Voor Hanna en Jan

Research in this thesis was funded by the National Institute of Mental Health, Grant number: R01 MH090553 (to prof. dr. R.A. Ophoff).

Financial support for the publication of this thesis was kindly provided by the Brain Center Rudolf Magnus and Rode Kruis Ziekenhuis.

ISBN 978-94-6380-100-3

Design: Bregje Jaspers, ProefschriftOntwerp.nl
Printed by: ProefschriftMaken, www.proefschriftmaken.nl

Pharmacological treatment and determinants of psychosis in patients with bipolar disorder

Medicamenteuze behandeling en determinanten van
psychose bij patiënten met bipolaire stoornis
(met een samenvatting in het Nederlands)

Proefschrift

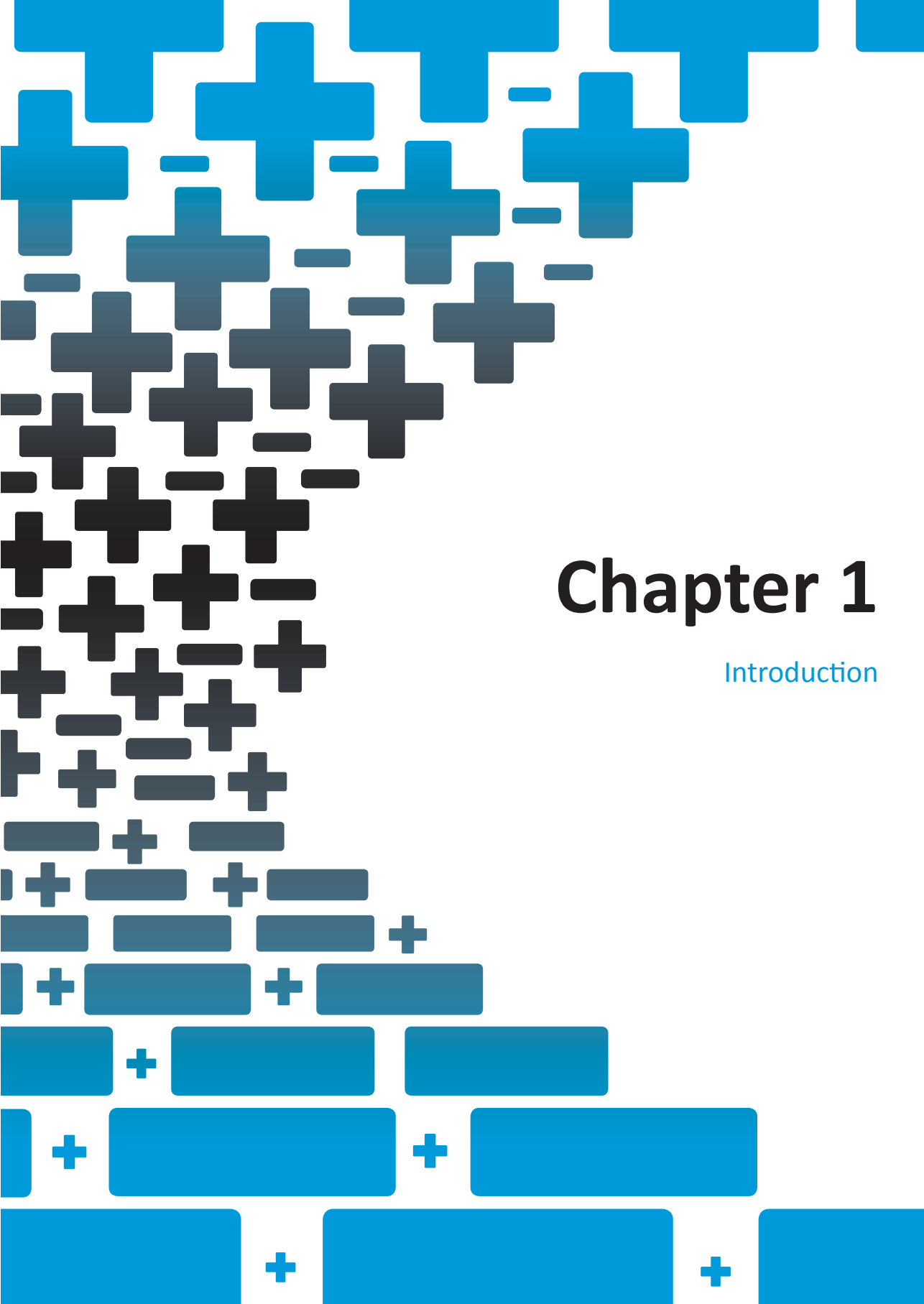
ter verkrijging van de graad doctor
aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op
vrijdag 21 december 2018
des middags te 12:45 uur

door

Annet Herma van Bergen
geboren op 12 augustus 1983
te Bunnik

Promotoren: Prof. dr. R.S. Kahn
Prof. dr. R.A. Ophoff
Copromotor: Dr. M.P.M. Boks

Table of contents



Chapter 1

Introduction

Introduction:

Bipolar disorder is a mental illness that is defined by recurrent episodes of depression and elevated mood (i.e. hypomanic and manic episodes). The broad spectrum of bipolar disorder is defined in several categories, of which bipolar type I and type II are the most typical forms, together affecting about 1% of the general population (Merikangas et al. 2011). Bipolar disorder is along with other psychiatric disorders, the leading cause of disease burden (expressed as years lived with disability) worldwide (GBD 2016 DALYs and HALE Collaborators, 2017). Despite the large impact of bipolar disorder, the underlying pathophysiology and the mechanism through which existing pharmacological treatment is effective remains largely unknown.
This thesis focuses on two topics:

1. *Psychotic symptoms in bipolar disorder to explore a potential psychotic subtype of the disorder.*
2. *Current and new developments in pharmacological treatment in bipolar disorder.*

In this chapter, the diagnostic criteria of bipolar disorder are described followed by a brief explanation of diagnostic heterogeneity within psychiatric disorders as an introduction of the first topic of this thesis. The second part describes the current status of pharmacological treatment of bipolar disorder.

1a. Classification and diagnostic criteria of bipolar disorder:

The spectrum of bipolar disorder refers to a group of affective disorders, which together are characterized by depressive and manic or hypomanic episodes. The spectrum is broad and the bipolar phenotype is defined solely to clinical features. The two main bipolar categories which are of interest for this thesis are bipolar disorder type I and type II. Bipolar disorder type I consists of manic episodes often alternated by depressive episodes. Bipolar disorder type II is distinguished from type I by the occurrence of (less severe) hypomanic instead of manic episodes. A depressive or (hypo)manic episode is diagnosed by strict criteria described in the Diagnostic and Statistical Manual of Mental Disorders (DSM).
Core symptoms of a (hypo)manic episode are a persistent elevated (euphoric) and expansive or irritable mood which at least lasts for a week. This change in mood is accompanied by symptoms like increased self-esteem, or grandiosity, decreased need for sleep, increased distractibility, psychomotor agitation and involvement in activities with painful consequences. Psychotic symptoms, consisting of hallucinations and delusions, can occur during a manic episode. By definition a hypomanic episode is not accompanied by psychotic symptoms and causes a lower level of distress or impairment in life compared to a manic episode. Bipolar disorder type I and Type II are best distinguished by the severity of manic symptoms. The majority of bipolar patients suffers the most from depressive episodes, which are more frequent and last longer than (hypo)manic episodes. A depressive

episode within the bipolar spectrum is not any different from an unipolar depression. Core symptoms are a persistent depressed mood and loss of interest, which lasts for more than two weeks and causes significant distress and impairment in life. In addition, depression is accompanied by symptoms like change in appetite or weight, insomnia or hypersomnia, agitation or psychomotor retardation, fatigue, feelings of worthlessness, diminished ability to concentrate and recurrent thoughts of death.
This thesis focuses on patients diagnosed with bipolar disorder type I and the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorder IV are used.

1b. Diagnostic heterogeneity:
Psychiatric disorders are considered separate diagnostic entities. Diagnostic heterogeneity is substantial in psychiatry and there is considerable overlap in symptoms indicating shared etiology. Bipolar disorder has a polygenic basis which is substantially shared with schizophrenia for instance (International Schizophrenia Consortium, 2009). In addition, psychotic and mood symptoms occur in bipolar disorder and schizophrenia patients and the presence of illness characteristics like cognitive impairment and childhood trauma are common in both disorders. Moreover, the mood and psychotic symptoms in both can respond to the same pharmaceutical treatment. The subcategories within the bipolar spectrum, bipolar disorder type I and type II, are very similar in symptom profile and these categories are mostly defined by the severity of symptoms. This can make it hard to classify and diagnose in clinical practice. Unravelling heterogeneity in diagnostics is important to make progress in detecting underlying biological mechanisms of psychiatric symptom groups. To date, the pathogenesis of bipolar disorder is poorly understood and reduction of the prevalence and burden of bipolar disorder is needed. Reducing heterogeneity by better characterizing the core clinical phenotype of bipolar disorder is essential to make progress in this search.
The first part of this thesis focuses on heterogeneity within psychiatric diagnostics by investigating psychotic symptoms in bipolar disorder to explore a potential subtype of the disorder **(chapter 2)**.

2. Pharmacological treatment
Pharmacological treatment is available for bipolar disorder, but more than a third of the patients does not or partly respond (Perlis & Ostacher 2006; Geddes & Miklowitz 2013). The current first choice of pharmacological treatment in bipolar disorder are mood stabilizers, of which the neurobiological mechanism remains largely unclear (Klein & Melton 1996; Williams et al. 2002). The high overlap in symptoms between psychiatric disorders has led to new developments in bipolar disorder treatment in the past decades, which mainly exist of repurposing drugs which are already registered for other psychiatric disorders. For instance,

antidepressants and antipsychotics can be added to a mood stabilizer depending on the state of mood (depressive or manic). The development of new pharmacological treatments for bipolar disorder depends mainly on identification of underlying biological mechanisms and by increasing the knowledge of the existing pharmacological treatment.

The second part of this thesis focuses on three pharmacological topics in the treatment of bipolar disorder with the aim to enlarge the knowledge of current psychopharmacological treatment by:

1. Investigating the effectiveness of lithium, a widely used mood stabilizer, in long-term treatment, in particular after a period of discontinuation (**Chapter 3**).
2. Reviewing the literature on cognitive effects of mood stabilizers and antipsychotics in addition to the development of new cognitive enhancing agents (**Chapter 4**).
3. Investigating epigenetic effects, a potential biological mechanism of action, of current available mood stabilizers, anti-psychotics and antidepressants (**Chapter 5**).

Part I: Psychotic symptoms in bipolar disorder

Approximately 56-70% of the patients diagnosed with bipolar disorder type I report lifetime psychotic symptoms (Goodwin FK & Jamison KR 1990; Keck et al. 2003; Bora et al. 2010; Upthegrove et al. 2015). Psychotic symptoms include hallucinations, delusions, catatonic behavior (marked disturbances in psychomotor movements) and thought disorder. Psychotic symptoms in bipolar disorder may occur during mood episodes but are not present during euthymia. Psychosis is a severe mental illness, which is not specific to bipolar disorder. Psychosis is a key symptom of schizophrenia which is a complex syndrome defined primarily as a psychotic disorder. Besides recurrent psychotic episodes, features of schizophrenia are cognitive decline, lack of initiative, lack of energy, social withdrawal, emotional flattening and poverty of speech which are more chronic of nature. The presence of mood episodes is not a required diagnostic criterion for schizophrenia but does often occur. Generally, schizophrenia is associated with a lower level of global functioning than bipolar disorder (Green 2006; Bowie et al. 2010). Bipolar disorder patients with a lifetime history of psychotic symptoms have been associated with characteristics that resemble features of schizophrenia like symptom severity and worse psychosocial outcome compared to bipolar patients without psychotic symptoms (Levy et al. 2013; Özyildirim et al. 2010). Apart from this, psychotic symptoms in bipolar disorders have been associated with features that indicate a more severe disease course: an earlier age of disease onset (Upthegrove et al. 2015), a higher frequency of mood episodes and hospitalizations (Glahn et al. 2007; Özyildirim et al. 2010; Simonsen et al. 2011; Levy et al. 2013), more comorbidity (Coryell et al. 2001) and a lower response to lithium (Maj et al. 2002; Maj 2003). In addition to psychosis and mood symptoms, schizophrenia and bipolar disorder overlap in more clinical characteristics, such as cognitive impairment (Green 2006). Cognitive impairment is a core

feature of schizophrenia (Kahn & Keefe 2013) and is also present in bipolar disorder, also throughout the euthymic phase but less severe (Martínez-Arán et al. 2004; Robinson et al. 2006; Arts et al. 2008; Bora et al. 2009; Vreeker et al. 2016). Some argue the existence of a psychosis continuum, which extends from bipolar disorder, to schizo-affective disorder and at the other end typical schizophrenia. It reflects an increasing level of severity including a decrease in level of global and cognitive functioning (van Os et al. 2000; Craddock et al. 2005; Internation Schizophrenia Consortium 2009). Evidence suggests that psychosis might have a negative impact on cognitive functioning (Glahn et al. 2007; Bora et al. 2007). The largest meta-analysis study investigating a history of psychotic symptoms and cognitive functioning in bipolar disorder shows that a history of psychosis is associated with greater severity of cognitive deficits. However, this effect is modest and the findings do not suggest a categorical distinction between bipolar disorder with and without psychotic symptoms (Bora et al. 2010).

An important risk factor of psychiatric disorders, psychosis specifically, is childhood trauma (Varese et al. 2012; Read et al. 2005). The presence of auditory hallucinations in particular have been associated with childhood trauma in psychotic patients (Daalman et al. 2012; Read et al. 1999). Whether this relation exists across diagnostic boundaries remains unclear. In bipolar disorder a relation between a history of hallucinations, mood congruent and abusive auditory hallucinations specifically, and childhood maltreatment was suggested in one study (Upthegrove et al. 2015). The study described in **Chapter 2**, provides data on demographical, clinical and neurocognitive characteristics in addition to the presence of childhood maltreatment in bipolar disorder type I patients. It is one of the largest and most comprehensive assessed bipolar type I samples (n=1,342) which gives the opportunity to investigate all these characteristics at once in relation to a history of psychotic symptoms.

*The hypothesis of **Chapter 2** states that patients with a history of psychotic symptoms have a more severe illness course, lower level of global functioning, lower level of cognitive functioning and higher levels of childhood maltreatment compared to patients without the presence of a history of psychotic symptoms.*

Part II: Pharmacological treatment in bipolar disorder

Treatment in bipolar disorder focuses on acute stabilization or maintenance therapy. In the acute phase the goal is to bring a patient with mania or depression to a symptomatic recovery with euthymic mood. The goals in the maintenance phase are relapse prevention, reduction of subthreshold symptoms, and enhanced social and occupational functioning. Pharmacotherapy is an essential part of treatment in bipolar disorder in each of these phases. Mood stabilizers are the first choice of treatment, but a wider range of drugs are effective in bipolar disorder treatment. For instance, acute mania is best treated by antipsychotics and bipolar depression can be treated by augmentation of antidepressants to a mood

stabilizer (Scherk et al. 2007; Sidor et al. 2011). In the STEP-BD cohort, 58% of patients with bipolar disorder type I and type II achieved recovery after pharmacological treatment. The STEP-BD cohort study was a multicenter study designed to evaluate longitudinal outcomes in 1469 bipolar disorder patients. Despite the currently available medication, 49% of the patients had recurrences in a 2-year interval. Twice as many of these recurrences were of depressive polarity rather than manic polarity (Perlis & Ostacher 2006). After initial onset, patients with bipolar disorder frequently have residual depressive symptoms (Judd et al. 2002). This highly contributes to impaired functioning and compromised quality of life in bipolar patients (Judd et al. 2005). Thereto, the side effects of pharmacological treatments can be wearing or even life threatening. Moreover, cognitive deficits in bipolar disorder are debated to be a possible side effect which adversely affects functional outcome in patients (Green 2006). Overall, success of the current pharmacological treatment is limited and there is still a lot to be achieved in treatment of bipolar disorder. Reducing the relapse rate and developing better treatment options for residual symptoms and cognitive dysfunction is the challenge for the future to improve the quality of life of bipolar patients. True advances in pharmacological treatment have been limited in the past decades due to the consequent absence of validated pharmacological targets of currently available medication in addition to the scarce knowledge of basic disease mechanisms.

First a general introduction of lithium treatment (1) is provided, which is followed by an introduction of the cognitive effects (2) and epigenetic effects (3) of psychotropic drugs.

1. Lithium:

Lithium, introduced by John Cade in 1949, remains the best established long-term treatment for bipolar disorder (Cade 1982). A meta-analysis of 770 bipolar disorder patients treated with lithium shows a decrease of a manic relapse by 38% and a decrease of a depressive relapse by 28% (Geddes et al. 2004). Treatment discontinuation is one of the most important predictor of relapse and poor outcome in bipolar patients (Maj 2000). Rapid discontinuation of lithium treatment specifically is associated with even a higher risk of relapse, also after many years of clinical stability (Baastrup et al. 1970; Klein et al. 1981; Mander & Loudon 1988; Mander 1986; Faedda et al. 1993; Baldessarini et al. 1997). This risk is not fully accounted for by the natural history of the disease. The nature of this withdrawal syndrome is unclear. Several studies suggest a decreased responsiveness for lithium after discontinuation in patients with an initial good response (Suppes et al. 1993; Klein et al. 1981). Whether this decrease in response really exists, is still largely questioned.

This decreased responsiveness for lithium is called lithium-discontinuation-induced refractoriness. Lithium response may dissipate by a tolerance process. The exact neurobiological mechanism through which lithium exerts its therapeutic effects remains unclear (Williams et al. 2002; Klein & Melton 1996). The neuroprotective and neurotrophic effects of lithium maintenance therapy are suggested to play a part in lithium-induced refractoriness, that are deteriorated when lithium is discontinued and reinstituted (Post

2012; Cakir et al. 2017). If a decreased effectiveness of lithium treatment after interruption exists than this may have serious clinical implications, because of the long-term effects on the subsequent course of illness.
The study in chapter 3 reviews the literature on this topic and pools relevant data to conduct a meta-analysis investigating if response to lithium is reduced, when reinstituted after a period of discontinuation.

The hypothesis of this study states that for an unselected group of bipolar patients lithium-discontinuation-induced refractoriness does not exist, which is consistent with clinical experience where high frequency of discontinuation and successful reinstitution of lithium is general practice.

2. Cognitive dysfunction and psychopharmacological treatment:
Cognitive function is impaired in bipolar disorder and schizophrenia independent of clinical state (Martínez-Arán et al. 2004; Kahn & Keefe 2013). In this thesis, cognition is described and investigated as a measure of cognitive performance such as memory, attention, acquisition of knowledge, processing speed, reasoning and executive function (Kahn & Keefe 2013). Current pharmacological treatment targets mainly mood stability in bipolar disorder and primarily psychotic symptoms in schizophrenia. It is important to investigate and elaborate potential cognitive enhancing pharmacological treatment, because cognitive dysfunction is associated with worse social and occupational functioning and a more severe course of illness (Zubieta et al. 2001; Martínez-Arán et al. 2004; Green 2006). In order to develop cognitive treatment, it is essential to investigate the nature of cognitive dysfunction and which factors contribute to cognitive decline in patients. Psychotic symptoms, independent of diagnosis, have been associated with worse cognitive impairment (Krabbendam et al. 2005). In schizophrenia, cognitive dysfunction develops already years before the onset of the illness, which is marked by the appearance of psychotic symptoms (Kahn & Keefe 2013). In bipolar disorder cognitive decline is described only after disease onset and appears to be less severe than in schizophrenia.
The reason for the cognitive decline in schizophrenia and bipolar disorder patients remains unknown. Several factors may influence cognitive decline in bipolar disorder, such as a history of psychosis (as mentioned in part I of this thesis) and severity of illness course. In addition, the side effects of drug treatment are debated as a cause of cognitive decline in bipolar disorder (Ferrier et al. 1999; Clark et al. 2002). In schizophrenia, cognitive dysfunction is assumed as a core feature of the illness and research is more directed towards the development of cognitive enhancing medication. **Chapter 4** provides an overview of studies on cognitive enhancing medication in schizophrenia. In addition, the indefinite cognitive effects of mood stabilizers and antipsychotics in patients with bipolar disorder are discussed.

The hypothesis of this study states that there is evidence to develop medication for cognition with cognitive enhancing effects.

3. Epigenetic effects of psychopharmacological treatment:

Bipolar disorder and schizophrenia are psychiatric disorders that are caused by complex interactions between biological and environmental factors. Not all disease risk can be explained by genetic variation (Cardno et al. 1999; O'Donovan et al. 2009; Craddock & Sklar 2013). This indicates that the environment plays an important role as well (Caspi & Moffitt 2006). For instance, it is known that childhood trauma increases the risk for several psychiatric disorders and children of mothers exposed to famine during pregnancy have been reported to have a higher incidence of schizophrenia (Brown & Susser 2008). Linking environmental risk factors to development of psychiatric disorders might be explained by a variety of biological processes named 'epigenetics' by Waddington in the early 20th century (Waddington 1942). Epigenetic mechanisms together form a stable heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence. Altering the DNA packaging influences the gene expression and ultimately the translation of DNA to proteins and molecules. DNA methylation is one of the epigenetic mechanisms and involves the addition of a methyl group to a DNA base (for review see Schubeler et al. (2015)) (See figure 1). The only DNA base known to be highly methylated in mammals is cytosine. The classical view is that methylated DNA represses gene activity (Irizarry et al. 2009). It appears now that hyper and hypomethylation can increase and decrease gene activity (van Eijk et al. 2012; Wagner et al. 2014).

There are several studies which demonstrate that environmental factors result in etiological changes in DNA methylation. Environmental factors like childhood trauma (Labonté et al. 2012; Vinkers et al. 2015) affect DNA methylation in the epigenome. Several studies on DNA methylation in psychotic disorders showed differences in brain tissue of psychotic patients compared to healthy controls. These differences consisted of epigenetic differentiated loci that are linked to genes of schizophrenia and early developmental processes (Jaffe et al. 2016; Melka et al. 2014; Mill et al. 2008; Pidsley et al. 2014). In utero exposure to stress showed the highest influence on changes in the epigenome with a long-lasting effect (Waterland & Michels 2007; Reik et al. 2001). Besides environmental factors such as childhood trauma or stress, psychotropic drugs like clozapine (antipsychotic medication) and valproic acid (mood stabilizer) also show the ability to change DNA methylation levels (Dong et al. 2016; Boks et al. 2012). Psychotropic medication can affect DNA methylation by altering the activity of DNA methyltransferases for instance, which are essential for initiating and maintaining DNA methylation (Bird 2002; Grayson & Guidotti 2013) during development and in adulthood (Roth & Sweatt 2009). At the moment it is too early to distinguish treatment effects from disease-related differences in DNA methylation and practical application is still far away. For a better understanding it is important to identify which factors are associated with epigenetic mechanisms and in addition to investigate how these factors influence these

epigenetic processes. The study described in **Chapter 5** investigates the influence of several types of psychotropic drugs (antipsychotics, moodstabilizers and antidepressants) on DNA methylation in bipolar disorder patients, as these patients use a wide variety of psychotropic medication.

The hypothesis of this study states that psychopharmacological agents cause alterations in DNA methylation signatures.

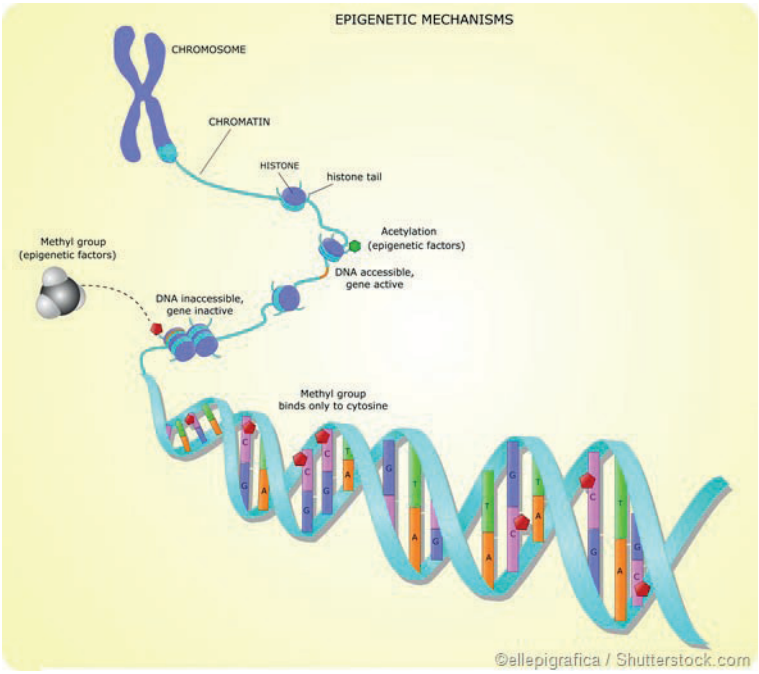


Figure 1. Epigenetic mechanisms

Outline

This thesis focuses on two topics in bipolar disorder patients. The first topic (**chapter 2**) of this thesis investigates demographical, neurocognitive and clinical characteristics of psychotic symptoms in bipolar disorder to explore a potential subtype of the disorder. The second topic of this thesis is pharmacological treatment in bipolar disorder (**chapter 3, 4 and 5**). Three topics are investigated:

- The effectiveness of the main and widely used mood stabilizer lithium after a period of discontinuation.
- The cognitive effects of current pharmacological agents and potential new psychotropic medication targeting enhancement of cognitive function in schizophrenia and bipolar disorder patients.
- The epigenetic effects of pharmacological agents in bipolar disorder patients as a potential new target treatment.

In summary this thesis consists of:

Part I:
The study described in **chapter 2** investigates demographic, clinical and neurocognitive characteristics of bipolar disorder with the presence of lifetime psychotic symptoms within a large cohort of bipolar disorder type I.

Part II:
The study described in **chapter 3** reviews the literature on the phenomenon described as lithium-discontinuation-induced refractoriness and pools relevant data to conduct a meta-analysis.

The study described in **chapter 4** reviews the literature on cognitive enhancing medication in schizophrenia and in addition discusses the cognitive effects of currently available mood stabilizers and antipsychotics in patients with bipolar disorder.

The study described in **chapter 5** investigates the influence of several types of psychotropic medication (antipsychotics, moodstabilizers and antidepressants) on genome wide DNA methylation levels in bipolar disorder patients.

Chapter 6 is a general discussion on the findings of chapters 2 to 5.

References

Arts B, Jabben N, Krabbendam L, Van Os J (2008). Meta-analyses of cognitive functioning in euthymic bipolar patients and their first-degree relatives. *Psychological Medicine* **38**, 771–785.

Baastrup PC, Poulsen JC, Schou M, Thomsen K, Amdisen A (1970). Prophylactic lihtium: double blind discontinuation in manic-depressieve and recurrent-depressive disorders. *The Lancet* **296**, 326–330.

Baldessarini RJ, Tondo L, Floris G, Rudas N (1997). Reduced morbidity after gradual discontinuation of lithium treatment for bipolar I and II disorders: A replication study. *American Journal of Psychiatry* **154**, 551–553.

Bird A (2002). DNA methylation patterns and epigenetic memory. *Genes & Development* **16**, 6–21.

Boks MP, de Jong NM, Kas MJH, Vinkers CH, Fernandes C, Kahn RS, Mill J, Ophoff RA (2012). Current status and future prospects for epigenetic psychopharmacology. *Epigenetics : official journal of the DNA Methylation Society* **7**, 20–8.

Bora E, Vahip S, Akdeniz F, Gonul AS, Eryavuz A, Ogut M, Alkan M (2007). The effect of previous psychotic mood episodes on cognitive impairment in euthymic bipolar patients. *Bipolar Disorders* **9**, 468–477.

Bora E, Yücel M, Pantelis C (2009). *Cognitive endophenotypes of bipolar disorder: A meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives*. *Journal of Affective Disorders* **113**, 1–20.

Bora E, Yücel M, Pantelis C (2010). Neurocognitive markers of psychosis in bipolar disorder: A meta-analytic study. Elsevier B.V. *Journal of Affective Disorders* **127**, 1–9.

Bowie CR, Depp C, McGrath JA, Wolyniec P, Mausbach BT, Thornquist MH, Luke J, Patterson TL, Harvey PD, Pulver AE (2010). Prediction of real-world functional disability in chronic mental disorders: a comparison of schizophrenia and bipolar disorder. *The American journal of psychiatry* **167**, 1116–24.

Brown AS, Susser ES (2008). Prenatal nutritional deficiency and risk of adult schizophrenia. *Schizophrenia Bulletin* **34**, 1054–1063.

Cade JF (1982). Lithium salts in the treatment of psychotic excitement. *Australasian Psychiatry* **16**, 129–133.

Cakir S, Yazıcı O, Post RM (2017). Decreased responsiveness following lithium discontinuation in bipolar disorder: A naturalistic observation study. *Psychiatry Research* **247**, 305–309.

Cardno AG, Marshall EJ, Coid B, Macdonald AM, Ribchester TR, Davies NJ, Venturi P, Jones LA, Lewis SW, Sham PC, Gottesman II, Farmer AE, McGuffin P, Reveley AM, Murray RM (1999). Heritability Estimates for Psychotic Disorders; The Maudsly Twin Psychosis Series. *Archives of General Psychiatry* **56**, 162–168.

Caspi A, Moffitt TE (2006). *Gene-environment interactions in psychiatry: Joining forces with neuroscience*. *Nature Reviews Neuroscience* **7**, 583–590.

Clark L, Iversen SD, Goodwin GM (2002). Sustained attention deficit in bipolar disorder. *The British journal of psychiatry : the journal of mental science* **180**, 313–319.

Coryell W, Leon AC, Turvey C, Akiskal HS, Mueller T, Endicott J (2001). The significance of psychotic features in manic episodes: A report from the NIMH collaborative study. *Journal of Affective Disorders* **67**, 79–88.

Craddock N, O'Donovan MC, Owen MJ (2005). The genetics of schizophrenia and bipolar disorder: dissecting psychosis. *Journal of medical genetics* **42**, 193–204.

Craddock N, Sklar P (2013). Bipolar Disorder 1 - Genetics of bipolar disorder. Elsevier Ltd *The Lancet* **381**, 1654–1662.

Daalman K, Diederen KMJ, Derks EM, Van Lutterveld R, Kahn RS, Sommer IEC (2012). Childhood trauma and auditory verbal hallucinations. *Psychological Medicine* **42**, 2475-2484.

Dong E, Tueting P, Matrisciano F, Grayson DR, Guidotti A (2016). Behavioral and molecular neuroepigenetic alterations in prenatally stressed mice: relevance for the study of chromatin remodeling properties of antipsychotic drugs. *Translational psychiatry* **6**, e711.

van Eijk KR, de Jong S, Boks MPM, Langeveld T, Colas F, Veldink JH, de Kovel CGF, Janson E, Strengman E, Langfelder P, Kahn RS, van den Berg LH, Horvath S, Ophoff RA (2012). Genetic analysis of DNA methylation and gene expression levels in whole blood of healthy human subjects. *BMC genomics* **13**, 636.

Faedda GL, Tondo L, Baldessarini RJ, Suppes T, Tohen M (1993). Outcome After Rapid vs Gradual Discontinuation of Lithium Treatment in Bipolar Disorders. *Archives of General Psychiatry* **50**, 448–455.

Ferrier IN, Stanton BR, Kelly TP, Scott J (1999). Neuropsychological function in euthymic patients with bipolar disorder. *The British journal of psychiatry : the journal of mental science* **175**, 246–51.

GBD 2016 DALYs and HALE Collaborators (2017). Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet* **390**, 1260–1344.

Geddes JR, Burgess S, Hawton K, Jamison K, Goodwin GM (2004). Long-term lithium therapy for bipolar disorder: systematic review and meta-analysis of randomized controlled trials. *The American journal of psychiatry* **161**, 217–22.

Geddes JR, Miklowitz DJ (2013). Treatment of bipolar disorder. *The Lancet* **381**, 1672–1682.

Glahn DC, Bearden CE, Barguil M, Barrett J, Reichenberg A, Bowden CL, Soares JC, Velligan DI (2007). The Neurocognitive Signature of Psychotic Bipolar Disorder. *Biological Psychiatry* **62**, 910–916.

Goodwin FK, Jamison KR (1990). *Manic-Depressive Illness*. Oxford University Press: New York.

Grayson DR, Guidotti A (2013). The dynamics of DNA methylation in schizophrenia and related psychiatric disorders. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **38**, 138–66.

Green MF (2006). Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. *The Journal of clinical psychiatry* **67**, 3–8.

International Schizophrenia Consortium (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **10**, 8192–8192.

Irizarry RA, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P, Cui H, Gabo K, Rongione M, Webster M, Ji H, Potash JB, Sabunciyan S, Feinberg AP (2009). The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. *Nature Genetics* **41**, 178–186.

Jaffe AE, Gao Y, Deep-Soboslay A, Tao R, Hyde TM, Weinberger DR, Kleinman JE (2016). Mapping DNA methylation across development, genotype, and schizophrenia in the human frontal cortex. *Nature Neuroscience* **19**, 40–47.

Judd LL, Akiskal HS, Schettler PJ, Endicott J, Maser J, Solomon D a, Leon AC, Rice JA, Keller MB (2002). The long-term natural history of the weekly symptomatic status of bipolar I disorder. *Archives of general psychiatry* **59**, 530–537.

