/ minor depressive disorder)
Controls	Trail: 128 placebo, 123 conventional treatment
Study design	Longitudinal & random trial
Cohorts	Korean Depression in ACS (K-DEPACS) study, hospitalized patients.
Biological Sample	Venous blood, leukocyte DNA
Purification of DNA extraction	QIAamp DNA Blood Mini Kit
DNA methylation methods/ Kits	Bisulfite conversion using EpiTech Bisulfite Kit, Pyrosequencing using the PSQ 96M System
Candidate genes vs. genome	BDNF exon VI , between -612 and -463
Methylation Validation	not mentioned
Genotyping	No
Gene expression	No
Analytical method of methylation difference	T-test and multivariate logistic regression models
Major findings	At baseline higher methylation percentage in MDD compared with no depressive. Higher BDNF methylation associated with prevalent depressive disorder at baseline and follow-up.
Category	BDNF

ID	19
First Author	Kimmel
Year	2016
Country	USA
Age	30.68 ± 6.32; 33; 32.7 ± 0.018
Sample Size	3 prospective cohort (two cohorts -women with previous diagnoses of mood disorder; one cohort -psychiatrically healthy women): 51/61/240, postpartum depression.
Cases	Not applicable
Diagnostic standard	DSM-IV
Controls	Not applicable
Study design	cohort
Cohorts	The Women's Mood Disorders Centre, Gene Expression Omnibus (GEO), and Franconian Maternal Health Evaluation Studies (FRAMES)
Biological Sample	Blood
Purification of DNA extraction	Not mentioned
DNA methylation methods/ Kits	Bisulfite conversion by EZ DNA Methylation Gold Kit and pyrosequencing using PyroMark MD system
Candidate genes vs. genome	OXTR
Methylation Validation	Not mentioned
Genotyping	Yes
Gene expression	Yes
Analytical method of methylation difference	Linear regression
Major findings	A PPD specific DNA methylation negatively correlates in the region with serum estradiol levels. Estradiol levels and OXTR DNA methylation exhibited a significant interaction to associate with the ratio of allopregnanolone to progesterone.
Category	OXTR

ID	20	21
First Author	Kleimann	Na
Year	2015	2014
Country	German	Korean
Age	47 ± 16.5	cases: 41.60 ± 11.8; controls: 40.72 ± 14.20
Sample Size	11 patients, treatment-resistant MDD (4 in remission, 6 in response)	117
Cases	Not applicable	45 (11 males/ 34 females)
Diagnostic standard	DSM IV	DSM IV, Anis I diagnosis
Controls	Not applicable	72 (21 males/51 females)
Study design	Perspective study	Case-control
Cohorts	not mentioned	Hospital outpatient
Biological Sample	Whole EDTA blood	Peripheral blood
Purification of DNA extraction	QIAamp DNA Blood Mini Kit	EZ DNA Methylation-Gold kit
DNA methylation methods/ Kits	Bisulfite convertion using EpiTect Bisulfite Kit, PCR and sequencing using BigDye Terminator Cycle Sequencing Kit	Bisulfite conversion, pyrosequencing, using PyroMark ID system with the Pyro Gold reagents kit
Candidate genes vs. genome	BDNF promoter exon I, IV, VI.	NR3C1 promoter, 5 CpG sites
Methylation Validation	not mentioned	not mentioned
Genotyping	No	No
Gene expression	No	No
Analytical method of methylation difference	Mixed linear models	Analysis of covariance (ANCOVA)
Major findings	Remitters had a significantly lower mean promoter methylation rate than non-remitters, especially exon I.	MDD had significantly lower methylation than healthy controls at 2 CPG sites (CpG 3,4)
Category	Treatment response	NR3C1

ID	22	23
First Author	Na	Nantharat
Year	2016	2015
Country	Korea	Thailand
Age	cases: 42.52 ± 11.42; controls: 40.34 ± 13.94	cases: 48.63 ± 8.43; controls: 48.00 ± 12.08
Sample Size	130	62
Cases	65 (11 males/ 54 females) recurrent MDD	29 (9 males /20 females)
Diagnostic standard	DSM IV, Anis I diagnosis	diagnosed by psychiatrists
Controls	65 (15 males/ 50 females)	33 (7 males /26 females)
Study design	Case-control	case-control
Cohorts	Hospital based	hospital based
Biological Sample	Peripheral blood samples	Peripheral blood samples
Purification of DNA extraction	Not mentioned	Illustrate blood genomic Prep Mini Spin Kit
DNA methylation methods/ Kits	Bisulfite conversion, pyrosequencing, using PyroMark ID system with the Pyro Gold reagents kit	Bisulfite pyrosequencing. PyroMark LINE-1 kit
Candidate genes vs. genome	BDNF promotor region at 4 CpG sites (CpG1 = − 675, CpG2 = − 682, CpG3 = − 686, and CpG4 = − 688)	NR3C1 promoter, 7 CpG sites.
Methylation Validation	not mentioned	Sodium bisulfite treatment using the EpiTect Bisulfite Kit
Genotyping	Yes	No
Gene expression	No	Yes
Analytical method of methylation difference	General linear model	Unpaired t-test
Major findings	Patients with MDD had significantly higher rates of methylation at CpG2 and CpG4 than healthy controls. No difference in naïve or on-medication patients.	Higher methylation levels at CpG 7 in MDD in female but no in male.
Category	BDNF; treatment response	NR3C1

ID	24	25
First Author	Okada	Philibert
Year	2014	2008
Country	Japan	USA
Age	cases: 40.3 ± 10.3; controls: 40.3 ± 10.5	males: 42.4 ± 8.5; females: 38.8 ± 6.8
Sample Size	100	192 (96 males /96 females)
Cases	50 (27 males/ 23 females)	Not applicable
Diagnostic standard	DSM IV	AAGA-II, DSM IV. Lifetime and current MD.
Controls	50 (27 males/ 23 females)	Not applicable
Study design	Case-control. Of 50 patients, 40 were followed-up for 6 weeks after treatment.	Longitudinal study
Cohorts	Control recruited by advertisement	Adoptees from Iowa Adoption Studies (IAS).
Biological Sample	Peripheral blood	Lymphoblast cell lines
Purification of DNA extraction	DNeasy Blood and Tissue Kits	DNA was prepared for the cell lines using cold protein precipitation
DNA methylation methods/ Kits	Bisulfite conversion using EZ DNA methylation kits; analyzed using a Mass ARRAY Compact System; methylation ratios were calculated using EpiTYPER software.	Bisulfite conversion, methylation ratios calculated by using a MassARRAY
Candidate genes vs. genome	SLC6A4 , 81 CpG	SLC6A4, 71 CpG residues
Methylation Validation	not mentioned	not mentioned
Genotyping	Yes	Yes
Gene expression	No	Yes
Analytical method of methylation difference	Mann-Whitney U test for methylation differences; Wilcoxon signed-rank test for antidepressant treatment	ANOVA
Major findings	Unable to distinguish between healthy controls, or between unmedicated patients and medicated patients No significant difference between unmedicated patients and healthy controls at any CpG unit. Pre-treatment methylation rate (CpG3) of SLC6A4 is associated with therapeutic responses to antidepressants in unmedicated patients with MD.	There is a trend to higher methylation with lifetime history of major depression, compared with alcohol dependence.
Category	SLC6A4; treatment response	SLC6A4

ID	26	27
First Author	Sabunciyan	Tseng
Year	2012	2014
Country	USA	Taiwan
Age	cases: 44.6 ± 10.6; controls: 48.2 ± 10.5	Severe MDD patients: 45.9±13.2; remitted MDD: 49.2±13.2; controls: 48.3±11.1
Sample Size	Pilot 65, blood 60, Replication 29	74
Cases	Pilot 39; blood 30; replication 16	49 (24 severe MDD, 25 remitted MDD)
Diagnostic standard	DSM IV	DSM IV
Controls	Pilot 26; blood 30, replication 13	25
Study design	Pilot-validation-replcation (brain, blood) , 17 regions for validation	Age-gender matched case-control, 4 compare group
Cohorts	Donated by the Stanley Medical Research Institute	not mentioned
Biological Sample	Postmortem frontal cortex; lymphoblastoid cell lines; postmortem brain	Peripheral leukocytes,
Purification of DNA extraction	MasterPure DNA Purification kit	not specified ("a commercial kit")
DNA methylation methods/ Kits	CHARM assay platform	ELISA-based for global DNA methylation profiling. MethylFlash methylated DNA quantification kit (for 5-mc), MethylFlash hydroxymethylated DNA quantification kit (for 5-hmc)
Candidate genes vs. genome	Genome-wide. 17 regions for validation	Genome wide, 5-hmc and 5-mc levels
Methylation Validation	Bisulfite pyrosequencing: Epitect Kit	not mentioned
Genotyping	No	No
Gene expression	Yes	No
Analytical method of methylation difference	T-test	Two-tailed t-test
Major findings	PRIMA1 significantly increased methylation in MDD in pilot, but not in replication.	Lower levels of 5-hmc and 5-mc in severe MDD than controls; no difference among severe and remitted patient.
Category	Genome	Genome

ID	28	29
First Author	Zhang	Zill
Year	2015	2012
Country	China	German
Age	MDD+suicide 14-71 (36.8±10.2); MDD 13-70 (35.3±11.0)	cases: 21-76 (45.8 ± 14.3); controls: 19-73 (46.2 ± 14.2)
Sample Size	125	162
Cases	50 (23 males /27 females) MDD + suicide	81 (30 males/ 51 females)
Diagnostic standard	DSM IV	DSM IV
Controls	75 (35 males /40 females) MDD	81 (40 males/ 41 females)
Study design	Case-control	Case-control
Cohorts	Hospital outpatient	Cases from inpatients; controls from general population
Biological Sample	Venous blood	Peripheral leukocytes.
Purification of DNA extraction	Wizard Genomic DNA Purification kit	Invisorb Blood Giga Kit
DNA methylation methods/ Kits	Bisulfite conversion, methylation-specific PCR	Bisulfite conversion, PCR and sequencing, EpiTect Bisulfite Kit
Candidate genes vs. genome	TPH2	Angiotensin Converting Enzyme (ACE) gene, CpG island -456 to -255, contains 25 CpG sites, 24 sequencing
Methylation Validation	not mentioned	Cycle sequencing: BigDye Terminator 3.1 Cycle Sequencing Kit
Genotyping	Yes	No
Gene expression	Yes	No
Analytical method of methylation difference	Pearson's correlation coefficient test and Fisher's exact test	Pearson's correlation coefficient test
Major findings	The TPH2 promoter was methylated in 36.0% of MDD + suicide patients, as compared with in 13.0% of MDD patients.	Depressive patients showed a hypermethylation pattern at all CpG sites compared to healthy controls; Statistical significant differences at three CpG sites (1, 5, 12) and a trend for significance at 5 CpG sites (7, 10, 11, 13, 21).
Category	others	others

ID	30	31	32
First Author	Alt	Guintivano	Bell
Year	2010	2014	2015
Country	Netherlands	USA	USA
Age	cases: 70.83 ± 16.04; controls: 72.67 ± 12.9	30.6	Maternal age was matched
Sample Size	12	93 with history of major depression or bipolar disorder	545
Cases	6, MDD without childhood abuse	Not applicable	269
Diagnostic standard	DSM IV	DSM-IV	Edinburgh Postnatal Depression Scale (EPDS)
Controls	6	Not applicable	276
Study design	Matched case-control	Longitudinal study; Discovery- replication	Nested matched case-control
Cohorts	Dutch Brain Bank	not mentioned	From a longitudinal study
Biological Sample	Post-mortem brain tissues	Blood	Whole blood
Purification of DNA extraction	QIAamp1 DNA Mini kit	Not mentioned	Not mentioned
DNA methylation methods/ Kits	Bisulphite conversion, pyrosequencing using PyroMark ID	Illumina's Infinium Human Methylation450 Beadchip Kit	Bisulfite conversion, pyrosequencing using PyroMark Gold Q24
Candidate genes vs. genome	GR promoter	Genome wide	OXTR (CpG site -934)
Methylation Validation	not mentioned	Bisulfite conversion using EZ DNA Methylation Gold Kit, pyrosequencing using PyroMark MD system	not mentioned
Genotyping	No	No	Yes
Gene expression	Yes	No	No
Analytical method of methylation difference	Mann-Whitney U-test	Logistic regression	Logistic regression
Major findings	No significant difference in methylation pattern between groups	CpG methylation levels at two loci within the HP1BP3 and TTC9B genes were identified as biomarkers predictive of PPD.	Methylation is not significantly associated with postpartum depression.
Category	NR3C1	Genome	OXTR

ID	33	34	35
First Author	Byrne	Chagnon	Córdova-Palomera
Year	2013	2015	2015
Country	Australia	Canada	Spain
Age	31-63	>=65	Concordant pairs: 22-54 (42.5±13); discordant pairs: 20-50 (37±10.9); healthy pairs: 19-39 (30.3±7.3)
Sample Size	24 pairs, 48 individuals	43	17 MZT pairs, 34 individuals
Cases	12 MZT pairs discordant for MDD	19 (anxiety and/or depression)	4 concordant & 6 discordant pairs
Diagnostic standard	DSM IV	DSM IV	SCIDI-I
Controls	12 MZT pairs concordant for no MDD	24	7 healthy pairs
Study design	Case-control twin study	case control	case-control twin study
Cohorts	Queensland Twin Registry	Population-based ESA study (Survey on Elders' Health)	General population-based UB-Twin Registry
Biological Sample	White blood cells	Saliva	Peripheral blood DNA
Purification of DNA extraction	not mentioned	Qiagen columns (DNA mini kit)	Not mentioned
DNA methylation methods/ Kits	Bisulphite conversion, Illumina Human Methylation 450 BeadChip	Bisulfite conversion, pyrosequencing using Pyromark 96, except for APOE analyzed on Illumina Beadchips	Bisulfite conversion using Illumina Infinium HumanMethylation450 Beadchip
Candidate genes vs. genome	Genome wide	BDNF, OXTR, SLC6A4, APOE	Genome wide
Methylation Validation	Replicate: EZ DNA methylation Kit	not mentioned	not mentioned
Genotyping	No	Yes	Yes
Gene expression	No	No	No
Analytical method of methylation difference	Two-sample t-test	T-test, except for BDNF used Wilcoxon-MannWhitney test	Ranking analysis
Major findings	No overall difference in mean global methylation between case and controls; the difference in mean methylation was significant in females within discordant pairs, but not in male.	BDNF & OXTR showed greater methylation in cases compared with controls; no difference with APOE and SLC6A4.	Hypomethylation in WDR26 gene associated with a lifetime diagnosis of depression.
Category	Genome	BDNF; SLC6A4; OXTR; others	Genome

ID	36	37
First Author	Haghighi	Haghighi
Year	2015	2014
Country	USA	USA
Age	Cases: 35.1 ± 11.8; controls: 36.9 ± 13.3	cases: 47±17; controls: 52±17
Sample Size	120	53
Cases	61	25 depressed-suicide
Diagnostic standard	SCID-I & SCID-I non-patient version	DSM IV
Controls	59	28
Study design	Case-control	Case-control
Cohorts	not mentioned	not mentioned
Biological Sample	Buffy coat	Orbital ventral prefrontal cortex
Purification of DNA extraction	QIAamp DNA Blood Mini Kit	Not mentioned
DNA methylation methods/ Kits	Bisulfite conversion by EpiTect Bisulfite Kit, pyrosequencing using PyroMark Q96 MD	Bisulfite conversion using Illumina Infinium HumanMethylation27 BeadChip
Candidate genes vs. genome	Main human LC-PUFA biosynthetic genes: Fads1, Fads2, ELovl5	Genome-wide, 20493 CpG sites.
Methylation Validation	not mentioned	Bisufite pyrosequencing: EpiTect Bisulfite kit; Selected sites validation (Eya2, Megf11, Lmna, Glud1, Erbb3, Slc18a2)
Genotyping	No	No
Gene expression	No	No
Analytical method of methylation difference	ANCOVA models	ANOVA
Major findings	MDD patients showed a lower methylation in Fads 2, but higher at Elovl5.	Increased age-related DNA methylation perturbations in prefrontal cortex in major depression suicide compared with nonpsychiatric controls.
Category	others	Genome

ID	38	39	40
First Author	Kaminsky	Melas	Reiner
Year	2014	2013	2015
Country	USA	Sweden	German
Age	not mentioned	Cases 23-74; controls 21-74	cases: 19-49; controls: 20-52
Sample Size	not mentioned	MAOA 174	85
Cases	Not applicable	MAOA 82	43 female (42 MD, Dysthymia 1)
Diagnostic standard	DSM-IV	DSM IV	SCID-I & SCID-II
Controls	not applicable	MAOA 92	42
Study design	Longitudinal	Case-control	Case-control
Cohorts	not mentioned	From a population-based longitudinal study	Inpatients from medical center; controls from flyers & posters
Biological Sample	Whole blood	Saliva	Leukocyte DNA
Purification of DNA extraction	Not mentioned	Not mentioned	QIAamp DNA Blood Mini Kit
DNA methylation methods/ Kits	not mentioned	Bisulfite-converted using EZ-96 DNA Methylation-Gold Kit, PCR and sequencing, EpiTyper software	Bisulfite conversion using EpiTect Bisulfite Kit, PCR and sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit
Candidate genes vs. genome	HP1BP3 and TTC9B	MAOA	OXTR exon 1 and 2
Methylation Validation	not mentioned	not mentioned	not mentioned
Genotyping	No	Yes	Yes
Gene expression	No	No	No
Analytical method of methylation difference	not mentioned	Non-parametric statistical analyses	Linear mixed effect model
Major findings	HP1BP3 and TTC9B predicted PPD with an area under the receiver operator characteristic curve (AUC) of 0.87. In a replication analysis, these biomarkers also functioned to segregate PPD status in women with depression during the antenatal period; however the prediction was in the opposite direction.	Overall MAOA methylation levels were decreased in depressed females compared to controls.	Depressed female patients had decreased OXTR exon1 DNA methylation compared to non-depressed women.
Category	others	others	OXTR

ID	41	42
First Author	Domschke	Domschke
Year	2014	2015
Country	German	German
Age	47.7±1.7	47.7±1.7
Sample Size	94 MDD patients	94 MDD patients
Cases	Not applicable	Not applicable
Diagnostic standard	CSID-I, BDI, and GAF	CSID-I, HAM-D-21, BDI, and GAF
Controls	Not applicable	Not applicable
Study design	Cohort	Cohort
Cohorts	MDD patients	MDD patients treated at the University
Biological Sample	Whole blood	Whole blood
Purification of DNA extraction	FlexiGene DNA Kit	FlexiGene DNA Kit
DNA methylation methods/ Kits	Sodium bisulfite converted using EZ-96 DNA methylation Kit, PCR and sequencing using Big Dye Terminator	Sodium bisulfite converted using EZ-96 DNA methylation Kit, PCR and sequencing using Big Dye Terminator
Candidate genes vs. genome	9 CpG sites in the 5-HTT transcriptional control region upstream of exon 1A	43 MAO-A CpG sites
Methylation Validation	As a control, commercially available fully methylated and fully non-methylated DNA were used in all experiments	As a control, commercially available fully methylated and fully non-methylated DNA was used in all experiments
Genotyping	Yes	Yes
Gene expression	No	No
Analytical method of methylation difference	Linear regression	Linear regression
Major findings	Hypomethylation of the 5-HTT transcriptional control region might impair antidepressant treatment response in Caucasian patients with MDD	It is not suggested that MAO-A DNA methylation major influence on antidepressant treatment response. However, the CpG-specific MAO-A gene hypomethylation might drive impaired antidepressant treatment response in females
Category	Treatment response	Treatment response

ID	43	44
First Author	Cordova-Palomera	Choi
Year	2015	2015
Country	Spain	Korea
Age	22-65	Cases: 41.9±11.3; controls: 41.2±13.9
Sample Size	34 (17 MZ twin pairs)	113
Cases	Not applicable	60
Diagnostic standard	Brief Symptom Inventory (BSI)	Hamilton Depression Rating Scale (HDRS-17), confirmed by SCID-I
Controls	Not applicable	53
Study design	Twin study (zygosity of the pairs was examined)	Case-control
Cohorts	General population	Patients from the outpatient psychiatric clinic; controls from community
Biological Sample	Peripheral blood	Peripheral blood samples
Purification of DNA extraction	Not mentioned	Not mentioned
DNA methylation methods/ Kits	Bisulfite conversion, bead array using The Illumina Infinium HumanMethylation450 (450K) BeadChip	Bisulfite conversion, pyrosequencing was performed on a PyroMark ID system using the Pyro Gold reagents kit
Candidate genes vs. genome	DEPDC7	BDNF promotor region at 4 CpG sites (− 675, − 682, − 686, and − 688)
Methylation Validation	Bisulfate pyrosequencing & replication in MDD post-mortem cerebellum samples	not mentioned
Genotyping	Yes	No
Gene expression	No	No
Analytical method of methylation difference	Linear regression model	Two-sample t-test
Major findings	A hypomethylation of cg09090376 in a co-twin would associated with an increase in his/her depressive symptom score	No significant differences in the BDNF DNA methylation status at the 4 CpG sites between MDD patients and healthy controls.
Category	others	BDNF

ID	45	46
First Author	Hohne	Khulan
Year	2015	2014
Country	German	Finland (Helsinki)
Age	30-42 (34.35 ± 3.43)	Cases: 64.0±2.9; controls: 62.9±2.5
Sample Size	116	166
Cases	61	83 Men with early-life stress (ELS)
Diagnostic standard	Munich version of the Composite International Diagnostic Interview (M-CIDI)	Beck Depression Inventory (BDI) and BDI II
Controls	55	83 Matched controls
Study design	Case-control	Case control cohort
Cohorts	From EDSP study - community sample	From the Helsinki Birth Cohort Study (HBCS)
Biological Sample	Peripheral blood cells	Peripheral blood
Purification of DNA extraction	Puregene whole blood DNA-extraction kit	QIAamp DNA Blood Maxi Kit
DNA methylation methods/ Kits	Bisulfite conversion, PCR and sequencing using EpiTYPER assay	Bisulphite conversion, EZ DNA methylation kit, bead array using illumina methylation 450k beadchip and infinium chemistry
Candidate genes vs. genome	Intro 7 of the KFBP5 gene (chr6:35, 666, 288-35, 666, 763, hg18)	Genome-wide
Methylation Validation	not mentioned	Pyrosequencing using PyroMark Q24Gold reagents on a PyroMark Q24 Pyrosequencer
Genotyping	Yes	No
Gene expression	Yes	No
Analytical method of methylation difference	Two-way ANCOVA	Linear modelling using Limma and methyAnalysis package and t-tests
Major findings	Subjects with the TT genotype and a life-time history of MD had a 10% higher DNA methylation rate than healthy controls with the same FKBP5 genotype.	Hypomethylation was identified in association with depressive symptoms.
Category	others	Genome

ID	47	48
First Author	Melas	Olsson
Year	2015	2010
Country	Sweden	Australia
Age	not mentioned	Adolocsents
Sample Size	44	150 (83 males /67 females)
Cases	17	25
Diagnostic standard	DSM, MDI	CIS-R (clinical interview schedule - revised)
Controls	27	125
Study design	Case control	Case control
Cohorts	From PART study	Population representative sample form VAHCS
Biological Sample	Saliva	Buccal cell
Purification of DNA extraction	Not mentioned	Not mentioned
DNA methylation methods/ Kits	Bisulfite-converted using EZ-96 DNA Methylation-Gold Kit, PCR and sequencing, EpiTyper software	Bisulfite conversion, Sequenom MassARRAY EpiTyping
Candidate genes vs. genome	MAOA, 7 CpG sites	799 bp CpG island 3' of the 5HTT promoter
Methylation Validation	not mentioned	Not mentioned
Genotyping	No	Yes
Gene expression	No	Yes
Analytical method of methylation difference	Linear regression; t-test for gender difference	Logistic regression
Major findings	Subjects with a history of depression were hypomethylated compared to controls. Depressed females were hypomethylated, but no significant association in males; females were hypermethylated at the MAOA region compared to males.	No association between depressive symptoms and either buccal cell 5HTT methylation or 5HTTLPR.
Category	others	SLC6A4

ID	49
First Author	van der Knaap
Year	2015
Country	Dutch
Age	16.2±0.7
Sample Size	954
Cases	Not applicable
Diagnostic standard	CIDI according to DSM-IV
Controls	Not applicable
Study design	Cross-sectional and prospective cohort
Cohorts	Adolescents from TRAILS - population-based
Biological Sample	Whole-blood samples
Purification of DNA extraction	Not mentioned
DNA methylation methods/ Kits	Methylation levels analyzed using EpiTYPER method; bisulfite conversion using EZ-96 DNA Methylation Kit, followed by PCR
Candidate genes vs. genome	NR3C1 & SLC6A4
Methylation Validation	Not mentioned
Genotyping	Yes
Gene expression	No
Analytical method of methylation difference	Logistic regression for diagnosis and linear regression for symptom scores
Major findings	NR3C1 methylation levels at NR3C1_1 were positively associated with the risk of a depressive disorder, and depressive symptom scores at follow-up, but became non-significant when accounting for the scores at baseline. SLC6A4 methylation levels were not associated with depression diagnosis, but were positively associated with depressive symptom scores at follow-up, and remained significant when accounting for the scores at baseline.
Category	NR3C1; SLC6A4

ID	50
First Author	Zhao
Year	2013
Country	USA
Age	55.1±2.8
Sample Size	84 Monozygotic twin pairs (43 pairs were discordant for MDD)
Cases	Not applicable
Diagnostic standard	Beck Depression Inventory II (BDI II), Life and Current Major Depression by DSM-III-R
Controls	Not applicable
Study design	Twin study
Cohorts	Emory Twin Studies drawn from Vietnam Era Twin Registry
Biological Sample	Peripheral blood leucocytes
Purification of DNA extraction	Not mentioned
DNA methylation methods/ Kits	Bisulfite conversion using EZ DNA methylation kit, pyrosequencing using PSQ96 HS System
Candidate genes vs. genome	20 CpG dinucleotides in the promoter region of the SLC6A4 from -213 to -69; 5-HTTLPR as confounder
Methylation Validation	Pyrosequencing assay on duplicate samples; Each experiment included non-CpG cytosines as internal controls; Unmethylated and methylated DNA as controls were added in each run.
Genotyping	Yes
Gene expression	No
Analytical method of methylation difference	Linear regression
Major findings	Variation in methylation level within the promoter region of the SLC6A4 is associated with variation in depressive symptoms. A 10% increase in mean DNA methylation level was associated with 4.4 increase in the difference in BDI scores.
Category	SLC6A4

ID	51	52
First Author	Bustamante	Tadic
Year	2016	2014
Country	USA	Not mentioned
Age	cases: 49.6±10.6; controls: 50.3±13.8	44.9±12.7
Sample Size	147 adults	39 MDD patients
Cases	65	Not applicable
Diagnostic standard	Patient Health Questionnaire (PHQ-9) consistent with DSM-IV	Hamilton Depression Rating Scale-21
Controls	82	Not applicable
Study design	Case-control	Cohort
Cohorts	From Detroit Neighborhood Health Study	Hospital patients treated for MDD
Biological Sample	Whole blood via venipuncture	Whole blood
Purification of DNA extraction	QIAamp DNA Blood Mini Kit & Life Sciences's Quickgene DNA Whole Blood Kit	QIAamp DNA Blood Mini Kit; BioMek NX liquid handling system
DNA methylation methods/ Kits	Bisulfite conversion using EpiTect Bisulfite Kit, pyrosequencing using PyroMark Q24 Assay Design Software	Bisulfite conversion, PCR and sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit
Candidate genes vs. genome	13 CpG sites within the promoter region of NR3C1	13 CpG sites within the BDNF exon IV promoter
Methylation Validation	Pyrosequencing using PyroMark Q24 Assay Design Software 2.0	not mentioned
Genotyping	No	No
Gene expression	Yes	Yes
Analytical method of methylation difference	Independent samples t-test	Linear mixed effect model
Major findings	MDD was not associated with DNA methylation in CpG sites 1-4 following FDR adjustment. DNA methylation was significantly lower over CpG sites 5-13 in those with vs. without MDD.	Antidepressant treatment did not significantly affect the methylation at BDNF promoter IV.
Category	NR3C1	Treatment response

ID	53	54
First Author	Nagy	Oh
Year	2015	2015
Country	Canada	Australia, The Netherlands, UK; and Canada
Age	cases: 41.0±2.6; controls: 41.3±5.9	18-75
Sample Size	121	260
Cases	76 subjects who died by suicide with MDD	133 (30 for prefrontal cortex; 103 for peripheral blood from MZ twins)
Diagnostic standard	DSM-IV	DSM-IV
Controls	45 subjects who died in accidents without axis I disorders	127 (30 for prefrontal cortex; 97 for peripheral blood from MZ twins)
Study design	Case-control	Case-control
Cohorts	Brain samples obtained from Douglas-Bell Canada Brain Bank	MZ twins from Australia, the Netherlands, and the UK; Prefrontal cortex samples from SMRI and QSBB
Biological Sample	Brain tissue	Peripheral blood from monozygotic twins; brain prefrontal cortex, and germline (sperm) samples
Purification of DNA extraction	Qiangen QIAamp	Not mentioned
DNA methylation methods/ Kits	Bisulfite converted using EpiTect Bisulfite kit, PCR and sequencing	Bisulfite conversion, pyrosequencing using Gold Q96 Reagents and Pyromark Q24
Candidate genes vs. genome	Genome wide	Genome wide
Methylation Validation	High-resolution melting and bisulfite Sanger sequencing	not mentioned
Genotyping	No	No
Gene expression	Yes	No
Analytical method of methylation difference	Mixed model regression and pairwise comparisons	not mentioned
Major findings	Significant differences (decrease) in the methylation patterns specific to astrocytic dysfunction associated with depressive psychopathology	Hypermethylated loci in the white blood cells of MDD twins; while the brain and the sperm showed higher proportions of hypomethylated regions in MDD patients compared with controls.
Category	Genome	Genome