Judd LL, Akiskal HS, Schettler PJ, Endicott J, Leon AC, Solomon DA, Coryell W, Maser JD, Keller MB (2005). Psychosocial disability in the course of bipolar I and II disorders: A prospective, comparative, longitudinal study. *Archives of General Psychiatry* **62**, 1322–1330.

Kahn RS, Keefe RSE (2013). Schizophrenia is a cognitive illness: time for a change in focus. *JAMA psychiatry* **70**, 1107–12.

Keck PE, McElroy SL, Havens JR, Altshuler LL, Nolen WA, Frye MA, Suppes T, Denicoff KD, Kupka R, Leverich GS, Rush AJ, Post RM (2003). Psychosis in bipolar disorder: Phenomenology and impact on morbidity and course of illness. *Comprehensive Psychiatry* **44**, 263–269.

Klein HE, Broucek B, Greil W (1981). Lithium withdrawal triggers psychotic states. *British Journal of Psychiatry* **139**, 255–256.

Klein PS, Melton DA (1996). A molecular mechanism for the effect of lithium on development. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 8455–8459.

Krabbendam L, Arts B, Van Os J, Aleman A (2005). Cognitive functioning in patients with schizophrenia and bipolar disorder: A quantitative review. *Schizophrenia Research* **80**, 137–149.

Labonté B, Suderman M, Maussion G, Navaro L, Yerko V, Mahar I, Bureau A, Mechawar N, Szyf M, Meaney MJ, Turecki G (2012). Genome-wide epigenetic regulation by early-life trauma. *Archives of General Psychiatry* **69**, 722–731.

Levy B, Medina AM, Weiss RD (2013). Cognitive and psychosocial functioning in bipolar disorder with and without psychosis during early remission from an acute mood episode: A comparative longitudinal study. *Comprehensive Psychiatry* **54**, 618–626.

Maj M (2000). The impact of lithium prophylaxis on the course of bipolar disorder: a review of the research evidence. *Bipolar disorders* **2**, 93–101.

Maj M (2003). The effect of lithium in bipolar disorder: a review of recent research evidence. *Bipolar disorders* **5**, 180–8.

Maj M, Pirozzi R, Bartoli L, Magliano L (2002). Long-term outcome of lithium prophylaxis in bipolar disorder with mood-incongruent psychotic features: A prospective study. *Journal of Affective Disorders* **71**, 195–198.

Mander AJ (1986). Is there a lithium withdrawal syndrome? *British Journal of Psychiatry* **149**, 498–501.

Mander AJ, Loudon JB (1988). Rapid recurrence of mania following abrupt discontinuation of lithium. *The Lancet* **332**, 15–17.

Martínez-Arán A, Vieta E, Colom F, Torrent C, Sánchez-Moreno J, Reinares M, Benabarre A, Goikolea JM, Brugué E, Daban C, Salamero M (2004). Cognitive impairment in euthymic bipolar patients: Implications for clinical and functional outcome. *Bipolar Disorders* **6**, 224–232.

Melka MG, Laufer BI, McDonald P, Castellani CA, Rajakumar N, O'Reilly R, Singh SM (2014). The effects of olanzapine on genome-wide DNA methylation in the hippocampus and cerebellum. *Clinical Epigenetics* **6**

Merikangas KR, Jin R, He J, Kessler RC, Lee S, Sampson NA, Viana MC, Andrade LH, Hu C, Karam EG, Ladea M, Medina-Mora ME, Ono Y, Posada-Villa J, Sagar R, Wells JE, Zarkov Z (2011). Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. *Archives of General Psychiatry* **68**, 241–251.

Mill J, Tang T, Kaminsky Z, Khare T, Yazdanpanah S, Bouchard L, Jia P, Assadzadeh A, Flanagan J, Schumacher A, Wang SC, Petronis A (2008). Epigenomic Profiling Reveals DNA-Methylation Changes Associated with Major Psychosis. *American Journal of Human Genetics* **82**, 696–711.

O'Donovan MC, Craddock NJ, Owen MJ (2009). Genetics of psychosis; insights from views across the genome. *Human Genetics* **126**, 3–12.

Van Os J, Hanssen M, Bijl RV, Ravelli A (2000). Strauss (1969) revisited: A psychosis continuum in the general population? *Schizophrenia Research* **45**, 11–20.

Özyildirim I, Çakir S, Yazici O (2010). Impact of psychotic features on morbidity and course of illness in patients with bipolar disorder. *European Psychiatry* **25**, 47–51.

Perlis R, Ostacher M (2006). Predictors of recurrence in bipolar disorder: primary outcomes from the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD). *American Journal of Psychiatry* **163**, 217–224.

Pidsley R, Viana J, Hannon E, Spiers H, Troakes C, Al-saraj S, Mechawar N, Turecki G, Schalkwyk LC, Bray NJ, Mill J (2014). Methylomic profiling of human brain tissue supports a neurodevelopmental origin for schizophrenia. *Genome Biology* **15**

Post RM (2012). Acquired lithium resistance revisited: Discontinuation-induced refractoriness versus tolerance. *Journal of Affective Disorders* **140**, 6–13.

Read J, Argyle N (1999). Hallucinations, delusions, and thought disorder among adult psychiatric inpatients with a history of child abuse. *Psychiatric Services* **50**, 1467–1472.

Read J, Van Os J, Morrison AP, Ross CA (2005). Childhood trauma, psychosis and schizophrenia: A literature review with theoretical and clinical implications. *Acta Psychiatrica Scandinavica* **112**, 330–350.

Reik W, Dean W, Walter J (2001). Epigenetic reprogramming in mammalian development. *Science* **293**, 1089–1093.

Robinson LJ, Thompson JM, Gallagher P, Goswami U, Young AH, Ferrier IN, Moore PB (2006). A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. *Journal of Affective Disorders* **93**, 105–115.

Roth TL, Sweatt JD (2009). Regulation of chromatin structure in memory formation. *Current Opinion in Neurobiology* **19**, 336–342.

Scherk H, Pajonk FG, Leucht S (2007). Second-Generation Antipsychotic Agents in the Treatment of Acute Mania. *Archives of General Psychiatry* **64**, 442.

Schübeler D (2015). Function and information content of DNA methylation. *Nature* **517**, 321–326.

Sidor MM, Macqueen GM (2011). Antidepressants for the acute treatment of bipolar depression: A systematic review and meta-analysis. *Journal of Clinical Psychiatry* **72**, 156–167.

Simonsen C, Sundet K, Vaskinn A, Birkenaes AB, Engh JA, Faerden A, Jónsdóttir H, Ringen PA, Opjordsmoen S, Melle I, Friis S, Andreassen OA (2011). Neurocognitive dysfunction in bipolar and schizophrenia spectrum disorders depends on history of psychosis rather than diagnostic group. *Schizophrenia Bulletin* **37**, 73–83.

Suppes T, Baldessarini RJ, Faedda GL, Tondo L, Tohen M (1993). *Discontinuation of maintenance treatment in bipolar disorder: Risks and implications*. *Harvard Review of Psychiatry* **1**, 131–144.

Upthegrove R, Chard C, Jones L, Gordon-Smith K, Forty L, Jones I, Craddock N (2015). Adverse childhood events and psychosis in bipolar affective disorder. *British Journal of Psychiatry* **206**, 191–197.

Varese F, Smeets F, Drukker M, Lieverse R, Lataster T, Viechtbauer W, Read J, Van Os J, Bentall RP (2012). Childhood adversities increase the risk of psychosis: A meta-analysis of patient-control, prospective-and cross-sectional cohort studies. *Schizophrenia Bulletin* **38**, 661–671.

Vinkers CH, Kalafateli AL, Rutten BPF, Kas MJ, Kaminsky Z, Turner JD, Boks MPM (2015). Traumatic stress and human DNA methylation: a critical review. *Epigenomics* **7**, 593–608.

Vreeker A, Boks MPM, Abramovic L, Verkooijen S, van Bergen AH, Hillegers MHJ, Spijker AT, Hoencamp E, Regeer EJ, Riemersma-Van der Lek RF, Stevens AWMM, Schulte PFJ, Vonk R, Hoekstra R, van Beveren NJM, Kupka RW, Brouwer RM, Bearden CE, MacCabe JH, Ophoff RA, GROUP Investigators (2016). High educational performance is a distinctive feature of bipolar disorder: a study on cognition in bipolar disorder, schizophrenia patients, relatives and controls. *Psychological Medicine* **46**, 807–818.

Waddington CH (1942). The epigenotype. *Endeavour*, 18–20.

Wagner JR, Busche S, Ge B, Kwan T, Pastinen T, Blanchette M (2014). The relationship between DNA methylation, genetic and expression inter-individual variation in untransformed human fibroblasts. *Genome Biology* **15**

Waterland RA, Michels KB (2007). Epigenetic Epidemiology of the Developmental Origins Hypothesis. *Annual Review of Nutrition* **27**, 363–388.

Williams RSB, Cheng L, Mudge AW, Harwood AJ (2002). A common mechanism of action for three mood-stabilizing drugs. *Nature* **417**, 292–295.

Zubieta JK, Huguelet P, O'Neil RL, Giordani BJ (2001). Cognitive function in euthymic Bipolar I Disorder. *Psychiatry Research* **102**, 9–20.

PART I

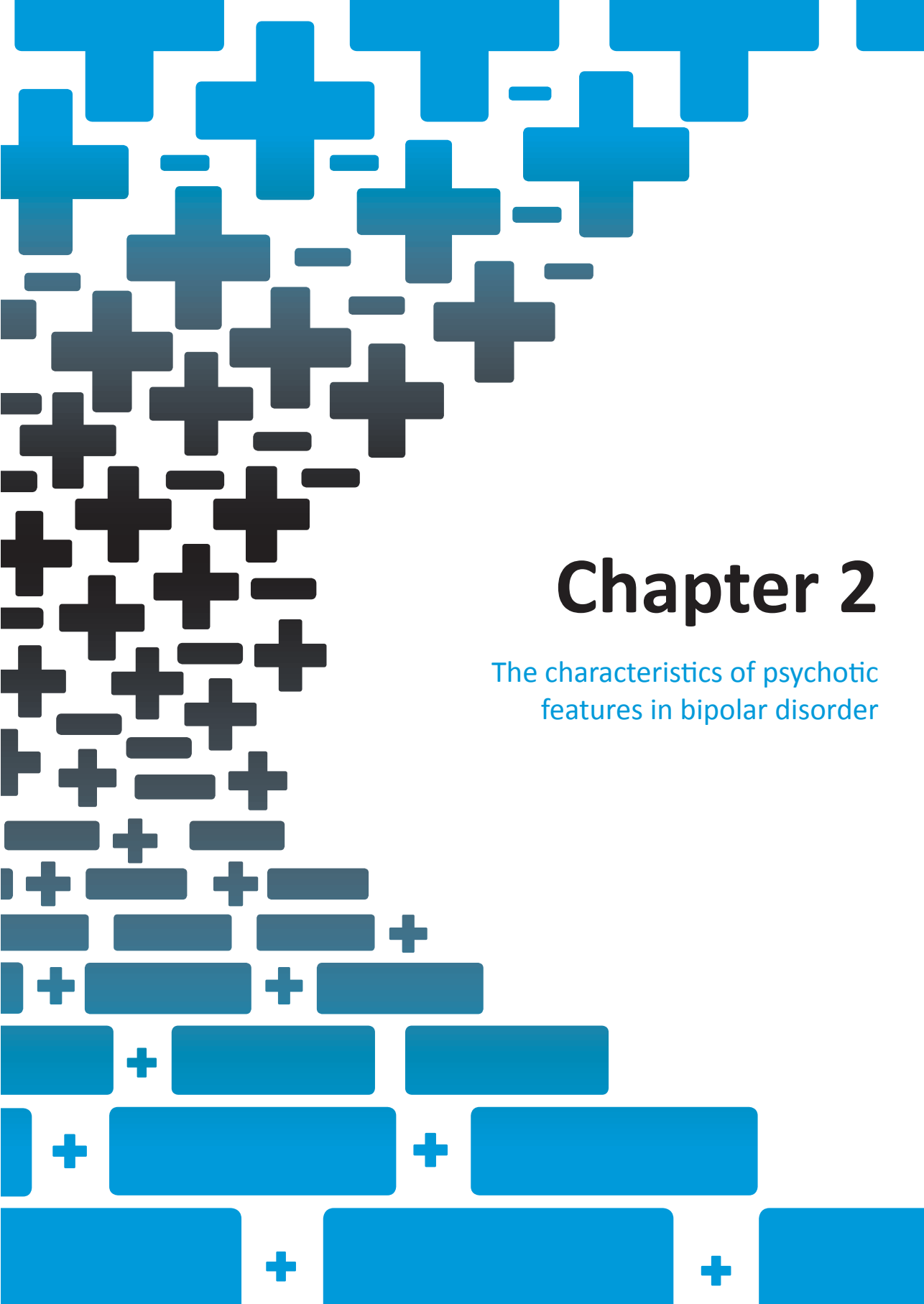
Determinants of psychosis in bipolar disorder

Annet H. van Bergen
Sanne Verkooijen
Annabel Vreeker
Lucija Abramovic

Manon H. Hillegers
Annet T. Spijker
Erik Hoencamp
Eline J. Regeer
Stefan E. Knapen
Rixt F. Riemersma - van der Lek
Robert Schoevers
Anja W. Stevens
Peter F.J. Schulte
Ronald Vonk
Rocco Hoekstra
Nico J. van Beveren
Ralph W. Kupka

Iris E.C. Sommer
Roel A. Ophoff
René S. Kahn
Marco P.M. Boks

Psychological Medicine, in press (published online: 10 October, 2018)



Chapter 2

The characteristics of psychotic features in bipolar disorder

Abstract

Background:
In a large and comprehensively assessed sample of patients with bipolar disorder type I (BDI), we investigated the prevalence of psychotic features and their relationship with life course, demographic, clinical, and cognitive characteristics. We hypothesized that groups of psychotic symptoms (Schneiderian, mood incongruent, thought disorder, delusions, and hallucinations) have distinct relations to risk factors.

Methods:
In a cross-sectional study of 1342 BDI patients, comprehensive demographical and clinical characteristics were assessed using the Structured Clinical Interview for DSM-IV (SCID-I) interview. In addition, levels of childhood maltreatment and intelligence quotient (IQ) were assessed. The relationships between these characteristics and psychotic symptoms were analyzed using multiple general linear models.

Results:
A lifetime history of psychotic symptoms was present in 73.8% of BDI patients and included delusions in 68.9% of patients and hallucinations in 42.6%. Patients with psychotic symptoms showed a significant younger age of disease onset ($\beta = -0.09$, $t = -3.38$, $p = 0.001$) and a higher number of hospitalizations for manic episodes ($F(11,338) = 56.53$, $p < 0.001$). Total IQ was comparable between groups. Patients with hallucinations had significant higher levels of childhood maltreatment ($\beta = 0.09$, $t = 3.04$, $p = 0.002$).

Conclusions:
In this large cohort of BDI patients, the vast majority of patients had experienced psychotic symptoms. Psychotic symptoms in BDI were associated with an earlier disease onset and more frequent hospitalizations particularly for manic episodes. The study emphasizes the strength of the relation between childhood maltreatment and hallucinations but did not identify distinct subgroups based on psychotic features and instead reported of a large heterogeneity of psychotic symptoms in BD.

Key words:
childhood trauma, cognitive functioning, delusions, formal thought disorder, hallucinations, mood incongruent symptoms, psychosis, Schneiderian symptoms

Introduction

The debate on overlap of psychotic symptomatology in schizophrenia and bipolar disorder (BD) from the perspective that these disorders may pose a diagnostic continuum with shared etiology (van Os and Reininghaus, 2016) is ongoing. Some argue that the psychosis continuum extends from BD, to schizoaffective disorder and at the other end typical schizophrenia, and reflect increasing level of severity (van Os et al., 2000; Craddock et al., 2005; The International Schizophrenia Consortium et al., 2009). Overlapping illness characteristics between these disorders are the presence of childhood trauma, high level of distress and cognitive impairment (Read et al., 2005; Green, 2006; Bora et al., 2010). Cognitive impairment in BD is reported during mania and depression and persists during the euthymic phase of the disorder (Martínez-Arán et al., 2004), however less severe than in schizophrenia (Krabbendam et al., 2005). The factors that are of influence on cognitive function in BD are still unclear but may inform of the relevance of intelligence quotient (IQ) in a psychosis continuum (Zammit et al., 2004; Robinson et al., 2006; Jabben et al., 2010). Particularly since cognitive impairment in schizophrenia is often considered a core feature of the illness that remains present in the absence of psychotic symptoms (Kahn and Keefe, 2013). Therefore, the question is whether BD patients with psychotic symptoms display similar cognitive deficits. Within the bipolar spectrum, a history of psychotic symptoms has been associated with several demographical and clinical characteristics including symptom severity, worse psychosocial outcome, lower response to lithium (Maj et al., 2002; Maj, 2003), more comorbidity (Coryell et al., 2001), earlier age of disease onset (Upthegrove et al., 2015), higher frequency of mood episodes, hospitalizations, and more severe cognitive impairment (Glahn et al., 2007; Özyildirim et al., 2010; Simonsen et al., 2011; Levy et al., 2013). Some of these characteristics resemble characteristics of schizophrenia and therefore feed the debate whether BD is part of a psychosis continuum and whether BD with psychotic symptoms may represent a distinct subtype of BD in level of severity (Potash et al., 2003). To answer this question, it is relevant to investigate how BD patients with psychosis differ from those without psychotic symptoms in cognitive and global functioning, disease course, and etiological factors such as history of childhood maltreatment. However, as the distinction psychosis v. nonpsychosis is broad, further investigation of types of psychotic symptoms (hallucinations, delusions, mood incongruent symptoms, Schneiderian symptoms, and formal thought disorder) could inform this debate from the perspective that these subgroups of psychotic symptoms may have distinct etiology (Upthegrove et al., 2015; Allardyce et al., 2018). Previous studies already showed the relevance of psychosis in BD type I (BDI). High frequencies of a lifetime history of psychotic symptoms were reported in BDI patients, ranging between 56% and 70% (Goodwin and Jamison, 1990; Keck et al., 2003; Bora et al., 2010; Upthegrove et al., 2015). Schneiderian symptoms (which include hallucinations of one's thoughts being spoken aloud, arguing or running commentary, and delusions of thought withdrawal, insertion, or broadcasting) may have some specificity

for schizophrenia according to some studies (Tandon and Greden,1987; O'Grady, 1990). Schneiderian symptoms have been reported in BD up to 20% and are associated with worse outcomes (Tohen et al., 1992; Carlson et al., 2012). In addition, mood incongruent symptoms in BD occur in the same frequency range of 20% (Fennig et al., 1996; Keck et al., 2003) and were associated with higher relapse risk, worse outcome (Tohen et al., 1992) and more frequent comorbid anxiety disorders (Keck et al., 2003). Formal thought disorder is not specific to schizophrenia either; thought disorder is common in mania with an average prevalence of 19% (Goodwin and Jamison, 1990) and rates are comparable to the rate in schizophrenia (McElroy et al., 1996; Dunayevich and Keck, 2000). Another point of interest are the determinants of these psychotic features in BD. Childhood trauma, regardless of its type, is known to increase the risk of schizophrenia and psychosis in general (Varese et al., 2012). One study suggests that childhood abuse is associated specifically with auditory hallucinations, but not with delusions, in BD (Upthegrove et al., 2015). But the relationship between childhood adversity and psychosis in BD is as yet inconclusive (Upthegrove et al., 2015). The current study is the most comprehensively characterized large sample of BDI patients (N = 1342) to date and provides a detailed description of psychotic symptoms subdivided into delusions, hallucinations, mood incongruent symptoms, Schneiderian symptoms, and formal thought disorder. The relationship of psychotic features with measures of disease course, neurocognitive functioning, and childhood maltreatment was analyzed. We hypothesize that patients with a history of psychotic symptoms have a more severe illness course (reflected by more comorbid psychiatric disorders, a higher number of episodes and hospitalizations, and younger age at disease onset), lower level of global functioning (reflected by marital and employment status, socioeconomic status, and general scale of global functioning), lower level of cognitive functioning (reflected by measures of IQ, premorbid IQ, and educational level), and higher levels of childhood maltreatment. In addition, we hypothesize that patients with Schneiderian and mood incongruent psychotic symptoms would have the most severe illness course if the hypothesis that BD with (specific) psychotic symptoms is part of a psychosis continuum with schizophrenia were to be true.

Methods

Study design and participants

Data were collected by the Dutch Bipolar Cohort (DBC) Study from June 2011 until April 2015. DBC is a National Institute of Mental Health funded collaborative study of the University of California Los Angeles (UCLA) and University Medical Center Utrecht (UMCU). The DBC investigated genetic and phenotypic information of patients with BDI, first-degree relatives, and controls. Patients were recruited in collaboration with several Dutch health care institutes: Altrecht Institute for Mental Health Care, GGZ InGeest, University Medical Center Groningen, Delta Center for Mental Health Care, Dimence, Parnassia Group (PsyQ),

and Reinier van Arkel. Inclusion criteria for all participants were: (1) age 18 years or older; (2) at least three Dutch-born grandparents; (3) a good understanding of Dutch language. Patients with a somatic illness that could have influenced the diagnosis of BD were excluded. The study was approved by the medical ethical committee of the UMCU and all participants gave written informed consent. Patients were recruited via clinicians (19.2%), the Dutch BD patient association (15.8%), pharmacies (33.6%), advertisements (6.9%), self-referral (5%), participated in previous studies of the UMCU (4.5%), or from miscellaneous undocumented resources (15.0%). More information on this cohort is provided in the study of Vreeker et al., (2016). For this study, a total of 3364 potential BDI patients were contacted and screened via a short interview by telephone. Clinical assessments were completed in 1575 patients. After exclusion of 23 patients with schizoaffective disorder, 86 patients with BD type II, 25 patients with recurrent depression, 11 patients with BD not otherwise specified, and 59 bipolar type I patients with incomplete data on lifetime psychotic symptoms, the total sample for analysis consisted of 1342 BDI patients. Sample characteristics are presented in Table 1.

Clinical assessments

The complete assessment consisted of a standardized clinical interview, neurocognitive tasks, and an Internet questionnaire. BDI diagnosis was assessed using the Structured Clinical Interview for DSM-IV (SCID-I) (First et al., 1997). The assessments were administered by one group of researchers of the UMCU. The team was supervised by two clinical psychiatrists (MB and AvB). All members were at least bachelor-level psychology or medical students. Training of the team consisted of a SCID-I and Wechsler Adult Intelligence Scale-III (WAIS-III) (Wechsler, 1997) training. 'Digit Symbol Coding', and 'Arithmetic' (Wechsler, 1997). The correlation of this combination of subtests with full-scale IQ has been shown to be high for both schizophrenia patients (R2 = 0.90) and controls (R2 = 0.86) (Blyler et al., 2000). The average test–retest reliability is 0.95–0.97 (Spreen et al., 1998). The National Adult Reading Test (NART Dutch version) was used to estimate the premorbid IQ level (Schmand et al., 1991; Bright et al., 2002). The NART is a single word, oral reading test consisting of 50 words testing previously obtained word knowledge. Reliability, test–retest reliability, and inter-rater reliability estimates of the NART are respectively 0.90, 0.92, and 0.88 (Spreen et al., 1998). The presence of traumatic experiences and maltreatment in childhood was assessed by the Childhood Trauma Questionnaire (CTQ) measuring emotional, physical and sexual abuse, and emotional and physical neglect (Bernstein et al., 1997). CTQ is a validated and widely used self-report instrument for both clinical and non-clinical populations. Correlations with therapists ratings of abuse were reported to be statistically significant ranging from 0.36 to 0.75 (Spreen et al.,1998). Although the CTQ is prone to recall bias (Lewinsohn and Rosenbaum, 1987), the validity of the 25 clinical CTQ items, including a Dutch translation, has been demonstrated in clinical and population samples (Bernstein et al., 2003; Thombs et al., 2009; Fergusson et al., 2011). In fact, there is also evidence that the retrospective assessment of childhood maltreatment tends to underestimate rather than

Table 1 : Demographical and clinical characteristics of BD with (BD P+) and without psychotic symptoms (BD P-)

	BD Total Sample (N= 1342)	BD P+ (N=990) 73.8%	BD P- (N=352) 26.2%	Statistics
Age Mean (sd)	49.5 (12.3)	48.2 (11.9)	53.1 (12.4)	**Beta=0.17, t=-6.22, p<0.001***
Gender male n (%)	580 (43.2%)	404 (40.8%)	176 (50.0%)	B=0.31, p=0.015, OR=1.36[1.06-1.75]
Marital Status n (%)	734.2 (54.7%)	528.2 (53.4%)	206 (58.5%)	B=-0.10, p=0.426, OR=0.90 [0.70-1.16]
Employment status n (%)	622.6 (46.4%)	466.2 (47.1%)	156.4 (44.4%)	$c^2(1)=0.68$, p=0.391
Global functioning Mean (sd)	65.3 (12.3)	65.1 (12.4)	65.9 (12.0)	Beta=-0.03, t=-1.08, p=0.282
Socio economic status mean (sd)	1.8 (1.5)	1.8 (1.5)	1.5 (1.5)	Beta=0.01, t=0.20, p=0.845
Mean level of education (sd)	5.0 (1.6)	5.0 (1.6)	4.7 (1.6)	**W $c^2(1)=12.28$, p<0.001, OR=0.67[0.54-0.84]***
Premorbid IQ Mean (sd)	106.1 (9.8)	106.4 (10.0)	105.1 (9.7)	Beta=0.08, t=2.71, p=0.007
Anxiety disorder (%)	345 (25.7%)	253 (25.6%)	92 (26.1%)	B=-0.13, p =0.380, OR=0.88[0.66-1.17]
Age at onset Mean (sd)	31.0 (10.6)	29.8 (10.0)	34.2 (11.5)	**Beta=-0.09, t=-3.38, p=0.001***
Nr. of episodes MANCOVA				F(2,1336)=5.64, p=0.005, Partial η^2=0.01
Nr. of depressive episodes Mean (sd)	3.8 (2.3)	3.7 (2.3)	4.1 (2.3)	F(1,1337)=5.15, p=0.026, Partial η^2<0.01
Nr. of manic episodes Mean(sd)	3.8 (1.9)	3.8 (1.9)	3.8 (2.1)	F(1,1337)=1.35, p=0.221, Partial η^2<0.01
Nr. of hospitalizations MANCOVA				**F(2,1337)=28.94, p<0.001, Partial η^2=0.04***
Nr. of hospitalizations for depressive episodes Mean (sd)	1.1 (1.5)	1.1 (1.6)	1.1 (1.5)	F(1,1338)=0.49, p=0.322, Partial η^2<0.01
Nr. of hospitalizations for manic episodes Mean (sd)	1.7 (1.9)	1.8 (1.7)	1.2 (1.6)	**F(1,1338)=56.53, p<0.001, Partial η^2=0.04***
Suïcide attempts (n=991) (%)	287 (29.0%)	219 (30.5%)	68 (24.9%)	B=0.25, p =0.133, OR=1.28[0.93-1.77]
Total IQ Mean (sd) (n=1060)	97.5 (14.0)	97.9 (14.3)	96.4 (13.3)	Beta=0.03, t=1.05, p=0.296
WAIS MANCOVA (n=1060)				F(4,1045)=4.00, p=0.003, Partial η^2<0.01
WAIS – Information Mean (sd)	10.6 (2.9)	10.7 (2.9)	10.3 (2.8)	F(1,1048)=7.20, p=0.007, Partial η^2<0.01
WAIS – Block Design Mean (sd)	9.8 (3.3)	9.9 (3.4)	9.6 (3.2)	F(1,1048)=0.18, p=0.673, Partial η^2<0.01
WAIS – Arithmetic Mean (sd)	9.4 (2.6)	9.3 (2.6)	9.5 (2.6)	F(1,1048)=2.63, p=0.105, Partial η^2<0.01
WAIS – Digit Symbol Mean (sd)	9.0 (2.7)	9.1 (2.7)	8.8 (2.8)	F(1,1048)=1.99, p=0.159, Partial η^2<0.01

Childhood trauma Total score Mean (sd)	42.2 (11.1)	42.5 (11.1)	41.8 (11.3)	Beta=0.05, t= 2.07, p=0.039
Trauma subtypes MANCOVA				F(5,1333)=1.02, p**=0.412, Partial η^2<0.01
Sexual abuse Mean (sd)	6.3 (3.0)	6.4 (3.2)	6.0 (2.7)	F(1,1337)=4.21, p=0.045, Partial η^2<0.01
Physical abuse Mean (sd)	5.8 (2.1)	5.8 (2.2)	5.9 (2.0)	F(1,1337)=0.44, p=0.451, Partial η^2<0.01
Emotional abuse Mean (sd)	8.6 (4.1)	8.7 (4.1)	8.3 (4.1)	F(1,1337)=2.25, p=0.146, Partial η^2<0.01
Physical neglect Mean (sd)	9.7 (2.2)	9.7 (2.1)	9.7 (2.4)	F(1,1337)=0.23, p=0.653, Partial η^2<0.01
Emotional neglect Mean (sd)	11.9 (4.8)	11.9 (4.8)	11.9 (4.8)	F(1,1337)=1.23, p=0.316, Partial η^2<0.01

* Significant between-group difference (p<0.0029). Bold fonts are used to highlight significance.

** Hotelling's trace

over-report real incidence rates (Schreier et al., 2009). Childhood maltreatment was also investigated in relation to gender differences and the risk for psychotic symptoms. The inter-rater reliability of the global assessment of functioning ranges from 0.53 to 0.95 (Rey et al., 1995; Startup et al., 2002).

Demographic characteristics

Marital and employment status was provided by the SCID-I. Socio-economical status was assessed by an Internet questionnaire based on the Family Affluence Scale (Currie et al., 2008). Information on educational performance was gathered by asking the participants their highest completed level of education based on the Dutch education system which consists of primary (4–12 years of age), secondary (low, intermediate, high preparatory vocational, and pre-university), and tertiary education (intermediate professional education, higher professional education, and university). Educational level was categorized in seven levels with university as highest level as previously reported (Vreeker et al., 2016). In addition, Global Assessment of Functioning was assessed using the SCID-I.

Clinical course

Information on clinical course was obtained by the self-report section B of the Questionnaire of Bipolar Disorders providing information on the number of manic and depressive episodes, number of hospitalizations for manic and depressive episodes and age at disease onset (Leverich et al., 2001). The number of hospitalizations for hypomanic and manic episodes or manic or hypomanic episodes were considered together, because the distinction is difficult to make in a retrospective assessment. Age of disease onset was defined as the age of first pharmacological treatment. This definition was chosen given the insidious onset of BDI and the high probability of recall bias in the retrospective assessment of first reported symptoms (Leverich et al., 2001; Suppes et al., 2001). Suicidal behavior, categorized if a person attempted to commit suicide ever (once or more) or never, was assessed using the suicide questions of the CASH (Andreasen et al., 1992).

Substance and medication use

Information on current cannabis use was derived from an online Cannabis Use Inventory questionnaire to asses current and last 2 years cannabis use (Schubart et al., 2011). Alcohol use was defined by the maximum total amount of glasses of alcohol per week in the past 12 months provided by the Composite International Diagnostic Interview (CIDI) (Robins et al., 1988), section B. Data on lifetime substance abuse and dependence were provided by sections J and L of the CIDI. The presence of a lifetime comorbid anxiety disorders was assessed by the SCID-I, section F. Information on current and lifetime use of mood stabilizers, antipsychotics, and antidepressants was assessed using a questionnaire on the use of psychotropic medication. Data on current and lifetime psychotropic medication use

were available in, respectively, 1240 and 922 BDI patients. In addition, current lithium use (n = 1342) was assessed using a lithium satisfactory questionnaire.

Statistical analyses

Differences between patients with and without lifetime psychotic symptoms were investigated for all selected demographical and clinical variables using logistic or linear regression with the presence of psychosis as a main indicator. In case of categorical measures, $\chi 2$ tests were performed. Correlated outcome measures, including WAIS subtasks and number of episodes and hospitalizations, were analyzed with a multivariate analysis of co-variance (MANCOVA) including post hoc analysis of co-variance. Analyses of all variables were adjusted for age and gender. Confounding analyses were conducted for comorbid anxiety disorder and socioeconomic status in the total set, and alcohol use, cannabis use and drug abuse and dependence in the available subset. Confounding was operationalized as those measures that have a significant association (all correlations above 0.7) with the main indicator and the outcome (psychotic symptoms) and that lead to a larger than 10% change in the β of the main indicator (Lee, 2014). All variables that matched this criterion were included as covariate. Unadjusted results are reported in online Supplementary Tables S1, S2A and B. Analyses of IQ measures were adjusted for premorbid IQ and a sensitivity analysis was conducted to investigate the role of missing values. To explore the nature of the psychotic symptoms, groups of symptoms (the presence of delusions, hallucinations, disorganized speech, Schneiderian, and mood incongruent symptoms) were used as indicators in one single model simultaneously in order to adjust for their dependencies. Assumptions were tested for all statistical analyses. In case of logistic regression, assumptions of multicollinearity were not violated in any of the analysis [all correlations <0.43 and variance inflation factor (VIF) <1.3]. In addition, the Hosmer–Lemeshow test for goodness of fit was violated not at the $p < 0.001$ level except in the case of employment status for which we performed a $\chi 2$ test. For linear regression analysis, no multicollinearity was present as determined by VIF and normality of residuals was established by the Shapiro–Wilk test. Socio-economic status was transformed in Z score and CTQ total score was log transformed to reach approximately normal distributions of all dependent variables. An ordinal regression was performed in case of educational level. The assumption of proportional odds was violated but outcomes were confirmed by six additional logistic regression analyses, with increasing level of education as split. For MANCOVA analysis homogeneity of covariance matrices was analyzed by the Box's M test with the threshold set at $p < 0.01$ and was violated for the childhood adversity scales and therefore the Hotelling's Trace is reported to provide a more robust type I error estimate. Standardized βs were obtained of six most relevant risk factors to allow comparisons of the effect size per psychotic symptom group as presented in Fig. 2. In an additional analysis to investigate which combination of risk factors provides the best classification of the psychosis v. nonpsychosis distinction, a forward stepwise logistic regression as implemented in SPSS was conducted with psychosis

as outcome and all demographical characteristics, number of episodes, age of disease onset, presence of comorbid anxiety disorder, level of premorbid IQ, total IQ, and childhood maltreatment as potential indicators. SPSS implements an algorithm whereby addition of each variable to the model is based on the likelihood ratio statistic, prioritizing the most statistically significant improvement of the fit (the cut-off point being 0.05). Subsequently, a logistic regression was performed to investigate the interaction with gender with childhood maltreatment on the outcome of psychotic symptoms (hallucinations). The differences in psychotropic medication use between BDI patients with and without psychotic symptoms were analyzed by a $\chi 2$ test. Bonferroni correction for the 17 statistical tests was applied, setting the threshold for statistical significance at $p < 0.0029$. Missing values were handled using multiple imputation (He, 2010) except for variables with over 15% missing such as in case of: alcohol use (n = 807), substance abuse (n = 976) and dependence (n = 1029), suicide attempt (n = 991), and IQ (n = 1066). These data were analyzed in the subset of complete data after establishing representativeness for the entire cohort. Finally, the results for IQ (WAIS) were checked for possible confounding of a current mood episode. Data analysis was performed in SPSS, version 22.

Results

Psychotic symptoms in BD

A total of 990 (73.8%) of the 1342 BDI patients had experienced psychotic symptoms at least once during their lifespan. All demographic and clinical variables and test statistics are listed in Table 1. The group of patients with a history of psychotic symptoms (BD P+) was significantly different to the group without a history of psychosis with respect to: a younger age, an earlier age of onset, more frequent hospitalizations for a manic episode, and a higher mean level of education. Additional analysis using six logistic regressions with increasing levels of educations as split yielded very similar results (data not shown). Total IQ did not differ significantly between the groups. The sensitivity analysis showed that participants with incomplete WAIS data had significantly lower educational level [$t(402) = -3.30$, $p = 0.001$], global functioning [$t(490) = -10.9$, $p < 0.001$], and premorbid IQ [$t(399) = -3.10$, $p = 0.003$] as compared with participants with complete data. In addition, participants with incomplete data were less frequently employed [$\chi 2(1) = 35.71$, $p < 0.001$] and married [$\chi 2(1) = 16.52$, $p < 0.001$] but did not differ in the prevalence of psychotic symptoms [$\chi 2(1) = 0.14$, $p = 0.713$]. A current mood episode was not related to the WAIS results. Total childhood maltreatment level was not significantly different between the two groups, nor were the levels of the five maltreatment subtypes. The optimal logistic regression to classify lifetime psychotic symptoms as outcome showed that a higher level of educational performance [B = 0.14, p = 0.002, OR 1.15 (1.05– 1.26)], less frequent depressive episodes [B = −0.12, p < 0.001, OR 0.89 (0.83–0.95)], being female [B = −0.32, p = 0.025, OR 0.72 (0.54–0.96)],

and a lower age of disease onset [B = −0.04, p < 0.001, OR = 0.96 (0.95–0.97)] significantly contributed to the classification. The Nagelkerke R2 of the optimal model was 0.09.

Prevalence of delusions and hallucinations

In the BD P+ group, 916 patients (92.5%) had experienced delusions. Within this group, 61.7% had a history of delusions of grandiosity, 61.5% delusions of reference, and 38.5% persecutory delusions. Other delusions, including somatic, erotomanic delusions, and delusions of jealousy and guilt, occurred in 39.9% of the psychotic patients. A history of hallucinations occurred in 58.0% of the BD P+ patients, of which 33.4% had a history of auditory hallucinations and 39.0% visual hallucinations, 20.9% of the BD P+ had both. Table 2 provides the rates of all reported psychotic symptoms and a comparison to other studies. A history of delusions and hallucinations occurred isolated in, respectively, 411 (42.0%) and 62 (6.3%) of the BD P+ group. The combination of a history of hallucinations and delusions was present in 505 (51.6%) of the BD P+ group. The bipolar patients with a history of delusions only (n = 411) reported delusions of grandiosity in 60.6% of the cases, delusions of reference also in 60.6%, and persecutory delusions in 35.0% of the patients compared with: delusions of grandiosity in 70.1%, delusions of reference in 69.5%, persecutory delusions in 46.1% in patients with both hallucinations and delusions [delusions of grandiosity: $\chi2(1) = 8.37$, $p = 0.004$, delusions of reference: $\chi2(1) = 8.02$, $p = 0.005$, persecutory delusions: $\chi2(1) = 11.64$, $p = 0.001$]. The overlap of all five psychotic symptom groups is displayed in Fig. 1.

Table 2: Comparison of rates of psychotic symptoms between this study and others

	BD Sample (N= 1342)	Literature
Psychotic symptoms	73.8%	58%-70% (Goodwin &Jamison, 1990; Upthegrove et al., 2015)
Delusions	68.9%	65% (Upthegrove et al., 2015)
Delusions of grandiosity	61.7%	35-60% (Dunayevic& Keck, 2000)
Delusions of persecutory	38.5%	18-65% (Dunayevic&Keck, 2000)
Hallucinations	42.7%	
Auditory hallucinations	24.6%	23% (Upthegrove et al., 2015)
Visual hallucinations	28.6%	14% (Upthegrove et al., 2015)
Mood incongruent symptoms	30.1%	20% (Fennig et al., 1996; Keck et al., 2003)
Schneiderian symptoms	21.2%	9-34% (Tohen et al., 1992; Carlson et al., 2012; Goodwin & Jamison, 1990; Keck et al., 2003)
Formal thought disorder	59.7%	9-84%(Goodwin & Jamison, 1990; Keck et al., 2003)

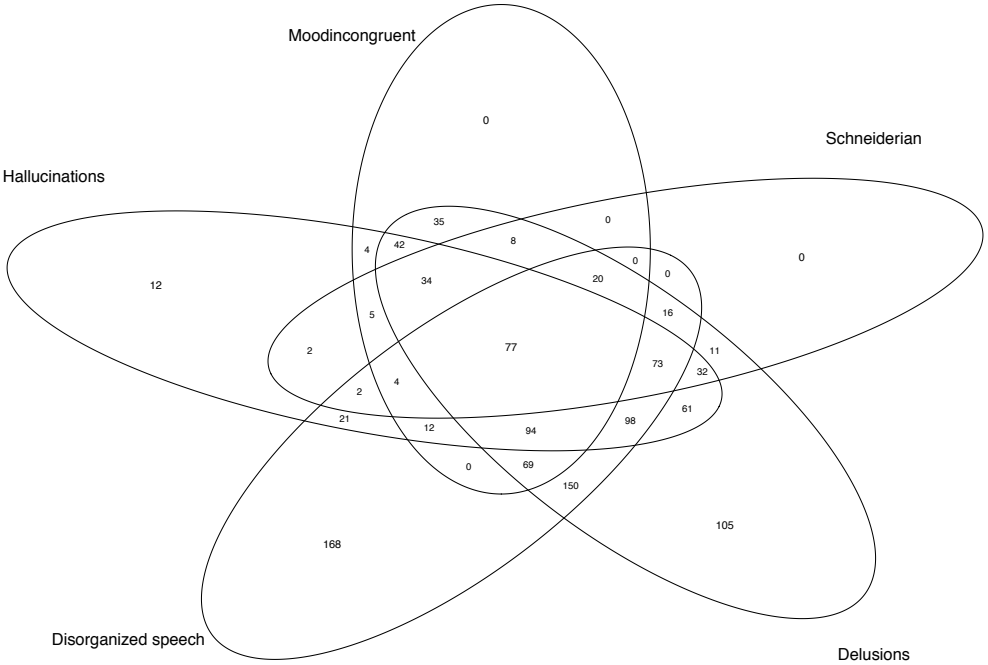


Figure 1. Venn diagram of overlap of patients with delusions/hallucinations/mood incongruent symptoms/ Schneiderian symptoms/disorganized speech, N=1,155

Determinants of delusions and hallucinations

Delusions

Patients with a history of delusions (n = 916, 68.9%) were significantly younger and had a significantly higher mean level of education and premorbid IQ compared with the overall BDI group. In addition, the presence of a history of delusions was significantly associated with more frequent hospitalizations for a (hypo)manic episode. Table 3 provides a complete overview of the clinical and demographic and neurocognitive features of delusions in BDI.

Hallucinations

A history of hallucinations was present in 567 (42.7%) patients. Patients with a history of hallucinations were more often female, suffered significantly more manic episodes, and childhood maltreatment. Particularly, auditory hallucinations were significantly associated with higher levels of childhood maltreatment ($\beta = 0.08$, $t = 2.66$, $p = 0.008$), in contrast to visual hallucinations ($\beta = 0.04$, $t = 0.02$, $p = 0.255$). Women reported significantly higher levels of childhood maltreatment ($t = 2.46$, $p = 0.014$) but no interaction between gender and childhood maltreatment on the risk for hallucinations was present (gender x childhood maltreatment $W= 0.08$, $B = 0.00$, $p = 0.782$). See Table 3 for a complete overview of the clinical, demographic, and neurocognitive features of BDI patients with lifetime hallucinations.

Determinants of mood incongruent symptoms, Schneiderian symptoms, and disorganized speech

The prevalence of a history of mood incongruent symptoms, Schneiderian symptoms, and disorganized speech in this BDI cohort was respectively 404 (30.1%), 284 (21.2%), and 801 (59.7%). Patients with a history of mood incongruent symptoms scored significantly higher on total IQ and patients with a history of disorganized speech had more frequent manic episodes. The presence of a history of Schneiderian symptoms showed no significant associations with any of the investigated variables. See Table 4 for a complete overview of the clinical, demographic, and neurocognitive features of BDI patients with a history of mood incongruent symptoms, Schneiderian symptoms, and disorganized speech. To provide an overview of the relationship between psychotic symptoms and the selected risk factors, we presented the standardized effect size (β) of the six most important risk factors for psychotic symptoms in Fig. 2.

Medication use

No significant differences between patients with or without psychosis was found for current use of antidepressants [$\chi2(1) = 2.2$, $p = 0.138$], mood stabilizers [$\chi2(1) = 1.9$, $p = 0.166$], antipsychotics [$\chi2(1) = 4.6$, $p = 0.060$] nor for a history of antidepressant [$\chi2(1) = 2.2$, $p = 0.073$] and mood stabilizers [$\chi2(1) = 1.5$, $p = 0.221$]. Also, current lithium use was not significantly different either between the groups [$\chi2(2) = 0.59$, $p = 0.751$]. As to be expected, lifetime use of antipsychotics in BDI patients with a history of psychotic symptoms was significantly more frequent [$\chi2(1) = 45.8$, $p < 0.001$].

Comorbid anxiety disorders and socio-economic status

All analyses of psychotic symptoms were adjusted for comorbid anxiety disorders and/or socio-economic status, based on our definition of potential confounding.

Substance use

In the subset (N = 922) with data on substance use, alcohol use, lifetime substance abuse, or dependence were not confounding the reported relations with lifetime psychotic symptoms. Similarly, alcohol and substance use did not confound the relations with delusions, hallucinations, mood incongruent symptoms, Schneiderian symptoms, and disorganized speech (all correlations below 0.7 and changes in β after inclusion as covariate <10%).

Table 3: Association of hallucinations and delusions with demographical and clinical characteristics in BD type I patients

	Test Statistics Delusions N=925 (68.9%)	Test Statistics Hallucinations N=572.6 (42.7%)
Age	**Beta=0.08, t=-5.35, p<0.001***	Beta=0.04, t=0.80, p=0.423
Gender	B=-0.06, p=0.651, OR=0.93[0.72-1.32]	**B=0.43, p=0.001, OR=1.54[1.18-1.99]***
Marital Status	B=-0.23, p=0.101, OR=0.80[0.53-1.03]	B=0.04, p=0.792, OR=1.04[0.79-1.47]
Employment status	B=0.13, p=0.367, OR=1.04[0.86-1.51]	B=-0.12, p=0.337, OR=0.87[0.68-1.16]
Global functioning	Beta=0.05, t=1.61, p=0.109,	Beta=-0.07, t=-2.219, p=0.029
Socio economic status	Beta=-0.01, t=-0.28, p=0.783	Beta=-0.03, t=-1.16, p=0.248
Mean level of education	**$Wc^2(1)=14.77$, p<0.001, OR=0.59[0.47-0.75]***	$Wc^2(1)=1.91$, p=0.184, OR=0.59[0.93-1.47]
Premorbid IQ	**Beta=0.12, t=3.66, p<0.001***	Beta=-0.03, t=-1.04, p=0.148
Anxiety disorder	B=-0.37, p=0.022, OR=0.69[0.51-0.95]	B=0.15, p =0.321, OR=1.16[0.87-1.56]
Age at onset	Beta=-0.07, t=-2.63, p=0.009	Beta=-0.04, t=-1.61, p=0.109
Nr. of episodes MANCOVA	F(2,1339)=5.72, p=0.005, Partial η^2=0.01	**F(2,1339)=6.30, p=0.002, Partial η^2=0.01***
Nr. of depressive episodes	F(1,1333)=11.15, p=0.001, Partial η^2<0.01	F(1,1333)=2.25, p=0.125, Partial η^2<0.01
Nr. of manic episodes	F(1,1333)=3.15, p=0.077, Partial η^2<0.01	**F(1,1333)=12.59, p<0.001, Partial η^2=0.01***
Nr. of hospitalizations MANCOVA	**F(2,1339)=20.86, p**<0.001, Partial η^2=0.03***	F(2,1339)=2.33, p**=0.115, Partial η^2<0.01
Nr. of hospitalizations for depressive episodes	F(1,1333)=1.95, p=0.179, Partial η^2<0.01	F(1,1333)=4.55, p=0.083, Partial η^2<0.01
Nr. of hospitalizations for manic episodes	**F(1,1333)=33.23, p<0.001, Partial η^2=0.02***	F(1,1333)=0.68, p=0.333, Partial η^2<0.01
Nr. of suicide attempts (n=991)	B=0.12, p=0.494, OR=1.13[0.80-1.60]	B=0.20, p =0.235, OR=1.22[0.88-1.70]
Total IQ	Beta=-0.012, t=-0.62, p=0.534	Beta=-0.01, t=-0.47, p=0.639
WAIS MANCOVA	F(4,974)=2.51, p=0.040, Partial η^2=0.01	F(4,974)=1.01, p=0.399, Partial η^2<0.01
WAIS – Information	F(1,981)=1.07, p=0.301, Partial η^2<0.01	F(1,981)=1.07, p=0.302, Partial η^2<0.01
WAIS – Block Design	F(1,981)=0.54, p=0.461, Partial η^2<0.01	F(1,981)=0.35, p=0.557, Partial η^2<0.01
WAIS – Arithmetic	F(1,981)=4.46, p=0.615, Partial η^2<0.01	F(1,981)=0.11, p=0.744, Partial η^2<0.01

WAIS – Digit Symbol	F(1,981)=0.94, p=0.332, Partial η^2<0.01	F(1,981)=2.27, p=0.132, Partial η^2<0.01
Childhood trauma Total score	Beta=-0.01, t=-0.25, p=0.803	**Beta=0.09, t=3.04, p=0.002***
Trauma subtypes MANCOVA	F(5,1328)=0.61, p**=0.691, Partial η^2<0.01	F(5,1328)=2.32, p**=0.045, Partial η^2<0.01
Sexual abuse	F(1,1332)=0.10, p=0.474, Partial η^2<0.01	F(1,1332)=1.06, p=0.321, Partial η^2<0.01
Physical abuse	F(1,1332)=2.01, p=0.171, Partial η^2<0.01	F(1,1332)=5.99, p=0.015, Partial η^2<0.01
Emotional abuse	F(1,1332)=0.09, p=0.822, Partial η^2<0.01	F(1,1332)=5.53, p=0.021, Partial η^2<0.01
Physical neglect	F(1,1332)=0.12, p=0.828, Partial η^2<0.01	F(1,1332)=1.24, p=0.283, Partial η^2<0.01
Emotional neglect	F(1,1332)=0.39, p=0.560, Partial η^2<0.01	F(1,1332)=8.41, p=0.004, Partial η^2<0.01

*Significant between-group difference (p<0.0029). Bold fonts are used to highlight significance.

** Lawley's Hotelling's Trace

Table 4: Association of Mood Incongruent symptoms, Schneiderian symptoms and Disorganized Speech with demographical and clinical characteristics in BD type I patients

	Test Statistics Mood Incongruent symptoms N=404 (30.1%)	Test Statistics Schneiderian symptoms N= 284 (21.2%)	Test Statistics Disorganized Speech N= 801 (59.7%)
Age	Beta=-0.01, t=-0.01, p=0.994	Beta=-0.01, t=-0.56, p=0.579	Beta=0.02, t=-2.58, p=0.012
Gender	B=0.21, p=0.124, OR=0.12[0.94-1.61]	B=0.24, p=0.132, OR=1.27[0.93-1.73]	B=-0.08, p=0.504, OR=0.92[0.73-1.17]
Marital Status	B=0.30, p=0.024, OR=0.14[0.98-1.83]	B=-0.16, p=0.309, OR=0.86[0.59-1.19]	B=0.01, p=0.958, OR=1.01[0.79-1.41]
Employment status	B=-0.18, p=0.203, OR=0.84[0.64-1.10]	B=-0.27, p=0.092, OR=0.76[0.56-1.05]	B=0.00, p=0.989, OR=1.00[0.79-1.27]
Global functioning	Beta=-0.01, t=-0.15, p=0.890	Beta=-0.09, t=-2.71, p=0.007	Beta=-0.06, t=-2.32, p=0.021
Socio economic status	Beta=0.02, t=0.68, p=0.498	Beta=0.04, t=0.1.40, p=0.161	Beta=0.03, t=1.26, p=0.211
Mean level of education	$Wc^2(1)=0.63$, p=0.383, OR=0.90[0.72-1.14]	$Wc^2(1)=0.19$, p=0.696, OR=1.05[0.81-1.37]	$Wc^2(1)=2.05$, p=0.165, OR=1.15[0.94-1.41]
Premorbid IQ	Beta=0.02, t=0.50, p=0.618	Beta=-0.05, t=-1.73, p=0.085	Beta=-0.04, t=-1.23, p=0.212
Anxiety disorder	B=0.10, p=0.499, OR=1.11[0.83-1.49]	B=0.39, p =0.018, OR=1.48[1.07-2.05]	B=0.22, p=0.094, OR=1.26[0.96-1.64]
Age at onset	Beta=0.01, t=0.19, p=0.852	Beta=-0.01, t=-0.27, p=0.791	Beta=0.00, t=-0.02, p=0.987
Nr. of episodes MANCOVA	F(2,1339)=0.05, p=0.951, Partial η^2<0.01	F(2,1339)=0.49, p=0.472, Partial η^2<0.01	**F(2,1339)=8.29, p<0.001, Partial η^2=0.01***
Nr. of depressive episodes	F(1,1333)=0.09, p=0.850, Partial η^2<0.01	F(1,1333)=0.03, p=0.859, Partial η^2<0.01	F(1,1333)=1.54, p=0.258, Partial η^2<0.01
Nr. of manic episodes	F(1,1333)=0.45, p=0.863, Partial η^2<0.01	F(1,1333)=0.75, p=0.384, Partial η^2<0.01	**F(1,1333)=16.14, p<0.001, Partial η^2=0.01***
Nr. of hospitalizations MANCOVA	F(2,1339)=1.27, p**=0.285, Partial η^2<0.01	F(2,1339)=2.71, p**=0.073, Partial η^2<0.01	F(2,1339)=0.80, p**=0.285, Partial η^2<0.01
Nr. of hospitalizations for depressive episodes	F(1,1333)=2.02, p=0.159, Partial η^2<0.01	F(1,1333)=0.64, p=0.432, Partial η^2<0.01	F(1,1333)=0.20, p=0.715, Partial η^2<0.01
Nr. of hospitalizations for manic episodes	F(1,1333)=0.13, p=0.570, Partial η^2<0.01	F(1,1333)=5.37, p=0.100, Partial η^2<0.01	F(1,1333)=1.09, p=0.348, Partial η^2<0.01
Nr. of suïcide attempts	B=-0.10, p=0.571, OR=0.91[0.65-1.27]	B=0.09, p =0.644, OR=1.09[0.75-1.58]	B=0.12, p=0.430, OR=1.13[0.83-1.54]
Total IQ (n=1060)	**Beta=0.09, t=3.30, p=0.001***	Beta=0.012, t=0.51, p=0.614	Beta=0.08, t=3.01, p=0.003
WAIS MANCOVA(n=1060)	F(4,974)=2.76, p=0.039, Partial η^2=0.01	F(4,974)=0.378, p=0.378, Partial η^2<0.01	F(4,974)=3.55, p=0.007, Partial η^2=0.01
WAIS – Information	F(1,981)=7.18, p=0.008, Partial η^2=0.01	F(1,981)=0.22, p=0.638, Partial η^2<0.01	F(1,981)=7.18, p=0.008, Partial η^2=0.01
WAIS – Block Design	F(1,981)=5.33, p=0.021, Partial η^2=0.01	F(1,981)=1.76, p=0.186, Partial η^2<0.01	F(1,981)=0.53, p=0.021, Partial η^2=0.01
WAIS – Arithmetic	F(1,981)=2.04, p=0.154, Partial η^2<0.01	F(1,981)=0.03, p=0.871, Partial η^2<0.01	F(1981)=2.04, p=0.154, Partial η^2<0.01
WAIS – Digit Symbol	F(1,981)=3.27, p=0.071, Partial η^2<0.01	F(1,981)=0.87, p=0.352, Partial η^2<0.01	F(1,981)=3.27, p=0.071, Partial η^2<0.01

Childhood trauma Total score	Beta=-0.02, t=-0.80, p=0.426	Beta=0.04, t=1.40, p=0.162	Beta=0.08, t=2.40, p=0.019
Trauma subtypes MANCOVA	F(5,1328)=2.87, p**=0.023, Partial η^2=0.01	F(5,1328)=1.02, p**=0.409, Partial η^2<0.01	F(5,1328)=4.86, p**=0.007, Partial η^2=0.02
Sexual abuse	F(1,1332)=1.95, p=0.177, Partial η^2<0.01	F(1,1332)=1.69, p=0.207, Partial η^2<0.01	F(1,1332)=7.51, p=0.010, Partial η^2=0.01
Physical abuse	F(1,1332)=0.07, p=0.814, Partial η^2<0.01	F(1,1332)=0.48, p=0.492, Partial η^2<0.01	F(1,1332)=11.22, p=0.002, Partial η^2=0.01
Emotional abuse	F(1,1332)=0.58, p=0.883, Partial η^2<0.01	F(1,1332)=0.58, p=0.469, Partial η^2<0.01	F(1,1332)=5.69, p=0.025, Partial η^2=0.01
Physical neglect	F(1,1332)=10.03, p=0.002, Partial η^2=0.01	F(1,1332)=3.48, p=0.066, Partial η^2<0.01	F(1,1332)=5.32, p=0.097, Partial η^2<0.01
Emotional neglect	F(1,1332)=1.04, p=0.323, Partial η^2<0.01	F(1,1332)=0.62, p=0.437, Partial η^2<0.01	F(1,1332)=0.48, p=0.526, Partial η^2<0.01

*Significant between-group difference (p<0.0029). Bold fonts are used to highlight significance.

**Lawley's Hotelling's Trace

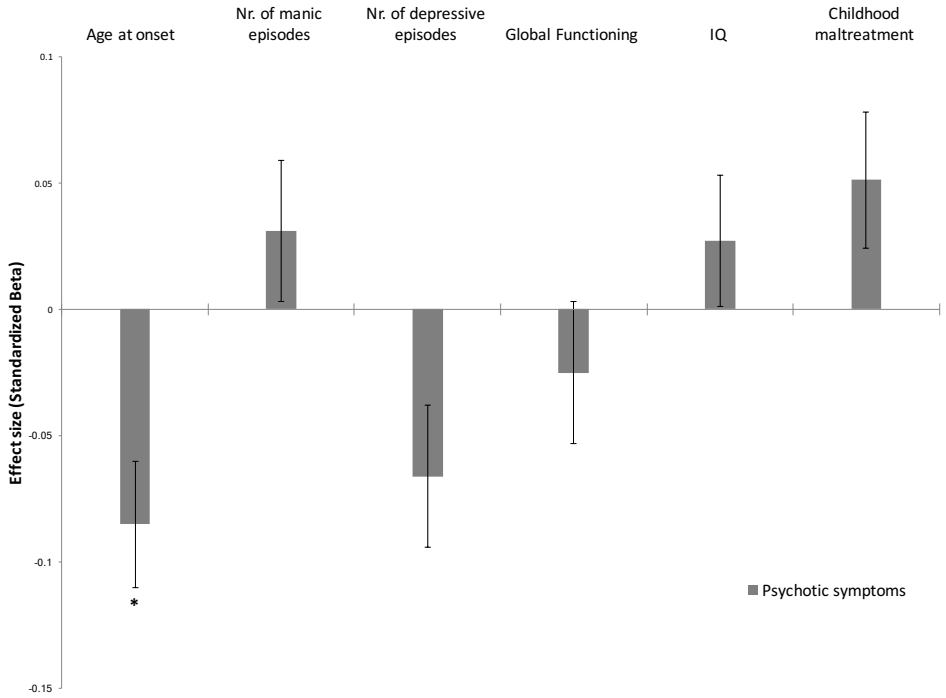


Figure 2A: Relationship between psychotic symptoms and age at onset, number of episodes, global functioning, IQ and childhood maltreatment.

(*Significantly associated with psychotic symptoms, p<0.0029, for graphical purposes standardized betas were obtained from separate binary logistic regressions)

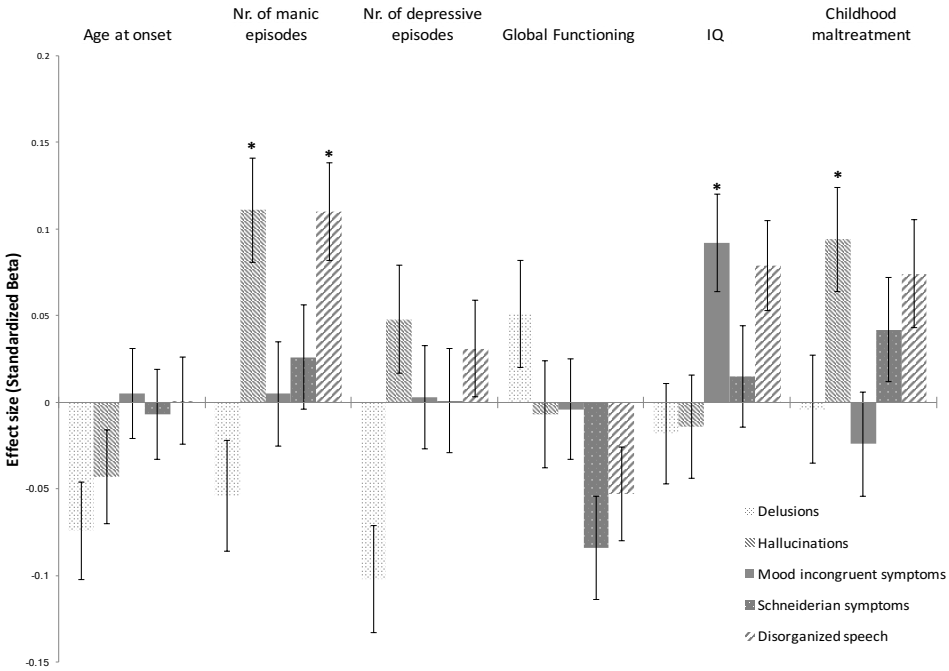


Figure 2B: Relationship between delusions/hallucinations/mood incongruent symptoms/Schneiderian symptoms/ disorganized speech and age at onset, number of episodes, global functioning, IQ and childhood maltreatment.

(*Significantly associated with psychotic symptoms, p<0.0029, for graphical purposes standardized betas were obtained from separate binary logistic regressions)

Discussion

In a large comprehensively characterized sample of 1342 BDI patients, we observed a high frequency of lifetime psychotic symptoms (73.8%) including delusions (68.9%), hallucinations (42.7%), mood incongruent symptoms (30.1%), Schneiderian symptoms (21.2%), and formal thought disorder (59.7%). Psychotic symptoms were associated with a more severe illness course, an earlier onset of disease, and more frequent hospitalizations. The characteristics of patients with different types of psychotic symptoms were considerably overlapping but were significantly different with respect to the level of childhood maltreatment. Auditory hallucinations stood out as the psychotic feature that was associated with higher levels of childhood maltreatment. Women were significantly more likely to have a history of hallucinations as compared with men.

Prevalences of (specific) psychotic symptoms

The reported prevalences in this study are in line with previous studies reporting on a history of psychotic symptoms (Goodwin and Jamison, 1990; Keck et al., 2003; Bora et al., 2010; Upthegrove et al., 2015) and the frequency of specific psychotic symptoms, including delusions (Dunayevich and Keck, 2000; Upthegrove et al., 2015), mood incongruent symptoms (Fennig et al., 1996; Keck et al., 2003), Schneiderian symptoms (Goodwin and Jamison, 1990; Keck et al., 2003; Carlson et al., 2012), and formal thought disorder (Goodwin and Jamison, 1990; Keck et al., 2003) (see Table 2). However, the observed frequency of visual hallucinations (28.6%) is much higher than the 14% for visual hallucinations reported by Upthegrove et al. (2015). This difference in frequency may reflect differences between the study populations or differences in the assessment of the hallucinations between studies. The reported rate of visual hallucinations in this BDI sample are comparable to those in schizophrenia (Bauer et al., 2011). In contrast to the prevalences of auditory hallucinations, Schneiderian symptoms and mood incongruent symptoms in our study are low compared with the rates reported in schizophrenia (Mueser et al., 1990; Baethge et al., 2005).

Demographic characteristics and life course

We found that woman were more likely to suffer from hallucinations compared with men [OR 1.54 (1.18-1.99)] in contrast to equivalent gender rates reported in several smaller studies (Keck et al., 2003; Bora et al., 2010; Özyildirim et al., 2010). However, the largest study by Upthegrove et al. (n = 2019) also reported more woman in the psychosis group (Upthegrove et al., 2015). Of note is that sex ratios in BD are nearly equal (Weissman et al., 1996; Hendrick et al., 2000) but for schizophrenia an excess of males that have a more severe disease course is reported (Aleman et al., 2003). In our study, the patients with a history of hallucinations (being more frequently female) suffer a more severe disease course, reflected by a more (hypo) manic episodes. This raises the question whether a misclassification has occurred whereby women with psychotic symptoms are diagnosed with BD rather than with

schizophrenia. Another potential explanation for the gender differences may be found in the association with childhood maltreatment. In general and also in this study, women report higher level of childhood maltreatment. The relation of childhood trauma with the risk for psychosis in affective disorders may be specific for women (Fisher et al., 2009). Our data did not support this explanation as no significant interaction between gender and childhood maltreatment on risk to develop psychotic symptoms was found. The association of childhood maltreatment with a history of auditory hallucinations in BDI is in agreement with previous studies that reported an association of hallucinations with early life events in BD (Hammersley et al., 2003; Upthegrove et al., 2015). This study replicates these reports and further provides evidence that the relationship between childhood adversity and psychosis in BD is particularly strong for auditory hallucinations. Such a relationship is reported in schizophrenia as well, unrelated to specific type of childhood adversity (Read et al., 2005; Varese et al., 2012), suggesting the relation is present across diagnostic boundaries of psychiatric disorders.

Clinical characteristics

Our study adds support for a more manic disease profile (as defined by more frequent hospitalizations for manic episodes) (Özyildirim et al., 2010) as characteristic of BDI patients with psychosis. The presence of psychosis is also accompanied by an earlier disease onset (Bora et al., 2010; Upthegrove et al., 2015), more frequent hospital admissions, mood episodes (Bora et al., 2010; Özyildirim et al., 2010; Upthegrove et al., 2015), and higher symptom severity (Coryell et al., 2001; Özyildirim et al., 2010). Of note is that the most recent genome wide association study (GWAS) of over 100 000 bipolar and schizophrenia patients conducted by the Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC) (Bipolar Disorder and schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018) demonstrated that bipolar patients with psychotic features have significantly higher schizophrenia polygenic risk scores than bipolar patients without psychotic features. Moreover, they showed that higher polygenic risk scores for schizophrenia in bipolar patients are associated with a more severe illness course reflected by more frequent hospitalizations and an earlier onset of the disease (Bipolar Disorder and schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018). This is consistent with our finding that BD patients with a history of psychotic symptoms have an earlier disease onset and more hospitalizations for a manic episode v. patients without psychotic symptoms. Together, this suggests that within the bipolar spectrum, a (genetic) differentiation may be present that clinically presents with psychotic features and a more severe disease course. In contrast to the association of psychosis to a manic and more severe disease profile, patients with mood incongruent and Schneiderian symptoms did not show differences in disease profile. Particularly, previous reports of more depressive episodes in BDI patients with mood incongruent symptoms (Tohen et al., 1992; Toni et al., 2001) could not be replicated. However, these were relatively small studies ($n \leqslant 155$) and

the other large study (Upthegrove et al., 2015) did not report on clinical characteristics in relation to a history of mood incongruent symptoms.