ID	55
First Author	Uddin
Year	2011
Country	USA
Age	cases: 43.5±11.9; controls: 46.2±18.7
Sample Size	100
Cases	33
Diagnostic standard	Patient Health Questionnaire (PHQ-9)
Controls	67
Study design	Case-control
Cohorts	Subset of participants in the Detroit Neighborhood Health Study (DNHS) - community sample
Biological Sample	Whole blood
Purification of DNA extraction	Not mentioned
DNA methylation methods/ Kits	Bisulfite conversion using EZ-96 DNA Methylation Kit, bead array using humanmethylation 27 (HM 27) DNA analysis beadchip
Candidate genes vs. genome	Genome wide
Methylation Validation	Four technical replicates were included - duplicate samples of two randomly selected individuals and duplicate samples of the control human methylated and unmethylated DNA.
Genotyping	No
Gene expression	Yes
Analytical method of methylation difference	Functional annotation cluster analyses
Major findings	Uniquely unmethylated gene sets distinguished between those with versus without lifetime depression. Some processes (e.g. brain development, tryptophan metabolism) showed increased methylation in those with depression, whereas others (e.g. lipoprotein) showed decreased methylation.
Category	Genome

ID	56	57
First Author	Walker	Osborne
Year	2016	2016
Country	Scotland	USA
Age	not mentioned	30.7 ± 6.3; 33; 32.7 ± 0.018
Sample Size	29	291
Cases	Affected carriers of the linked haplotype (ALH=10; BD=5, MDD=5)	51 High risk women
Diagnostic standard	Not mentioned	Beck Depression Inventory and the Edinburgh Postnatal Depression Scale
Controls	Unaffected carriers of the linked haplotype (ULH=10); unaffected, non-haplotype-carring married-in controls (Mis=9)	240 Women without previous psychiatric diagnosis
Study design	Case-control	Case-control
Cohorts	Members of a large family multiply affected by BD and MDD	From two prospective cohorts designed to study PPD and two cohorts where DNA was taken long after pregnancy.
Biological Sample	Blood	Blood
Purification of DNA extraction	Nucleon BACC2 Genomic DNA Extraction Kit	Not mentioned
DNA methylation methods/ Kits	Sodium bisulphite using the EZ-96 DNA Methylation Kit, bead array using the Infinium HumanMethylation450 BeadChip	Illumina Human Methylation 450 (HM450) bead array for 51 women with mood disorders (existing data); Bisulfite conversion pyrosequencing using PyroMark MD system for the rest of samples
Candidate genes vs. genome	Genome wide	Genome wide
Methylation Validation	not mentioned	not mentioned
Genotyping	No	No
Gene expression	Yes	Yes
Analytical method of methylation difference	T-test	Linear regression
Major findings	Nominally significant differences in DNA methylation were observed.	For N=51, first trimester antenatal gene expression levels of HP1BP3 and TTC9B predicted PPD status. For N=240, DNA methylation variations could predict PPD status.
Category	Genome	Genome

CHAPTER 5 – PREDICTORS OF FUNCTIONAL IMPROVEMENT IN CHILDREN AND ADOLESCENTS AT A PUBLICALLY FUNDED SPECIALIST OUTPATIENT CLINIC

A version of this chapter as a manuscript entitled **“**Predictors of functional improvement in children and adolescents at a publicly funded specialist outpatient treatment clinic in a Canadian Prairie City” authored by Li, Muzi; D'Arcy, Carl; Meng, Xiangfei, has been submitted online to Child and Adolescent Mental Health for publication review.

5.1 Abstract

Objectives. Children's mental health problems substantially impact their daily functioning. For children and adolescents we (1) document the impact of mental health treatment on functioning, and (2) identify predictors of functional improvement.

Methods. Clinic anonymized data from a regional publicly funded specialist outpatient treatment clinic (N=645 children, ages 6-11 and 682 adolescents, ages 12-17) were analyzed. Outcome was assessed with the Child and Adolescent Functional Assessment Scale (CAFAS) - a global measure of impairment/functioning with eight domain subscales. Non-parametric tests were used to compare median scores at baseline and exit. Logistic regression was used to examine predictors of improvement. Comparisons between children and youth were conducted. A typical treatment cycle involves 8-12 sessions.

Results. CAFAS Total Scores at exit showed a significant decrease from initial scores for both age groups, indicating that client functioning had improved. Initial level of dysfunction, length of treatment and the presence of pervasiveness of behavioural impairment (PBI) were shared predictors for functional improvement among in both age groups. Primary presenting problem, caregiver support and area of residence were only associated with outcome among children.

Conclusion. Our findings clearly indicate that current mental health treatment significantly improved overall functioning in children and adolescents. Clients with a high initial level of dysfunction and PBI require longer treatment to reach an acceptable level of functioning. Shortening the length of treatment cycles may improve the efficiency of resource use but will be detrimental to some clients. Personalized treatment should be tailored to the specific characteristics and needs of clients.

Key words: CAFAS, functional improvement, child and adolescent, outpatient treatment

5.2 Introduction

Children's mental health problems substantially impact their functioning in various aspects of life, especially social and cognitive development. Children with mental health issues tend to have a lower self-worth, negative feelings, poor performance in school, and be involved in unhealthy lifestyle later. Good mental health is as important as good physical health, and is essential for the individuals themselves and people surrounding them (Kids Mental Health, 2015).

Outpatient psychiatric treatment, as the most common form of treatment for children and adolescents, has been consistently found to be positive on psychiatric symptoms (Angold, Costello, Burns, Erkanli, & Farmer, 2000; Burns, Hoagwood, & Mrazek, 1999; Waddell, McEwan, Shepherd, Offord, & Hua, 2005). It contains a large number of therapeutic approaches, including individual and group psychotherapy, school-based services, therapeutic foster care, and focused family-support programs (Kazdin, & Weisz, 1998). A systematic review identified outpatient treatment as either "well-established" or "probably efficacious" treatment for such mental disorders as disruptive behaviour disorders, anxiety disorders, and autism (Burns etc., 1999). A significant dose-response relationship was also found between the number of treatment sessions received and improvement in symptoms at follow-up. Eight or more sessions was suggested as being required to produce such positive effects (Angold etc., 2000).

Studies have documented that the Child and Adolescent Functional Assessment Scale (CAFAS) is a useful tool for assessing degree of impairment in functioning (Boydell, Barwick, Ferguson, & Haines, 2005; Hodges, 2000). It is used to assess functioning of children and adolescents entering and exiting from the mental health care. It consists eight subscales: School, Home, Community, Behaviour Toward Others, Mood/Emotions, Self-Harm, Substance Use, and Thinking. Change in scores on CAFAS subscales is informative as it is important to know whether clients improve generally as well as in specific domains when exiting from treatment (Rohr, Bartlett, & Duncan, 2014). Complementary scales allow the assessment of caregiver resources, *Family/Social Support* and *Material Needs* subscales are used to examine the extent to which the clients' functioning was disrupted due to limitations in the family's psychosocial resources and caregiving ability. Having an impaired caregiving environment can decrease the probability of successful treatment outcomes (Xue, Hodges, & Wotring, 2004).

It is important to allow clinicians and health managers to know their clients, the effectiveness of their services in improving client functioning, and the factors that may affect the effectiveness of their services. It is also important to understand special needs for children and youth separately since they are at different development stages. Parents or caregivers play a large role in children's life during their development of behaviours, social skills, cognition, and emotions that can affect their life-long health. Similarly, the transition from childhood to adulthood can further subject adolescents to a variety of psychological and social pressures. They experience puberty with both physical and emotional changes, including hair, breasts, establishing identity, etc.

Studies have reported the wide use of the CAFAS scale in clinical settings, for instance, a province wide Ontario (Canada) study found a high reliability in the use of the scale in health practitioners 1 and 3-years after training (Barwick, Boydell, Cunningham & Ferguson, 2014). Another study of the scale indicated its usefulness in case formulation and in tracking changes in functioning over time (Boydell et al., 2005). However, little research can be found on whether different social-demographic and clinical characteristics are associated with changes in clients' functioning (Walrath, Mandell, & Leaf, 2001). Knowledge of the predicators of functional change are important for clinicians, decision-makers, and administrators as they need to know what determinants are associated with better functioning improvements and apply personalized care towards clients' needs improving mental health outcomes for all.

This study aims to fill the information gap by answering the following questions: (1) were the CAFAS subscales good indicators for clients' overall functioning impairment? (2) What, if any, changes occurred by the time clients exited treatment? (3) What factors were associated with improvement in client functioning? And, (4) are there any difference between children and youth in answers to the previous questions?

These issues are explored using clinical data from a publicly funded specialized provider of a range of outpatient mental health treatment services for children and youth in a mid-sized Canadian prairie city.

5.3 Methods

5.3.1 Context

Though federally mandated, provincial governments in Canada take the responsibility of most health services delivered within their provinces with the services provided varying somewhat from province to province. These services include almost all hospital and physician services, prescription drug subsidies, a significant proportion of nursing home, community care services and preventive public health services. Saskatchewan is a prairie province in Canada. These public funded health services are available at no direct cost to the clients. Saskatoon is a city in central Saskatchewan. It is the largest city in the province with a population of 222,189 in 2011. The Saskatoon Health Region (SHR) is the largest health region in the province, and about 30% of the province's population resides within the region's geography. SHR is an integrated health delivery agency providing a comprehensive range of services and programs including hospital, long-term care, public health, home care, mental health and addition services, and prenatal and palliative care. In 2015 it served approximately 342,362 residents in urban and rural areas in central Saskatchewan (Saskatoon Health Region, 2015).

Child and Youth Mental Health and Addictions Services (CYMHAS) is the major agency in the Saskatoon Health Region involved in the provision of a continuum of outpatient treatment services to children and youth (and their families) who require mental health treatment. Children and adolescents are generally referred for assessment and treatment from a variety of sources: parents, schools, health professionals, mental health centres, departments of juvenile justice, and social services. The common and over-arching treatments include Cognitive Behavioural Therapy, exposure therapies, play therapy, parent education, behavioural therapy, pet therapy, art therapy, and neuro-sequential model of therapeutics. Treatment modalities vary between clinicians and depend on the clients' presenting concerns.

The Child and Adolescent Functional Assessment Scale (CAFAS) was widely used as a functioning assessment tool within the CYMHAS during the study period. In general, the prototypical treatment cycle (episode) at the time was conceived as using the CAFAS at: an initial assessment, a 3-month assessment, a 9-month assessment, followed by an exit assessment. Some me patients can and do terminate treatment earlier than anticipated, whereas others continue the treatment over a longer time period. The CAFAS assessments are conducted by

social workers, counsellors, psychologists, etc. These clinicians do not prescribe medications.

5.3.2 Population Studied - Data Source

We analysed data for all those CYMHAS clients aged 6 to 17 years old, and enrolled for treatment between 2011 and 2014 for their first treatment episode (cycle). Those with incomplete records of initial and exit assessments were excluded from this study.

The data was extracted and anonymised from individual client clinical (CAFAS) and administrative (Administrative and Management Information System, AMIS) data files.

5.3.2.1 CAFAS

The Child and Adolescent Functional Assessment Scale (CAFAS) consists of eight subscales (School, Home, Community, Behaviour Toward Others, Mood/Emotions, Self-Harm, Substance Use, and Thinking) and two caregiver resources sub-scales (Family/Social Support and Material Needs). Each of subscales is scored 0 to 30, indicating minimal/none to severe impairment. Higher scores indicated greater impairment. A Total Score is summed up by the scores from the eight subscales. The Total Score was further divided into five levels of dysfunction: 1 (scored 0 to 10) – no impairment; 2 (scored 20 to 40) – outpatient treatment; 3 (scored 50 to 90) – additional services beyond outpatient care; 4 (scored 100 to 130) – more intensive than outpatient care and/or multiple sources of supportive care; and, 5 (scored 140 and above) – intensive treatment.

5.3.2.2 AMIS

CYMHAS's AMIS – Administrative and Management Information System contains detail socio-demographic and some clinical data. It was matched with CAFAS data via clients' unique identification number. However, due to a large proportion of missing values in youth age group, AMIS predictors were only used in the analysis of predictors of functioning and changes in functioning for the child age group.

5.3.3 Measures

5.3.3.1 Outcome

We defined our outcome as the improvement in level of dysfunction between intake and exit, which was calculated by the following formula and coded as a dichotomous variable (1 =

with improvement, 0 = without improvement):

Improvement in level of dysfunction = level of dysfunction at intake – level of dysfunction at exit

5.3.3.2 Predictors

Social demographic variables. Majority of the social demographic variables were retrieved from AMIS including information on living arrangement, area of residence, number of addresses, referral source, and parental involvement in capacity development. Information on age and gender was available and retrieved from the CAFAS dataset.

Clinical variables. Information on primary presenting problem and number of presenting problems were obtained from the AMIS dataset. The CAFAS dataset provided client information on initial and exit CAFAS Total Scores and sub-scales scores, length of stay in treatment, number of episodes (treatment cycles), Pervasive Behavioural Impairment (PBI), which was defined as moderately or severely impaired on all three relevant subscales: School, Home, and Behaviour Towards Others (scored 30 or 20), caregiver resources – family/social support which assesses the extent to which the client's functioning was disrupted due to limitations in the family's psychosocial resources, and caregiver resources – material needs, which examined whether the client's needs exceed the caregiver's ability to provide.

5.3.4 Statistical Analysis

All the analyses were conducted separately for each age group – child (aged 6-11 years) and youth (aged 12-17). Children and youth are experiencing different developmental stages both mentally and physically, thus their mental health treatment needs are different. The cut-off point of age group is based on the definition of children and youth from the Centres for Disease Control and Prevention (CDC) (CDC, 2015).

Descriptive analyses were used to understand clients' characteristics. Confirmatory factor analyses (CFAs) were applied to test whether and to what extent the eight subscales were consistently predicting the overall level of dysfunction. Because the subscale variables were ordinal, CFAs were conducted using robust maximum likelihood estimation with the Satorra and Bentler (S-B) scaled chi-squared tests (Satorra, & Bentler, 1994). This adjustment for non-normality also allowed us to obtain robust results for standard errors, p-values, confidence intervals, and goodness-of-fit statistics. Model fitting was evaluated by using the following fit

statistics: S-B chi-square, comparative fit index (CFI), Tucker Lewis Index (TLI), root mean square error of approximation (RMSEA), Akaike information criterion (AIC), and Bayesian Information Criterion (BIC). By convention, higher CFI and TLI values (≥ 0.90), and lower chi-square values, RMSEA (≤ 0.08), AIC, and BIC values indicate better fit (Hu, & Bentler, 1998, 1999).

Because the subscale and total scores showed non-normality, non-parametric tests were used to gauge the changes in total and each subscale score and their significances. Higher CAFAS scores indicate more severe impairment. More negative differences were expected when comparing initial and exit scores. Asymptotic significances were used unless there were less than a total of 25 positive and negative differences, in which cases exact significances were employed.