Neurocognitive characteristics

The relationship between cognitive function and psychotic symptoms was ambiguous. A higher educational performance in the psychosis group but the absence of significant differences in IQ are in contrast to most studies that reported no differences between BD with or without psychotic symptoms for these measures (Glahn et al., 2006, 2007; Savitz et al., 2009; Simonsen et al., 2011; Aminoff et al., 2013). However, one previous study also showed a higher level of premorbid functioning BDI patients with a history of psychotic symptoms (Selva et al., 2007). The largest study to date on cognitive function in 774 bipolar patients showed greater severity of cognitive deficits in those with psychotic symptoms (Bora et al., 2010) in accordance with similar findings in schizophrenia (MacCabe, 2008; Kahn and Keefe, 2013). An explanation of these discrepancies may be found in previous reports of increased educational performance in BD patients particularly in those with a tendency toward manic episodes (MacCabe et al., 2010; Vreeker et al., 2016). There also may be influence of the presence of an academic environment or pressure for academic achievement, which the current study did not take into account. Sampling bias provides a likely explanation, particularly considering the bias in this study for drop out in participating in the IQ measurements for those with low educational level.

Limitations

Strength of our study lies in the very comprehensive assessment in a large sample of BDI patients although the retrospective and the cross-sectional data collection poses an inherent limitation. A further limitation is that the measures of reliability of all used psychometric tests were limited to reporting general reliability statistics. However, all instruments are widely used, have a longstanding record of validity, and were used by one team of well-trained collaborators in one single university hospital. Despite the fact that we cannot rule out rater variability, there is also no reason to assume this variation is systematic and has led to bias. The self-report online assessment in our study, consisting of the CTQ and medical questionnaire, is reported to be fairly equivalent to paper–pencil versions (Prescott et al., 2000; Vallejo et al., 2007; Vleeschouwer et al., 2014). Despite multivariate analysis, residual confounding may remain as we did not adjust for several unmeasured potentially confounding factors, such as the number of psychotic episodes, the age of onset of psychosis, and comorbid disorders other than anxiety disorders. Also, whereas the current selection of clinical characteristics is comprehensive and constitutes the most relevant items, it is by no means exhaustive and other measures may have additional value for identifying distinct subgroups of patients. Multiple testing was handled by using a Bonferroni correction avoiding type I error inflation and report more reliable findings albeit at the expense of power. Finally, despite our large sample, we cannot be sure that our population is representative although

there also is no reason to assume bias, particularly considering the predominantly non-clinical recruitment.

Summary

Overall, we showed in a large well-characterized sample of 1342 bipolar type I patients that 73.8% of the patients presented a history of psychotic symptoms including delusions, hallucinations, formal thought disorder, mood incongruent, and Schneiderian symptoms. The uniqueness of this study is the comprehensive data collection, including demographic, clinical, and neurocognitive characteristics in a large cohort of bipolar type I patients. This study is the most comprehensive analysis of determinants and characteristics of psychotic symptoms in BD to date. Overall, our findings suggest that psychotic symptoms in BD are associated with a more severe, predominantly manic illness course. BDI patients suffering from distinct psychotic symptoms (including hallucinations, delusions, formal thought disorder, mood incongruent and Schneiderian symptoms) showed interesting difference in disease course and history of childhood maltreatment. Hallucinations stood out by its association with a history of childhood maltreatment. Nevertheless, the overlap between patients with a particular symptom type was large as can also be seen in the Venn diagram (Fig. 1). Moreover, a classifier built from all characteristics could accurately predict just about 8% of the cases showing that the current set of risk factors does not provide a good distinction between the psychosis and non-psychosis group. In summary, our results do not point to a clear categorical distinct psychotic subtype but do support a differentiation in severity within BDI based on psychosis vulnerability (Bipolar Disorder and schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018). In future research, the role of distinct risk factors such as trauma in relation to specific psychotic symptoms could be better investigated by prospective studies across psychiatric diagnostic boundaries. This combined with recent genetic insight may provide a lead in further unravelling the etiology of psychosis across psychiatric disorders.

References:

Aleman A, Kahn RS and Selten JP (2003) Sex differences in the risk of schizophrenia: evidence from meta-analysis. *Archives of General Psychiatry* **60**, 565–571.

Allardyce J, Leonenko G, Hamshere M, Pardiñas AF, Forty L, Knott S, Gordon-Smith K, Porteous DJ, Haywood C, Di Florio A, Jones L, McIntosh AM, Owen MJ, Holmans P, Walters JTR, Craddock N, Jones I, O□Donovan MC and Escott-Price V (2018) Association between schizophrenia-related polygenic liability and the occurrence and level of mood-incongruent psychotic symptoms in bipolar disorder. *JAMA Psychiatry* **75**, 28–35.

Aminoff SR, Hellvin T, Lagerberg TV, Andreassen OA and Melle I (2013) Neurocognitive features in subgroups of bipolar disorder. *Bipolar Disorders* **15**, 272–283.

Andreasen NC, Flaum M and Arndt S (1992) The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnosis and psychopathology. *Archives of General Psychiatry* **49**, 615–623.

Baethge C, Baldessarini RJ, Freudenthal K, Streeruwitz A, Bauer M and Bschor T (2005) Hallucinations in bipolar disorder: characteristics and comparison to unipolar depression and schizophrenia. *Bipolar Disorders* **7**, 136–145.

Bauer SM, Schanda H, Karakula H, Olajossy-Hilkesberger L, Rudaleviciene P, Okribelashvili N, Chaudhry HR, Idemudia SE, Gscheider S, Ritter K and Stompe T (2011) Culture and the prevalence of hallucinations in schizophrenia. *Comprehensive Psychiatry* **52**, 319–325.

Bernstein DP, Ahluvalia T, Pogge D and Handelsman L (1997) Validity of the Childhood Trauma Questionnaire in an adolescent psychiatric population. *Journal of The American Academy of Child and Adolescent Psychiatry* **36**, 340–348.

Bernstein DP, Stein JA, Newcomb MD, Walker E, Pogge D, Ahluvalia T, Stokes J, Handelsman L, Medrano M, Desmond D and Zule W (2003) Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse & Neglect* **27**, 169–190.

Bipolar Disorder and schizophrenia Working Group of the Psychiatric Genomics Consortium* (2018) Genomic dissection of bipolar disorder and schizophrenia, including 28 subphenotypes. *Cell* **173**, 1705–1715.

Blyler CR, Gold JM, Iannone VN and Buchanan RW (2000) Short form of the WAIS-III for use with patients with schizophrenia. *Schizophrenia Research* **46**, 209–215.

Bora E, Yücel M and Pantelis C (2010) Neurocognitive markers of psychosis in bipolar disorder: a meta-analytic study. *Journal of Affective Disorders* **127**, 1–9.

Bright P, Jaldow E and Kopelman MD (2002) The National Adult Reading Test as a measure of premorbid intelligence: a comparison with estimates derived from demographic variables. *Journal of the International Neuropsychological Society: JINS* **8**, 847–854.

Carlson GA, Kotov R, Chang SW, Ruggero C and Bromet EJ (2012) Early determinants of four-year clinical outcomes in bipolar disorder with psychosis. *Bipolar Disorders* **14**, 19–30.

Coryell W, Leon AC, Turvey C, Akiskal HS, Mueller T and Endicott J (2001) The significance of psychotic features in manic episodes: a report from the NIMH collaborative study. *Journal of Affective Disorders* **67**, 79–88.

Craddock N, O'Donovan MC and Owen MJ (2005) The genetics of schizophrenia and bipolar disorder: dissecting psychosis. *Journal of Medical Genetics* **42**, 193–204.

Currie C, Molcho M, Boyce W, Holstein B, Torsheim T and Richter M (2008) Researching health inequalities in adolescents: the development of the Health Behaviour in School-Aged Children (HBSC) family affluence scale. *Social Science & Medicine* **66**, 1429–1436.

Dunayevich E and Keck PE (2000) Prevalence and description of psychotic features in bipolar mania. *Current Psychiatry Reports* **2**, 286–290.

Fennig S, Bromet EJ, Tanenberg Karant M, Ram R and Jandorf L (1996) Mood-congruent versus mood-incongruent psychotic symptoms in first admission patients with affective disorder. *Journal of Affective Disorders* **37**, 23–29.

Fergusson DM, Horwood LJ and Boden JM (2011) Structural equation modeling of repeated retrospective reports of childhood maltreatment. *International Journal of Methods in Psychiatric Research* **20**, 93–104.

First MB, Spitzer RL, Gibbon M and Williams JBW (1997) *Structured Clinical Interview for DSM-IV Axis I Disorders-Patient Edition (SCID-I/P, version 2.0).* Washington, D.C.: American Psychiatric Press, Inc.

Fisher H, Morgan C, Dazzan P, Craig TK, Morgan K, Hutchinson G, Jones PB, Doody GA, Pariante C, McGuffin P, Murray RM, Leff J and Fearon P (2009) Gender differences in the association between childhood abuse and psychosis. *The British Journal of Psychiatry* **194**, 319–325.

Glahn DC, Bearden CE, Cakir S, Barrett JA, Najt P, Monkul ES, Maples N, Velligan DI and Soares JC (2006) Differential working memory impairment in bipolar disorder and schizophrenia: effects of lifetime history of psychosis. *Bipolar Disorders* **8**, 117–123.

Glahn DC, Bearden CE, Barguil M, Barrett J, Reichenberg A, Bowden CL, Soares JC and Velligan DI (2007) The neurocognitive signature of psychotic bipolar disorder. *Biological Psychiatry* **62**, 910–916.

Goodwin FK and Jamison KR (1990) *Manic-Depressive Illness.* New York: Oxford University Press.

Green MF (2006). Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. *The Journal of Clinical Psychiatry* **67**, 3–8.

Hammersley P, Dias A, Todd G, Bowen-Jones K, Reilly B and Bentall RP (2003) Childhood trauma and hallucinations in bipolar affective disorder: preliminary investigation. *The British Journal of Psychiatry : The Journal of Mental Science* **182**, 543–547.

He Y (2010) Missing data analysis using multiple imputation: getting to the heart of the matter. *Circulation: Cardiovascular Quality and Outcomes* **3**, 98–105.

Hendrick V, Altshuler LL, Gitlin MJ, Delrahim S and Hammen C (2000) Gender and bipolar illness. *Journal of Clinical Psychiatry* **61**, 393–396.

Jabben N, Jabben N, Arts B, van Os J and Krabbendam L (2010) Neurocognitive functioning as intermediary phenotype and predictor of psychosocial functioning across the psychosis

continuum: studies in schizophrenia and bipolar disorder. *Journal of Clinical Psychiatry* **71**, 764–774.

Kahn RS and Keefe RSE (2013) Schizophrenia is a cognitive illness: time for a change in focus. *JAMA Psychiatry* **70**, 1107–1112.

Keck PE, McElroy SL, Havens JR, Altshuler LL, Nolen WA, Frye MA, Suppes T, Denicoff KD, Kupka R, Leverich GS, Rush AJ and Post RM (2003) Psychosis in bipolar disorder: phenomenology and impact on morbidity and course of illness. *Comprehensive Psychiatry* **44**, 263–269.

Krabbendam L, Arts B, Os J and Aleman A (2005) Cognitive functioning in patients with schizophrenia and bipolar disorder: a quantitative review. *Schizophrenia Research* **80**, 137–149.

Lee PH (2014) Is a cutoff of 10% appropriate for the change-in-estimate criterion of confounder identification? *Journal of Epidemiology* **24**, 161–167.

Leverich GS, Nolen WA, Rush AJ, McElroy SL, Keck Jr PE, Denicoff KD, Suppes T, Altschuler LL, Kupka R, Kramlinger KG and Post RM (2001) The Stanley Foundation Bipolar Treatment Outcome Network: I. Longitudinal methodology. *Journal of Affective Disorders* **67**, 33–44.

Levy B, Medina AM and Weiss RD (2013) Cognitive and psychosocial functioning in bipolar disorder with and without psychosis during early remission from an acute mood episode: a comparative longitudinal study. *Comprehensive Psychiatry* **54**, 618–626.

Lewinsohn PM and Rosenbaum M (1987) Recall of parental behavior by acute depressives, remitted depressives, and nondepressives. *Journal of Personality and Social Psychology* **52**, 611–619.

MacCabe JH (2008) Population-based cohort studies on premorbid cognitive function in schizophrenia. *Epidemiologic Reviews* **30**, 77–83.

MacCabe JH, Lambe MP, Cnattingius S, Sham PC, David AS, Reichenberg A, Murray RM and Hultman CM (2010) Excellent school performance at age 16 and risk of adult bipolar disorder: National cohort study. *The British Journal of Psychiatry* **196**, 109–115.

Maj M (2003) The effect of lithium in bipolar disorder: a review of recent research evidence. *Bipolar Disorders* **5**, 180–188.

Maj M, Pirozzi R, Bartoli L and Magliano L (2002) Long-term outcome of lithium prophylaxis in bipolar disorder with mood-incongruent psychotic features: a prospective study. *Journal of Affective Disorders* **71**, 195–198.

Martínez-Arán A, Vieta E, Colom F, Torrent C, Sanchez-Moreno J, Reinares M, Benabarre A, Goikolea JM, Brugue E, Daban C and Salamero M (2004) Cognitive impairment in euthymic bipolar patients: implications for clinical and functional outcome. *Bipolar Disorders* **6**, 224–232.

McElroy SL, Keck PE and Strakowski SM (1996) Mania, psychosis, and antipsychotics. *Journal of Clinical Psychiatry* **57**, 14–26.

Mueser KT, Bellack AS and Brady EU (1990) Hallucinations in schizophrenia. *Acta Psychiatrica Scandinavica* **82**, 26–29.

O'Grady JC (1990) The prevalence and diagnostic significance of schneiderian first-rank symptoms in a random sample of acute psychiatric in-patients. *British Journal of Psychiatry* **156**, 496–500.

Özyildirim I, Çakir S and Yazici O (2010) Impact of psychotic features on morbidity and course of illness in patients with bipolar disorder. *European Psychiatry* **25**, 47–51.

Potash JB, Yen-Feng C, MacKinnon DF, Miller EB, Simpson SG, McMahon FJ, McInnis MG and DePaulo JR (2003) Familial aggregation of psychotic symptoms in a replication set of 69 bipolar disorder pedigrees. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics* **116B**, 90–97.

Prescott A, Bank L, Reid JB, Knutson JF, Burraston BO and Eddy JM (2000) The veridicality of punitive childhood experiences reported by adolescents and young adults. *Child Abuse and Neglect* **24**, 411–423.

Read J, van Os J, Morrison AP and Ross CA (2005) Childhood trauma, psychosis and schizophrenia: a literature review with theoretical and clinical implications. *Acta psychiatrica Scandinavica* **112**, 330–350.

Rey JM, Starling J, Wever C, Dosseter DR and Plapp JM (1995) Inter-rater reliability of global assessment of functioning in a clinical setting. *Journal of Child Psychology and Psychiatry* **36**, 787–792.

Robins LN, Wing J, Wittchen HU, Helzer JE, Babor TF, Burk J, Farmer A, Jablenski A, Pickens R, Regier DA, Sartorius N and Towle LH (1988) The Composite International Diagnostic Interview. *Archiver of General Psychiatry* **45**, 1069–1077.

Robinson LJ, Thompson JM, Gallagher P, Goswami U, Young AH, Ferrier IN and Moore PB (2006) A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. *Journal of Affective Disorders* **93**, 105–115.

Savitz J, van der Merwe L, Stein DJ, Solms M and Ramesar R (2009) Neuropsychological status of bipolar I disorder: impact of psychosis. *British Journal of Psychiatry* **194**, 243–251.

Schmand B, Bakker D, Saan R and Louman J (1991) The Dutch Reading Test for Adults: a measure of premorbid intelligence level. *Tijdschrift Voor Gerontologie En Geriatrie* **22**, 15–19.

Schreier A, Wolke D, Thomas K, Horwood J, Hollis C, Gunnell D, Lewis G, Thompson A, Zammit S, Duffy L, Salvi G and Harrison G (2009) Prospective study of peer victimization in childhood and psychotic symptoms in a nonclinical population at age 12 years. *Archives of General Psychiatry* **66**, 527–536.

Schubart CD, Sommer IEC, van Gastel WA, Goetgebuer RL, Kahn RS and Boks MPM (2011) Cannabis with high cannabidiol content is associated with fewer psychotic experiences. *Schizophrenia Research* **130**, 216–221.

Selva G, Salazar J, Balanzá-Martínez V, Martínez-Arán A, Rubio C, Daban C, Sánchez-Moreno J, Vieta E and Tabarés-Seisdedos R (2007) Bipolar I patients with and without a history of psychotic symptoms: do they differ in their cognitive functioning? *Journal of Psychiatric Research* **41**, 265–272.

Simonsen C, Sundet K, Vaskinn A, Birkenaes AB, Engh JA, Færden A, Jónsdóttir H, Ringen PA, Opjordsmoen S, Melle I, Friis S and Andreassen OA (2011) Neurocognitive dysfunction in bipolar and schizophrenia spectrum disorders depends on history of psychosis rather than diagnostic group. *Schizophrenia Bulletin* **37**, 73–83.

Spreen O, Strauss E and Sherman EM (1998) *A Compendium of Neuropsychological Tests. Administration Norms And Commentary.* New York: Oxford University Press 2006, 1216.

Startup M, Jackson MC and Bendix S (2002) The concurrent validity of the Global Assessment of Functioning (GAF). *The British Journal of Clinical Psychology* **41**, 417–422.

Suppes T, Leverich GS, Keck Jr PE, Nolen WA, Altshuler LL, McElroy SL, Rush AJ, Kupka R, Frye MA, Bickel M and Post RM (2001) The Stanley Foundation Bipolar Treatment Outcome Network – II. Demographics and illness characteristics of the first 261 patients. *Journal of Affective Disorders* **67**, 45–59.

Tandon R and Greden JF (1987) Schneiderian first rank symptoms: reconfirmation of high specificity for schizophrenia. *Acta Psychiatrica Scandinavica* **75**, 392–396.

The International Schizophrenia Consortium* (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **10**, 8192.

Thombs BD, Bernstein DP, Lobbestael J and Arnstz A (2009) A validation study of the Dutch Childhood Trauma Questionnaire-Short Form: factor structure, reliability, and known-groups validity. *Child Abuse & Neglect* **33**, 518–523.

Tohen M, Tsuang MT and Goodwin DC (1992) Prediction of outcome in mania by mood-congruent or mood-incongruent psychotic features. *American Journal of Psychiatry* **149**, 1580–1584.

Toni A, Perugi G, Mata B, Madaro D, Maremmani I and Akiskal HS (2001) Is mood-incongruent manic psychosis a distinct subtype? *European Archives of Psychiatry and Clinical Neuroscience* **251**, 12–17.

Upthegrove R, Chard C, Jones L, Gordon-Smith K, Forty L, Jones I and Craddock N (2015) Adverse childhood events and psychosis in bipolar affective disorder. *British Journal of Psychiatry* **206**, 191–197.

Vallejo MA, Jordán CM, Díaz MI, Comeche MI and Ortega J (2007) Psychological assessment via the internet: a reliability and validity study of online (vs paper-and-pencil) versions of the General Health Questionnaire-28 (GHQ-28) and the Symptoms Check-List-90-Revised (SCL-90-R). *Journal of Medical Internet Research* **9**, e2.

van Os J and Reininghaus U (2016) Psychosis as a transdiagnostic and extended phenotype in the general population. *World Psychiatry* **15**, 118–124.

van Os J, Hanssen M, van Bijl R and Ravelli R (2000) Strauss (1969) revisited: a psychosis continuum in the general population*? Schizophrenia Research* **45**, 11–20.

Varese F, Smeets F, Drukker M, Lieverse R, Lataster T, Viechtbauer W, Read J, van Os J and Bentall RP (2012) Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control, prospective-and cross-sectional cohort studies. *Schizophrenia Bulletin* **38**, 661–671.

Vleeschouwer M, Schubart CD, Henquet C, Myin-Germeys I, van Gastel WA, Hillegers MHJ, van Os J, Boks MPM and Derks EM (2014) Does assessment type matter? A measurement invariance analysis of online and paper and pencil assessment of the Community Assessment of Psychic Experiences (CAPE). *PLoS ONE* **9**, e84011.

Vreeker A, Boks MPM, Abramovic L, Verkooijen S, van Bergen AH, Hillegers MHJ, Spijker AT, E. Hoencamp E, Regeer EJ, Riemersma- Van der Lek RF, A. W. M. M. Kupka RW, Brouwer RM, Bearden CE, Stevens AW, Schulte PF, Vonk R, Hoekstra R, van Beveren NJ, MacCabe JH and Ophoff RA and GROUP Investigators* (2016) High educational performance is a distinctive

feature of bipolar disorder: a study on cognition in bipolar disorder, schizophrenia patients, relatives and controls. *Psychological Medicine* **46**, 807–818.

Wechsler D (1997) *WAIS-III Administration and Scoring Manual*, 3rd Edn. San Antonio, TX: Psychological Corporation/Harcourt Brace.

Weissman MM, Bland RC, Canino GJ, Faravelli C, Greenwald S, Hwu H, Joyce PR, Karam EG, Lee C, Lellouch J, Lépine J, Newman SC, Rubio- Stipec M, Welss JE, Wickrmamaratne PJ, Wittchen H and Yeh E (1996) Cross-national epidemiology of major depression and bipolar disorder. *JAMA* **276**, 293–299.

Zammit S, Allebeck P, David AS, Dalman C, Hemmingsson T, Lundberg J and Lewis G (2004) A longitudinal study of premorbid IQ score and risk of developing Schizophrenia, bipolar disorder, severe depression, and other nonaffective psychoses. *Archives of General Psychiatry* **61**, 354–360.

Suplemental material

Supplemental Table S1 : Demographical and clinical characteristics of BD with (BD P+) and without psychotic symptoms (BD P-), unadjusted results

	Statistics
Age Mean (sd)	**Beta=0.17, t=-6.46, p<0.001***
Gender male n (%)	B=0.37, p=0.003, OR=1.45[1.13-1.85]
Marital Status n (%)	B=-0.21, p=0.095, OR=0.81 [0.63-1.04]
Employment status n (%)	$c^2(1)$=0.68, p=0.391
Global functioning Mean (sd)	Beta=-0.03, t=-1.01, p=0.312
Socio economic status mean (sd)	**Beta=0.10, t=3.62, p<0.001***
Mean level of education (sd)	**W $c^2(1)$=12.87, p<0.001, OR=0.67[0.54-0.84]***
Premorbid IQ Mean (sd)	Beta=0.06, t=2.14, p=0.033,
Anxiety disorder (%)	W= 0.05, B=-0.03, p =0.318, OR=0.97[0.74-1.28]
Age at onset Mean (sd)	**Beta=-0.18, t=-6.40, p<0.001***
Nr. of episodes MANCOVA	F(2,1339)=5.11, p=0.007, Partial η^2=0.01
Nr. of depressive episodes Mean (sd)	F(1,1340)=5.56, p=0.023, Partial η^2<0.01
Nr. of manic episodes Mean(sd)	F(1,1340)=0.31, p=0.588, Partial η^2<0.01
Nr. of hospitalizations MANCOVA	**F(2,1339)=22.24, p<0.001, Partial η^2=0.03***
Nr. of hospitalizations for depressive episodes Mean (sd)	F(1,1340)=0.07, p=0.821, Partial η^2<0.01
Nr. of hospitalizations for manic episodes Mean (sd)	**F(1,1340)=40.69, p<0.001, Partial η^2=0.03***
Suïcide attempts (n=991) (%)	B=0.28, p =0.083, OR=1.32[0.96-1.82]
Total IQ Mean (sd) (n=1060)	Beta=0.05, t=1.55, p=0.120
WAIS MANCOVA (n=1060)	F(4,1061)=3.56, p=0.007, Partial η^2=0.01
WAIS – Information Mean (sd)	F(1,1064)=4.46, p=0.035, Partial η^2<0.01
WAIS – Block Design Mean (sd)	F(1,1064)=1.74, p=0.186, Partial η^2<0.01
WAIS – Arithmetic Mean (sd)	F(1,1064)=1.11, p=0.293, Partial η^2<0.01
WAIS – Digit Symbol Mean (sd)	F(1,1064)=2.77, p=0.096, Partial η^2<0.01
Childhood trauma Total score Mean (sd)	Beta=0.04, t= 1.24, p=0.214
Trauma subtypes MANCOVA	F(5,1336)=1.57, p**=0.179, Partial η^2<0.01
Sexual abuse Mean (sd)	F(1,1340)=3.53, p=0.068, Partial η^2<0.01
Physical abuse Mean (sd)	F(1,1340)=0.05, p=0.844, Partial η^2<0.01
Emotional abuse Mean (sd)	F(1,1340)=2.81, p=0.104, Partial η^2<0.01
Physical neglect Mean (sd)	F(1,1340)=0.11, p=0.766, Partial η^2<0.01
Emotional neglect Mean (sd)	F(1,1340)=0,01, p=0.949, Partial η^2<0.01

*Significant between-group difference (p<0.0029)

** Hotelling's trace

Supplemental Table S2A: Association of hallucinations and delusions with demographical and clinical characteristics in BD type I patients, unadjusted results

	Test Statistics Delusions N=925 (68.9%)	Test Statistics Hallucinations N=572.6 (42.7%)
Age	**Beta=-0.18, t=-6.15, p<0.001***	Beta=0.04, t=1.43, p=0.154
Gender	B=-0.02, p=0.882, OR=0.98[0.75-1.27]	**B=0.42, p=0.001, OR=1.53[1.18-1.97]***
Marital Status	B=-0.34, p=0.013, OR=0.71[0.54-0.93]	B=0.07, p=0.614, OR=1.07[0.83-1.38]
Employment status	B=0.38, p=0.005, OR=1.46[1.12-1.91]	B=-0.21, p=0.108, OR=0.81[0.63-1.05]
Global functioning	Beta=0.07, t=2.02, p=0.044	Beta=-0.08, t=-2.34, p=0.020
Socio economic status	**Beta=0.10, t=3.23, p=0.001***	Beta=-0.06, t=-2.03, p=0.042
Mean level of education	**W c^2(1)=22.04, p<0.001, OR=0.58[0.46-0.73]***	W c^2(1)=2.76, p=0.089, OR=1.22[0.97-1.53]
Premorbid IQ	**Beta=0.12, t=3.41, p=0.001***	Beta=-0.04, t=-1.23, p=0.220
Anxiety disorder	B=-0.31, p=0.049, OR=0.74[0.54-0.99]	B=0.17, p =0260, OR=1.18[0.88-1.58]
Age at onset	**Beta=-0.17, t=-5.29, p<0.001***	Beta=-0.13, t=-0.83, p=0.407
Nr. of episodes MANCOVA	**F(2,1335)=9.16, p<0.001, Partial η^2=0.01***	**F(2,1335)=7.07, p=0.001, Partial η^2=0.01***
Nr. of depressive episodes	**F(1,1336)=17.51, p<0.001, Partial η^2<0.01***	F(1,1336)=4.43, p=0.036, Partial η^2<0.01
Nr. of manic episodes	F(1,1336)=7.09, p=0.008, Partial η^2<0.01	**F(1,1336)=13.89, p<0.001, Partial η^2=0.01***
Nr. of hospitalizations MANCOVA	**F(2,1335)=18.33, p<0.001, Partial η^2=0.03***	F(2,1335)=3.84, p=0.027, Partial η^2<0.01
Nr. of hospitalizations for depressive episodes	F(1,1336)=6.60, p=0.013, Partial η^2<0.01	F(1,1336)=7.61, p=0.008, Partial η^2<0.01
Nr. of hospitalizations for manic episodes	**F(1,1336)=22.19, p<0.001, Partial η^2=0.02***	F(1,1336)=0.99, p=0.326, Partial η^2<0.01
Nr. of suïcide attempts (n=991)	B=0.08, p=0.641, OR=1.09[0.77-1.53]	B=0.24, p =0.145, OR=1.27[0.92-1.70]
Total IQ	Beta=0.05, t=1.39, p=0.164	Beta=-0.05, t=-1.39, p=0.165
WAIS MANCOVA	F(4,1017)=2.07, p=0.083, Partial η^2<0.01	F(4,1017)=1.02, p=0.394, Partial η^2<0.01
WAIS – Information	F(1,1020)=4.87, p=0.028, Partial η^2<0.01	F(1,1020)=0.01, p=0.932, Partial η^2<0.01
WAIS – Block Design	F(1,1020)=0.28, p=0.597, Partial η^2<0.01	F(1,1020)=0.16, p=0.443, Partial η^2<0.01
WAIS – Arithmetic	F(1,1020)=0.04, p=0.846, Partial η^2<0.01	F(1,1020)=2.31, p=0.129, Partial η^2<0.01

WAIS – Digit Symbol	F(1,1020)=1.43, p=0.233, Partial η^2<0.01	F(1,1020)=1.75, p=0.186, Partial η^2<0.01
Childhood trauma Total score	Beta=0.04, t=-1.47, p=0.143	**Beta=0.12, t=3.68, p<0.001***
Trauma subtypes MANCOVA	F(5,1332)=1.64, p**=0.170, Partial η^2<0.01	F(5,1332)=3.07, p**=0.011, Partial η^2=0.01
Sexual abuse	F(1,1336)=0.25, p=0.719, Partial η^2<0.01	F(1,1336)=2.31, p=0.181, Partial η^2<0.01
Physical abuse	F(1,1336)=5.09, p=0.027, Partial η^2<0.01	F(1,1336)=5.52, p=0.015, Partial η^2<0.01
Emotional abuse	F(1,1336)=0.50, p=0.507, Partial η^2<0.01	F(1,1336)=8.62, p=0.004, Partial η^2<0.01
Physical neglect	F(1,1336)=0.83, p=0.407, Partial η^2<0.01	F(1,1336)=1.56, p=0.224, Partial η^2<0.01
Emotional neglect	F(1,1336)=3.26, p=0.075, Partial η^2<0.01	F(1,1336)=12.13, p<0.001, Partial η^2<0.01

*Significant between-group difference (p<0.0029)

** Hotelling's trace

Supplemental Table S2B: Association of Mood Incongruent symptoms, Schneiderian symptoms and Disorganized Speech with demographical and clinical characteristics in BD type I patients, unadjusted results

	Test Statistics Mood Incongruent symptoms N=404 (30.1%)	Test Statistics Schneiderian symptoms N= 284 (21.2%)	Test Statistics Disorganized Speech N= 801(59.7%)
Age	Beta=-0.02, t=-0.70, p=0.487	Beta=0.06, t=-2.07, p=0.039	**Beta=-0.13, t=-4.55, p<0.001***
Gender	B=0.22, p=0.100, OR=1.25[0.96-1.63]	B=0.28, p=0.067, OR=1.33[0.98-1.81]	B=-0.02, p=0.861, OR=0.98[0.78-1.23]
Marital Status	B=0.29, p=0.031, OR=1.33[1.03-1.73]	B=-0.20, p=0.189, OR=0.82[0.61-1.10]	B=-0.07, p=0.545, OR=0.93[0.73-1.18]
Employment status	B=-0.16, p=0.217, OR=0.85[0.66-1.10]	B=-0.22, p=0.143, OR=0.80[0.60-1.08]	B=0.13, p=0.283, OR=1.13[0.90-1.42]
Global functioning	Beta=-0.08, t=-0.29, p=0.776	**Beta=-0.11, t=-3.15, p=0.002***	Beta=-0.08, t=-2.59, p=0.010
Socio economic status	Beta=0.03, t=0.82, p=0.413	Beta=0.07, t=2.18, p=0.029	**Beta=0.11, t=3.68, p<0.001***
Mean level of education	W $c^2(1)$=0.54, p=0.416, OR=0.90[0.72-1.15]	W $c^2(1)$=0.20, p=0.749, OR=1.04[0.80-1.36]	W $c^2(1)$=1.01, p=0.390, OR=1.09[0.89-1.33]
Premorbid IQ	Beta=0.02, t=0.59, p=0.558	Beta=-0.05, t=-1.60, p=0.110	Beta=-0.04, t=-1.23, p=0.219
Anxiety disorder	B=0.12, p=0.405, OR=1.13[0.85-1.52]	B=0.43, p =0.009, OR=1.54[1.12-2.12]	B=0.26, p=0.053, OR=1.29[0.99-1.69]
Age at onset	Beta=0.01, t=-0.29, p=0.771	Beta=-0.04, t=-1.40, p=0.163	Beta=-0.07, t=-2.41, p=0.016
Nr. of episodes MANCOVA	F(2,1335)=0.07, p=0.911, Partial η^2<0.01	F(2,1335)=0.49, p=0.618, Partial η^2<0.01	**F(2,1335)=8.57, p<0.001, Partial η^2=0.01***
Nr. of depressive episodes	F(1,1336)=0.19, p=0.731, Partial η^2<0.01	F(1,1336)=0.32, p=0.582, Partial η^2<0.01	F(1,1336)=1.36, p=0.262, Partial η^2<0.01
Nr. of manic episodes	F(1,1336)=0.04, p=0.869, Partial η^2<0.01	F(1,1336)=0.93, p=0.349, Partial η^2<0.01	**F(1,1336)=14.14, p<0.001, Partial η^2=0.01***
Nr. of hospitalizations MANCOVA	F(2,1335)=1.43, p=0.247, Partial η^2<0.01	F(2,1335)=1.57, p=0.221, Partial η^2<0.01	F(2,1335)=0.91, p=0.440, Partial η^2<0.01
Nr. of hospitalizations for depressive episodes	F(1,1336)=2.02, p=0.159, Partial η^2<0.01	F(1,1336)=0.40, p=0.538, Partial η^2<0.01	F(1,1336)=1.60, p=0.312, Partial η^2<0.01
Nr. of hospitalizations for manic episodes	F(1,1336)=0.25, p=0.631, Partial η^2<0.01	F(1,1336)=3.11, p=0.085, Partial η^2<0.01	F(1,1336)=0.10, p=0.942, Partial η^2<0.01
Nr. of suicide attempts	B=-0.09, p=0.588, OR=0.91[0.65-1.27]	B=0.15, p =0145, OR=1.27[0.92-1.76]	B=0.15, p=0.323, OR=1.16[0.86-1.57]
Total IQ (n=1060)	Beta=0.08, t=2.52, p=0.012	Beta=-0.04, t=-1.13, p=0.259	Beta=0.07, t=2.36, p=0.018
WAIS MANCOVA(n=1060)	F(4,1017)=1.88, p=0.111, Partial η^2=0.01	F(4,1017)=2.36, p=0.051, Partial η^2<0.01	F(4,1017)=3.48, p=0.008, Partial η^2=0.01
WAIS – Information	F(1,1020)=2.16, p=0.142, Partial η^2=0.01	F(1,1020)=2.64, p=0.104, Partial η^2<0.01	F(1,1020)=0.61, p=0.434, Partial η^2<0.01
WAIS – Block Design	F(1,1020)=5.48, p=0.019, Partial η^2=0.01	F(1,1020)=0.65, p=0.421, Partial η^2<0.01	F(1,1020)=1.22, p=0.269, Partial η^2=0.01
WAIS – Arithmetic	F(1,1020)=0.55, p=0.460, Partial η^2<0.01	F(1,1020)=3.28, p=0.071, Partial η^2<0.01	F(1,1020)=4.06, p=0.044, Partial η^2<0.01

WAIS – Digit Symbol	F(1,1020)=4.18, p=0.041, Partial η^2<0.01	F(1,1020)=1.96, p=0.162, Partial η^2<0.01	F(1,1020)=12.01, p=0.001, Partial η^2=0.01
Childhood trauma Total score	Beta=-0.02, t=-0.74, p=0.461	Beta=0.05, t=1.45, p=0.148	Beta=0.04, t=1.91, p=0.059
Trauma subtypes MANCOVA	F(5,1332)=3.15, p**=0.010, Partial η^2=0.01	F(5,1332)=1.03, p**=0.411, Partial η^2<0.01	F(5,1332)=4.71, p=0.003, Partial η^2=0.01
Sexual abuse	F(1,1336)=2.19, p=0.113, Partial η^2<0.01	F(1,1336)=2.45, p=0.149, Partial η^2<0.01	F(1,1336)=6.54, p=0.015, Partial η^2<0.01
Physical abuse	F(1,1336)=0.15, p=0.732, Partial η^2<0.01	F(1,1336)=0.23, p=0.638, Partial η^2<0.01	F(1,1336)=8.32, p=0.007, Partial η^2=0.01
Emotional abuse	F(1,1336)=0.16, p=0.388, Partial η^2<0.01	F(1,1336)=1.75, p=0.198, Partial η^2<0.01	F(1,1336)=6.45, p=0.016, Partial η^2=0.01
Physical neglect	F(1,1336)=10.75, p=0.002, Partial η^2=0.01	F(1,1336)=2.49, p=0.121, Partial η^2<0.01	F(1,1336)=3.11, p=0.099, Partial η^2<0.01
Emotional neglect	F(1,1336)=0.1.07, p=0.312, Partial η^2<0.01	F(1,1336)=0.43, p=0.516, Partial η^2<0.01	F(1,1336)=0.19, p=0.789, Partial η^2<0.01

*Significant between-group difference (p<0.0029)

** Hotelling's trace

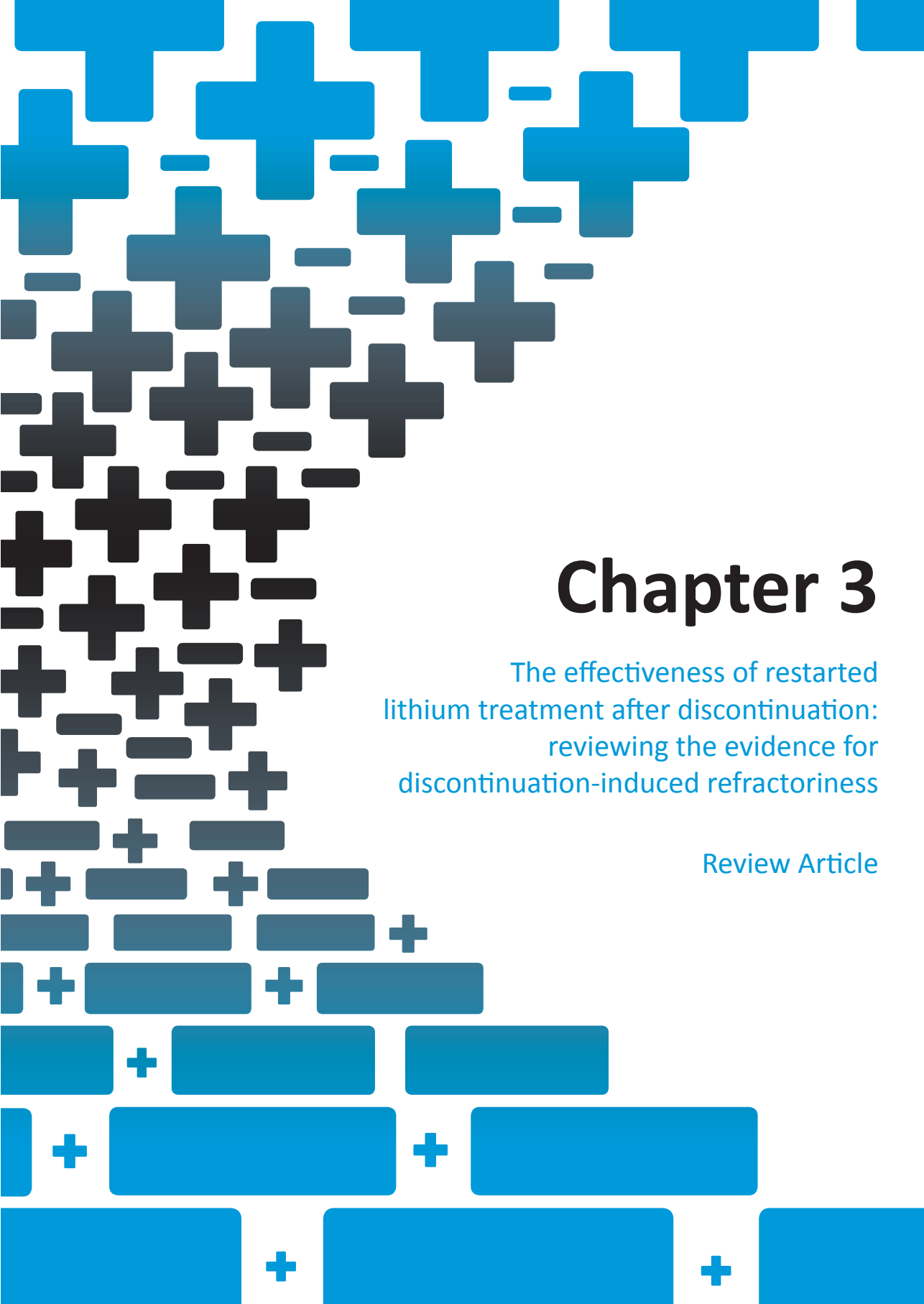
PART II

Pharmacological treatment in bipolar disorder

Annet H. van Bergen*
Christine de Vries*
Eline J. Regeer
Elsje Benthem
Ralph W. Kupka
Marco P.M. Boks

*equal contribution

Bipolar Disorders 2013: 15: 645–649



Chapter 3

The effectiveness of restarted lithium treatment after discontinuation: reviewing the evidence for discontinuation-induced refractoriness

Review Article

Abstract

Objectives:
We sought to determine whether the risk of relapse in patients with bipolar disorder is higher after discontinuation and restart of lithium treatment as compared to continuous lithium treatment in these same patients.

Methods:
We conducted literature searches in the Pubmed, Embase, Cochrane, and PsycINFO databases with cross-reference checks. Relevant data were extracted and pooled for meta-analysis.

Results:
Five relevant studies were included for review, of which three studies qualified for the meta-analysis and included a total of 212 analyzed cases. Two studies found lithium to be less effective after discontinuation and reintroduction and three studies found no decreased effectiveness. The pooled odds ratio for the occurrence of one or more relapses after interruption of lithium treatment compared to continuous treatment was 1.40 (95% confidence interval: 0.85–2.31; p = 0.19).

Conclusions:
Although studies are scarce, review and meta-analysis of the available literature does not provide convincing evidence that lithium is less effective when treatment is discontinued and restarted, compared to uninterrupted treatment.

Key words:
bipolar disorder, discontinuation, effectiveness, lithium, refractoriness, restarting

Introduction

In 1949, the efficacy of lithium salts for the treatment of mania was first described by Cade (Cade 1982). The prophylactic effect of lithium has also been known for decades and lithium is still the preferred treatment for bipolar disorder in treatment guidelines today (Chou 2004). It is considered the best evaluated and most effective prophylactic maintenance treatment (Fountoulakis 2010; Maj 2000; Baldessarini & Tondo 2000). Although many patients continue lithium treatment for years, there are various reasons for lithium discontinuation. Some patients wish to stop taking medication and some forget or refuse their medication (Murray 1994), and pregnancy or side effects such as cognitive impairment, weight gain, dermatologic reactions, and renal or thyroid dysfunction are valid reasons to consider the discontinuation of lithium (Suppes et al. 1993).

However, discontinuation of lithium treatment is associated with a higher risk of relapse, even after many years of clinical stability (Baastrup et al. 1970; Klein et al. 1981; Mander & Loudon 1988; Mander 1986; Faedda et al. 1993). According to Suppes et al. (Suppes et al. 1993), who reviewed the literature on this subject, this risk is not fully accounted for by the natural history of the illness. Furthermore, recurrences increased soon after rapid discontinuation, but were delayed or limited if lithium was slowly tapered (Baldessarini et al. 1997). The nature of this withdrawal syndrome is unclear and the evidence of its existence remains inconclusive due to a lack of studies with appropriate methodology (Schou 1993).

A second problem can occur when lithium is reinstituted after a relapse. This phenomenon was first described by Garver et al. (1984) in 1984 in schizophrenia patients treated with lithium. Although the majority of patients will respond to renewed treatment with lithium (Baastrup & Mogens 1967; Coryell et al. 1998; Grof & Müller-Oerlinghausen 2009), there are several reports of patients who fail to respond once lithium is restarted. In 1992, Post et al. (1992) described four patients who were successfully treated with lithium for 6–15 years but developed refractoriness to lithium after discontinuing and restarting treatment. Bauer (1994) also reported a patient who discontinued lithium after 12 years of successful treatment. Six months later the patient relapsed and treatment was restarted, but the patient remained unresponsive to lithium, carbamazepine, and a combination of these medications. More case reports on *lithium-discontinuation-induced refractoriness* (Post et al. 1992) followed, including three cases described by Oostervink et al. (2000) and one by Appleby et al. (2006).

These reports of decreased effectiveness of lithium treatment after interruption may have serious clinical implications, since they suggest that discontinuation may have long-term effects on the subsequent course of illness. Recently, Post (2012) discussed several explanations for this phenomenon, one of which was that the occurrence of a new episode in the absence of lithium may be more damaging to the brain than previous episodes, and thus cause refractoriness. Other hypotheses are that lithium may be neuroprotective or that the mere occurrence of a new episode may change the course of illness.

However, several recent studies that examined discontinuation-induced refractoriness presented contradictory results. The aim of this study was therefore to investigate the effectiveness of restarted lithium treatment in patients with bipolar disorder compared to previous continuous treatment in these same patients. A literature search was conducted to collect the best available evidence for review and meta-analysis.

With regard to long-term lithium treatment, four distinct periods can be distinguished. The first period is the *pre-lithium phase*, in which patients suffer from episodes, but lithium treatment has not yet been initiated. The second period, *initial lithium treatment*, begins when lithium is started, and ends with its discontinuation. The third period, the *discontinuation period*, lasts until lithium is restarted. Finally, the fourth period, the *reintroduction period*, begins after restarting lithium treatment (see Supplementary Fig. 1). Discontinuation-induced refractoriness is the phenomenon that lithium is less effective in the reintroduction period compared to the second period when lithium was first introduced.

Methods

Search strategy and selection

The PubMed, Embase, Cochrane library, and PsycINFO databases were searched in order to identify relevant published articles in scientific journals. The search terms were *bipolar, mania, manic, manic-depressive, manic-depression, and lithium, along with restarted, restart, restarting, interrupted, interrupting, interrupt, resumed, resume, resuming, resumption, discontinuation, discontinuing, discontinue, discontinued, discontinuous, discontinuation-induced, continuous, continue, continued, continuously, continuing, temporary, temporarily, on and off, reinstituted, reinstituting, reinstitute, reinstitution, or lithium-discontinuation-induced*. The search field was set at title and/or abstract. The titles and abstracts of the papers were screened and inclusion criteria included: (i) relevance in terms of the domain and determinant of the research question and (ii) human studies including subjects >18 years of age. Case reports or case series, opinion papers, and reviews were excluded. Subsequently, the full text of the remaining articles was screened, using the same inclusion and exclusion criteria. In addition, the references of relevant papers were screened for informative publications and authors were contacted if potentially useful data had been recorded but not published. Screening was performed by two authors. Supplementary Figure 2 shows the process of selection of relevant papers. A baseline summary of the selected studies is given in Table 1.

Table 1: Study characteristics

Study	N	Design	Duration second period	Duration fourth period	In favor of hypothesis
Baldessarini *(1999)*	130	Prospective cohort	Mean 4.2 SD=3.9 yrs	Mean 4.0 yrs SD=3.7	no
Coryell *(1998)*	28	Prospective cohort		≥ 6 mo	no
Koukopoulos *(1995)*	89	Prospective cohort	Mean 12.2 range: 4-24.6 yrs	Mean 13.5 yrs	yes
Maj *(1995)*	54	Prospective cohort	Mean 5.9 SD=3.7 yrs	≤ 1 yr	yes
Tondo *(1997)*	86	Prospective cohort	Mean 4.6 SD=3.7 yrs	Mean 4.5 yrs	no

SD = standard deviation

Meta-analysis

The second (initial treatment) and fourth (reintroduction) treatment periods were compared, using all relevant data regarding the occurrence of one or more relapses in these periods, as extracted from the selected papers. These data were pooled using Comprehensive Meta-Analysis© version 2 (Biostat, Englewood, NJ, USA). Odds ratios were calculated, including 95% confidence intervals, and the level of significance was set at $p < 0.05$.

Results

After the initial search on 15 December 2011, 1,703 articles were retrieved. After screening, five papers qualified for review (Table 1) (Coryell et al. 1998; Baldessarini et al. 1999; Koukopoulos et al. 1995; Maj et al. 1995; Tondo et al. 1997). A reference cross-check was performed and no further relevant papers were identified.

Results per study

Tondo et al. (1997) evaluated 86 patients with bipolar I or II disorder, diagnosed according to the DSM-IV criteria, who discontinued and restarted lithium treatment. In a review focusing on the risks and implications of discontinuation of lithium treatment, Baldessarini et al. (1999) presented reanalyzed and updated data from this study. A total of 130 patients were included, unselected for the response to lithium in the initial treatment period (the second period). Morbidity was rated according to the number of episodes of mania or depression per year, the number of hospitalizations per year, and the percentage of time ill in affective episodes. In the initial lithium treatment period, lasting on average 4.6 years, an average of 0.90 events per year were recorded [standard deviation (SD) = 1.17], compared to an average of 2.25 (SD = 2.91) in the first (pre-lithium) period. The duration of the reintroduction

period averaged 4.1 years, in which 0.94 episodes per year (SD = 1.25) were recorded. The average time in an episode was 4.5% greater in the reintroduction period compared to the initial lithium treatment period (23.1 versus 18.6%, respectively; p = 0.089); the time with depression increased significantly (by 4.5%; p = 0.024). In the reintroduction period, fewer participants experienced no relapses (28.5 versus 20.0% in the second period). The likelihood of receiving short-term (<3 months) supplemental antipsychotic or antidepressant drugs was similar in the two periods (51.5 versus 42.9%, respectively; χ^2= 1.70, not significant). Finally, neither the severity of pre-lithium morbidity nor the rapidity of discontinuing lithium showed significant relationships to morbidity. Coryell et al. (1998) reported 28 patients with bipolar disorder who recovered from an episode after initial lithium treatment, relapsed after discontinuation of lithium (third period), and subsequently restarted their medication (fourth period). To assess the effectiveness of lithium, Kaplan–Meier survival curves were constructed, which began at the ninth week after the reintroduction of lithium, i.e., the first week after the eight weeks defined as the recovery period. The endpoint was recurrence of another affective episode. After reintroduction, 27 subjects recovered while taking lithium. The recurrence rate after two years in the second period was 45.0%; after reintroduction it was 32.9%. Furthermore, the pre- and post-discontinuation episodes did not differ in the likelihood that additional medications were used, nor did the mean values for available lithium levels differ significantly. Koukopoulos et al. (Koukopoulos et al. 1995) described 375 patients with bipolar I or II disorder, or unipolar depression, who were treated with prophylactic treatments for at least five years. Of these patients, 110 discontinued lithium once or more because they felt well, or because of pregnancy or minor side effects; 89 relapsed and lithium was restarted. Subsequently, 13 patients showed refractoriness to lithium, ranging from two to seven years (mean 4.2 years). In eight cases, the refractoriness continued after seven years. Furthermore, it was not limited to lithium treatment only in some patients, but included all anti-manic and anti-depressive treatments, including electroconvulsive therapy. Additional information about the effect of lithium treatment could not be retrieved from this article. Maj et al. (1995) reported 54 patients with bipolar I disorder. All showed a complete response to lithium during initial treatment, defined as the absence of manic or major depressive episodes during at least two years of treatment. All patients temporarily discontinued lithium treatment, for reasons other than recurrence of the illness or the occurrence of serious side effects. Lithium prophylaxis was reintroduced after one or more affective episodes following discontinuation. During the initial treatment period, all 54 patients were complete responders to lithium and experienced no relapses. After reintroduction, 10 of the 54 patients experienced at least one relapse. The only significant baseline difference compared to the 44 others was the duration of lithium treatment before discontinuation (mean 8.4 years, SD = 4.9 versus mean 5.4 years, SD = 3.1, respectively; p < 0.05).

Meta-analysis

The participants in the Tondo et al. study (Tondo et al. (1997) are included in the population of the Baldessarini et al. study (Baldessarini et al. (1999), and therefore only the latter was included for meta-analysis. Koukopoulos et al. (1995) did not publish the rate of relapse in the second and fourth periods; therefore, this study did not qualify for meta-analysis. Because of the different study methods, only the crude measure of one or more relapses could be used for meta-analysis. In the study of Baldessarini et al. (1999), 93 subjects (71.5%) experienced one or more relapses during the initial treatment, versus 104 (80.0%) after reintroduction. In the study of Coryell et al. (1998), 13 subjects (45.0%) experienced one or more relapses during initial treatment and nine subjects (32.9%) experienced one or more relapses after reintroduction. Maj et al. (1995) found that, of the 54 patients who experienced no relapses during initial treatment, 10 (18.5%) experienced at least one relapse after reintroduction. The results of the meta-analysis are shown in Table 2 and Figure 1. The pooled fixed odds ratio for the occurrence of one or more relapses after interruption of lithium treatment was 1.40 (95% confidence interval: 0.85–2.31; p = 0.19).

Table 2: Meta-analysis; results for each study

Study	Statistics for each study			
	Odds ratio [95% CI]	p-value	Second period*	Fourth period*
Baldessarini (*1999)*	1,591 [0.896-2.286]	0,113	93/130	104/130
Coryell (*1998)*	0,557 [0.194-1.719]	0,323	13/28	9/27
Maj (*1995)*	25,719 [1.466-451.138]	0,026	0/54	10/54
Pooled	1,400 [0.849-2.309]	0,188	-	-

CI = confidence interval

*Amount of persons with the occurrence of one or more relapses in the second (initial) and fourth (reintroduction) treatment periods.

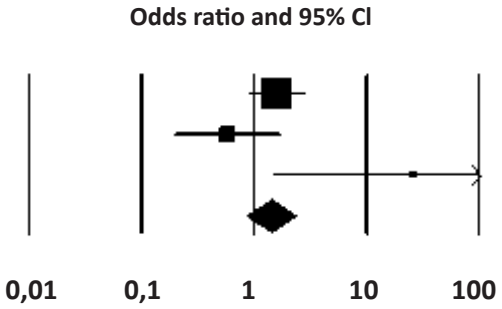


Figure 1: Meta-analysis of the pooled results.

CI = confidence interval

Discussion

The literature on the effectiveness of lithium after discontinuation and reintroduction of maintenance treatment presents conflicting results. In several case reports, a total of 23 cases of lithium-discontinuation- induced refractoriness were described. However, in studies that presented methodologically superior data, a significantly increased risk of relapse after interruption of lithium treatment was found only in one of three studies. After pooling of the data for these studies, which included a total of 212 cases, the risk of relapse was found to be not significantly increased after lithium was restarted.

Two studies (Coryell et al. 1998; Baldessarini et al. 1999) included subjects who were unselected for their initial response to lithium treatment, while one study (Maj et al. 1995) was stricter and only selected patients who experienced no relapses at all in their first period of lithium treatment. This selection towards lithium responders increases the likelihood of finding reduced efficacy after discontinuation; due to the extreme first outcome, regression to the mean is likely to occur on the second measurement. Therefore, there is reason to assume that the effects of discontinuation are smaller in an unselected group of bipolar disorder patients. Moreover, publication bias is likely to play a role in the case reports and positive studies on discontinuation- induced refractoriness.

Our review is limited by the paucity of studies on this subject and the crude measure used to establish discontinuation, in which the lengths of the treatment and discontinuation periods are not taken into account. The information on concomitant medications and the blood levels of lithium were not available in all the selected studies. Another limitation of the review is the heterogeneity of the included studies, although all studies were conducted in the western world: two of three cohorts were from Europe and one from the USA. It is therefore difficult to draw firm conclusions. Nevertheless, review and meta-analysis of the

available literature do not provide convincing evidence that lithium is less effective when treatment is discontinued and subsequently restarted, compared to continuous treatment. Our findings do not rule out the possibility of the existence of discontinuation refractoriness in selected subgroups and it may be of interest to investigate the characteristics of these subgroups. Larger prospective studies are needed in order to correctly inform patients who consider discontinuing lithium treatment and to make evidence-based recommendations about the duration of lithium prophylaxis.

References

Appleby B, Wise T, Isaac A (2006). A case of refractoriness to lithium therapy following its discontinuation in a previously responsive patient. *Harvard Review of Psychiatry* **14**, 330–332.

Baastrup PC, Mogens S (1967). Lithium As a Prophylactic Agent: Its Effect Against Recurrent Depressions and Manic-Depressive Psychosis. *Archives of General Psychiatry* **16**, 162–172.

Baastrup PC, Poulsen JC, Schou M, Thomsen K, Amdisen A (1970). Prophylactic lithium: double blind discontinuation in manic-depressive and recurrent-depressive disorders. *The Lancet* **296**, 326–330.

Baldessarini RJ, Tondo L (2000). Does lithium treatment still work?: Evidence of stable responses over three decades. *Archives of General Psychiatry* **57**, 187–190.

Baldessarini RJ, Tondo L, Floris G, Rudas N (1997). Reduced morbidity after gradual discontinuation of lithium treatment for bipolar I and II disorders: A replication study. *American Journal of Psychiatry* **154**, 551–553.

Baldessarini RJ, Tondo L, Viguera AC (1999). Discontinuing lithium maintenance treatment in bipolar disorders: Risks and implications. *Bipolar Disorders* **1**, 17–24.

Bauer M (1994). Refractoriness induced by lithium discontinuation despite adequate serum lithium levels. *American Journal of Psychiatry* **151**, 1522.

Cade JF (1982). Lithium salts in the treatment of psychotic excitement. *Australasian Psychiatry* **16**, 129–133.

Chou JC-Y (2004). Review and update of the American Psychiatric Association Practice Guideline for Bipolar Disorder. *Primary Psychiatry* **11**, 73–84.

Coryell W, Solomon D, Leon AC, Akiskal HS, Keller MB, Scheftner WA, Mueller T (1998). Lithium discontinuation and subsequent effectiveness. *American Journal of Psychiatry* **155**, 895–898.

Faedda GL, Tondo L, Baldessarini RJ, Suppes T, Tohen M (1993). Outcome After Rapid vs Gradual Discontinuation of Lithium Treatment in Bipolar Disorders. *Archives of General Psychiatry* **50**, 448–455.

Fountoulakis KN (2010). An update of evidence-based treatment of bipolar depression: Where do we stand? *Current Opinion in Psychiatry* **23**, 19–24.

Garver DL, Hirschowitz J, Fleishmann R, Djuric PE (1984). Lithium response and psychoses: A double-blind, placebo-controlled study. *Psychiatry Research* **12**, 57–68.

Grof P, Müller-Oerlinghausen B (2009). A critical appraisal of lithium's efficacy and effectiveness: The last 60 years. *Bipolar Disorders* **11**, 10–19.

Klein HE, Broucek B, Greil W (1981). Lithium withdrawal triggers psychotic states. *British Journal of Psychiatry* **139**, 255–256.

Koukopoulos A, Reginaldi D, Minnai G, Serra G, Pani L, Johnson FN (1995). The long-term prophylaxis of affective disorders. *Advances in Biochemical Psychopharmacology* **49**, 127-147.

Maj M (2000). The impact of lithium prophylaxis on the course of bipolar disorder: a review of the research evidence. *Bipolar disorders* **2**, 93–101.

Maj M, Pirozzi R, Magliano L (1995). Nonresponse to reinstituted lithium prophylaxis in previously responsive bipolar patients: Prevalence and predictors. *American Journal of Psychiatry* **152**, 1810–1811.

Mander AJ (1986). Is there a lithium withdrawal syndrome? *British Journal of Psychiatry* **149**, 498–501.

Mander AJ, Loudon JB (1988). Rapid recurrence of mania following abrupt discontinuation of lithium. *The Lancet* **332**, 15–17.

Murray J (1994). Lithium maintenance therapy for bipolar I patients: possible refractoriness to reinstitution after discontinuation. *Psychological Reports* **74**, 355–361.

Oostervink F, Nolen WA, Hoenderboom AC, Kupka RW (2000). Risk of inducing resistance upon stopping and restarting lithium after long-term usage. *Ned.Tijdschr.Geneeskd.* **144**, 401–404.

Post RM (2012). *Acquired lithium resistance revisited: Discontinuation-induced refractoriness versus tolerance*. *Journal of Affective Disorders* **140**, 6–13.

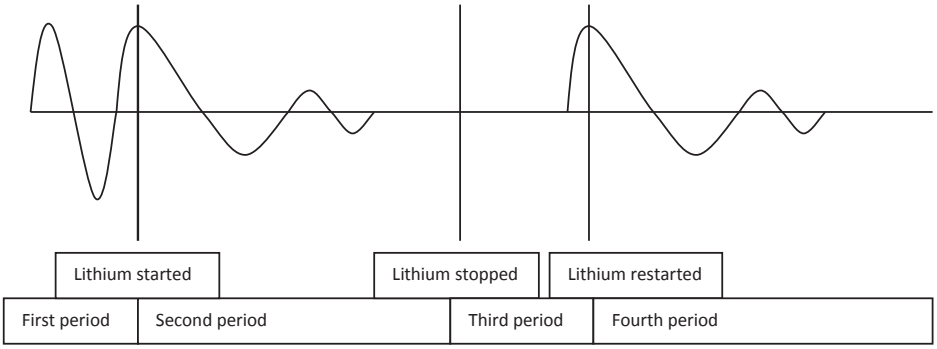
Post RM, Leverich GS, Altshuler L, Mikalauskas K (1992). Lithium-discontinuation-induced refractoriness: Preliminary observations. *American Journal of Psychiatry* **149**, 1727–1729.

Schou M (1993). Is there a lithium withdrawal syndrome? An examination of the evidence. *British Journal of Psychiatry* **163**, 514–518.

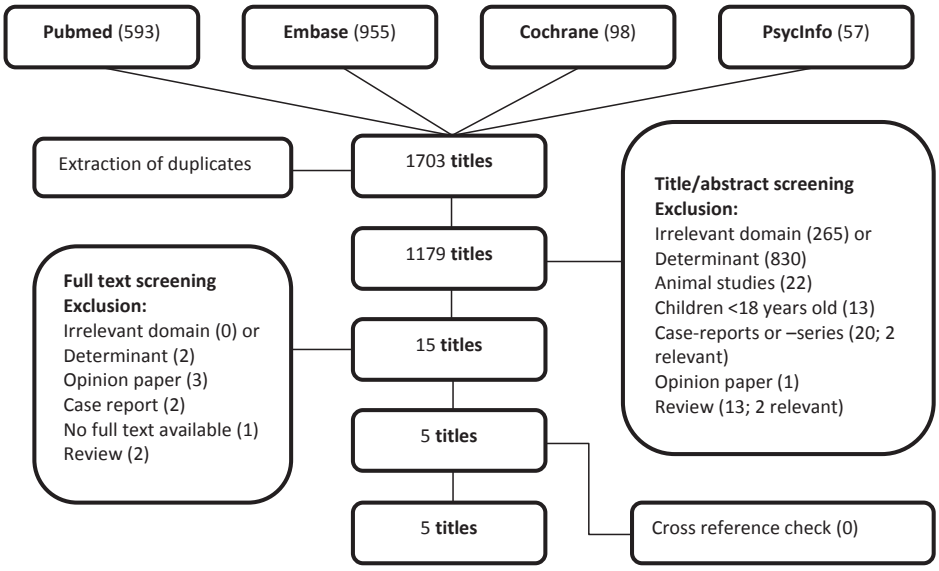
Suppes T, Baldessarini RJ, Faedda GL, Tondo L, Tohen M (1993). Discontinuation of maintenance treatment in bipolar disorder: Risks and implications. *Harvard Review of Psychiatry* **1**, 131–144.

Tondo L, Baldessarini RJ, Floris G, Rudas N (1997). Effectiveness of restarting lithium treatment after its discontinuation in bipolar I and bipolar II disorders. *American Journal of Psychiatry* **154**, 548–550.

Supplemental figures



Supplemental figure 1: the four treatment periods

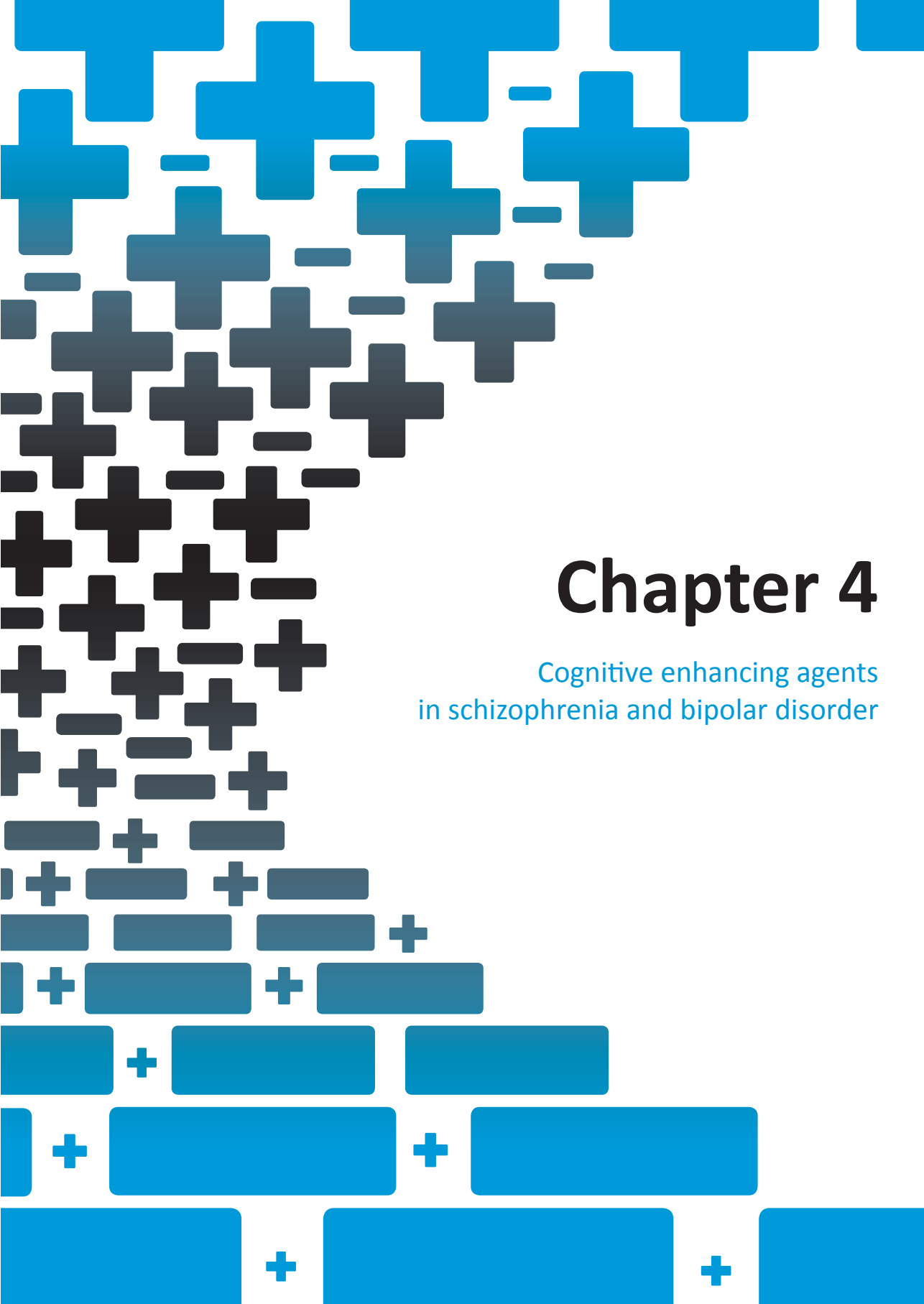


Supplemental figure 2: flow chart search and selection

Annet H. Van Bergen*
Annabel Vreeker*
René S. Kahn

*equal contribution

European Neuropsychopharmacology 2015; 25: 969-1002



Chapter 4

Cognitive enhancing agents in schizophrenia and bipolar disorder

Abstract

Cognitive dysfunction is a core feature of schizophrenia and is also present in bipolar disorder (BD). Whereas decreased intelligence precedes the onset of psychosis in schizophrenia and remains relatively stable thereafter; high intelligence is a risk factor for bipolar illness but cognitive function decreases after onset of symptoms. While in schizophrenia, many studies have been conducted on the development of cognitive enhancing agents; in BD such studies are almost non-existent. This review focuses on the pharmacological agents with putative effects on cognition in both schizophrenia and bipolar illness; specifically agents targeting the dopaminergic, cholinergic and glutamatergic neurotransmitter pathways in schizophrenia and the cognitive effects of lithium, anticonvulsants and antipsychotics in BD. In the final analysis we conclude that cognitive enhancing agents have not yet been produced convincingly for schizophrenia and have hardly been studied in BD. Importantly, studies should focus on other phases of the illness. To be able to treat cognitive deficits effectively in schizophrenia, patients in the very early stages of the illness, or even before – in the ultra-high risk stages – should be targeted. In contrast, cognitive deficits occur later in BD, and therefore drugs should be tested in BD after the onset of illness. Hopefully, we will then find effective drugs for the incapacitating effects of cognitive deficits in these patients.