Multivariable logistic regressions were used to examine determinants associated with functioning improvement. Again, the goodness of fit was tested. Due to the large proportion of missing values (approximately 85%), AMIS variables were not included in the model for youth group and were analysed separately using chi-square tests. For the purpose of comparison of factors between children and youth, the model for child age group was assessed in two ways: AMIS variables included and not included. We used Stata 14 software (StataCorp, 2015) for all the analyses.

5.4 Results

5.4.1 Sample Description

A total number of 1,327 children (645) and youth (682) met inclusion criteria and their data were included in the analysis. Table 5-1 presents the socio-demographic and clinical characteristics of the clients. Overall, the majority of the subjects lived in the West or Southwest of Saskatoon, lived with family of origin with one stable address, were referred by health professionals, families or guardians, or schools, had only one treatment episode (cycle), had mostly behavioural concerns or anxiety, received services for 15 months or less, did not have issues with regard to both caregiver resources (family support and material needs) and PBI, and had a Total Score of 90 or less at intake, indicating minimal to moderate level of dysfunction. Their caregivers were not involved in any parental capacity developmental programs.

Table 5-1 Population characteristics by age group (total number of cases may vary due to missing values)

Categorical variables	Child (6–11 years) n	Child (6–11 years) Percent	Adolescent (12–17 years) n	Adolescent (12–17 years) Percent	Total n	Total Percent
Gender						
Male	392	60.8%	296	43.4%	688	51.8%
Female	253	39.2%	386	56.6%	639	48.2%
Area of residence						
Northeast	67	11.6%	11	10.9%	78	11.5%
East Centre	57	9.9%	15	14.9%	72	10.6%
South	71	12.3%	13	12.9%	84	12.4%
Southwest	108	18.7%	11	10.9%	119	17.6%
North	80	13.9%	12	11.9%	92	13.6%
West	129	22.4%	30	29.7%	159	23.5%
Rural & Prince Albert	65	11.3%	9	8.9%	74	10.9%
Number of addresses						
1	456	79.0%	77	76.2%	533	78.6%
2	95	16.5%	12	11.9%	107	15.8%
3+	26	4.5%	12	11.9%	38	5.6%
Living arrangement						
Family of origin	549	86.5%	99	85.3%	648	86.3%
Foster homes & other	39	6.2%	9	7.8%	48	6.4%
Not provided	47	7.4%	8	6.9%	55	7.3%
Primary presenting problem						
Aggressive behavior	59	10.4%	12	11.5%	71	10.6%
Anxiety	137	24.2%	30	28.8%	167	25.0%
Relationship difficulties	33	5.8%	4	3.8%	37	5.5%
Cognitive difficulties	37	6.5%	4	3.8%	41	6.1%
Behavioral concern	176	31.2%	28	26.9%	204	30.5%
Traumatic events	64	11.3%	13	12.5%	77	11.5%
Depression	44	7.8%	13	12.5%	57	8.5%
Other	15	2.7%	0	0.0%	15	2.2%
Number of problems						
1	195	34.5%	22	21.2%	217	32.4%
2	164	29.0%	38	36.5%	202	30.2%
3+	206	36.5%	44	42.3%	250	37.4%
Referral source						
Professionals	204	32.1%	38	32.8%	242	32.2%
Client family/guardian	290	45.7%	55	47.4%	345	45.9%
Justice	12	1.9%	3	2.6%	15	2.0%
School	115	18.1%	16	13.8%	131	17.4%
Other	14	2.2%	4	3.4%	18	2.4%
Initial total score						
Low (0 – 40)	354	54.9%	237	34.8%	591	44.5%
Medium (50 – 90)	235	36.4%	318	46.6%	553	41.7%
High (100+)	56	8.7%	127	18.6%	183	13.8%
PBI						
No	569	88.2%	626	91.8%	1,195	90.1%
Yes	76	11.8%	56	8.2%	132	9.9%

Categorical variables	Child (6–11 years)		Adolescent (12–17 years)		Total	
	n	Percent	n	Percent	n	Percent
Length of stay (month)						
0-3	104	16.1%	242	35.5%	346	26.1%
3-6	128	19.8%	109	16.0%	237	17.9%
6-9	136	21.1%	185	27.1%	321	24.2%
9-12	77	11.9%	45	6.6%	122	9.2%
12-15	75	11.6%	52	7.6%	127	9.6%
15-18	41	6.4%	10	1.5%	51	3.8%
18-21	34	5.3%	21	3.1%	55	4.1%
21-24	22	3.4%	4	0.6%	26	2.0%
24+	28	4.3%	14	2.1%	42	3.2%
Caregiver resources – Family support						
Minimal (0)	386	62.6%	300	51.0%	686	56.9%
Mild (10)	129	20.9%	173	29.4%	302	25.1%
Moderate (20) to Severe (30)	102	16.5%	115	19.6%	217	18.0%
Caregiver resources – Material needs						
Minimal (0)	570	92.8%	547	93.0%	1,117	92.9%
Mild (10)	34	5.5%	34	5.8%	68	5.7%
Moderate (20) to Severe (30)	10	1.6%	7	1.2%	17	1.4%
Number of treatment episodes						
1	524	81.2%	565	82.8%	1,089	82.1%
2	100	15.5%	108	15.8%	208	15.7%
3+	21	3.3%	9	1.3%	30	2.3%
Parental capacity development						
No						
Yes	526	82.8%	102	87.9%	628	83.6%
	109	17.2%	14	12.1%	123	16.4%

SD, Standard Deviation; PBI, Pervasive Behavioral Impairment

5.4.2 Contribution of CAFAS subscale scores to overall level of dysfunction at intake

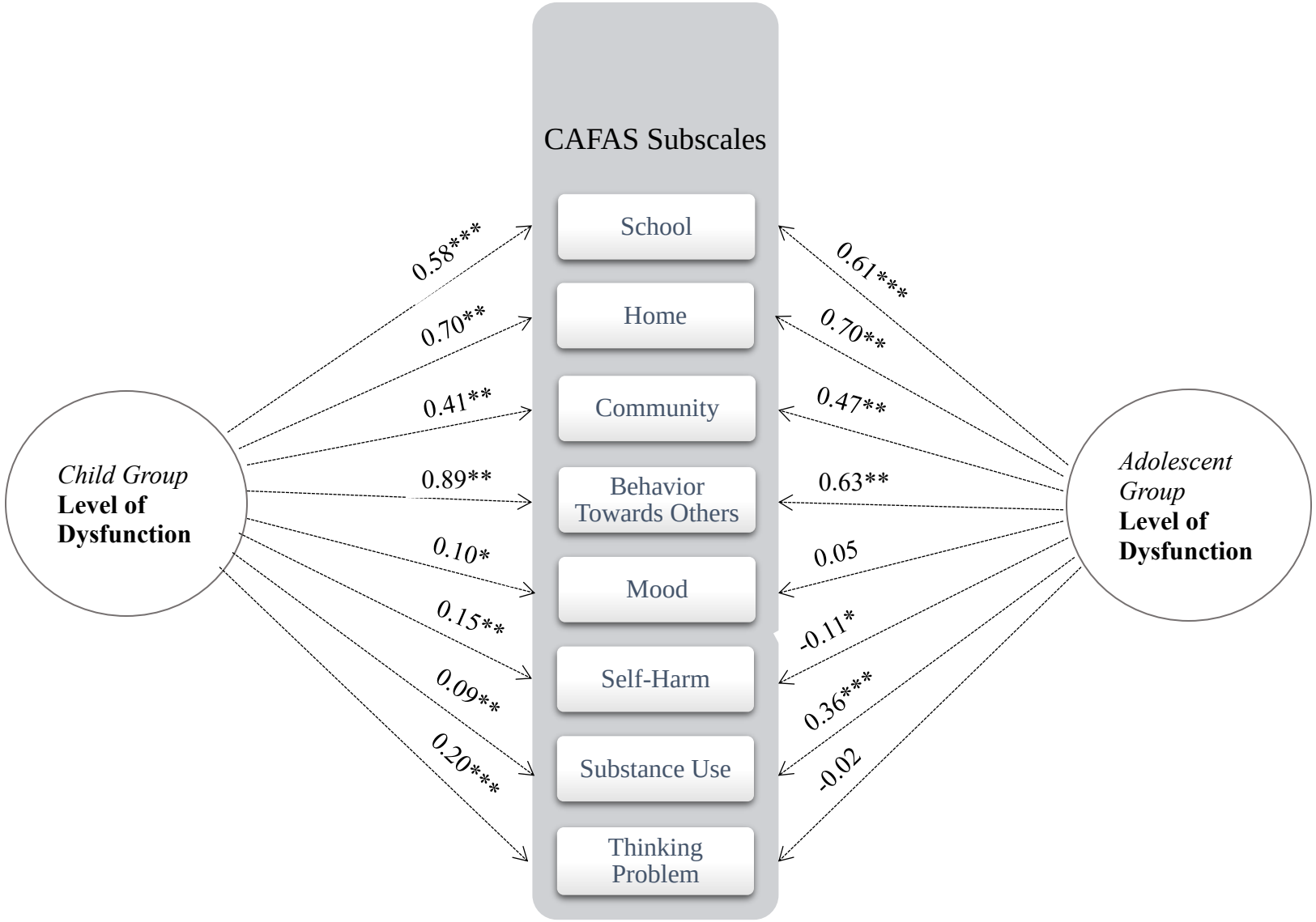
Figure 5-1 shows the relationship of subscales and level of dysfunction for children and adolescents. While all eight subscales were positively and significantly predicting children's overall level of dysfunction. There was no statistically significant relationship for 'Mood' and 'Thinking Problem' domains among adolescents. 'School', 'Home', and 'Behaviour Towards Others' subscales were top three domains that had the strongest impact to the overall level of dysfunction in both groups. 'Mood', 'Self-Harm', and 'Substance Use' subscales had the least impact on overall dysfunction score among children. 'Substance Use' subscale also had a relatively low association with youth's level of dysfunction compared with other significant subscales. It is noteworthy that 'Self-Harm' subscale negatively predicted the overall level of dysfunction in youth.

5.4.3 Difference in CAFAS Total Score between Initial and Exit Assessment

Figure 5-2 graphs the differences in the distribution of Total Score at initial assessment and at exit for child and adolescent client populations separately. Clearly evidence is that substantial improvement has occurred for the majority of clients in both age groups. However, it is also evident that there is still a number of clients that remain substantially dysfunctional after a single treatment episode. It is also obvious that those with higher initial Total Score have a greater potential for improvement.

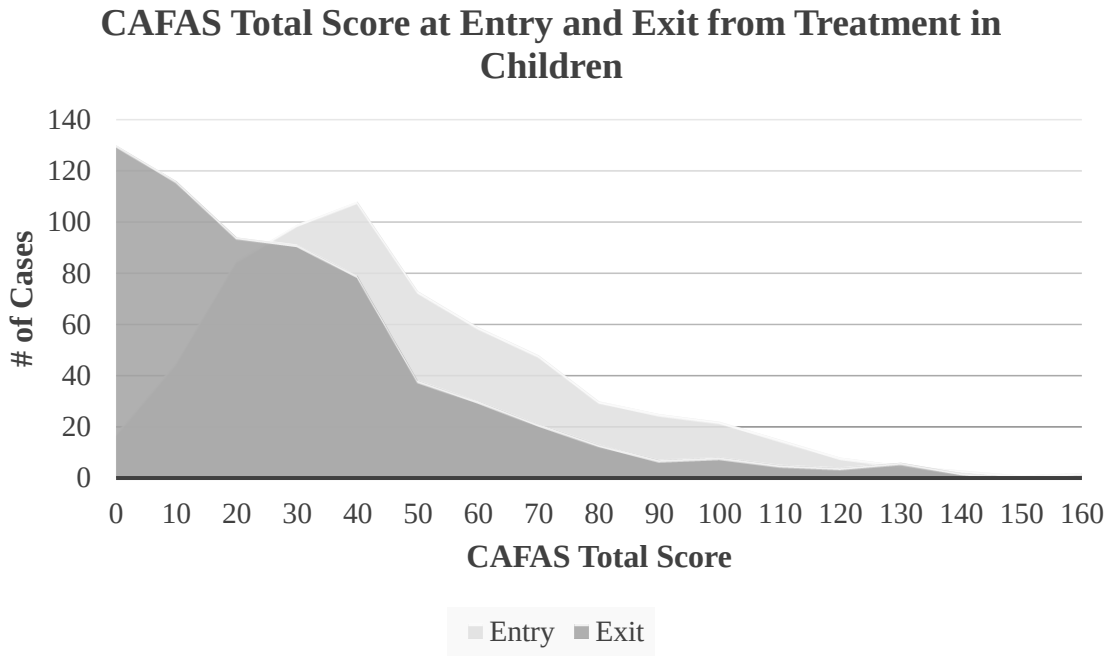
CAFAS defines *a clinically meaningful and reliable difference* in Total Score (dysfunction) as a reduction in Total Score of 20 or more points. A client has to have score 20 or more at intake to be included in the analysis, 583 (out of 645) children and 645 adolescents (out of 682) qualified. Of those, 52% of children and 56% of adolescents were deemed to have made a clinically significant and meaningful change in functioning.