Key words:
schizophrenia, bipolar disorder, cognition, cognitive impairment, pharmacology, drug development

Introduction

Cognitive dysfunction is a core feature of schizophrenia; in fact schizophrenia may arguably be considered a cognitive disorder (Kahn and Keefe, 2013). Indeed, cognitive decline precedes the onset of psychosis by almost a decade (Maccabe et al., 2008; Elvevag and Goldberg, 2000; van Oel et al., 2002; Reichenberg et al., 2010); after the onset of psychosis cognitive deficits remain present and may even progress further (Hedman et al., 2013). Cognitive dysfunction has a clear detrimental influence on socio-vocational outcome in schizophrenia patients (Green, 1996; Green et al., 2000), making cognitive enhancement an important target for treatment.

Bipolar disorder (BD), classified as mood disorder, has several clinical characteristics with schizophrenia in common and both disorders partly share a genetic background (International Schizophrenia Consortium, 2009; Owen and Craddock, 2009). However, in stark contrast with schizophrenia, premorbid BD patients demonstrate normal or even higher premorbid cognitive functioning compared to controls (Zammit et al., 2004; Gale et al., 2013; MacCabe et al., 2010). Yet, cognitive dysfunction does occur after the onset of illness in many BD patients (Trotta et al., 2014). Accumulating evidence suggests that cognitive dysfunction is also found in euthymic BD patients (Martinez-Aran et al., 2004b; McIntosh et al., 2005; Toulopoulou et al., 2006). The reason for the apparent cognitive decline in BD remains elusive.

An extensive number of agents have been examined in schizophrenia patients targeting several neurotransmitter pathways associated with cognitive function. To date, cognitive enhancing agents have hardly been studied in BD; research focuses on the indefinite cognitive effects of mood-stabilizing agents in these patients.

In this review, we will focus on two topics:

1. The cognitive effects of pharmacological agents targeting the dopaminergic, cholinergic and glutamatergic neurotransmitter pathways with a concise overview of the cognitive effects of other agents in schizophrenia.
2. The effects of lithium, anticonvulsants and antipsychotics on cognitive function in BD.

In the context of this review the term cognitive function means any measure of cognitive performance such as memory, attention, acquisition of knowledge, processing speed, reasoning and executive function (Kahn and Keefe, 2013).

Schizophrenia

Antipsychotics

Most of the currently used antipsychotic agents are antagonists of the dopamine D2 receptor. The cognitive effects of antipsychotic drugs, both first generation and second

generation, have been unclear (Mishara and Goldberg, 2004; Woodward et al., 2005; Carpenter and Gold, 2002) since findings have been mostly based on small samples. Two more recent and larger studies suggest that antipsychotics do not have a material effect on cognitive dysfunction in schizophrenia, assessed by extensive cognitive test batteries (Keefe et al., 2007; Davidson et al., 2009). The first trial (CATIE) included 817 chronic schizophrenia patients randomly assigned to treatment by pherphenazine, olanzapine, quetiapine, ziprasidone and risperidone. The cognitive enhancing effect was modest in all five treatment groups (*z*-score range: 0.12–0.26), with no significant difference between the groups (Keefe et al., 2007). Subsequently, the EUFEST trial investigated the cognitive effects of haloperidol, zisprasidone, quetiapine, amisulpride and olanzapine in 286 first-episode schizophrenia or schizophreniform disorder patients. At the 6 month follow-up period cognitive test scores improved with an effect size ranging from 0.33 to 0.56 in all five treatment groups, with no significant difference between the groups (Davidson et al., 2009). Interestingly, although in both studies cognitive improvement was related to reduction in (psychotic) symptoms, this explained less than 4% of the variance in cognitive change in each of the studies (Davidson et al., 2009; Keefe et al., 2007). Thus, although dopamine antagonists enhance cognitive function in both first-episode and chronic schizophrenia patients the effect size is limited and much smaller than their antipsychotic effect (Leucht et al., 2013). Moreover, the often claimed superiority of second generation over first generation antipsychotics (Woodward et al., 2005; Mishara and Goldberg, 2004) does not hold up in larger trials (Davidson et al., 2009; Keefe et al., 2007). Table 1 demonstrates detailed information on the reported trials.

Dopamine agonists

The revised dopamine hypothesis suggests that decreased dopamine D(1) activity in the prefrontal cortex – clinically expressed as negative symptoms and cognitive dysfunction – leads to increased activity of dopamine at D2 receptors in the mesolimbic system – clinically expressed as psychosis (Davis et al., 1991). Indeed, decreased D1 receptor signaling in the prefrontal cortex has been linked to cognitive deficits in schizophrenia (Goldman-Rakic et al., 2004). Thus, it would make sense to enhance D1 function in schizophrenia patients. Surprisingly only two D1 receptor agonists have been studied in schizophrenia patients, SKF-38939 and dihydrexidine. Both agents were tested in randomized double-blind placebo-controlled trials, but showed no beneficial cognitive effect in schizophrenia patients (respectively N=10, N=20) (see Table 1) (Davidson et al., 1990; George et al., 2007). Interestingly, in a randomized double-blind placebo-controlled study in 16 patients with a schizotypal personality disorder dihydrexidine was reported to improve verbal, but not visual working memory (Rosell et al., 2014).

Another way to increase the release of dopamine is by the administration of dopamine agonists (psychostimulants). Two randomized double-blind placebo-controlled add-on trials investigated the cognitive effects of D-amphetamine in chronic schizophrenia or schizoaffective patients using an extensive cognitive test battery (see Table 1). Significant

improvements were found in speed of processing (Pietrzak et al., 2010), spatial working memory, language production, executive function, visual attention and vigilance (Barch and Carter, 2005; Pietrzak et al., 2010).

In conclusion, antipsychotics, the most widely studied drugs in schizophrenia but not developed to enhance cognition, have a small, generally positive, effect on cognitive function in schizophrenia. Dopamine agonists, hypothesized to increase prefrontal dopamine function and through that cognitive dysfunction, have only been tested in a few small studies and effects are unclear at this stage. Theoretically at least, there still is a good case to be made for the development of these agents in the treatment of cognitive deficits in schizophrenia.

Glutamatergic drugs

The finding that the anesthetic Phencyclidine (PCP) mimics schizophrenia by causing positive as well as negative symptoms and cognitive deficits in healthy individuals, indicates the relevance of the N-methyl-D-aspartate (NMDA) glutamate system in schizophrenia (Javitt and Zukin, 1991; Javitt, 2007). More specifically, data from animal and human studies suggest that NMDA receptor hypofunction may underlie the negative symptoms and cognitive deficits of schizophrenia (Kahn and Sommer, 2014; Anticevic et al., 2012; Javitt, 1999).

The NMDA receptor requires simultaneous co-activation of two ligands; glutamate and either glycine or D-serine. The glycine site agonists that have been investigated in schizophrenia patients are glycine, D-serine, D-alanine and D-cycloserine (Lane et al., 2005; Lane et al., 2010; Heresco-Levy et al., 2004; Tsai et al., 2004; Tsai et al., 2006; Heresco-Levy et al., 2005; Tuominen et al., 2005). The first pilot studies on the effects of glycine were promising, as the agent was associated with beneficial effects on both negative symptoms and cognitive impairments (Heresco-Levy et al., 1996; Heresco-Levy et al., 1999; Javitt et al., 1994). However, a major limitation of all these studies is the cognitive assessment through the Positive and Negative Symptom Scale (PANSS), which is an observational rating scale that in fact does not measure cognitive function. The CONSIST study was the first and the largest randomized double-blind, placebo-controlled add-on trial that used a standardized cognitive test battery to investigate the cognitive effects of glycine site agonists in 157 chronic schizophrenia patients; no positive cognitive effects for glycine and D-cycloserine were found (see Table 1) (Buchanan et al., 2007). In addition, no cognitive effects were shown in two double-blind placebo-controlled add-on trials of D-cycloserine obtained by standardized cognitive test batteries in chronic schizophrenia patients (see Table 1) (Goff et al., 1999; Goff et al.,2005). For D-serine, contrasting findings have been reported. Whereas a large randomized placebo-controlled add-on trial in 195 chronic schizophrenia or schizoaffective disorder patients showed no cognitive enhancing effects (Weiser et al., 2012), a smaller open-label three dose-level add-on study in 42 chronic schizophrenia or schizoaffective patients found a beneficial effect on composite cognitive score for the glycine site agonist, but only at high dose (Kantrowitz et al., 2010). In both D-serine studies

(Weiser et al., 2012; Kantrowitz et al., 2010) cognitive function was assessed by a cognitive test battery as specified by the National Institute of Mental Health on Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS). The test battery assesses seven cognitive domains consisting of attention/vigilance, reasoning and problem solving, speed of processing, social cognition, verbal learning and memory, visual learning and memory, and working memory (Buchanan et al., 2005).

Another way to stimulate the NMDA receptor is to increase availability of glycine by a glycine reuptake inhibitor, which inhibits the glycine transporter-1 (Gly-1). The current Gly-1 inhibitors are sarcosine and bitopterin. The cognitive effect of sarcosine has only been studied in clinical trials using PANNS as cognitive measure and therefore difficult to interpret (Tsai et al., 2004; Lane et al., 2010). In a randomized placebo-controlled add-on trial of bitopterin in 231 chronic schizophrenia patients, bitopterin did not enhance cognitive function measured by a computerized test battery (see Table 1) (Umbricht et al., 2014a).

Memantine, an uncompetitive antagonist of the NMDA receptor, has been registered for cognitive enhancement in Alzheimer's disease. Three add-on trials, the largest including 138 chronic schizophrenia patients (Lieberman et al., 2009), reported no beneficial cognitive effect for memantine in chronic schizophrenia patients (see Table 1) (Lieberman et al., 2009; Lee et al., 2012; Krivoy et al., 2008).

A novel approach to enhance the NMDA receptor function is by administration of a D-amino acid oxidase (DAAO) inhibitor. DAAO is an enzyme in the central nervous system and is responsible for degrading D-serine, the endogenous co-agonist of the NMDA receptor. It is suggested that expression and activity of the enzyme DAAO is increased in schizophrenia patients (Madeira et al., 2008; Verall et al., 2010; Boks et al., 2007). Through inhibiting DAAO activity D-serine levels increase, which may be effective in enhancing the NMDA receptor function. Recently, the first randomized double-blind placebo-controlled add-on trial on the cognitive effect of a DAAO inhibitor, sodium benzoate, in 52 chronic schizophrenia patients, reported significant improvement on the composite cognitive score of the MATRICS test battery (Lane et al., 2013).

Two other relevant glutamatergic agents are ampakine and lamotrigine. Ampakine is an AMPA receptor modulator that enhances NMDA channel opening. The only and relatively large (n=105) randomized placebo-controlled add-on trial on the cognitive effect of ampakine in chronic schizophrenia patients reported no cognitive improvement after eight weeks measured by a standardized cognitive test battery (see Table 1) (Goff et al., 2008). Lamotrigine, an anticonvulsant, is a frequently used mood stabilizer that inhibits glutamate release through different mechanisms (Anand et al., 2000; Large et al., 2005). Two randomized double-blind placebo-controlled add-on trials in schizophrenia patients (study 1 N=217; study 2 N=212) reported no beneficial effects for lamotrigine on executive functions, verbal fluency, attention, verbal memory, working memory, and motor speed measured by the Brief Assessment of Cognition in Schizophrenia (Goff et al., 2007).

In conclusion, the cognitive effects of glutamatergic agents are equivocal and all conducted trials were limited by targeting chronic schizophrenia patients only. Since cognitive decline in schizophrenia precedes the first psychotic episode, patients in the very early stages of the illness should be targeted in future research.

Cholinergic drugs

The cholinergic system is an important target of research on cognitive enhancing drugs due to its role in attention, memory and processing speed (Furey et al., 2000; Wallace and Bertrand, 2013). Acetylcholine, a neurotransmitter of the cholinergic system, exerts its effect on two receptor classes: the nicotinic and muscarinic receptor sites. It is suggested that the pathophysiology in schizophrenia results from impaired expression and function of the nicotinic and muscarinic receptors (Breese et al., 2000; Crook et al., 2001).

Part of the research on cognitive enhancing cholinergic agents focuses on the alpha 4 and 7 subtypes of the nicotinic acetylcholine receptors (nAChRs). Post-mortem brain studies in schizophrenia showed decreased alpha 7 receptor expression in the inhibitory interneurons of the hippocampus (Freedman et al., 1995). In addition, nicotine is heavily abused in schizophrenia patients; approximately 40-80% of the patients smokes (Kuman and Postma, 2005) and they extract more nicotine than other smokers (Olincy et al., 1997). In healthy individuals nicotine has been reported to improve attention, learning and memory (Ernst et al., 2001; Lawrence et al., 2002; Levin et al., 2006). This effect is limited by tachyphylaxis (Harris et al., 2004) and therefore treatment effects of nicotinic agents in schizophrenia patients are influenced by smoking. A recent review by D'Souza and Markou (2012) suggests that placebo-controlled nicotine administration in chronic schizophrenia patients via nasal spray, gum or transdermal patch is associated with improvements in working memory (Sacco et al., 2005; Smith et al., 2006), attention and novelty detection (Barr et al., 2008; Harris et al., 2004; Jubelt et al., 2008; Sacco et al., 2005; Smith et al., 2006) (see Table 1). Both patients using tobacco (Sacco et al., 2005; Smith et al., 2006; Harris et al., 2004) and non-tobacco using patients (Barr et al., 2008; Harris et al., 2004; Jubelt et al., 2008) were studied; nevertheless all trials reported improvement in cognitive function of nicotine versus placebo. Another nicotinic agent is DMXB-A, which is an alpha 4 and 7 partial agonist. Two randomized double-blind placebo-controlled cross-over add-on trials were conducted to the cognitive effect of DMXB-A in chronic schizophrenia patients. The first trial (N=12) reported a significant improvement on composite score of the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), which assesses immediate and delayed memory, attention, language, and visuospatial skills (Olincy et al., 2006). However, the second trial (N=31) reported no beneficial cognitive effect of DMXB-A, this time assessed by the MATRICS test battery (see Table 1) (Freedman et al., 2008). Other recently investigated alpha 7 agonists are tropisetron, TC-5619 and RG-3487. A randomized double-blind placebo-controlled add-on trial in 33 chronic schizophrenia patients reported a significant beneficial effect of tropisetron on sustained visual attention in non-tobacco using patients

only. However, the cognitive domain of simultaneous and delayed perceptual matching was significantly improved in the placebo group, which consisted of tobacco using and non-tobacco using patients (Shiina et al., 2010). In this study cognitive function, memory, attention and executive function was assessed by the Cambridge Neuropsychological Test Automated Battery (CANTAB). In addition, TC-5619 showed promising results in a randomized placebo-controlled add-on trial in 185 chronic schizophrenia patients; the composite score of the Groton Maze Learning Test, which tests executive functioning, was significantly improved. Secondary analysis of tobacco using schizophrenia patients revealed a significant enhancement in favor of TC-5619 in working memory tested by the Cogstate Schizophrenia Battery (CSSB) (Lieberman et al., 2013). Finally, the alpha 7 nicotinic agent RG-3487 did not have a beneficial effect on cognitive function assessed by the MATRICS test battery in a randomized placebo-controlled add-on trial in 215 chronic schizophrenia patients (Umbricht et al., 2014b).

A relatively newly developing field concerns cholinesterase inhibitors. Several of these drugs, such as rivastigmine and donepezil, are effective in treating cognitive deficits in mild Alzheimer's dementia. Cholinesterase inhibitors act by blocking the acetylcholinesterase enzymes, which metabolize acetylcholine. The first randomized placebo-controlled add-on trials (Sharma et al., 2006; Chouinard et al., 2007; Freudenreich et al., 2005; Friedman et al., 2002; Tugal et al., 2004; Fagerlund et al., 2007) and an open-label add-on trial (Buchanan et al., 2003) conducted to examine the cognitive effect of cholinesterase inhibitors included relatively small samples ($n \leq 40$) and reported negative findings (see Table 1). Also, the largest randomized double-blind placebo-controlled add-on trial in 245 chronic patients with schizophrenia or schizoaffective disorder showed no cognitive enhancing effect for donepezil, measured by an extensive cognitive battery (see Table 1) (Keefe et al., 2008). Furthermore, four clinical trials have been conducted to the cognitive effect of galantamine in schizophrenia patients. Galantamine is a non-selective cholinesterase inhibitor and a modulator of the nicotinic receptor. Three randomized double-blind placebo-controlled add-on trials reported significant beneficial effects on different subdomains of cognitive function, involving delayed memory and attention (Schubert et al., 2006), visual recognition (Lee et al., 2007), processing speed and verbal memory (Buchanan et al., 2008) in chronic schizophrenia patients. However, the fourth randomized double-blind placebo-controlled add-on study did not find a beneficial cognitive effect of galantamine in 32 chronic schizophrenia or schizoaffective disorder patients (Lindenmayer and Khan, 2011). In all four trials cognitive function was assessed by an extensive cognitive test battery (see Table 1). In conclusion, galantamine may be a promising cognitive enhancing agent, as the reported studies showed cognitive enhancing effects on several subdomains in chronic schizophrenia patients. Considering that the cognitive effects of galantamine have not been investigated in first-episode schizophrenia patients, it could be expected that the agent exerts more pronounced effects on cognitive function in an earlier phase of the illness.

The muscarinic receptor is the second receptor, which is part of the cholinergic system. The muscarinic receptor has five subtypes. The M1 subtype is highly expressed in the cortex, striatum and hippocampus; brain regions responsible for learning, cognition and memory (Melancon et al., 2013). One muscarinic agent, xanomelanine, has been tested in chronic schizophrenia patients. This type 1 and 4 muscarinic agonist had a significant positive effect on short-term memory function and verbal learning in a randomized placebo-controlled add-on trial in 20 chronic patients with schizophrenia or schizoaffective disorder (Shekhar et al., 2008). The reported beneficial cognitive effects of xanomelanine have never been replicated in a sample of schizophrenia patients.

Other agents

Research in the field of cognitive enhancing drugs has focused mainly on the above described neurotransmitter pathways. Other receptors that have been studied are GABA, noradrenergic, serotergic, histamanergic and the cannaboid receptor.

MK-0777, a GABA alpha 2 and 3 agonist, has failed to show a beneficial cognitive effect compared to placebo in a double-blind, randomized add-on trial in 60 chronic schizophrenia patients, measured by the MATRICS test battery (Buchanan et al., 2011). A randomized placebo-controlled add-on study in 73 chronic schizophrenia patients revealed no cognitive enhancing effect for the partial 5HT1a agonist buspiron after six months measured by an extensive cognitive test battery (see Table 1) (Sumiyoshi et al., 2007). Additionally, tandospirone, a serotonin-5-HT1a partial agonist, has been shown to improve verbal memory and executive function in 26 chronic schizophrenia patients in a randomized placebo-controlled open-label add-on trial assessing executive function and verbal memory (Sumiyoshi et al., 2001). Finally mianserine, tested in a randomized placebo-controlled double-blind add-on trial in 24 chronic schizophrenia patients, had a beneficial effect on the memory subtest of the Automated Neuropsychological Assessment Metrics, which assesses learning, memory and sustained attention, but not on executive function measured by the Wisconsin Card Sorting Test (WCST) (Poyurovsky et al., 2003). Furthermore, ABT-288, a histamanergic type 3 (H3) antagonist, and MK-0249, an H3 inverse antagonist, are agents that modulate the histamanergic receptor. ABT-288 and MK-0249 did not have a significant cognitive effect in randomized placebo-controlled add-on trials in 214 and 55 chronic schizophrenia patients, respectively (Haig et al., 2014; Egan et al., 2013). In addition, the noradrenergic agents atomoxetine, a selective norepinephrine reuptake inhibitor, (Friedman et al., 2008; Kelly et al., 2009), and guanfacine, an alpha-2 noradrenergic agonist (Friedman et al., 2001), did not have a beneficial effect on cognitive measures in randomized double-blind placebo-controlled add-on trials in chronic schizophrenia patients either (see Table 1). One randomized double-blind placebo-controlled add-on trial investigated the cognitive effect of the cannaboïd receptor modulating agent rimonibant in 14 schizophrenia patients and reported no beneficial influence on cognition assessed by the RBANS (Boggs et al., 2012).

Pregnenolone and modafinil are agents with an uncertain mechanism. Pregnenolone is an endogenous neurosteroid, which positively modulates NMDA receptors (Bowlby, 1993 and Wu et al., 1991). A double-blind placebo-controlled add-on trial showed that pregnenolone improved visual and sustained attention and executive functions measured by the CANTAB in 60 recent-onset schizophrenia or schizoaffective disorder patients (Kreinin et al., 2014). Two other randomized double-blind placebo-controlled add-on trials in respectively 18 and 120 chronic schizophrenia patients found that pregnenolone was not associated with a beneficial cognitive effect measured by the MATRICS test battery (Marx et al., 2009; Marx et al., 2014).

Modafinil is a novel stimulant that inhibits dopamine and norepinephrine transporters, leading to increased dopamine and norepinephrine efflux in cortical and other brain regions (Minzenberg and Carter, 2008). Modafinil is associated with significant cognitive enhancing effects in healthy subjects (Turner et al., 2003). However, the majority of randomized placebo-controlled add-on trials conducted in schizophrenia patients reported no benefit on cognitive function (see Table 1) (Turner et al., 2004; Sevy et al., 2005; Freudenreich et al., 2009; Kane et al., 2010; Bobo et al., 2011). Interestingly, one randomized double-blind placebo-controlled cross-over add-on trial reported improved verbal and spatial working memory with modafinil treatment in 40 patients with a first psychotic episode compared to placebo (Scoriels et al., 2012).

Anti-inflammatory drugs

New findings suggest a role of the immune system in the etiology of schizophrenia (Fineberg and Ellman, 2013; Drexhage et al., 2011; Chew et al., 2013; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Sommer et al., 2014). It has been suggested that anti-inflammatory drugs may be effective in symptom reduction in schizophrenia. Although indeed, several studies indicate that anti-inflammatory drugs, such as aspirin and N-acetylcysteine, reduce (some of the) symptoms in schizophrenia (Laan et al., 2010; Berk et al., 2008) this effect does not extend to cognition (see Table 1) (Laan et al., 2010; Javitt et al., 2012; Chaudhry et al., 2012; Sommer et al., 2014). However, a recent randomized double-blind placebo-controlled minocycline add-on trial reported a slight enhancing effect in the attention subdomain measured by the MATRICS test battery in 92 early stage schizophrenia patients (Liu et al., 2014).

Bipolar disorder

Lithium

Lithium is the first mood-stabilizing agent used for treatment of BD (Hartigan, 1963). It is particularly effective in both long-term maintenance (Young and Hammond, 2007; BALANCE Investigators and collaborators et al., 2010; Baldessarini and Tondo, 2000) as well as in the

treatment of acute mania (Stokes et al., 1971). Yet, the mechanisms underlying the mood-stabilizing effects remain poorly understood.

Multiple studies have investigated the cognitive effect of lithium, but findings have been equivocal. The first longitudinal study assessing the effect of lithium treatment on memory in patients with affective disorders (n=53) reported increased immediate and delayed memory scores after 12 months of prophylactic lithium use (Smigan and Perris, 1983). Another longitudinal study in 18 BD patients found no significant effect of lithium on memory function assessed by the Wechsler Memory Scale (WMS) and the Benton Visual retention Test after 6 years (Engelsmann et al., 1988). A cross-sectional study showed lower verbal and visual–verbal memory for both 20 BD patients on lithium and 20 medication-free BD patients compared with 20 healthy controls (see Table 2) (Lopez-Jaramillo et al., 2010). In addition, a longitudinal study examined BD patients (of whom 33 used lithium at any given moment) at two-month interval over a period of two years. Lithium use at baseline and duration of lithium use were both positively associated with motor speed. Negative (short-term) effects of lithium on basic information processing were also found. However, in general, no significant cognitive effects of lithium were reported (see Table 2 for detailed information) (Arts et al., 2011). In a cross-sectional study in 119 elderly euthymic BD patients, lithium use was not associated with impaired cognitive function when controlled for risk factors like age and cardiovascular disease (see Table 2) (Schouws et al., 2010). In addition, another study reported Alzheimer's disease in 5% of lithium-treated BD patients as opposed to 33% in BD patients, which were not treated with lithium (Nunes et al., 2007). Although the above mentioned studies suggest a mild, positive cognitive effect of lithium in BD, there have also been findings suggesting that lithium has subtle negative cognitive effects (Wingo et al., 2009; Pachet and Wisniewski, 2003). A meta-analysis of 12 studies showed that lithium use was associated with small impairments in immediate verbal learning and memory and creativity in euthymic patients with an affective disorder and healthy volunteers. The other investigated cognitive domains, delayed verbal memory, visual memory, attention, executive function, processing speed and psychomotor performance, were not affected. Additionally, long-term lithium use was associated with moderate impairments in psychomotor performance in patients with an affective disorder. It was suggested that negative cognitive effects of lithium were a function of duration of treatment and appear to be minor (Wingo et al., 2009). A cross-sectional study in 230 individuals with varying psychiatric disorders from 47 families found that treatment with lithium and antipsychotic medication was related to lower executive and verbal recognition memory (see Table 2 for the cognitive test battery and sample information) (Savitz et al., 2008). Another cross-sectional study found that 33 BD patients on lithium or valproic acid had greater response latency in affective processing and impaired sustained attention compared to both 32 unmedicated BD patients and 52 healthy controls (Holmes et al., 2008).

The inconsistent findings regarding the cognitive effects of lithium are partly the result of methodological flaws. Pachet and Wisniewski (2003) addressed these methodological

flaws in their review and concluded that lithium is associated with mild impairments in psychomotor speed and verbal memory. Interestingly, a subgroup of lithium-using patients may not suffer from these cognitive impairments. A recent cross-sectional study showed that 13 excellent lithium responders had similar cognitive function as 60 matched healthy controls measured by the CANTAB (Rybakowski and Suwalska, 2010). Patients in which the effect of lithium was not optimal (*n*=47) scored lower on the subtests of the CANTAB as compared with controls. These findings are supported by another cross-sectional study in BD patients that found lower executive functioning measured by the WCST for lithium non-responders (*n*=7) but not for lithium responders (*n*=23) compared with matched controls (*n*=30) (Rybakowski et al., 2009). In conclusion, findings on the cognitive effects of lithium are contradictory, but tend to be mildly negative, at least in patients who do not have an optimal lithium response. Studies conducted so far included relatively small samples and mostly cross-sectional designs. Therefore, longitudinal studies on the cognitive effects of lithium in larger BD samples are sorely warranted.

Anticonvulsants

Anticonvulsant drugs have become important adjunctive and alternative treatments to lithium in BD (Okuma et al., 1981) but the cognitive effects are unclear. Some studies reported similar cognitive function for BD patients on either anticonvulsant or lithium treatment (see Table 2) (Senturk et al., 2007; Joffe et al., 1988). However, there appears variability in the cognitive effect of different types of anticonvulsants and lithium. A cross-sectional study in 159 BD patients on five types of anticonvulsants or lithium found that patients on lamotrigine and oxcarbazepine exerted the best scores on an extensive cognitive test battery, followed by patients on lithium. Patients on valproic acid, carbamazepine and topiramate had the lowest scores (see Table 2) (Gualtieri and Johnson, 2006). Another cross-sectional study showed that BD patients treated with lamotrigine (*n*=15) had better phonemic verbal fluency, but did not significantly differ on immediate verbal memory, executive functions, attention and working memory compared with patients treated with other anticonvulsants (*n*=18) (Daban et al., 2006). Also, several open-label studies showed that lamotrigine positively affects self-reported cognitive function (Khan et al., 2004; Kaye et al., 2007) (see Table 2).

The paucity of studies, the lack of prospective randomized controlled trials and the contradictory results make it impossible to draw conclusions on the cognitive effects of anticonvulsants in BD patients.

Antipsychotics

Few studies have investigated the cognitive effect of antipsychotic agents in BD patients, but the findings generally suggest that antipsychotics negatively influence cognitive function. The previously mentioned study by Arts et al. (2011) showed that use of second generation

antipsychotics had negative effects on motor speed and basic information processing in 24 BD patients (Arts et al., 2011).

Two cross-sectional studies (respectively *N*=43 and *N*=40 BD patients) found that antipsychotic treatment was associated with significant underperformance on IQ, general memory and working memory (Donaldson et al., 2003), psychomotor function, verbal fluency, verbal learning, memory and recognition memory, executive function and attention (see Table 2) (Jamrozinski et al., 2009).

One cross-sectional study specifically focused on the cognitive effects of subtypes of antipsychotics in BD patients. BD patients on risperidone (*n*=30), quetiapine (*n*=12), olanzapine (*n*=26), unmedicated BD patients (*n*=16) and healthy controls (*n*=35) were compared on executive functioning, attention/concentration, mental tracking, verbal learning and verbal memory. BD patients treated with atypical antipsychotics demonstrated significantly lower cognitive function compared with unmedicated BD patients and controls (see Table 2) (Torrent et al., 2011). Few randomized controlled trials have been conducted on the cognitive effect of adding antipsychotics to treatment as usual in BD patients. Pooled data of two 3-week randomized controlled trials in 249 patients with acute mania (trial 1 *N*=139; trial 2 *N*=110) showed that olanzapine significantly improved cognitive function, but measured this with the PANSS (Shi et al., 2004). In addition, cognitive improvement was highly associated with improvement in manic symptoms. Another small randomized placebo-controlled trial showed that adding quetiapine to mood stabilizers did not improve cognitive function measured by an extensive cognitive test battery in euthymic BD patients (*n*=5), whereas placebo did (*n*=9) (see Table 2) (Rakofsky et al., 2014).

In summary, studies on the cognitive effects of antipsychotics in BD patients have been carried out in relatively small samples and predominantly conducted in cross-sectional designs, but point to a negative cognitive effect. Notably, the presence of psychotic symptoms may act as a confounding factor in these studies, since a history of psychotic symptoms may be associated with reduced cognitive function in BD patients (Toulopoulou et al., 2006; Martinez-Aran et al., 2004a). Prospective randomized studies are needed to resolve this issue.

Conclusion

Despite extensive efforts on the development of cognitive enhancing drugs, to date no putative agents with such properties have been produced for schizophrenia. Antipsychotics appear to mildly improve cognitive function in schizophrenia patients (more in first episode than in chronic patients), but these agents do not improve cognitive function to any meaningful degree. Also, cognitive enhancing effects by dopamine agonists and glutamatergic drugs have been reported. However, results are inconclusive and at best suggest an improvement

in cognitive subdomains. Cholinergic agents, in particular galantamine and nicotine, appear to have the most promising cognitive enhancing effects in schizophrenia patients.