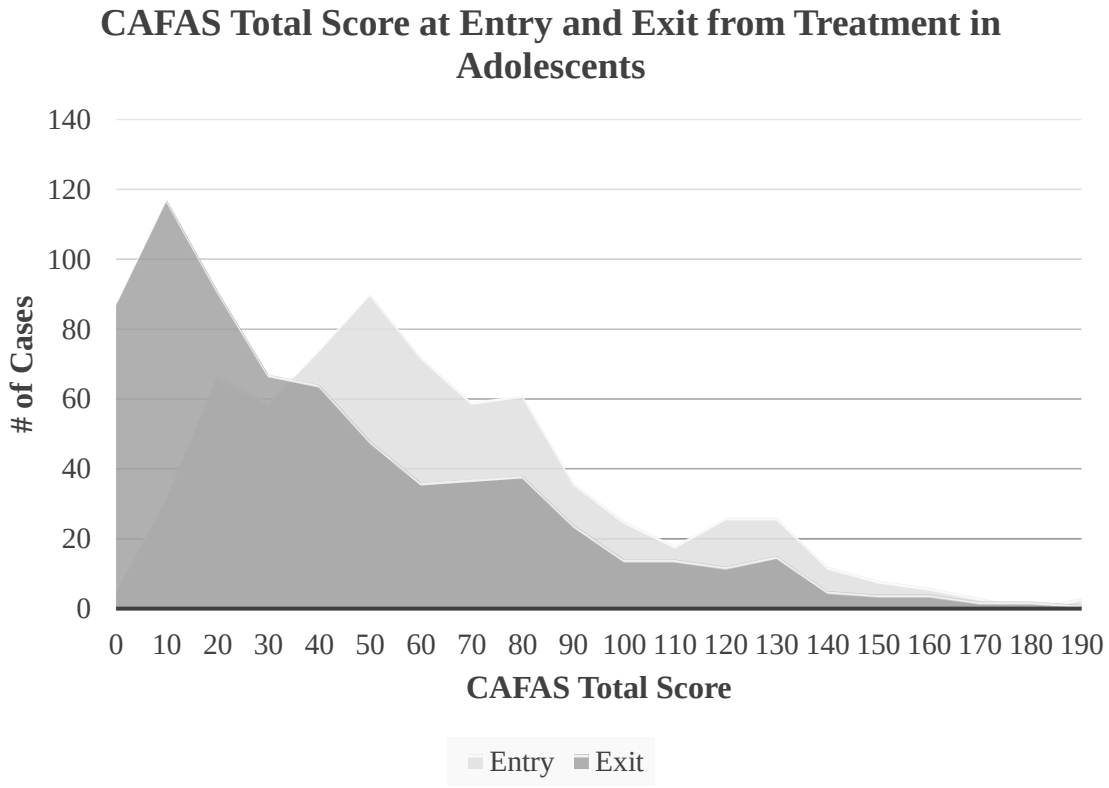
Figure 5-1 Confirmatory factor analysis by age group



*p<0.05; **p<0.01; ***p<0.001

Figure 5-2 Area graph of the distribution of initial and exit CAFAS Total Scores for both child and adolescent age groups





5.4.4 Difference in Total and Subscale Scores of CAFAS between Initial and Exit Assessments

Non-parametric tests (sign tests) were used to compare the differences in total and subscale scores between initial and exit assessment for both children and adolescents (see Table 5-2). The total score and seven of the eight subscale (except 'Substance Use') scores at exit elicited a statistically significant median decline in functioning impairment compared to initial assessment scores among children. 'School', 'Home', 'Behaviour Towards Others', and 'Mood' subscales showed more improvement. The exact significance test was used for 'Substance Use' due to the small number of negative and positive differences, and the p-value was 0.25.

For adolescents, the total score and all the subscale scores at exit demonstrated a significant median decrease in functioning impairment, compared to initial assessment scores. 'Mood' and 'Self-Harm' subscales declined more than other subscales.

5.4.5. Predictors of Improvement in Level of Dysfunction among Children with All AMIS Variables Included In the Model

There were 76.7% (495/645) children with completed records for all variables included in the model. Clients who had medium to high initial total score, no initial PBI, stayed 12 month or longer for the treatment, had a diagnosis of anxiety, relationship difficulties, and depression as primary presenting problems, and who lived in the north and northeast of Saskatoon and rural areas and Prince Albert (a neighbouring city) were more likely to have improvement in level of dysfunction (Table 5-3).

Table 5-2 Median differences, total and subscale scores from entry to exit for children and adolescents (N=1,327)

	Children (6-11 years, n=645)				Adolescent (12-17 years, n=682)			
	Negative differences [1] (better)	Positive differences [2] (worse)	Ties [3]	Z score	Negative difference [1] (better)	Positive difference [2] (worse)	Ties [3]	Z score
Total score	389	57	199	-15.7***	457	96	129	-15.3***
School subscale	205	40	400	-10.5***	187	63	432	-7.8***
Home subscale	207	23	415	-12.1***	198	61	423	-8.5***
Community subscale	24	9	612	-2.4*	72	23	587	-4.9***
Behavior subscale	233	32	380	-12.3***	168	49	465	-8.0***
Mood subscale	247	31	367	-12.9***	301	47	334	-13.6***
Self-Harm subscale	60	10	575	-5.9***	211	16	455	-12.9***
Substance subscale	3	0	642	0.250 [a]	83	49	550	-2.9**
Thinking subscale	27	8	610	-3.0**	59	27	596	-3.3**

[1] Exit score < initial score; [2] Exit score > initial score; [3] Exit score = initial score; [a] Exact sign test was used
*p-value<0.05; **p-value<0.01; ***p-value<0.001

Table 5-3 Comparison of predictors for improvement in level of dysfunction by age group

		Child (AMIS variables included in the model)	Child (Amis variables excluded)	Adolescent (Amis variables excluded)
Logistic regression				
Variable	Categories	OR (95% CI)	OR (95% CI)	OR (95% CI)
Initial total score	Low (0 – 40)	1	1	1
	Medium (50 – 90)	4.3 (2.7 – 7.0)***	3.7 (2.5 – 5.4)***	2.4 (1.6 – 3.5)***
	High (100+)	9.9 (3.1 – 31.9)***	5.8 (2.4 – 14.1)***	7.2 (3.5 – 14.9)***
Initial PBI	Yes	1	1	1
	No	3.8 (1.5 – 9.7)**	1.6 (0.8 – 3.4)	3.1 (1.3 – 7.4)**
Length of stay (month)	0-3	1	1	1
	3-6	2.0 (1.0 – 4.0)	1.5 (0.8 – 2.6)	1.4 (0.8 – 2.4)
	6-9	1.8 (0.9 – 3.6)	2.0 (1.1 – 3.6)*	1.9 (1.2 – 2.9)**
	9-12	2.1 (0.9 – 4.6)	2.6 (1.3 – 5.1)**	1.8 (0.9 – 3.6)
	12-15	4.3 (1.9 – 9.5)***	4.3 (2.2 – 8.5)***	2.6 (1.3 – 5.1)**
	15-18	4.4 (1.6 – 12.1)**	4.4 (1.9 – 9.9)***	6.9 (1.2 – 40.9)*
	18-21	4.0 (1.4 – 12.0)*	5.1 (2.1 – 12.8)***	5.6 (1.5 – 20.5)*
	21-24	1.9 (0.6 – 6.5)	1.6 (0.6 – 4.4)	1.2 (0.2 – 9.3)
	24+	4.2 (1.4 – 13.1)*	3.8 (1.5 – 9.9)**	1.8 (0.5 – 6.1)
Caregiver resources – Family support	Minimal (0)	1	1	1
	Mild (10)	0.7 (0.4 – 1.1)	0.5 (0.4 – 0.9)**	0.9 (0.6 – 1.3)
	Moderate (20) to Severe (30)	1.0 (0.6 – 1.9)	0.6 (0.4 – 1.0)	0.6 (0.4 – 1.0)
Number of address	1	1		
	2	1.6 (0.9 – 2.8)		
	3 or more	1.7 (0.6 – 5.1)		
Primary presenting problem	Behavioural concern	1		
	Aggressive behaviour	2.0 (1.0 – 4.2)		
	Anxiety	2.5 (1.4 – 4.4)**		
	Relationship difficulties	4.0 (1.6 – 10.0)**		
	Cognitive difficulties	1.5 (0.6 – 3.6)		
	Traumatic events	1.4 (0.6 – 3.0)		

		Child (AMIS variables included in the model)	Child (Amis variables excluded)			Adolescent (Amis variables excluded)		
	Depression	3.1 (1.3 – 7.1)*						
	Other	1.7 (0.5 – 6.2)						
			Chi-square test					
			Not improved	Improved	Total	Not improved	Improved	Total
Area of residence	West	1	76 (58.9%)	53 (41.1%)	129 (100%)			
	Northeast	2.4 (1.1 – 4.9)*	33 (49.3%)	34 (50.7%)	67 (100%)			
	East Centre	1.7 (0.8 – 3.8)	29 (50.9%)	28 (49.1%)	57 (100%)			
	South	1.9 (1.0 – 3.9)	34 (47.9%)	37 (52.1%)	71 (100%)			
	Southwest	1.1 (0.6 – 2.1)	74 (68.5%)	34 (31.5%)	108 (100%)			
	North	2.2 (1.1 – 4.5)*	39 (48.8%)	41 (51.2%)	80 (100%)			
	Rural & Prince Albert	3.0 (1.4 – 6.6)**	26 (40.0%)	39 (60.0%)	65 (100%)			
			Chi-square value: 18.3**					

PBI, Pervasive Behavioural Impairment; OR, odds ratio; CI, Confidence Interval; *p<0.05; **p<0.01; ***p<0.001

5.4.6 Comparison of Predictors between Children and Adolescents with AMIS Variables Analysed Separately

Due to a large proportion of missing values of AMIS variables in the adolescent group, AMIS variables were excluded from model building process and were analysed separately by using chi-square tests. For the purpose of comparison, a model was also built for children without AMIS variables. In the regression models, 95.7% (617/645) of children and 85.8% (585/682) of adolescents with completed records of CAFAS variables were included in the analysis. Initial total score and length of stay were associated with the improvement in level of dysfunction for both groups, while initial PBI showed significant association only in the youth group and caregiver family support only in the children group. Children living in North, Northeast, South, and rural of Saskatoon and Prince Albert were more likely to improve their level of dysfunction at exit, whereas no significant differences were identified among adolescents.

5.4.7 Comparison of predictors in children with and without AMIS variables included in the model

The predictors of improvement in level of dysfunction were analysed via two different methods for children – including and not including AMIS variables in the model. Both methods indicated that initial total score, length of stay, and area of residence were significantly associated with the improvement in level of dysfunction. Initial PBI and primary presenting problem did not show significant impacts in the model without AMIS variables and separate chi-square tests, respectively. Children had mild impairment on their caregiver family support initial score (10), were more likely to have a decreased level of dysfunction compared to those who had a minimal score (0) in the model with CAFAS variables only.

5.5 Discussion

This study evaluated the structure of the CAFAS as a tool assessing client functioning is a

variety of aspects of life , examined the effectiveness of treatment offered by the Saskatoon Mental Health and Addiction Services, and identified the determinants of the desirable outcome. The CAFAS subscales values generally well predicted the overall level of dysfunction for both children and adolescents. The total and most of the subscales scores (except the 'Substance Use' in child group which as low to begin with) at exit significantly decreased compared to their initial scores, which means client functioning had been improved by the time they exited treatment. Common and unique characteristics were associated with functional improvements for both children and adolescents.

Our finding is consistent with a report in which statistically significant declines in scores were observed for most of the subscales, while the 'Substance Use' subscale did not show significant results for either age groups (Rohr et al., 2014). A similar finding was also observed in children with serious emotional disturbances, indicating that significant functional improvement was found from baseline to 6-month assessment (Walrath et al., 2001).

CAFAS scores have been reported as significant predicators of treatment and service utilization (Hodges & Wong, 1997; Bates, Furlong, & Green, 2006). Bates et al. (2001) in his review also pointed out the merit of the CAFAS used as a tool for making treatment eligibility decisions and documenting the outcomes.

Pervasiveness of behavioural impairment (PBI) is one of the shared predictors for improvements in functioning among children and adolescents. This finding fits with the existing literatures. Pervasive behavioural problems was the strongest predictor of poor outcome for 'School' and 'Home' domains, and social interactions (Xue et al., 2004). Loeber (1982) also demonstrated that cross-setting consistency was associated with greater stability of problems.

Notably, we did not find the difference between children and adolescents with regard to their initial total score, PBI, and length of stay in treatment as predictors of improvement. The significance levels of 'length of stay' categories across the three models generally shows that the

association between the improvement in level of dysfunction and length of stay was getting stronger with the increase of length of stay to a specific point in time (around 18-21 months) (see Table 3). However, staying longer than that in the treatment did not play a role in achieving a better outcome.

Caregiver resources (family support), primary presenting problem, and area of residence were unique factors related to the improvement in functioning in the child age group. Children's mental and psychological development may rely more on parents and caregivers than the adolescent age group do. Family's psychosocial resources (family support), living environment and social economic status (reflected by area of residence) play greater roles in children's mental health and their recovery from dysfunction in comparison to adolescents.

5.5.1 Strengths and Limitations

Although this is a prospective case cohort study using standardized assessment of functioning, few limitations should be noted. Firstly, our analysis was limited to clients' first treatment cycle. We did not report on the predictors or effectiveness of treatment in subsequent treatment cycles. Secondly, data analysed here were limited to the variables already collected for standard administrative and clinical purposes. As a result we did not have enough information to include ethnicity and number of services/visits in the analysis. Nor did we have access to the data on whether or not these clients came back for subsequent treatment of similar problems or new emerging problems. Thirdly, the CAFAS was not administered to all clients with behavioural consults and children under 6 years old not attending school. Clients younger than 6 years old were generally not administered by the CAFAS. It was estimated that the CAFAS is administer to approximately 75-80% of all clients seen by Saskatoon Child and Youth Mental Health and Addictions Services. Finally, the analysis concerning predictors of functioning only dealt with clients who had completed their first treatment cycle (episode) using their initial and exit assessment records, which means in-process improvements were not considered (e.g. 3-month

and/or 9-month assessment). Also, there is potential selection bias in terms of only analyzing data on clients who stayed in treatment. We do not have information on those who left program with incomplete record.

5.6 Conclusion

Our findings provide robust evidence clearly indicating that current child and adolescent mental health services effectively improved clients' functioning. Treatment does make a difference. Clients with a high level of dysfunction at intake and pervasive behavioural problems needed a longer period for treatment in order to reach favourable outcome. Shortening the length of each treatment cycle may improve the efficiency of resource use but at the expense of clients that need more time to achieve a more optimal functional improvement. Personalized treatment services is what is required. Further studies on predictors of functioning improvement using CAFAS data are warranted.

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CHAPTER 6 – STIMULANT USE AND DEVELOPMENT OF BIPOLAR AFFECTIVE DISORDER: A 10-YEAR OUTCOME STUDY USING ADMINISTRATIVE HEALTH CARE DATA FILES OF REGULAR PRACTICE SETTINGS

A version of this chapter will be submitted to an ADHD journal for publication review.

6.1 Abstract

Introduction. There has been controversy concerning the use of stimulant medications, a standard treatment for attention deficit hyperactivity disorder (ADHD), and the development of bipolar affective disorder (BPAD). Previous studies on the subject have had various limitations suggesting a need for prospective, longitudinal investigations to better understand the relationship between stimulant use, ADHD, and development of BPAD. In regular practice does the treatment of ADHD patients with stimulants lead to BPAD?

Method. Health administrative data for the Canadian province of Saskatchewan were used for a prospective cohort study. All children and adolescents aged 5 to 17 years of age who were diagnosed and treated for ADHD with stimulant medications during 1989-1990 comprised the inception cohort. These exposures were followed-up for 10 years to determine BPAD occurrence, and were matched with two age-gender-region of residence comparisons. A total of 1,918 exposures and comparisons comprised the cohorts. Analyses used Penalized Maximum Likelihood Estimation.

Results. Prescription of stimulants for ADHD was a significant risk factor of BPAD in unconditional analysis (OR 2.67, 95% CI 1.66-4.35). However, stimulant use became to be a protective factor for BPAD after adjusting for comorbid psychiatric disorders (OR 0.48, 95% CI 0.24-0.98).

Conclusion. The findings reflect the impact of the nature of the initial disease on further disease progression. This study, consistent with some previous research, indicates that stimulant use by itself does not lead to the development of BPAD but rather the severity of the initial disease is an indicator of future disease trajectory.

Key words (3): ADHD treatment, stimulant medication, bipolar affective disorder (BPAD)

6.2 Introduction

Prescription of psychotropic medications to treat psychiatric disorders in children and adolescents has steadily increased (Zito, et al., 2003) (Alessi-Severini, Biscontri, Collins, Sareen, & Enns, 2012). Anti-depressants and anti-psychotics are the most commonly used for pediatric population (Merikangas, He, Rapoport, Vitiello, & Olfson, 2013).