Whereas in schizophrenia the goal of research is to develop new putative cognitive enhancing agents, BD research still focuses on the cognitive effects of current available mood-stabilizers and antipsychotics. Cognitive enhancing agents in BD have not been proposed so far. There are some indications that lithium may act as a cognitive enhancing agent in a subgroup of BD patients with an excellent lithium response. However, the research methodology in BD so far is flawed using, as it does, mostly cross-sectional designs. Clearly, prospective randomized (placebo-)controlled trials are necessary to investigate the cognitive effects of medication in BD. In fact, the study of cognitive dysfunction (and its treatment) in BD is severely underdeveloped despite the relevance of cognitive dysfunction in the later stage of the illness.

In schizophrenia, the majority of clinical trials testing the efficacy of putative cognitive enhancing agents have focused on chronic patients. As cognitive decline precedes the first psychotic episode by many years, these studies may have barked up the wrong tree. Therefore, the focus of research should be on patients in an earlier phase of the illness, preferably when the first cognitive deficits appear. Since it is difficult to identify the first cognitive problems in the general population, targeting a population with increased vulnerability for schizophrenia may be more appropriate. Monitoring an ultrahigh risk population provides the opportunity to administer cognitive enhancing agents if the first cognitive deficits appear. In contrast, studies examining putative cognitive enhancing agents in BD patients should focus on the illness stage that occurs after the onset of (mood) symptoms, since cognitive function in BD decreases after the onset of illness. Hopefully, these recommendations will accelerate the development of cognitive-enhancing drugs for schizophrenia and bipolar disorder patients. They are sorely needed.

Table 1. Overview of cognitive enhancing agents in schizophrenia

Author	Agent and Dose	Design and follow up	Participants	Cognitive measures	Outcome
	Antipsychotics				
Keefe et al 2007 CATIE trial	Pherphenazine (20,3mg/d) Olanzapine (19,6mg/d) Quetiapine (528,3mg/d) Risperidone (3,9mg/d) Ziprasidone (121mg/d)	Randomized double-blind trial 18 months	N = 817 schizophrenia patients (mean age total group = 40.9 yrs, SD = 10.8 pherphenazine: N = 149 olanzapine: N = 211 quetiapine: N = 181 risperidone: N = 183 ziprasidone: N = 93)	- Controlled word association test (COWAT) - Category Instances score - Wechsler Intelligence Scale for Children (WISC-III) mazes score - Letter number sequencing - Hopkins Verbal Learning test (HVLT) - Wechsler Adult Intelligence Test–Revised Edition (WAIS-R) Digit symbol - Grooved Pegboard - Continuous Performance Test - Visuospatial Working Memory - Wisconsin Card Sorting Test (WCST)	Significant enhancement on composite score for all five treatment groups Olanzapine z = 0.13 Perphenazine z = 0.25 Quetiapine z = 0.18 Risperidone z = 0.26 Ziprasidone z = 0.12
Davidson et al 2009 EUFEST trial	Haloperidol (2,5mg/d) Olanzapine (12mg/d) Quetiapine (458mg/d) Ziprasidone (98mg/d) Amisulpiride (455mg/d)	Randomized open-label trial 6 months	N =286 first-episode schizophrenia or schizophreniform disorder (haloperidol: N = 52, mean age = 26.0 yrs, SD = 5.8 olanzapine: N = 74, mean age = 26.1 yrs SD = 5.6 quetiapine: N = 60, mean age = 26.2 yrs, SD = 5.2 ziprasidone: N = 45, mean age = 25.6 yrs, SD = 5.9 amisulpiride: N = 55, mean age = 24.7 yrs, SD = 4.2)	- The Rey Auditory Verbal Learning Test (RAVLT) - Trail Making Test A+B - Wechsler Adult Intelligence Scale-III (WAIS-III) Digit Symbol Test - Purdue Pegboard Test (PPT)	Significant enhancement on composite score for all five treatment groups Haloperidol Cohen's d= 0.43 Olanzapine Cohen's d= 0.56 Quetiapine Cohen's d= 0.51 Ziprasidone Cohen's d= 0.49 Amisulpiride Cohen's d= 0.33
	Dopamine Agonists				
Davidson et al 1990	SKF-38393 (500mg/d)	Randomized double-blind placebo-controlled cross-over add-on trial 4 weeks	N= 10 schizophrenia patients, only male (mean age = 43.7 yrs)	- WCST - Vocabulary subtest WAIS-R	No significant effects
George et al 2007	Dihydrexidine (20 mg subcutaneously)	Randomized double-blind cross-over add-on trial 2 single doses	N = 20 schizophrenia patients (mean age = 39.5 yrs, SD = 10.5)	- Trail making test A-D - COWAT - HVLT	No significant effects
Rosell et al 2014	Dihydrexidine (15mg/150ml intravenously over 30 minutes)	Randomized double-blind placebo-controlled add-on trial 2 single doses	N = 16 medication free patients with schizotypal personality disorder (mean age total group = 35.9 yrs, SD = 12.2 N = 8 placebo, N = 8 dihydrexidine)	- N-Back - Pased auditory serial addition test	Significant enhancing effect on: - Pased auditory serial addition test (Cohen's d= 1.14)

Barch and Carter 2005	D-Amphetamine (0,25mg/kg)	Randomized double-blind placebo-controlled add-on trial 2 single doses	N = 10 schizophrenia patients (mean age = 40.3 yrs, SD = 8.7) N = 22 healthy controls (mean age = 36.6 yrs, SD = 5.7)	- Structured language production interview - Single-trial Stroop task - Dual-task Stroop task - Single task spatial working memory - Dual task spatial working memory	Significant enhancing effect on: -Language production, reaction times on spatial working memory and Stroop tasks in both groups -Working memory accuracy in schizophrenia patients
Pietrzak et al 2010	D-Amphetamine (10mg per dose)	Randomized double-blind placebo-controlled cross-over add-on trial 2 single doses	N = 32 schizophrenia patients (mean age = 43.3 yrs, range 24-55)	- Detection task (DET) - Identification task (IDN) - One card learning (OCL) - Grozon Maze learning test (GMLT)	Significant enhancing effect on: -DET (Dunlop's d= 0.42) - IDN (Dunlop's d = 1.01) - GMLT (Dunlop's d = 1.44)
	Glutamatergic Drugs				
Buchanan et al 2007 CONSIST study	Glycine (60g/d) D cycloserine (50mg/d)	Randomized double-blind parallel group add-on trial 16 weeks	N = 157 schizophrenia patients (N=53 d cycloserine mean age = 44.4 yrs, SD = 10.4 N= 52 glycine mean age = 42.6 yrs, SD = 10.8) n =52 placebo (mean age = 43.4 yrs, SD = 11.4	- WAIS-III digit symbol and symbol search - Phonemic verbal fluency - Categorical verbal fluency - Grooved pegboard - Continuous Performance Test - RAVLT immediate and delayed - Rey discrimination index - Brief Visual Spatial Memory Test immediate and delayed - Letter number span forward - WAIS-III letter number sequencing - Spatial working memory - WCST	No significant effects
Goff et al 1999	D cycloserine (50mg/d)	Randomized double-blind placebo-controlled add-on trial 8 weeks	N = 46 schizophrenia patients (N = 23 d cycloserine mean age = 46.8 yrs, SD = 12.3 N = 23 placebo mean age = 41.2 yrs, SD = 8.1)	- Sternberg Item Recognition Paradigm - Stroop test - Miller Selfridge test - Verbal fluency - Digit span	No significant effects
Goff et al 2005	D Cycloserine (50mg/d)	Randomized placebo-controlled add-on trial 6 months	N = 55 schizophrenia patients (N = 27 d cycloserine mean age = 45.9 yrs, SD = 7.4 N = 28 placebo mean age = 47.0 yrs, SD = 8.6)	- California Verbal Learning Test (CVLT) - Vocabulary - Information - Digit Span - Block design tests WAIS III - Stroop Test - Finger Tapping - WCST	No significant effects
Weiser et al 2012	D serine (2g/d)	Randomized double-blind placebo-controlled add-on trial 16 weeks	N = 190 schizophrenia patients (N = 98 placebo mean age = 39.8 yrs, SD = 12.3 N = 92 d serine mean age = 39.4 yrs, SD = 12.0)	- Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) test battery	No significant effects

Kantrowitz et al 2010	D serine (30mg/kg, 60mg/kg, or 120mg/kg)	Open-label trial of 3 dose-level add-on comparison trial 4 weeks	N = 47 schizophrenia patients or schizoaffective disorder (N = 12 d serine 30mg/kg mean age = 41.7 yrs, SD = 11.4 N = 19 d serine 60mg/kg mean age = 43.5 yrs, SD = 9.4 N = 16 d serine 120mg.kg mean age = 43.2 yrs, SD = 9.6)	- MATRICS test battery	Significant enhancing effect on: - speed of processing domain for 30mg/kg dose (Cohen's d = 0.25) - composite score for dose 60mg/kg (Cohen's d=1.1) and 120 mg/kg (Cohen's d= 0.99)
Umbricht et al 2014	Bitopterin (10mg/d, 30mg/d or 60mg/d)	Randomized placebo-controlled 3 dose-level add-on trial 8 weeks	N = 215 schizophrenia patients (N = 54 bitopterin 5mg mean age = 40.1 yrs, SD = 8.3 N = 53 bitopterin 15 mg mean age = 39.6 yrs, SD = 9.6 N = N = 54 bitopterin 50 mg mean age = 40.5 yrs, SD = 8.9 N = 54 placebo mean age = 38.1 yrs, SD = 9.9)	- MATRICS test battery	No significant effects
Lieberman et al 2009	Memantine (20mg/d)	Randomized placebo-controlled add-on trial 8 weeks	N = 136 schizophrenia patients (N = 69 memantine mean age = 40.9 yrs, SD = 9.8 N = 67 placebo mean age = 40.1 yrs, SD = 11.3)	- Brief Assessment of Cognition in Schizophrenia (BACS)	No significant effects
Lee et al 2012	Memantine (20mg/d)	Placebo-controlled add-on trial 12 weeks	N = 26 schizophrenia patients (N = 15 memantine mean age = 44.3 yrs, SD = 4.3 N = 11 placebo mean age = 43.4 yrs, SD = 3.9)	- HVLT - Rey complex figure test (RCFT) - The Digit symbol substitution test (DSST) WAIS III - Digit span forward and backward test - Trail making test A - Stroop Color-Word Test - Verbal fluency test	No significant effects
Krivoy et al 2008	Memantine (20mg/d)	Open-label add-on trial 6 weeks	N = 7 schizophrenia patients (mean age = 39.8 yrs, SD = 15.0)	- Neurobehavioral cognitive examination - Clock drawing test	No significant effects
Lane et al 2013	DAAO (1g/d)	Randomized double-blind placebo-controlled add-on trial 6 weeks	N = 52 schizophrenia patients (N = 25 DAAO mean age = 38.4 yrs, 9.7 N = 27 placebo mean age = 36.3 yrs, SD = 7.9)	- MATRICS test battery	Significant enhancing effect on: - Composite score (Cohen's d= 0.67) including speed of processing (Cohen's d=0.65) and visual learning and memory (Cohen's d=0.7)

Goff et al 2008	Ampakine (900mg/d)	Randomized placebo-controlled add-on trial 8 weeks	N = 105 schizophrenia patients (N = 51 ampakine mean age = 42.0 yrs, SD = 9.3 N = 54 placebo mean age = 43.7 yrs, SD = 11)	- Trail making test - Verbal fluency - Degraded-stimulus Continuous Performance Test - Faces and Family Pictures subtests from WMS-III - WCST - Letter and Category Fluency - Letter-Number Span - Grooved Peg Board	No significant effects
Goff et al 2007	Lamotrigine (100-400mg/d)	Data of 2 Randomized double-blind add-on trials 12 weeks	N = 415 schizophrenia patients (Trial 1: N = 209 mean age = 41.0 yrs, SD = 9.8 Trial 2 : N = 206 mean age = 41.6 yrs, SD = 10.6)	- BACS	No significant effects
	Cholinergic Drugs				
Barr et al 2008	Nicotine patch (14mg)	Randomized placebo-controlled cross-over add-on trial Single dose, test 3 hour before and after patch application.	N = 28 schizophrenia patients, non-tobacco users (mean age = 47.0 yrs, SD = 8.0) N = 32 healthy controls, non-tobacco users (mean age = 40.0 yrs, SD = 11)	- Stroop Color-Word Test - Continuous Performance Test Identical Pairs	Significant enhancing effect on: - hit reaction of the CPT-IP in both groups (ES using partial eta^2 = 0.26) - Stroop test in schizophrenia patients (ES using partial eta^2 = 0.18)
Sacco et al 2005	Nicotine (>15 cigarettes /d)	Neuropsychological assessment after overnight abstinence and after reinstatement of nicotine, add-on 3 visits	N= 25 schizophrenia patients, tobacco users (mean age = 42.5 yrs, SD = 9.4) N= 25 healthy controls, tobacco users (mean age = 41.9 yrs, SD = 10.9)	- Visuospatial working memory (VSWM) - Continuous Performance Test (CPT) - Word Serial Position Test - Stroop Color-Word Test - WCST	Significant enhancing effect on: - VSWM (Cohen's d = 0.9) and CPT (Cohen's d = 0.58) in smoking schizophrenia patients
Smith et al 2006	Nicotine nasal spray (4 puffs)	Randomized double-blind placebo-controlled add-on trial Neuropsychological testing after overnight nicotine abstinence and after nasal spray, 4 visits	N = 27 schizophrenia patients or schizoaffective patients, male tobacco users (mean age = 37.6 yrs, SD = 8.3)	- CPT - Automated neuropsychocological Assessment Metrics (ANAM) - Dot test - Verbal portion of RANDT memory scale	Significant enhancing effect on: - CPT, hit reaction time (ES using partial eta^2 = 0.21) - ANAM, accuracy (ES using partial eta^2 = 0.17)
Harris et al 2004	Nicotine gum (6mg)	Administration of the effect nicotine gum and placebo gum after two hour nicotine abstinence, add-on	N = 10 schizophrenia patients, tobacco users N = 10 schizophrenia patients, non-tobacco users (mean age total group = 43.9 yrs, range 33-51)	- Repeatable battery for the assessment of neuropsychological status (RBANS)	Significant enhancing effect on: - attentional subtest RBANS for schizophrenia patients non-tobacco users Significant decreasing effect on: - attentional subtest RBANS for schizophrenia patients tobacco users

Jubelt et al 2008	Nicotine patch (7mg)	Two single doses of nicotine or placebo, add-on	N = 10 schizophrenia patients, non-tobacco users (mean age = 46.0 yrs, SD = 10.0) N = 10 healthy controls, non-tobacco users (mean age = 35.0 yrs, SD = 2.0)	- CPT - Identical Pairs - Stroop Color-Word Task - Letter number sequencing - Grooved Pegboard	Significant enhancing effect on: - recognition of false alarms and reaction time for new items (Cohen's d= 0.71)
Olincy et al 2006	DMXB-A (first dose: 150mg, 75mg Second dose: 75mg, 37,5mg)	Randomized double-blind placebo-controlled cross-over 2-dose-level add-on trial One single dose	N = 12 schizophrenia patients (mean age = 44.5 yrs, range 20-58)	- RBANS	Significant enhancing effect on: - composite score (ES = 0.51)
Freedman et al 2008	DMXB-A (150mg or 300mg /d)	Randomized double-blind placebo-controlled cross-over add-on 2-dose-level trial 4 weeks	N = 31 schizophrenia patients (Age range total group 22-60 yrs)	- MATRICS test battery - RBANS	No significant effects
Shiina et al 2010	Tropisetron (10mg/d)	Randomized double-blind placebo-controlled add-on trial 8 weeks	N = 33 schizophrenia patients (N = 16 tropisetron, 30 % tobacco users mean age = 35.0 yrs, SD = 6.8 N = 17 placebo, 25% tobacco users mean age = 35.2 yrs, SD = 8.5)	- Cambridge Neuropsychological Test Automated Battery (CANTAB)	Significant enhancing effect on: - sustained visual attention in non-tobacco users of the tropisetron group - simultaneous and delayed perceptual matching in placebo group
Lieberman et al 2013	TC-5619 (25mg/d)	Randomized placebo-controlled add-on trial 12 weeks	N = 185 schizophrenia patients (N = 94 TC-5619, 48% tobacco users mean age = 36.3 yrs N = 91 placebo, 45% tobacco users mean age = 36.3 yrs)	- GMLT - CogState Schizophrenia Battery (CSSB) - Trail making test A+B - DSST	Significant enhancing effect on: - composite score GMLT (Cohen's d= 0.40) measured by a - attention-concentration of the CSSB - working memory in CSSB in smokers only
Umbricht et al 2014	RG-3487 (5,15 or 50mg/d)	Randomized placebo-controlled 3-dose-level add-on trial 8 weeks	N = 215 schizophrenia patients (N = 54 RG-3487 5mg, 74 % tobacco users mean age = 40.1 yrs, SD = 8.3 N = 53 RG-3487 15mg, 77% tobacco users mean age = 39.6 yrs, SD = 9.6 N = 54 RG-3487 50mg, 81% tobacco users mean age = 40.5 yrs, SD = 8.9 N = 54 placebo, 76 % tobacco users mean age = 38.1 yrs, SD = 9.9)	- MATRICS test battery	No significant effects

Sharma et al 2006	Rivastigmine (6mg/d)	Randomized placebo-controlled double-blind add-on trial 24 weeks	N = 21 schizophrenia patients (N = 11 rivastigmine mean age = 42.6 yrs, SD = 8.9 N = 10 placebo mean age = 46.8 yrs, SD = 13.0)	- California Verbal Learning Test (CVLT): total learning trials 1–5 - WCST - Trail Making Test A+B - Verbal Fluency: category and phonological fluency - WAIS-III letter number, Digit symbol - Dot Test - Continuous Performance Test - Finger Tapping Test: total score	No significant effects
Chouinard et al 2007	Rivastigmine (9mg/d)	Randomized cross-over add-on trial 12 weeks	N = 20 schizophrenia patients (mean age = 28.9 yrs, SD = 7.9)	- CANTAB - RBANS	No significant effects
Buchanan et al 2003	Donepezil (10mg/d)	Open-label add-on trial 6 weeks	N = 15 schizophrenia patients (mean age = 43.1 yrs, SD = 6.6)	- RCFT - Benton visual retention test (BVRT) - WAIS III digit symbol test - Gordon Diagnostic System - continuous performance test	No significant effects
Freudenreich et al 2005	Donepezil (5mg/d or 10mg/d)	Randomized double-blind placebo-controlled 2-dose-level add-on trial 8 weeks	N = 36 schizophrenia patients (mean age = 48.7 yrs, range 24-64)	- Digit span, WAIS III - HVLT - Trail making test A+B - Benton Oral Word Association Test - Grooved Pegboard test	No significant effects
Friedman et al 2002	Donepezil (5mg/d or 10mg/d)	Randomized double-blind placebo-controlled 2-dose-level add-on trial 12 weeks	N = 36 schizophrenia patients (N = 18 donepezil mean age = 50.3 yrs, SD = 10.1 N = 18 placebo Mean age = 48.8 yrs, 11.1)	- CPT - Trail Making test A+B - WCST - RAVLT - Digit Span Distraction Test - Simple spatial Working memory	No significant effects
Tugal et al 2004	Donepezil (5mg/d)	Randomized double-blind placebo-controlled cross-over add-on trial 12 weeks	N = 12 schizophrenia patients (group placebo-donepezil mean age = 38.0 yrs, SD = 10.2 Group donepezil-placebo mean age = 29.2 yrs, SD = 5.9)	- WCST - Wechsler memory scale revised (WMS-R) - Verbal fluency - Trail making test A+B	No significant effects
Fagerlund et al 2007	Donepezil (10mg/d)	Randomized double-blind placebo-controlled add-on trial 16 weeks	N = 11 schizophrenia patients (N = 7 donepezil mean age = 33.2 yrs, range 23.2-43.0 N = 4 placebo mean age = 35.0 yrs, range 28.2-40.9)	- CANTAB	No significant effects

Keefe et al 2008	Donepezil (5-10mg/d)	Randomized double-blind placebo-controlled add-on trial 12 weeks	N = 245 schizophrenia or schizoaffective patients (N = 121 donepezil mean age = 40.9 yrs, SD = 9.7 N = 124 placebo mean age = 39.7 yrs, SD = 9.0)	- Controlled word association test (COWAT) - Category Instances score - Wechsler Intelligence Scale for Children (WISC-III) mazes score - Letter number sequencing - Hopkins Verbal Learning test (HVLT) - Wechsler Adult Intelligence Test–Revised Edition (WAIS-R) Digit symbol - Grooved Pegboard - Continuous Performance Test - Visuospatial Working Memory - Wisconsin Card Sorting Test (WCST)	No significant effects
Schubert et al 2006	Galantamine (24mg/d)	Randomized double-blind add-on trial 8 weeks	N= 14 schizophrenia patients or schizoaffective patients (N = 8 galantamine mean age = 48.3 yrs, SD = 6.9 N = 6 placebo mean age = 46.8 yrs, SD = 8.8)	- RBANS	Significant enhancing effect on: - Composite score consisting of attention and delayed memory
Lee et al 2007	Galantamine (16 mg/d)	Randomized double-blind placebo-controlled add-on trial 12 weeks	N = 24 schizophrenia patients (N = 12 galantamine mean age = 39.5 yrs, SD = 3.2 N = 12 placebo mean age = 41.5 yrs, SD = 3.2)	- HVLT - Korean version of Mini-Mental State Exam - RCFT - Digit Span forward and backward test - Digit Symbol Substitution Test - Stroop Color-Word Test - Trail making test A - Verbal fluency test - Boston Naming Test	Significant enhancing effect on: - RCFT (recognition)
Buchanan et al 2008	Galantamine (24mg/d)	Randomized double-blind placebo-controlled add-on trial 12 weeks	N = 86 schizophrenia patients (N = 42 galantamine mean age = 49.9 yrs, SD = 9.2 N = 44 placebo mean age = 49.5 yrs, SD = 9.9)	- WAIS-III letter-number, digit symbol and symbol search - BACS, number sequencing - CVLT - Grooved Pegboard - CPT - Brief Visuospatial Memory Test - Gordon Diagnostic System (GDS) - CPT	Significant enhancing effect on: - Digit symbol and verbal memory WAIS-III - GDS distractibility test

Lindenmayer et al 2011	Galantamine (24mg/d)	Randomized double-blind placebo-controlled add-on trial 12 months	N = 32 schizophrenia patients (N = 15 galantamine mean age = 41.9 yrs, SD = 10.8 N = placebo mean age = 38.5 yrs, SD = 12.2)	- CPT - Flanker Continuous Performance Test - Face Memory Test - CPT- Identical Pairs - Object Working Memory - Penn Emotional Acuity Test - Set Shifting Test - Strategic Target Detection Test - Tapping Speed Test - Workstation Orientation - Word List Memory	No significant effects
Shekhar et al 2008	Xanomelanine (225 mg/d)	Randomized placebo-controlled add-on trial 4 weeks	N = 20 schizophrenia or schizoaffective disorder patients (N = 10 xanomelanine mean age = 43.3 yrs, SD = 9.3 N = 10 placebo mean age = 42.1 yrs, SD = 9.3)	- CPT- Identical Pairs - Stroop Color-Word Test - Wechsler Memory Scale WAIS-III (WMS) - Trail Making Test A+B - HVLT-revised - Shipley Institute of Living Vocabulary Test - Finger Tapping Test - Brief Visuospatial Memory Test-Revised (BVMT)	Significant enhancing effect on: - WMS: digit span, story recall - HVLT-revised: learning total - BVMT: delayed memory
	Other Agents				
Buchanan et al 2011	MK0777 (6mg/d or 16mg/d)	Randomized double-blind placebo-controlled 2-dose-level add-on trial 4 weeks	N = 60 schizophrenia patients (N = 18 MK0777 6mg/d mean age = 43.3 yrs, SD = 9.3 N = 21 MK0777 18mg/d mean age = 44.9 yrs, SD = 8.7 N = 21 placebo mean age = 40.0 yrs, SD = 10.9)	- MATRICS test battery	No significant effects
Sumiyoshi et al 2007	Buspiron (30mg/d)	Randomized double-blind placebo-controlled add-on trial 6 months	N = 73 schizophrenia patients (N = 36 buspiron mean age = 40.5 yrs, SD = 11.8 N = 37 placebo mean age = 39.7 yrs, SD = 12.5)	- Digit Symbol Substitution Test (DSST) WAIS-Revised - Controlled Word association Test - Category Instance Generation Test - CVLT - Auditory Consonants Trigram (ACT) - WCST	Significant enhancing effect on: - DSST at 3 month follow-up only (Cohen's d = 0.32)
Sumiyoshi et al 2001	Tandospirone (30mg/d)	Randomized placebo-controlled open-label add-on trial 4 weeks	N = 26 schizophrenia patients (N = 15 tandospirone mean age = 27.8 yrs, SD = 6.3 N = 11 placebo mean age = 31.8 yrs, SD = 9.4)	- WCST - Verbal memory composite score from WMS-R	Significant enhancing effect on: - WCST (ES= 0.63) - WMS-R (ES = 0.70)
Poyorovsky et al 2003	Mianserine (15mg/d)	Randomized double-blind placebo-controlled add-on trial 4 weeks	N = 24 schizophrenia patients (N = 11 mianserine mean age = 42.5 yrs, SD = 12.9 N = 13 placebo mean age = 45.5 yrs, SD = 7.5)	- ANAM - WCST	Significant enhancing effect on: - ANAM, memory (Cohen's d= 1.16)

Haig et al 2014	ABT-288 (10mg/d or 25mg/d)	Randomized double-blind placebo-controlled 2-dose-level add-on trial 12 weeks	N = 213 schizophrenia patients (N = 72 ABT-288 10mg mean age = 43.9 yrs, SD = 9.5 N = 69 ABT-288 25 mg mean age = 42.9 yrs, SD = 9.8 N = 72 placebo mean age = 43.0 yrs, SD = 9.1)	- MATRICS test battery	No significant effects
Egan et al 2013	MK-0249 (10mg/d)	Randomized placebo-controlled cross-over add-on trial 4 weeks	N = 55 schizophrenia patients (mean age = 31.6 yrs, SD = 7.9)	- BACS	No significant effects
Friedman et al 2008	Atomoxetine (80mg/d)	Randomized placebo-controlled add-on trial 8 weeks	N = 15 schizophrenia patients (N = 7 atomoxetine, age was not reported N = 8 placebo, age was not reported)	- BACS	No significant effects
Kelly et al 2009	Atomoxetine (80mg/d)	Randomized double-blind placebo-controlled add-on trial 8 weeks	N = 22 schizophrenia or schizoaffective patients (N = 10 atomoxetine mean age = 48.9 yrs, SD = 5.7 N = 12 placebo mean age = 49.1 yrs, SD = 8.5)	- WAIS III Letter Number Sequencing, Number Sequencing Test, Digit Symbol - Grooved Pegboard - Letter Fluency Test - Woodcock Johnson Planning - CVLT - Brief Visuospatial Memory test - Distractibility version of the - GDS - CPT	No significant effects
Friedman et al 2001	Guanfacine (2mg/d)	Randomized double-blind placebo-controlled add-on parallel trial 8weeks	N = 39 schizophrenia patients (N = 19 guanfacine mean age = 49.1 yrs, SD = 11 N = 20 placebo mean age = 47.3 yrs, SD = 10.4)	- Simple Spatial Working Memory Test - CPT-computerized - Trail Making Part A+B - RAVLT - Digit Span Distraction Test - Verbal Fluency	No significant effects secondary analysis; spatial working memory test performance and CPT reaction time significantly enhanced in subjects treated with SGA only.
Boggs et al 2012	Rimonibant (20mg/d)	Randomized double-blind placebo-controlled add-on trial 16 weeks	N = 14 schizophrenia patients (N = 7 rimonibant mean age = 45.9 yrs, SD = 6.9 N = 7 placebo mean age = 44.9 yrs, SD = 12.2)	- RBANS - Iowa Gambling Task - N-Back	No significant effects
Kreinin et al 2014	Pregnenolone (50mg/d)	Randomized double-blind placebo-controlled add-on trial 8 weeks	N = 53 schizophrenia patients, recent onset (N = 25 pregnenolone mean age = 26.9 yrs, SD = 5.2 N = 27 placebo mean age = 27.8 yrs, SD = 6.0)	- CANTAB	Significant enhancing effect on: - Matching to Sample Visual Search task (Cohen's d= 0.42) - Rapid Visual Information Processing, - Stockings of Cambridge - Spatial Working Memory

Marx et al 2009	Pregnenolone (up to 500mg/d)	Randomized double-blind placebo-controlled add-on trial 8 weeks	N = 18 schizophrenia patients (N = 9 pregnenolone mean age = 52.7 yrs, SD = 6.3 N = 9 placebo mean age = 49.a. SD = 12.2)	- MATRICS test battery - BACS	No significant effects
Marx et al 2014	Pregnenolone (up to 500mg/d)	Randomized placebo-controlled add-on trial 8 weeks	N = 111 schizophrenia patients (N = 56 pregnenolone mean age = 36.8 yrs, SD = 8.4 N = 55 placebo mean age = 39.0 yrs, SD = 8.7)	- MATRICS test battery	No significant effects
Turner et al 2004	Modafinil (200mg/d)	Randomized double-blind placebo-controlled cross-over add-on trial 2 drugs doses	N = 20 schizophrenia patients (mean age = 43.0 yrs, SD = 9.0)	- CANTAB	Significant enhancing effect on: - Digit span - 'One-Touch' Tower of London spatial planning tas- latency - Attentional set shifting-total
Sevy et al 2005	Modafinil (up to 200mg/d)	Randomized double-blind placebo-controlled add-on trial 8 weeks	N = 20 schizophrenia or schizoaffective patients (N = 10 modafinil mean age = 35.9 yrs, SD = 9.4 N = 10 placebo mean age = 38.9 yrs, SD = 10.0)	- CPT - Letter number span - Oculomotor delayed response test - Delayed Match to sample Task - COWAT - RAVLT	No significant effects
Freudenreich et al 2009	Modafinil (up to 300mg/d)	Randomized double-blind placebo-controlled add-on trial 8 weeks	N = 35 schizophrenia patients (N = 16 modafinil mean age = 44.2 yrs, SD = 12.0 N = 19 placebo mean age = 46.4 yrs, SD = 6.4)	- CPT - HVLT - WCST - Grooved Pegboard - Trail making test - Faces and family pictures subsets WMS-III - WAIS-III Letter number sequencing - Letter and category fluency	No significant effects
Kane et al 2010	Armodafinil (50mg/d, 100mg/d or 200mg/d)	Randomized double-blind placebo-controlled 3-dose-level add-on trial 4 weeks	N = 60 schizophrenia patients (mean age total group = 43.2 yrs, SD = 7.8 N = 14 armodafinil 50mg N = 14 armodafinil 100mg N = 12 armodafinil 200mg)	- MATRICS test battery	No significant effects
Bobo et al 2011	Armodafinil (150mg/d)	Randomized double-blind placebo-controlled add-on trial 6 weeks	N = 58 schizophrenia or schizoaffective patients (N = 29 armodafinil mean age = 44.0 yrs, SD = 14.6 N = 29 placebo mean age = 38.8 yrs, SD = 11.7)	- CPT, Identical Pairs and Flanker version - WCST - COWAT - Auditory Consonant Trigram - Category Fluency Test - Face Memory Test - Strategic Target Detection Test - Auditory Number Sequencing - Digit Span (forward, backward) - Penn's Emotional Acuity Test - Spatial Working Memory Test	No significant effects

Scoriels et al 2012	Modafinil (200mg/d)	Randomized placebo-controlled double-blind cross-over add-on trial Single dose	N = 40 first psychotic episode patients (mean age = 25.0 yrs, SD = 2.0)	- CANTAB: Spatial Working Memory task, the digit span test Paired Associates Learning - Stop Signal Task (SST) or motor inhibitory control - Information Sampling Test - HVLT - Intra/Extradimensional set shifting task Rapid - visual information processing test - Category fluency	Significant enhancing effect on: - Digit span Backward (ES = 0.24) - Spatial Working Memory Task (strategy score Cohen's d = 0.23, Errors Cohen's d = 0.30) - SST: discrimination errors (Cohen's d= 0.30)
	Anti-inflammatory Drugs				
Laan et al 2010	Aspirin (1000mg/d)	Randomized double blind placebo-controlled add-on trial 3 months	N = 70 schizophrenia patients (N = 33 aspirin mean age = 31.6 yrs, SD = 8.9 N 37 placebo mean age = 30.6 yrs, SD = 9.2)	- RAVLT - Continuous Performance test - Purdue Pegboard test - Trail making test	No significant effects
Javitt et al 2012	Davunetide (5mg/d or 30mg/d)	Randomized double-blind placebo-controlled two-dose add-on trial 12 weeks	N = 55 schizophrenia patients (N = 17 minocylcine 5mg mean age = 43.2 yrs, SD = 10.5 N = 19 minocycline 30mg mean age = 45.2 yrs, SD = 8.2 N = 19 placebo mean age = 41.4 yrs, SD = 10.4)	- MATRICS test battery - UCSD = Performance-based Skills Assessment	No significant effects
Chaudry et al 2012	Minocycline (200mg/d)	Randomized double-blind placebo-controlled add-on trial 1 year	N = 144 early psychosis patients (N = 71 minocycline mean age = 25.9 yrs, SD = 7.0 N = 73 placebo mean age = 26.6 yrs, SD = 8.3)	- CANTAB	No significant effects
Liu et al 2014	Minocycline (200mg/d)	Randomized double-blind placebo-controlled add-on trial 16 weeks	N = 79 schizophrenia patients, early stage (N = 39 minocycline mean age = 27.0 yrs, SD = 5.7 N = 40 placebo mean age = 27.7 yrs, SD = 7.3)	- MATRICS test battery	Significant enhancing effect on: - CPT