Most recently, the dispensing of psychotropic drugs for mental health disorders has increased in North America. From 2010 to 2013 in Canada, prescription of anti-psychotics to children and adolescents increased 33% (from 34 to 45 prescriptions per 1000) and that of anti-depressants raised 63% (from 34 to 55 per 1000) (Arora, et al., 2016). In Saskatchewan the prescription of anti-depressants for children and adolescents increased from 5.9 per 1,000 in 1983 to 15.4 per 1,000 in 2007 (Meng, D'Arcy & Tempier, 2014). The number of prescriptions dispensed for depression also increased in adolescents from 2005 to 2014 in the United States (Mojtabai, Olfson, & Han, 2016).

6.2.1 ADHD as A Common Mental Health Issue and Its Comorbidity

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common mental health disorders in children and adolescents, and is characterized by inattention, hyperactivity and impulsivity (Phelan, 1993) (Barkley, 1998). Diagnosis of ADHD can result in a range of adverse effects. Children with ADHD, compared with controls, are more impaired in reading, arithmetic achievement, repeating grades, needing extra help etc. Their family environments were also more impaired in terms of cohesion and conflict (Biederman et al., 1996a).

Affective and conduct disorders are the most common comorbid diagnosis with ADHD (Cuffe, et al., 2001) (Thapar, Harrington, & McGuffin, 2001). Biederman et al. comprehensively reviewed comorbidity from a 4-year follow-up study and found that remission rates were higher for children with major depression, multiple anxiety disorders and conduct disorder (Biederman

et al., 1996b). In addition, the occurrence of anxiety disorders, major depression, bipolar disorder, and substance abuse disorders all increased from baseline to the end of the 4-year follow-up (Biederman et al., 1996a).

Various studies have reported that children and adolescents with ADHD are at higher risk of subsequently developing Bipolar Affective Disorder (BPAD), and children with comorbidity of ADHD and BPAD appear to have an earlier onset of other illness compared to their counterparts who are not comorbid (Barkley, 1998; Faraone, Biederman, Wozniak, Mundy, Mennin, & O'Donnell, 1997). It also found that among a group of children who were all diagnosed with bipolar disorder on the basis of the presence of elation or grandiosity, prepubertal children who had ADHD along with BPAD, were significantly younger than those without comorbid ADHD (Geller, et al., 2000).

6.2.2 Bipolar Affective Disorder (BPAD)

Bipolar affective disorder (BPAD) is a serious psychiatric illness that is thought to occur in approximately 1% of children (Lewinsohn, klein, & Seeley, 1995). Those who develop Bipolar Disorder as children and young adolescents frequently have the low recovery and high relapse rates characteristic of adults with a severe form of the disorder (Geller, et al., 2001). Young individuals with BPAD often struggle with significant psychiatric co-morbidity, frequent suicide attempts, and poor family, peer, and educational functioning (Reichart, Nolen, Wals, & Hillegers, 2000).

6.2.3 Stimulant Use and Concerns of Its Iatrogenic Effects

Stimulant medications are prescribed primarily for the treatment of ADHD. The short-term efficacy of stimulant medications (methylphenidate, dextroamphetamine and magnesium pemoline) in the treatment of ADHD has been well established (Sadock & Sadock, 1999) (Barkley, 1998) (Robin, 1998). Stimulant medication has been shown to have a robust and positive effect on academic performance in school, short term outcomes, ADHD symptoms and

quality of life, even though long-term studies with controls are warranted (Craig, Davies, Schibuk, Weiss, & Hechtman, 2015).

However, stimulant use has been fraught with controversy both within professional circles and the public arena due to the possibility of iatrogenic effects. DelBello et al. (2001) have confirmed that prior treatment with stimulants is related to earlier age of onset of BPAD independent of ADHD. It has also hypothesized that the very low rates of childhood bipolar disorder in The Netherlands is related to the low rates in that country of prescribing of stimulants and antidepressants to children (Reichart, Nolen, Wals, & Hillegers, 2000).

On the other hand, more recent studies found that stimulant treatment in children and adolescents offers protection against the development of a range of psychiatric problems (Rasmussen, Palmstierna, & Levander, 2015) (Jain, Jain, & Islam, 2011). Biederman, Monuteaux, Spencer, Wilens, & Faraone (2009) in their longitudinal study reported that ADHD patients who received stimulant treatment were significantly less likely to subsequently develop depressive, disruptive behavior and anxiety disorders compared with those who were not treated with stimulants.

The limits of previous studies on this topic have led to the need for prospective, longitudinal investigations in "real word settings" to better understand the relationship between stimulant use, ADHD, and development of BPAD. The purpose of this study is to determine whether there is an increased diagnosis of BPAD in children and adolescent treated with stimulants for ADHD and comorbidities in regular practice settings.

6.3 Method

6.3.1 Context

Though federally mandated, provincial governments in Canada take the responsibility of most health services delivered within their provinces with the services provided varying somewhat from province to province. These services include almost all hospital and physician

services, prescription drug subsidies, a significant proportion of nursing home, and public health (Marchildon & O'Fee, 2007). There is a single payer for these health services. Saskatchewan is a prairie province in Canada. The Saskatchewan Health Care data files which record basic administrative, basic clinical and payment for services, were used for this study. These data files are a unique and comprehensive source of data on health care utilization of the population. Through the use of a unique identifier, they also allow for the tracking of the health utilization of individuals over time. The value of these data files for epidemiological research has been well recognized (Rawson, D'Arcy, & Blackburn, 1992) (Strand & Downey, 1994). These data files have been used for a range of important epidemiological studies (Spitzer, et al., 1992).

Data from the *Physician Services* data file which tracks fee-for-service physician utilization and diagnoses; the *Mental Health Services* data file which tracks the use of provincially funded direct mental health services; the *Hospital Services* data file which tracks hospital stays and length of stay; and the *Prescription Drug* data file which tracks prescription drug usage used in this study. Linkage between the data files allowed for the development of individual patient profile through time. A dummy-identified linked health use dataset was released for research purposes.

6.3.2 Study Design

This is a prospective cohort study which consist of all children and adolescents aged 5 to 17 years of age who were diagnosed and treated for ADHD *and/or* prescribed a stimulant medication during the two year period January 1, 1989 to December 31, 1990.

This exposure group was then followed up for a further ten years to assess the outcome of treatment for ADHD, particularly focusing on the occurrence of Bipolar Mood Disorder. The follow-up period was from inception into the cohort to ten years or December 31, 2000.

An age-gender-residence (Regional Health Authority - RHA) matched comparison group was also be picked. Two comparisons were selected for each exposed subject. Each comparison

was age-gender-RHA matched to each exposed subject but had not had a diagnosis or treatment for ADHD and had not been prescribed a stimulant medication during the inception and follow-up period. Those comparisons who during the follow-up period were diagnosed with ADHD and/or prescribed a stimulant were deleted from the study.

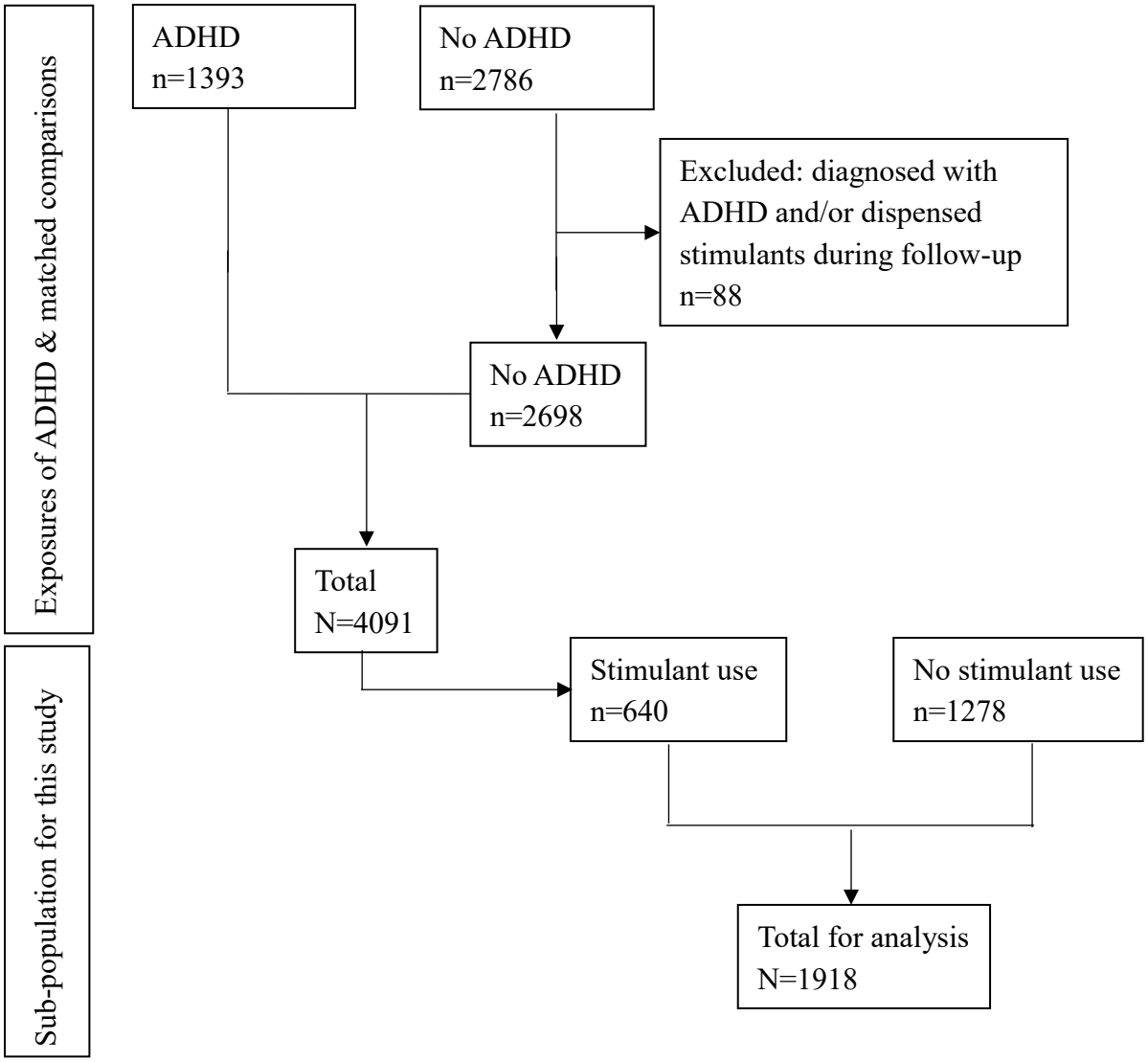
6.3.3 Study Population

A total number of 1,393 ADHD subjects and 2,786 matched comparisons were initially enrolled. Surprisingly, a significant number of those diagnosed with ADHD did not have a stimulant medication registered as being dispensed to them (753/1,393; 54.1%). In this study, we were only interested in subjects who were both *diagnosed ADHD and prescribed and dispensed a stimulant* medications during inception period, which is 45.9% (640/1,393) of all ADHD cases.

Same matching strategies were applied to generate a subpopulation of exposures and comparisons for ADHD and stimulant use for this study. The inclusion criteria for exposures are a diagnosis of ADHD (DSM III-R 314.01) in the Physician Services or the Mental Health Services data files and prescription and dispensed a stimulant medication (methylphenidate, dextroamphetamine, or magnesium pemoline) in the Prescription Drug data file, during the period January 1, 1989 to December 31, 1990.

Comparisons were age-gender-region matched individuals who received treatment during the inception inception period but were not prescribed stimulants during the period of the study from 1989 to 2000. Figure 6-1 shows a flowchart of the subject selection process.

Figure 6- 1 Flowchart of subject selection



6.3.4 Statistical Analysis

Descriptive analyses were employed to understand demographic characteristics of the study population.

Inference analyses were also conducted by using Penalized Maximum Likelihood Estimation (the Firth Method) which was designed for analyzing rare events with logistic regression (Williams, 2016). Two regression models were fitted: (1) Association between stimulant use, individual diagnosis of mental illnesses, and diagnosis of BPAD; (2) whether stimulant use and comorbidity of other psychiatric diagnoses with ADHD would increase the risk of diagnosis of BPAD.

Variables in inference analyses were mainly the diagnoses of psychiatric disorders and prescriptions of psychotropic medications. The outcome variable was bipolar diagnosis (yes/no). Predictor variables in the first model were all dichotomous (yes/no), including *ADHD diagnosis, conduct/anti-social diagnosis, other depression diagnosis, substance abuse, anti-depressant prescription, mood stabilizer prescription, anti-psychotic prescription, and other psychotropic prescription*. The variable "*comorbid ADHD"* replaced individual comorbid diagnosis of mental illness in the second model and were coded into four categories: no diagnosis, ADHD only, other diagnosis without ADHD, and ADHD comorbid.

6.4 Results

6.4.1 Characteristics of Study Population

A total number of 1,918 children and adolescents were included in the analysis. Of which, 640 (33.4%) had at least one stimulant prescription (exposure group), and 1,278 (66.6%) had no prescription stimulant medicines (comparison group) during the inception and follow-up period.

Table 6-1 presents the socio-demographic and clinical characteristics of the study population. Age, gender, and region of residence were proportionally distributed among subjects with and without stimulant use due to matching. The majority of the subjects had no diagnosis of BPAD, other depression, and substance abuse or prescription of psychotropic medications (anti-depressant, mood stabilizer, anti-psychotic, or other psychotropic medications except stimulants) in exposure and comparison groups, respectively. However, more exposures (428/640, 66.9%) had been diagnosed conduct/anti-social disorder, while most comparisons had not (1,168/1,278, 91.4%). Subjects prescribed stimulants were more likely to be diagnosed psychiatric disorders and be prescribed psychotropic medications.

Table 6-1 Characteristics of study population by exposure of stimulant use

	No stimulant (n=1,278)	Stimulant use (n=640)	Total (N=1,918)	P-value[a]
Scale variables (Mean ± SD)				
Age at index (year)	9.7 ± 2.7	9.6 ± 2.7	9.7 ± 2.7	0.961[b]
Categorical variables (n, %)				
Gender				0.990
Male	1,112 (87.0%)	557 (87.0%)	1,669 (87.0%)	
Female	166 (13.0%)	83 (13.0%)	249 (13.0%)	
Regional Health Authority (RHA)				1.000[c]
Sun County	54 (4.2%)	27 (4.2%)	81 (4.2%)	
Five Hills	51 (4.0%)	26 (4.1%)	77 (4.0%)	
Cypress	26 (2.0%)	13 (2.0%)	39 (2.0%)	
Regina Qu'Appelle	148 (11.6%)	74 (11.6%)	222 (11.6%)	
Sunrise	29 (2.3%)	15 (2.3%)	44 (2.3%)	
Saskatoon	716 (56.0%)	358 (55.9%)	1,074 (56.0%)	
Heartland	76 (6.0%)	38 (5.9%)	114 (5.9%)	
Kelsey Trail	36 (2.8%)	18 (2.8%)	54 (2.8%)	
Parkland	92 (7.2%)	46 (7.2%)	138 (7.2%)	
Prairie North	38 (3.0%)	19 (3.0%)	57 (3.0%)	
Mamawetan Churchill River	2 (0.2%)	1 (0.2%)	3 (0.2%)	
Keewatin Yatthe	10 (0.8%)	5 (0.8%)	15 (0.8%)	
Any ADHD diagnosis				0.000[c]
No	969 (75.8%)	0 (0.0%)	969 (50.5%)	
Yes	309 (24.2%)	640 (100.0%)	949 (49.5%)	
Death				0.549[c]
No death recorded	1,271 (99.5%)	635 (99.2%)	1,906 (99.4%)	
Death recorded	7 (0.6%)	5 (0.8%)	12 (0.6%)	
Any bipolar diagnosis				0.000
No	1,248 (97.7%)	601 (93.9%)	1,849 (96.4%)	
Yes	30 (2.4%)	39 (6.1%)	69 (3.6%)	
Any conduct/anti-social diagnosis				0.000
No	1,168 (91.4%)	212 (33.1%)	1,380 (72.0%)	
Yes	110 (8.6%)	428 (66.9%)	538 (28.1%)	
Any other depression diagnosis				0.000
No	1,031 (80.7%)	348 (54.4%)	1,379 (71.9%)	
Yes	247 (19.3%)	292 (45.6%)	539 (28.1%)	
Any substance abuse				0.000
No	1,222 (95.6%)	558 (87.2%)	1,780 (92.8%)	
Yes	56 (4.4%)	82 (12.8%)	138 (7.2%)	
Any anti-depressant prescription				0.000
No	1,168 (91.4%)	473 (73.9%)	1,641 (85.6%)	
Yes	110 (8.6%)	167 (26.1%)	277 (14.4%)	

	No stimulant (n=1,278)	Stimulant use (n=640)	Total (N=1,918)	P-value[a]
Any mood stabilizer prescription				0.000
No	1,240 (97.0%)	573 (89.5%)	1,813 (94.5%)	
Yes	38 (3.0%)	67 (10.5%)	105 (5.5%)	
Any anti-psychotic prescription				0.000
No	1,251 (97.9%)	544 (85.0%)	1,759 (93.6%)	
Yes	27 (2.1%)	96 (15.0%)	123 (6.4%)	
Any other psychotropic prescription				0.000
No	1,151 (90.1%)	431 (67.3%)	1,582 (82.5%)	
Yes	127 (9.9%)	209 (32.7%)	336 (17.5%)	
ADHD and comorbidity				0.000[c]
No diagnosis	792 (62.0%)	0 (0.0%)	792 (41.3%)	
ADHD only	162 (12.7%)	132 (20.6%)	294 (15.3%)	
Other diagnosis without ADHD	177 (13.9%)	0 (0.0%)	177 (9.2%)	
ADHD comorbid	147 (11.5%)	508 (79.4%)	655 (34.2%)	

[a] Chi-square test was employed unless noted; [b] 2-tailed t-test; [c] Fisher's exact test

Table 6-2 Predictors for diagnosis of Bipolar Affective Disorder

Variable		Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Stimulant prescription				
	Yes	2.67 (1.66-4.35)***	0.57 (0.28-1.16)	0.48 (0.24-0.98)*
	No	Reference	Reference	Reference
ADHD diagnosis				N/A
	Yes	4.94 (2.66-9.16)***	1.48 (0.63-3.46)	
	No	Reference	Reference	
Other depression				N/A
	Yes	20.74 (10.04-42.81)***	7.78 (3.52-17.22)***	
	No	Reference	Reference	
Conduct/anti-social disorder			n.s.	N/A
	Yes	3.72 (2.29-6.05)***		
	No	Reference		
Substance abuse			n.s.	N/A
	Yes	6.05 (3.50-10.46)***		
	No	Reference		
Comorbidity			N/A	
	ADHD only	0.73 (0.12-4.47)		0.45 (0.07-3.05)
	Other diagnosis without ADHD	6.30 (2.07-19.16)**		2.63 (0.78-8.82)
	ADHD comorbid	13.49 (5.59-32.59)***		4.76 (1.60-14.23)**
	No diagnosis	Reference		Reference
Anti-depressant prescription				
	Yes	17.13 (10.05-29.20)***	4.02 (2.17-7.44)***	5.17 (2.77-9.64)***
	No	Reference	Reference	Reference
Mood stabilizer prescription				
	Yes	32.28 (19.00-54.83)***	8.89 (4.79-16.52)***	10.33 (5.56-19.20)***
	No	Reference	Reference	Reference
Anti-psychotic prescription				
	Yes	13.53 (8.06-22.71)***	2.65 (1.36-5.16)**	2.47 (1.27-4.80)**
	No	Reference	Reference	Reference
Other psychotropic prescription			n.s.	n.s.
	Yes	3.65 (2.24-5.96)***		
	No	Reference		

OR, odds ratio; CI, confidence interval; n.s., not significant; *p<0.05; **p<0.01; ***p<0.001

6.4.2 Predictors for Diagnosis of Bipolar Affective Disorder (BPAD)

In unconditional analysis, any psychiatric diagnoses significantly predicted the occurrence of BPAD (Table 6-2). The odds ratios (ORs) were: ADHD [OR 4.94, 95% confidence interval (CI) 2.66-9.16], other depression (OR 20.74, 95% CI 10.04-42.81), conduct/anti-social disorder (OR 3.72, 95% CI 2.29-6.05), and substance abuse (OR 6.05, 95% CI 3.50-10.46). It also shows that subjects diagnosed with psychiatric disorders without ADHD (OR 6.30, 95% CI 2.07-19.16) and comorbid with ADHD (OR 13.49, 95% CI 5.59-32.59) were more likely to develop BPAD compared with those without any psychiatric diagnosis.

Prescription of any psychotropic drugs, including stimulants, were also risk factors of development of BPAD. The odds ratios (ORs) were: stimulant (OR 2.67, 95% CI 1.66-4.35), anti-depressant (OR 17.13, 95% CI 10.05-29.20), mood stabilizer (OR 32.28, 95% CI 19.00-54.83), anti-psychotics (OR 13.53, 95% CI 8.06-22.71), and other psychotropic medication (OR 3.65, 95% CI 2.24-5.96).

Two regression models were fitted to explore the association between stimulant use and the occurrence of BPAD: individual diagnoses of psychiatric disorders were included in Model 1; and these individual diagnoses were recoded and replaced by comorbidity in Model 2. Prescriptions of psychotropic medications were included in both models.

In Model 1, stimulant use appeared to be a protective but non-significant factor for BPAD (OR 0.57, 95% CI 0.28-1.16) and the diagnosis of ADHD was a non-significant risk factor for BPAD (OR 1.18, 95% CI 0.63-3.46). The subjects with diagnosis of ‘other depression’ were almost 8 times more likely to develop BPAD compared with those without that diagnosis (OR 7.78, 95% CI 3.52-17.22). The prescription of anti-depressant (OR 4.02, 95% CI 2.17-7.44), mood stabilizer (OR 8.89, 95% CI 4.79-16.52), and anti-psychotics (OR 2.65, 95% CI 1.36-5.16) also predicted BPAD. These associations were diminished compared with those in unconditional analysis due to the adjustment for all diagnoses of psychiatric disorders and psychotropic

prescriptions. Diagnosis of conduct/anti-social disorder, substance abuse, and 'other psychotropic' medications were not significantly related to BPAD.

The redefined comorbid ADHD variable included in Model 2 showed that the subjects with comorbid ADHD were approximately 5 times more likely to develop BPAD than those without any psychiatric diagnosis (OR 4.76, 95% CI 1.60-14.23). In addition, prescription of stimulants demonstrated a protective effect on the future development of BPAD (OR 0.48, 95% CI 0.24-0.98). The prescription of anti-depressants (OR 5.17, 95% CI 2.77-9.64), mood stabilizers (OR 10.33, 95% CI 5.56-19.20), and anti-psychotics (OR 2.47, 95% CI 1.27-4.80) still positively predicted BPAD.

6.5 Discussion

Our study used population-based provincial administrative health care data files to investigate the association between prescription of stimulant medications for ADHD and subsequent onset of BPAD. We found that stimulant prescription had protective effects on the development of BPAD. In contrast, subjects with ADHD and comorbid for other psychiatric disorders and those prescribed anti-depressant, mood stabilizer, and anti-psychotic medications were at much greater risk of subsequently developing BPAD.

6.5.1 Stimulant Use and BPAD

Prescription of stimulants for ADHD was shown as a significant risk factor of BPAD in unconditional analysis. This is consistent with some studies indicating that prior treatment with stimulants is related to earlier onset of BPAD independent of ADHD (DelBello, et al., 2001).

With adjustment for several specific psychiatric diagnoses and other psychotropic medications, stimulant use tended to be a protective factor for BPAD, although the association was not significant. However, this protective effect became significant when the redefined comorbid psychiatric disorder variable and the use of other psychotropic medications were taken into account. This is consistent with more recent studies among children and adolescents with

ADHD that showed stimulant treatment offers protection against the development of BPAD (Biederman, Monuteaus, Spencer, Wilens, & Faraone, 2009) (Gibson, 2009).

6.5.2 ADHD and Comorbidity

In unconditional analysis, the diagnosis of ADHD was found to be a risk factor of the development of BPAD but not when it was adjusted for the diagnosis of other psychiatric disorders and the dispensing of non-stimulant psychotropic medication. Our finding in unconditional analysis is consistent with previous studies (West, et al., 1995; DelBello, et al., 2001).

In model building, we found that subjects with ADHD and comorbid with other mental disorders were almost 5 times more likely to develop BPAD compared with those without any psychiatric diagnosis. Biederman, Newcorn, & Sprich (1991) proposed that "attention deficit hyperactivity disorder is most likely a group of conditions with potentially different etiologic and modifying risk factors and different outcomes rather than a single homogeneous clinical entity". It was also claimed in a longitudinal study that children with comorbid diagnosis of ADHD and conduct disorder or major depression at baseline were more likely to develop bipolar disorder than those with ADHD alone and normal controls (Biederman et al., 1996a).

We propose that ADHD is more likely to be persistent when it is comorbid with other psychiatric disorders. It has been confirmed that when combined with conduct problems, ADHD is a more severe genetic variant (Thapar, Harrington, & McGuffin, 2001). A longitudinal twin study was conducted and indicated that children with persistent ADHD had more mental health issues, including generalized anxiety disorder, major depressive episode, conduct disorder, and marijuana dependence (Agnew-Blais, et al., 2016).

6.5.3 ADHD and Prescription of Other Psychotropic Medications

Besides the prescription of stimulants, we found that use of other psychotropic medications, such as anti-depressant, mood stabilizer, and anti-psychotic, were also associated

with BPAD. These prescription drugs can be used for treatment of corresponding disorders or comorbidities, or can also be alternative options for better outcomes if symptoms of bipolar disorders have shown up.

Many authors have called attention to the difficulty of distinguishing between ADHD and BPAD in children and adolescents (West, McElroy, Strakowski, Keck, & McConville, 1995) (Faraone S. V., Biederman, Mennin, & Russell, 1998) (Wozniak, Biederman, & Richards, 2001). This might be important, because it has been found that symptoms of ADHD were stabilized only in subjects whose mania was well treated (Wozniak, Biederman, & Richards, 2001) (Spencer, et al., 2001). Biederman, Russell, Soriano, Wozniak, & Faraone (1998) discovered no disadvantage to the use of adjunctive medication, such as selective serotonin reuptake inhibitors (SSRIs) and stimulants, to treat comorbid symptoms, but children did better if they received a mood stabilizer than an antidepressant or stimulant when symptoms of mania were present.

6.5.4 Strengths and Limitations

This study should be considered in light of several strengths and limitations. The first strength is that a large number of population exposure group from regular practice settings were analyzed. Second, the exposures and matched comparisons were followed-up for 10 years, which is sufficient for a longitudinal study on mental health outcomes to reasonably expect the occurrence of an outcome in question and to examine the association between the exposure and outcome, if it exists.

Some limitations also need to be noted. First, limited information was provided from administrative data files. For example, only age, gender, and region of residence were recorded as socio-demographic characteristics. Additionally, since we do not have information on clients who were lost of follow-up leading to the possibility of some selection bias.

More details are needed to better understand the relationship between stimulant use and bipolar disorder, and to explore socio-demographic determinants of the outcome. More detailed

clinical information is also desired, such as diagnosis and severity of ADHD, number of hospital/physician's visits, and number of comorbidity. These can be helpful describing the population under study, severity of disease, and identifying a dose-response relationship. Second, diagnoses can vary between general practitioners and specialists. One-time diagnosis or prescription might be the least severe cases, but can also be an error judgement. In this patient population, approximately 7.6% (55/728) were prescribed stimulant medications only once. Finally, Penalized Maximum Likelihood Estimation for logistic regression was applied for analysis due to low number of outcome events. As a result, statistical model diagnosis was not available to examine the degree of model fit.

6.6 Conclusion

This study using administrative health data from everyday practice provides robust evidence that stimulant use in children and adolescents appears to be protective against the future development of bipolar disorder. In contrast, anti-depressant, mood stabilizer, and anti-psychotic use and the additional diagnoses of depression and comorbid ADHD are predictive of BPAD.

The findings no doubt reflect the impact of the nature of the initial disease on further disease progression. Given the current controversies regarding the use of stimulant medications in children, this study indicates that stimulant use by itself does not lead to the development of BPAD but rather the severity of the initial disease itself and comorbidity serve as an indicator of the development of future disease.

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CHAPTER 7 – CONCLUSION AND POLICY AND PROGRAM IMPLICATIONS

Mental illness is a leading cause of disability in Canada (Mental Health Commission of Canada, 2014; Lim, Jacobs, Ohinmaa, Schopflocher, & Dewa, 2008). They account for nearly a quarter (23%) of Years of Life Lost (YLL) due to disability and 13% of YLL due to disability and premature mortality in Canada. It is estimated that 1 in 5 Canadians experiences a mental health or addiction problem every year (Smetanin, et al., 2011).

Mental illness also causes a heavy economic burden. The costs in Canada were estimated at $51 billion per year, which included health care costs, lost productivity, and reductions in health-related quality of life (Lim, Jacobs, Ohinmaa, Schopflocher, & Dewa, 2008; Smetanin, et al., 2011). People with mental illness and addictions are more likely to suffer from comorbidity of other mental or chronic health conditions (Scott, et al., 2011). Conversely, depression and anxiety disorders may be a concomitant consequence of the burden of chronic diseases or conditions, such as long-term medical conditions and coronary heart disease (Patten, 2001; Frasure-Smith & Lesperance, 2005).

In order to reduce the burden of mental and behavioral disorders, the World Health Organization (WHO, 2001) suggested that a public health approach would be the most appropriate method to respond to the multifaceted etiology, widespread stigma and discrimination, and significant treatment gap across the world. There are a number of actions that can be achieved, such as formulating policies, assuring universal access to mental health services (including health promotion and prevention), ensuring adequate care and protection of human rights, promoting healthy lifestyles and reducing risk factors, as well as enhancing research into the causes of mental disorders, the development of effective treatment, and the evaluation of mental health systems, etc. (WHO, 2001).

The core of public health is the prevention of disease, particularly primary and secondary prevention. In order to prevent and intervene in the development of mental illness, knowledge of

its nature – risk factors, course and outcome – is needed. Prevention should not be harmful or iatrogenic. The treatment of some psychiatric conditions and population groups have been considered as carrying significant risks for iatrogenesis.

Why Study Children and Adolescents? It is reported that 70% of mental health problems have their onset during childhood or adolescence (Government of Canada, 2006). Young people aged 15 to 24 are more likely to experience mental illness and/or substance abuse than any other age group (Pearson, Janz, & Ali, Statistics Canada Catalogue no.82-624-X, 2015). The usage of health services for mental illness among children and adolescents increased from 1996/97 to 2009/10. In addition, a life course epidemiology links adult health and disease risk to physical or social exposures during gestation, childhood, adolescence, earlier in adult life, or across generations. Early life conditions and experiences, such as poverty, adverse experience, and poor early growth, may make individuals more susceptible to developing adult risk factors and/or chronic diseases. Therefore, the life course strategies for prevention of chronic conditions suggest intervening as early as possible before damage and disability set in (Factor-Litvak & Susser, A life course approach to chronic disease epidemiology, 2004).

The primary goal of this thesis is to contribute to our understanding of mental health issues in children and adolescents by applying various epidemiological methods and data sources to provide a possible basis for future health prevention planning and policy decision-making. In this context, this thesis explores in separate chapters research targeting on primary and secondary prevention and potential iatrogenic effects on psychoactive medication use in children adolescents. Finally, the implications of this research for mental health policy and intervention in children and adolescents' mental illness/health are identified.

7.1 Major Findings of This Thesis

At the primary prevention level in Chapter 3 of this thesis using systematic review and meta-analysis methods examined relationship between early childhood maltreatment and the

latter onset of depression and anxiety disorders and the potential for disease reduction if exposure to such risk factors was decreased. While the literature supports a strong relationship between childhood maltreatment and mental illness, most studies were cross-sectional and/or use recall to assess maltreatment and are thus prone to temporality and recall bias. In addition, research on the potential prospective impact of maltreatment reduction on the incidence of psychiatric disorders is scarce. Electronic databases and grey literature from 1990 to 2014 were searched for English-language cohort studies with criteria for depression and/or anxiety and non-recall measurement of childhood maltreatment. Systematic review with meta-analysis synthesized the results. Study quality, heterogeneity, and publication bias were examined. Initial screening of titles and abstracts resulted in 199 papers being reviewed. Eight high-quality articles met eligibility criteria. Population attributable fractions (PAFs) estimated potential preventive impact. Physical abuse, sexual abuse, and neglect were found to all significantly increase the risk for depression and anxiety. The pooled odds ratio (OR) between any type of maltreatment and depression was 2.03 [95% confidence interval (CI) 1.37–3.01] and 2.70 (95% CI 2.10–3.47) for anxiety. For specific types of maltreatment and depression or anxiety disorders, the ORs were: physical abuse (OR 2.00, 95% CI 1.25–3.19), sexual abuse (OR 2.66, 95% CI 1.88–3.75), and neglect (OR 1.74, 95% CI 1.35–2.23). PAFs suggest that over one-half of global depression and anxiety cases are potentially attributable to self-reported childhood maltreatment. A 10–25% reduction in maltreatment could potentially prevent 31.4–80.3 million depression and anxiety cases worldwide. PAFs also suggest that over one-third of Canadian depression and anxiety cases are potentially attributable to childhood maltreatment. A large number of cases could potentially be prevented by reducing the exposure to maltreatment. This review provides robust evidence of childhood maltreatment increasing the risk for depression and anxiety, and reinforces the need for effective programs and policies to reduce its occurrence.

Chapter 4 of this thesis also targeting primary prevention explores that epigenetic changes, especially DNA methylation changes that are generally associated with depression.

Studies have consistently found that genetic and psychosocial environment substantially contribute to the risk of depression (Saveanu & Nemeroff, 2012; Silberg, et al., 1999; Rice, Harold, & Thapar, 2001), although inconsistent findings were identified in different subgroups of candidate genes (e.g. BDNF, SLC6A4, NR3C1, OXTR, and others) and genome-wide studies. However, replications of these research findings have been hampered by phenotypic and genetic heterogeneities, even in large-scale genome wide association studies (Lewis, et al., 2010; Shi, et al., 2011; Akula, et al., 2010; Muglia, et al., 2010). It is now generally accepted that the pathogenesis of depression not only includes genetic, psycho-socio environmental factors, their interactions, but also involves epigenetic modifications, especially with altered DNA methylations, which have been identified as an etiological and diagnostic biomarker for many mental disorders in a number of studies (Dempster, et al., 2011; Fuchikami, et al., 2011; Walker, et al., 2016; Kaminsky, et al., 2012). Both genetic and environmental factors can affect the extent of DNA methylation. It may also integrate the impact of both genetic and environmental factors in the potential downstream functional outcomes on a phenotype (Lienert, et al., 2011; Schadt, 2009).

Gene expression and epigenetics. A number of gene have been putatively linked to major depression. A gene is a string of DNA encoding information and hiding in a cell's nucleus. Gene expression refers to the process of synthesizing the information in a gene to produce functional gene products which can be proteins or non-proteins, such as transfer RNA (tRNA) or small nuclear RNA (snRNA). Gene expression consists from several steps, including transcription, RNA splicing, translation, and post-translational modification. Genes are expressed by being transcribed into message RNA (mRNA), and then be translated into protein via tRNA (Wikipedia, 2016).

The regulation of gene expression is crucial to an organism's development. It ensures the genetic information in DNA is properly interpreted and allows the genotype to give rise to organism's phenotype. Genes can interact with and respond to organism's environment. External

environmental factors or endocrine signals (Nguyen, Nioi, & Pickett, 2009) may cause modification of regulatory proteins (Paul, 2008) and intracellular signals (Los, Maddika, Erb, & Schulze-Osthoff, 2009), then further affect regulation of gene expression.

Epigenetics refers to the external changes in a chromosome, which affects transcription and gene expression, and alters heritable phenotype. Epigenetic modifications of gene expression include alterations in DNA methylation – the addition of a methyl group which prevents certain genes from being expressed, and histone modifications (Dalton, Kolshus, & McLoughlin, 2014; Rettner, 2013; Ennis, 2014). Histones are proteins that DNA wraps around. Modifications that squeeze DNA tightly make the DNA cannot be "read" by the cell; on the contrary, relaxed histones can make the DNA accessible to be "read" (Rettner, 2013). Epigenetic modifications can be potentially caused by many outside stimulus from chemicals to lifestyle factors, such as Bisphenol A (BPA), exercise, and child abuse and other forms of early trauma (Ennis, 2014). DNA methylation is the most studied epigenetic modification, and can change the activity (turning genes "on" or "off") of a DNA segment without change in the DNA's sequence (Dalton, Kolshus, & McLoughlin, 2014).

A number of systematic reviews on susceptible genes and gene by environment interactions provide a comprehensive list of putative genetic and environmental risk factors for major depressive disorder (MDD) (Levinson, 2006; Lohoff, 2010; Shyn & Hamilton, 2010; Saveanu & Nemeroff, 2012; Cohen-Woods, Craig, & McGuffin, 2013; Dunn, et al., 2015). However, there has been little compilation of our knowledge of DNA methylation and depression. Furthermore, there has been no comprehensive review of epigenetic studies in depression critically exploring experimental methodologies and verification of laboratory testing in humans, which may significantly affect the accuracy and validity of results.

To fill this information gap, and provide critical update on the latest findings of DNA methylation in depression, we aimed to: 1) systematically synthesize major findings on DNA

methylation and depression; 2) compare similarities and differences across different studies, including experimental and laboratory factors and statistical analyses, which might partially explain some inconsistencies of results; and, 3) comment on the challenges and opportunities for future studies.

Electronic databases and grey literatures up to June 2016 were searched for English-language studies with clear criteria for diagnosis of depression. Fifty-seven articles met our eligibility criteria and were included in this review along with a summary of study characteristics. We grouped the findings into etiological and treatment studies according to the following genomic attributes: (1) *BDNF*; (2) *SLC6A4*; (3) *NR3C1*; (4) *OXTR*; (5) *other candidate genes*; (6) *genome-wide*; and, (7) *treatment response*. Majority of the studies were recently published and from developed countries. Whole blood and saliva samples were the most studied common tissues. Bisulfite conversion, along with pyrosequencing, was widely used to test DNA methylation level across all studies. High heterogeneity existed among the studies in terms of experimental and statistical methodologies and study designs. Given such heterogeneity it is recommended that a systematic review without meta-analysis be undertaken. Inconsistent findings were identified in each study subgroup. Majority of the studies on *BDNF* (10/11) and nearly half of studies on *SLC6A4* (5/11) showed that an increased DNA methylation was associated with depression. Significant (with both hyper- and hypo-methylation) and insignificant relationships were found in all other subgroups. However, this review generally supports the finding that DNA methylation changes are associated with depression. It is suggested that more longitudinal studies using standardized experimental and laboratory methodologies are needed in future epigenetic studies to enable more systematic comparisons and quantitative synthesis.

In Chapter 5 the effect of early treatment of mental health and emotional problems in children and adolescent mental health problems in a publicly funded mental health treatment services is examined. Children and adolescents' mental health problems substantially impact

their daily functioning. We sought to: (1) understand the impact of treatment in public mental health services had on functional improvement; (2) identify predictors of functional improvement in the various domains of children and youths' lives; and (3) make suggestions regarding improving the effectiveness of services.

Clinical data from the Child and Youth Mental Health and Addictions Services of the Saskatoon Health Region (N=645 children and 682 youths) for the year 2011-2014 were examined. The outcome measure used was the established Child and Adolescent Functional Assessment Scale (CAFAS) - a global measure of impairment/functioning with eight domain subscales. Non-parametric tests were used to compare median scores at baseline and exit. Logistic regression models were fitted to examine predictors of improvement. Comparisons between children and youth were conducted.

CAFAS Total Scores at exit form treatment showed a significant decrease from initial scores for both the child and adolescent age groups, indicating that client functioning had improved. Initial levels of dysfunction, length of treatment and pervasiveness of behavioural impairment (PBI) were shared predictors for functional improvement among all clients. Primary presenting problem, caregiver support and area of residence (west side of the City of Saskatoon) were associated with the outcome among children only.

Our findings clearly indicate that current mental health services significantly improved child and adolescent functioning for most but not all clients. However, those with an initial high level of dysfunction and high PBI score require more treatment to reach an appropriate outcome. Shortening the length of each treatment cycle may improve the efficiency of resource use but can be detrimental to some clients. Personalized treatment should be tailored to the specific characteristics and needs of clients.

Finally, in Chapter 6 the question of whether the use of stimulants to treat Attention Deficit and Hyperactivity Disorder (ADHD) has iatrogenic results in the development of

Bipolar Affective Disorder (BPAD) is examined in a 10-year outcome study using administrative health care data files of physicians in regular practice settings in the Province of Saskatchewan. There has been controversy in the literature concerning the use of stimulant medications, a standard treatment for ADHD, and the development of BPAD. Previous studies on the subject have had various limitations suggesting a need for prospective, longitudinal investigations of everyday practice to better understand the relationship between stimulant use, ADHD, and development of BPAD. In regular practice, does the treatment of ADHD patients with stimulants lead to BPAD?

Health administrative data for the Canadian province of Saskatchewan were used for a prospective cohort study. All children and adolescents aged 5 to 17 years of age who were diagnosed and treated for ADHD with stimulant medications during 1989-1990 comprised the inception cohort. These exposure subjects were followed-up for 10 years to determine BPAD occurrence, and were matched with two age-gender-region of residence comparisons. A total of 1,918 exposures and comparisons comprised the cohorts analyses used Penalized Maximum Likelihood Estimation.

It was found that prescription of stimulants for ADHD was a significant risk factor of BPAD in unconditional analysis (OR 2.67, 95% CI 1.66-4.35). However, it became to be a protective factor for BPAD after adjusting for comorbidity of psychiatric disorders, particularly co-morbid depression. (OR 0.48, 95% CI 0.24-0.98).

The study finding suggest findings that it is the impact of the nature of the initial disease rather than the use of stimulants that leads to progression of some initial exposure to Bipolar Disorder. This study, consistent with some previous research, indicates that stimulant use by itself does not lead to the development of BPAD but rather the severity of the initial disease is an indicator of future disease trajectory.

7.2 Policy Implications and Future Research

The two systematic reviews in this thesis reveal the association between childhood maltreatment and epigenetics and the development of depression and/or anxiety. They reinforce the importance of life course strategies for prevention and the need for effective programs and policies to reduce the occurrence of mental illnesses. Pregnancy and early life experience and environment, which may lead to epigenetic changes, play an essential role in lifetime mental wellbeing. Decreasing the amount of maltreatment and other adverse experience in childhood and during pregnancy should be the target for mental illness prevention and mental health promotion.

This thesis also indicates that current child and adolescent mental health services effectively improved most clients' functioning. Severity of mental problems can affect the effectiveness of treatment. Shortening the length of each treatment cycle may improve the efficiency of resource use but at the expense of clients that need more time to achieve a more optimal functional improvement. Personalized treatment services are required to meet clients' specific needs and maximize the cost effectiveness of services.

Given the current controversies regarding the use of stimulant medications in children, this thesis indicates that stimulant use in children and adolescents appears to be protective against the future development of bipolar disorder. However, the severity of the initial disease itself and comorbidity serve as an indicator of the development of future disease.

From a research perspective future epidemiological studies should be more linked to epigenetics and adopt longitudinal study designs to trace the change overtime. To allow for a systematic comparison of studies, an agreed upon consistent set of standards involving a minimum set for items for the execution and reporting of epigenetic studies is warranted.

7.3 Conclusion

There are four take-way messages from this research are that:

1) Early childhood maltreatment is a significant causal factor in the development of future depression and anxiety, and that the incidence of depression and anxiety could be significantly reduced by reducing the prevalence of various types of childhood mal treatment.
2) Alterations in gene expression (epigenetic events) can lead to increased risk of depression. Such epigenetic events can be the result of in utero exposure of fetus to a variety of negative stressors.
3) Early treatment of mental health problems in children and adolescents is beneficial in increasing their positive functioning in and across life domains. Early intervention is likely to change the trajectory of disease progression.
4) Stimulant medication used to treat children and adolescents for ADHD does not have negative (iatrogenic) effects leading to an increased risk of developing BPAD.

The clear public health message is that early reduction in risk factor exposure in utero and in childhood and adolescence and the early treatment of mental health problems has a very positive effect in reducing the onset and further development of psychiatric disease and mental health problems.

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