*ES= effect size; N=The number of patients that are included in the analyses; yrs=years; SD=standard deviation

Table 2. Overview of cognitive effects of lithium, anticonvulsants and antipsychotics in bipolar disorder

Author	Agent and Dose	Design and follow-up	Participants	Cognitive measures	Outcome
	Lithium				
Smigan and Perris 1983	Lithium (mean serum levels approximately 0.6 mmol/l)	Follow-up (4 and 12 months)	N = 53 patients with affective disorders (median age total group = 42 yrs, N = 20 bipolar disorder (BD) N = 16 unipolar N = 10 cycloid psychosis N = 5 recurrent depression N = 2 schizo-affective psychoses)	- 30 Figure Test, subtest Cronhol-Molander test battery - 30 Word-Pair Test, subtest Cronhol-Molander test battery - 30 Person-Data Test, subtest Cronholm-Molander test battery - 30 Face Test, subtest Face Memory Test	Increased immediate (z=-2.0) and delayed (z=-2.5) scores on the 30 Person-Data Test after 4 months Increased immediate (4 months: z=-3.4; 12 months z=-3.3) and delayed (4 months: z=-2.0; 12 months: z=-2.3) scores on the 30 Face Test after 4 and 12 months
Engelsmann et al. 1988	Lithium (mean serum levels 0.61 mEq/liter)	Follow-up (6 years)	N = 18 BD patients (mean age = 45.8 yrs, SD = 13.7)	- Wechsler Memory Scale (WMS) - Benton Visual Retention Test (BVRT)	No significant re-testing differences after 6 years
López-Jaramillo et al. 2010	Lithium (serum levels 0.6-1.2 mEq/L)	Cross-sectional	N = 20 euthymic patients with bipolar disorder on lithium (median age = 38.5 yrs) N = 20 euthymic patients with bipolar disorder on no medication (median age = 40 yrs) N = 20 healthy controls (median age = 39.5 yrs)	- TMT A and B - Stroop test-interference - Semantic verbal fluency - Phonological verbal fluency - Continuous Visual Performance Test - Rey Figure-immediate recall - Test for associative memory with semantic increase - Wisconsin Card Sorting Test (WCST; abbreviated version) - WMS - Wechsler Adult Intelligence Scale - California Verbal Learning Task (CVLT)	No significant difference between BD patients on lithium and unmedicated BD patients BD patients on lithium had lower scores than healthy controls on: - the cued recall (ES=1.00) and cued delayed recall (ES=0.90) of the test for associative memory with semantic increase - recognition of logical memory (ES=1.35) of the WMS - free short recall (ES=0.99), cued short recall (ES=0.87) and cued delayed recall (ES=1.81) of the CVLT Unmedicated BD patients had lower scores than healthy controls on: - the cued short recall (ES=0.85) and cued delayed recall (ES=0.89) of the test for associative memory with semantic increase - the backward digits (ES=0.79) and recognition of logical memory (ES=1.29) of the WMS - the free short recall (ES=1.28), cued short recall (ES=0.78) and cued delayed recall (ES=0.80) of the CVLT

Arts et al. 2011	- Lithium (mean serum levels=0.76) - Valproic acid (mean serum levels=76.7) - Carbamazepine (mean serum levels=9.0) - Lamotrigine (mean serum levels=2.7) - Second-generation antipsychotics (no serum levels reported)	Naturalistic study of 2 years (two monthly intervals)	N = 39 patients with bipolar spectrum disorder (mean age at baseline = 44.7 yrs, SD = 7.9) N = 61 controls (mean age at baseline = 45.3 yrs, SD = 8.7)	- Subtest Mental Rotation of the Groningen Intelligence Test (GIT) - Subtest Word Analogies (GIT) - Subtest Mental Arithmetic (GIT) - Visual Verbal Learning Test - Continuous performance test (CPT-HQ version) - Flanker CPT - Tapping Speed test - Digit Span Forward and Digit Span Backward (WAIS-III)	Lithium was not associated with significant effects on cognitive function. Duration of lithium and lithium use at baseline was significantly positively associated with Tapping rates on the Tapping speed test. Negative effects of lithium in the last 2 months before interview occasion on the Flanker CPT The use of anticonvulsants was not significantly associated with cognitive test scores The use of antipsychotics was negatively associated with scores on the Tapping speed test and the Flanker CPT
Schouws et al. 2010	Lithium (doses not reported)	Cross-sectional	N = 119 euthymic BD patients (mean age total group = 70.4 yrs, SD = 7.2; N=94 bipolar disorder type I; bipolar disorder type II, N=19; rapid cycling disorder, N=6)	- Mini Mental State Examination (MMSE) - Digit Span (WAIS-III) - TMT A and B - The Amsterdam Short Term Memory Test - The 10 Words test - Figure Copying subtest of the Amsterdam Dementia Screening Test - Clock Drawing - Modified version of the Stroop Color Word Test - Mazes (1 to 4) subtest of the Wechsler Intelligence Scale for Children - Rule Shift Cards subtest of the Behavorial Assessment of the Dysexecutive Syndrome - Control Oral Word Association Test (COWAT) - Animal and Occupation Naming subtest of the GIT - Auditory verbal learning test	Lithium was not associated with any cognitive measure when controlled for risk factors

Savitz et al. 2008	- Lithium - Mood stabilizers other than lithium - Antipsychotics (doses not reported)	Cross-sectional study	N = 230 largely euthymic participants from 47 families (mean age total group = 47.9 yrs, SD = 17.3 N = 49 patients with BD-I mean age = 47.8 yrs, SD = 15.1 N = 19 patients with BD-II mean age = 36.7 yrs, SD = 14.9 N = 44 patients with recurrent major depression mean age = 47.5 yrs, SD = 15.6 , N=33 patients with a single lifetime episode of depression, mean age = 51.9 yrs, SD = 17.2 N = 20 patients with another DSM-IV diagnosis mean age=not reported N = 65 unaffected relatives, mean age = 50.1 yrs, SD = 19.5)	- General knowledge subtest of the South African Wechsler Adult Intelligence Scale - Digit span (forward and reverse) - Controlled Oral Word Association Test (COWAT) - RCF - Stroop Color-Word Task - Rey Auditory Verbal Learning Test (RAVLT) - WCST	Lithium use was significantly associated with poorer performance on: - Stroop Color-Word task - recognition memory of the RAVLT Antipsychotics were significantly associated with: - poorer performance on the Stroop Color-Word task - poorer performance on the recognition memory of the RAVLT - a greater number of perseverative errors on the WCST
Holmes et al. 2008	- Lithium (serum level 0.6-1.2 mEq/L) - Valproic acid (serum level 50-125 mg/mL)	Cross-sectional study	N = 33 patients with bipolar depression on lithium or valproic acid (mean age = 41.1 yrs, SD = 10.9) N = 32 patients with bipolar depression who were unmedicated (mean age = 35.3 yrs, SD = 8.7) N = 52 healthy controls (mean age = 37.0 yrs, SD = 10.1)	- Rapid Visual Information Processing (CANTAB) - Pattern Recognition Memory (CANTAB) - Spatial Working Memory (CANTAB) - Wechsler Abbreviated Scale of Intelligence - Affective Shift	Medicated BD patients made significantly more omission errors in the happy shift condition of the Affective Shift than unmedicated BD patients and healthy controls Reaction time on the Affective Shift was significantly slower in medicated patients compared with unmedicated patients and controls. Medicated BD patients made significantly more omission errors in the CANTAB Rapid Visual Information Processing than healthy controls and had longer response latency than unmedicated BD patients and HC
Rybakowski and Suwalska 2010	Lithium (serum levels between 0.5 and 0.8 mmol/l)	Cross-sectional study	N = 60 euthymic patients with bipolar disorder (mean age = 52.6 yrs, SD = 10.2 N = 13 excellent lithium responders mean age = 51.3 yrs, SD = 12.1 N = 47 non-excellent lithium responders mean age = 52.9 yrs, SD = 9.8) N = 60 matched controls (mean age = 52.1 yrs, SD = 13.6)	Cambridge Neuropsychological Test Automated Battery (CANTAB): - Spatial Working Memory (SWM) - Spatial Span (SSP) - Stockings of Cambridge (SOC) - Rapid Visual Information (RVP); RVP A' and RVP B'	Excellent lithium responders did not significantly differ from controls on the CANTAB subtests Non-excellent lithium responders had significantly poorer scores than controls on: - the SSP span length - SWM between errors - SWM strategy - RVP A' - RVP mean latency - SOC mean initial thinking time Non-excellent lithium responders had significant lower scores on the SSP span length than excellent lithium responders

Rybakowski et al. 2009	Lithium (serum levels were not reported)	Cross-sectional study	N = 30 euthymic patients with bipolar disorder (mean age = 54 yrs, SD = 6 N = 6 excellent lithium responders N = 17 partial responders N = 7 non-responders) N = 30 matched healthy controls	Wisconsin Card Sorting Test: - The percentage of perseverative errors (WCST-P) - The percentage of non-perseverative errors (WCST-NP) - The number of correctly completed categories (WCST-CC) - The percentage of conceptual level responses (WCST-%conc) - The set of the first category (WCST-1st cat)	Non-responders had significantly poorer scores than lithium responders and controls on: - WCST-P - WCST-%conc
	Anticonvulsants				
Senturk et al. 2007	- Lithium (600-1800 mg/day and serum levels=0.63 mEq/L) - Valproate (500-2000 mg/day and serum levels 70.07µg/mL)	Cross-sectional	N = 28 euthymic patients with bipolar disorder (N = 17 patients on lithium monotherapy mean age = 34.9 yrs, SD = 10.3 N = 11 patients on valproate monotherapy, mean age = 27.9 yrs, SD = 7.3) N=29 healthy controls (mean age = 28.5 yrs, SD = 6.3)	- Perseverative errors (WCST) - Non-perseverative errors (WCST) - Categories completed (WCST) - Logical Memory Subscale (WMS) - Digit Symbol Substitution (WAIS-R) - Arithmetic (WAIS-R) - Block design (WAIS-R)	Both patients on lithium and valproate had significantly worse scores on the Logical Memory Subscale compared with controls (ES=0.17). The patient groups did not differ from each other
Joffe et al. 1988	- Lithium (900 to 1500 mg daily and serum levels between 0.7 and 0.9 mmol/l) - Carbamazepine (800 to 1200 mg daily and blood levels within the therapeutic range)	Cross-sectional	N = 34 euthymic patients with bipolar disorder (N = 18 lithium mean age = 36.4 yrs, SD = 11.5 N = 18 carbamazepine mean age = 39.4 yrs, SD = 10.6 N = 12 medication free mean age = 35.8 yrs, SD = 7.8) N = 15 normal controls (mean age = 33.3 yrs, SD = 11.5)	- The Digit Symbol Test of the WAIS - TMT - The Cancellation Test - The Test of Selected Reminding and Restricted Reminding (Buschke)	No significant difference was found on any of the cognitive tests between patients on lithium, patients on carbamazepine, medication free patients and healthy controls
Gualtieri and Johnson 2006	- Carbamazepine - Lamotrigine - Oxcarbazepine - Topiramate - Valproic acid - Lithium (doses were not reported)	Cross-sectional	N = 159 patients with bipolar disorder (N = 16 Carbamazepine mean age = 43.2 yrs N = 38 Lamotrigine mean age = 42.1 yrs N = 30 Lithium mean age = 30.6 yrs N = 19 Oxcarbazepine mean age = 36.5 yrs Topiramate N = 19 mean age = 41.1 yrs Valproic acid N = 37 mean age = 41.2 yrs)	CNS Vital Signs (CNSVS), composed of: - Verbal and visual memory - Finger tapping - Symbol-digit coding - Stroop Test - Shifting Attention Test - Continuous Performance Test	Patients on lamotrigine had the best scores on the (summarized) scores of CNSVS, followed in order by patients on oxcarbazepine, lithium, topiramate, valproic acid and carbamazepine

Daban et al. 2006	- Lamotrigine - Carbamazepine - Valproate (doses were not reported)	Cross-sectional	N = 33 euthymic patients with bipolar disorder (N = 15 lamotrigine mean age = 43.8 yrs, SD = 7.7 N = 18 carbamazepine or valproate mean age = 40.3 yrs, SD = 8.6)	- CVLT - WCST - Verbal Fluency - Stroop Test - TMT - Digits (WAIS)	Patients on lamotrigine had significantly higher scores on the phonemic task of the verbal fluency compared with patients on other anticonvulsants (Cohen's d=1.01)
Khan et al. 2004	Lamotrigine (target dose=200 mg/day and minimum dose = 100 mg/day)	Two trials in the open-label phase, 8- to 16-week	N = 664 patients with bipolar disorder type I (N = 480 patients with an index depressive episode, mean age = 42.2 yrs, SD = 12.2 N = 184 patients with an index hypomanic/ manic/mixed episode, mean age = 40.7 yrs, SD = 11.8)	- 4 items of the Medical Outcomes Study Cognitive Scale (MOS-Cog) - AB-Neurological Assessments Scale (AB-NAS)	Improved MOS-cog and AB-NAS scores
Kaye et al. 2007	Lamotrigine (200 mg/d)	Open-label trial, assessment of cognitive function during 2 periods (baseline and after 12 weeks)	N = 912 patients with bipolar disorder type I (mean age = 42.2 yrs, SD = 13.1)	- MOS-Cog	Improved MOS-Cog scores (independent of concomitant valproate, antipsychotics or antidepressants)
	Antipsychotics				
Donaldson et al. 2003	- Antipsychotics (mean dose 384.5 mg chlorpromazine equivalents (CPZ)) (Typical and atypical antipsychotics) - Lithium (mean dose 936.3 mg) - Sodium valproate (mean dose 833.3 mg) - Carbamazepine (mean dose 800.0 mg)	Cross-sectional	N = 43 euthymic patients with bipolar disorder type I (mean age = 42.9 yrs, SD = 11.1 N = 22 patients on antipsychotics)	- Digit Span (WAIS-R) - Vocabulary (WAIS-R) - Arithmetic (WAIS-R) - Similarities (WAIS-R) - Picture completion (WAIS-R) - Picture arrangement (WAIS-R) - Block Design (WAIS-R) - The National Adult Reading Test - WMS	Use of antipsychotics was associated with: - lower IQ (as measured by the WAIS-R; r=-0.57) - lower WMS scores (r=-0.3) - lower working memory index scores (r=-0.32)

Jamrozinsky et al. 2009	Antipsychotics (doses between 100 and 1600 units with an average of 601.67 CPZ units) (type of antipsychotic was not indicated)	Cross-sectional	N = 40 euthymic patients with bipolar disorder type I (mean age total group = 43.5 yrs, SD = 12.2 N = 18 antipsychotic medication mean age = 44.2 yrs, SD = 10.4 N = 22 patients without antipsychotics mean age = 42.8 yrs, SD = 13.6) N = 40 healthy controls (mean age = 41.3 yrs, SD = 11.9)	- Regensburger Wortflüssigkeitstest (RWT) - Verbaler Lern- und - Merkfähigkeitstest (VLMT) - WCST - TOL - Focused attention, subtest of the Attention Test Battery (TAP) - TMT (Zahlenverbindungstest)	Patients without antipsychotic treatment did not differ from healthy controls on the cognitive measures Patients without antipsychotic treatment performed significantly better than patients on antipsychotics on: - TMT - RWT semantic fluency - VLMT - WCST number of trials - TOL, score - TAP hits -false positives Patients on antipsychotics had significantly lower scores than controls on: - TMT - RWT semantic fluency - VLMT verbal learning and recognition - WCST number of trials - TOL - TAP

Torrent et al. 2011	- Quetiapine (404.1 mg/day) - Olanzapine (7.7 mg/day) - Risperidone (3.7 mg/day)	Cross-sectional study	N = 84 euthymic BD patients (N = 12 quetiapine mean age = 45.6 yrs, SD = 5.7 N = 26 olanzapine mean age = 41.2, SD = 14.2 N = 30 risperidone mean age = 38.0 yrs, SD = 10.5 N = 16 drug-free mean age = 42.1 yrs, SD = 14.6) N = 35 healthy controls (mean age = 39.1 yrs, SD = 12.1)	- WCST - Stroop Color-Word InterferenceTest - Controlled Oral Word Association Test (FAS) including the animal naming subtests - Digit subtest (WAIS) - TMT - CVLT	All the medicated groups had significantly lower scores on animal naming than controls and unmedicated patients Patients using risperidone and olanzapine had significantly lower scores than controls on: - FAS - TMT-A - CVLT Patients using olanzapine had significantly lower scores than controls on: - TMT-B - Digits backward The risperidone group had lower scores on the Stroop test compared with controls The olanzapine and risperidone groups performed significantly worse on the verbal measures than the unmedicated group The olanzapine group had significantly lower scores on the recognition task of the CVLT than the unmedicated group
Shi et al. 2004	Olanzapine (5-20 mg/day)	Two double-blind, randomized, placebo-controlled trials, 3 weeks	N = 244 patients with bipolar disorder type I (N = 124 olanzapine-treated patients mean age = 39.4 yrs, N = 120 placebo-treated patients mean age = 38.8 yrs)	- PANSS cognition score	Patients on olanzapine had increased PANSS cognition scores relative to patients on placebo
Rakofsky et al. 2014	Quetiapine extended release (200-400 mg)	6 week randomized placebo-controlled add-on trial	N = 5 euthymic BD patients on quetiapine extended release N = 9 euthymic BD patients on placebo (mean age was not reported)	- The MATRICS version of the Continuous Performance Test Identical Pairs (CPT-IP) - the Brief Assessment of Cognition in Affective Disorders (BAC-A) - the Brief UCSD Performance-Based Skills Assessment (UPSA-B)	Patients on quetiapine extended release did not show significantly improved cognitive function after 6 weeks Patients on placebo had significantly improved scores from baseline on: - CPT-IP - BAC-A components (digit sequencing, Token Motor Task, symbol coding) - BAC-A composite score - UPSA-B

*ES= effect size; N=The number of patients that are included in the analyses; BD=bipolar disorder; yrs=years; SD=standard deviation

References

Anand A, Charney DS, Oren DA, Berman RM, Hu XS, Cappiello A, Krystal JH (2000). Attenuation of the Neuropsychiatric Effects of Ketamine With Lamotrigine. *Archives of General Psychiatry* **57**, 270.

Anticevic A, Gancsos M, Murray JD, Repovs G, Driesen NR, Ennis DJ, Niciu MJ, Morgan PT, Surti TS, Bloch MH, Ramani R, Smith M a, Wang X-J, Krystal JH, Corlett PR (2012). NMDA receptor function in large-scale anticorrelated neural systems with implications for cognition and schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 16720–5.

Arts B, Jabben N, Krabbendam L, Van Os J (2011). A 2-year naturalistic study on cognitive functioning in bipolar disorder. *Acta Psychiatrica Scandinavica* **123**, 190–205.

BALANCE Investigators and collaborators, Geddes JR, Goodwin GM, Rendell J, Azorin JM, Cipriani A, Ostacher MJ, Morriss R, Alder N, Juszczak E (2010). Lithium plus valproate combination therapy versis monotherapy for relapse prevention in bipolar I disorder (BALANCE): a randomised open-label trial. *Lancet* **375**, 385-395.

Baldessarini RJ, Tondo L (2000). Does lithium treatment still work?: Evidence of stable responses over three decades. *Archives of General Psychiatry* **57**, 187–190.

Barch DM, Carter CS (2005). Amphetamine improves cognitive function in medicated individuals with schizophrenia and in healthy volunteers. *Schizophrenia Research* **77**, 43–58.

Barr RS, Culhane MA, Jubelt LE, Mufti RS, Dyer MA, Weiss AP, Deckersbach T, Kelly JF, Freudenreich O, Goff DC, Evins AE (2008). The effects of transdermal nicotine on cognition in nonsmokers with schizophrenia and nonpsychiatric controls. *Neuropsychopharmacology* **33**, 480–490.

Berk M, Copolov D, Dean O, Lu K, Jeavons S, Schapkaitz I, Anderson-Hunt M, Judd F, Katz F, Katz P, Ording-Jespersen S, Little J, Conus P, Cuenod M, Do KQ, Bush AI (2008). N-Acetyl Cysteine as a Glutathione Precursor for Schizophrenia-A Double-Blind, Randomized, Placebo-Controlled Trial. *Biological Psychiatry* **64**, 361–368.

Bobo W V., Woodward ND, Sim MY, Jayathilake K, Meltzer HY (2011). The effect of adjunctive armodafinil on cognitive performance and psychopathology in antipsychotic-treated patients with schizophrenia/schizoaffective disorder: A randomized, double-blind, placebo-controlled trial. *Schizophrenia Research* **130**, 106–113.

Boggs DL, Kelly DL, McMahon RP, Gold JM, Gorelick DA, Linthicum J, Conley RR, Liu F, Waltz J, Huestis MA, Buchanan RW (2012). Rimonabant for neurocognition in schizophrenia: A 16-week double blind randomized placebo controlled trial. *Schizophrenia Research* **134**, 207–210.

Boks MPM, Rietkerk T, van de Beek MH, Sommer IE, de Koning TJ, Kahn RS (2007). Reviewing the role of the genes G72 and DAAO in glutamate neurotransmission in schizophrenia. *European Neuropsychopharmacology* **17**, 567–572.

Bowlby MR (1993). Pregnenolone sulfate potentiation of N-methyl-D-aspartate receptor channels in hippocampal neurons. *Molecular pharmacology* **43**, 813–819.

Breese CR, Lee MJ, Adams CE, Sullivan B, Logel J, Gillen KM, Marks MJ, Collins AC, Leonard S (2000). Abnormal regulation of high affinity nicotinic receptors in subjects with schizophrenia. *Neuropsychopharmacology* **23**, 351–364.

Buchanan RW, Conley RR, Dickinson D, Ball MP, Feldman S, Gold JM, McMahon RP (2008). Galantamine for the treatment of cognitive impairments in people with schizophrenia. *The American journal of psychiatry* **165**, 82–9.

Buchanan RW, Davis M, Goff D, Green MF, Keefe RSE, Leon AC, Nuechterlein KH, Laughren T, Levin R, Stover E, Fenton W, Marder SR (2005). A summary of the FDA-NIMH-MATRICS workshop on clinical trial design for neurocognitive drugs for schizophrenia. In *Schizophrenia Bulletin* vol 31, pp5–19.

Buchanan RW, Javitt DC, Marder SR, Schooler NR, Gold JM, McMahon RP, Heresco-Levy U, Carpenter WT (2007). The Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST): The efficacy of glutamatergic agents for negative symptoms and cognitive impairments. *American Journal of Psychiatry* **164**, 1593–1602.

Buchanan RW, Keefe RS, Lieberman JA, Barch DM, Csernansky JG, Goff DC, Gold JM, Green MF, Jarskog LF, Javitt DC, Kimhy D, Kraus MS, McEvoy JP, Mesholam-Gately RI, Seidman LJ, Ball MP, McMahon RP, Kern RS, Robinson J, Marder SR (2011). A randomized clinical trial of MK-0777 for the treatment of cognitive impairments in people with schizophrenia. *Biological Psychiatry* **69**, 442–449.

Buchanan RW, Summerfelt A, Tek C, Gold J (2003). An open-labeled trial of adjunctive donepezil for cognitive impairments in patients with schizophrenia. *Schizophrenia Research* **59**, 29–33.

Carpenter WT, Gold JM (2002). Another view of therapy for cognition in schizophrenia. *Biological Psychiatry* **51**, 969–971.

Chaudhry IB, Hallak J, Husain N, Minhas F, Stirling J, Richardson P, Dursun S, Dunn G, Deakin B (2012). Minocycline benefits negative symptoms in early schizophrenia: a randomised double-blind placebo-controlled clinical trial in patients on standard treatment. *Journal of psychopharmacology (Oxford, England)* **26**, 1185–93.

Chew LJ, Fusar-Poli P, Schmitz T (2013). Oligodendroglial alterations and the role of microglia in white matter injury: Relevance to schizophrenia. *Developmental Neuroscience* **35,** 102–129.

Chouinard S, Stip E, Poulin J, Melun J-P, Godbout R, Guillem F, Cohen H (2007). Rivastigmine treatment as an add-on to antipsychotics in patients with schizophrenia and cognitive deficits. *Current Medical Research and Opinion* **23**, 575–583.

Crook JM, Tomaskovic-Crook E, Copolov DL, Dean B (2001). Low muscarinic receptor binding in prefrontal cortex from subjects with schizophrenia: A study of brodmann's areas 8, 9, 10, and 46 and the effects of neuroleptic drug treatment. *American Journal of Psychiatry* **158**, 918–925.

D'Souza MS, Markou A (2012). Schizophrenia and tobacco smoking comorbidity: NAChR agonists in the treatment of schizophrenia-associated cognitive deficits. *Neuropharmacology* **62**, 1564–1573.

Daban C, Martínez-Arán A, Torrent C, Sánchez-Moreno J, Goikolea JM, Benabarre A, Comes M, Colom F, Vieta E (2006). Cognitive functioning in bipolar patients receiving lamotrigine: Preliminary results. *Journal of Clinical Psychopharmacology* **26**, 178–181.

Davidson M, Galderisi S, Weiser M, Werbeloff N, Fleischhacker WW, Keefe RS, Boter H, Keet IPM, Prelipceanu D, Rybakowski JK, Libiger J, Hummer M, Dollfus S, López-Ibor JJ, Hranov LG, Gaebel W, Peuskens J, Lindefors N, Riecher-Rössler A, Kahn RS (2009). Cognitive effects of antipsychotic drugs in first-episode schizophrenia and schizophreniform disorder: a randomized, open-label clinical trial (EUFEST). *American Journal of Psychiatry* **166**, 675–682.

Davidson M, Harvey PD, Bergman RL, Powchik P, Kaminsky R, Losonczy MF, Davis KL (1990). Effects of the D-1 Agonist SKF-38393 Combined with Haloperidol in Schizophrenic Patients. *Archives of General Psychiatry* **47**, 190–191.

Davis KL, Kahn RS, Ko G, Davidson M (1991). Dopamine in schizophrenia: A review and reconceptualization. *American Journal of Psychiatry* **148**, 1474–1486.

Donaldson S, Goldstein LH, Landau S, Raymont V, Frangou S (2003). The Maudsley Bipolar Disorder Project: The effect of medication, family history, and duration of illness on IQ and memory in bipolar I disorder. *Journal of Clinical Psychiatry* **64**, 86–93.

Drexhage RC, Weigelt K, van Beveren N, Cohen D, Versnel MA, Nolen WA, Drexhage HA (2011). Immune and Neuroimmune Alterations in Mood Disorders and Schizophrenia. *International Review of Neurobiology* **101**, 169–201.

Egan MF, Zhao X, Gottwald R, Harper-Mozley L, Zhang Y, Snavely D, Lines C, Michelson D (2013). Randomized crossover study of the histamine H3 inverse agonist MK-0249 for the treatment of cognitive impairment in patients with schizophrenia. *Schizophrenia Research* **146**, 224–230.

Elvevag B, Goldberg TE (2000). Cognitive Impairment in Schizophrenia Is the Core of the Disorder. *Critical Reviews™ in Neurobiology* **14**, 21.

Engelsmann F, Katz J, Ghadirian AM, Schachter D (1988). Lithium and memory: a long-term follow-up study. *Journal of clinical psychopharmacology* **8**, 207–12.

Ernst M, Heishman SJ, Spurgeon L, London ED (2001). Smoking history and nicotine effects on cognitive performance. *Neuropsychopharmacology* **25**, 313–319.

Fagerlund B, Søholm B, Fink-Jensen A, Lublin H, Glenthøj BY (2007). Effects of donepezil adjunctive treatment to ziprasidone on cognitive deficits in schizophrenia: A double-blind, placebo-controlled study. *Clinical Neuropharmacology* **30**, 3–12.

Fineberg AM, Ellman LM (2013). *Inflammatory cytokines and neurological and neurocognitive alterations in the course of schizophrenia*. *Biological Psychiatry* **73**, 951–966.

Freedman R, Hall M, Adler LE, Leonard S (1995). Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. *Biological Psychiatry* **38**, 22–33.

Freedman R, Olincy A, Buchanan RW, Harris JG, Gold JM, Johnson L, Allensworth D, Guzman-Bonilla A, Clement B, Ball MP, Kutnick J, Pender V, Martin LF, Stevens KE, Wagner BD, Zerbe GO, Soti F, Kem WR (2008). Initial phase 2 trial of a nicotinic agonist in schizophrenia. *American Journal of Psychiatry* **165**, 1040–1047.

Freudenreich O, Henderson DC, Macklin EA, Evins AE, Fan X, Cather C, Walsh JP, Goff DC (2009). Modafinil for clozapine-treated schizophrenia patients: a double-blind, placebo-controlled pilot trial. *The Journal of clinical psychiatry* **70**, 1674–80.

Freudenreich O, Herz L, Deckersbach T, Evins AE, Henderson DC, Cather C, Goff DC (2005). Added donepezil for stable schizophrenia: A double-blind, placebo-controlled trial. *Psychopharmacology* **181**, 358–363.

Friedman JI, Adler DN, Howanitz E, Harvey PD, Brenner G, Temporini H, White L, Parrella M, Davis KL (2002). A double blind placebo controlled trial of donepezil adjunctive treatment to risperidone for the cognitive impairment of schizophrenia. *Biological Psychiatry* **51**, 349–357.

Friedman JI, Adler DN, Temporini HD, Kemether E, Harvey PD, White L, Parrella M, Davis KL (2001). Guanfacine treatment of cognitive impairment in schizophrenia. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **25**, 402–9.

Friedman JI, Carpenter D, Lu J, Fan J, Tang CY, White L, Parrella M, Bowler S, Elbaz Z, Flanagan L, Harvey PD (2008). A pilot study of adjunctive atomoxetine treatment to second-generation antipsychotics for cognitive impairment in schizophrenia. *Journal of Clinical Psychopharmacology* **28**, 59–63.

Furey ML, Pietrini P, Haxby J V. (2000). Cholinergic enhancement and increased selectivity of perceptual processing during working memory. *Science* **290**, 2315–2319.

Gale CR, Batty GD, Mcintosh AM, Porteous DJ, Deary IJ, Rasmussen F (2013). Is bipolar disorder more common in highly intelligent people A cohort study of a million men. *Molecular Psychiatry* **18**, 190–194.

George MS, Molnar CE, Grenesko EL, Anderson B, Mu Q, Johnson K, Nahas Z, Knable M, Fernandes P, Juncos J, Huang X, Nichols DE, Mailman RB (2007). A single 20 mg dose of dihydrexidine (DAR-0100), a full dopamine D1agonist, is safe and tolerated in patients with schizophrenia. *Schizophrenia Research* **93**, 42–50.

Goff DC, Herz L, Posever T, Shih V, Tsai G, Henderson DC, Freudenreich O, Evins AE, Yovel I, Zhang H, Schoenfeld D (2005). A six-month, placebo-controlled trial of d-cycloserine co-administered with conventional antipsychotics in schizophrenia patients. *Psychopharmacology* **179**, 144–150.

Goff DC, Keefe R, Citrome L, Davy K, Krystal JH, Large C, Thompson TR, Volavka J, Webster EL (2007). Lamotrigine as Add-On Therapy in Schizophrenia. *Journal of Clinical Psychopharmacology* **27**, 582–589.

Goff DC, Lamberti JS, Leon AC, Green MF, Miller AL, Patel J, Manschreck T, Freudenreich O, Johnson SA (2008). A placebo-controlled add-on trial of the Ampakine, CX516, for cognitive deficits in schizophrenia. *Neuropsychopharmacology* **33**, 465–472.

Goff DC, Tsai G, Levitt J, Amico E, Manoach D, Schoenfeld DA, Hayden DL, McCarley R, Coyle JT (1999). A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. *Archives of General Psychiatry* **56**, 21–27.

Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams G V, Castner PSGSA, Svensson TH, Siever LJ, Williams G V, Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams G V, Castner PSGSA, Svensson TH, Siever LJ, Williams G V, Goldman-Rakic PS, Castner SA, Svensson

TH, Siever LJ, Williams GV (2004). Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. *Psychopharmacology* **174**, 3–16.

Green MF (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *The American journal of psychiatry* **153**, 321–30.

Green MF, Kern RS, Braff DL, Mintz J (2000). Neurocognitive Deficits and Functional Outcome in Schizophrenia: Are We Measuring the 'Right Stuff'? *Schizophrenia Bulletin* **26**, 119–136.

Gualtieri CT, Johnson LG (2006). Comparative neurocognitive effects of 5 psychotropic anticonvulsants and lithium. *MedGenMed : Medscape general medicine* **8**, 46.

Haig GM, Bain E, Robieson W, Othman AA, Baker J, Lenz RA (2014). A randomized trial of the efficacy and safety of the H3 antagonist ABT-288 in cognitive impairment associated with schizophrenia. *Schizophrenia Bulletin* **40**, 1433–1442.

Harris JG, Kongs S, Allensworth D, Martin L, Tregellas J, Sullivan B, Zerbe G, Freedman R (2004). Effects of nicotine on cognitive deficits in schizophrenia. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **29**, 1378–1385.

Hartigan GP (1963). The Use of Lithium Salts in Affective Disorders. *The British Journal of Psychiatry* **109**, 810–814.

Hedman AM, van Haren NEM, van Baal CGM, Kahn RS, Hulshoff Pol HE (2013). IQ change over time in schizophrenia and healthy individuals: A meta-analysis. *Schizophrenia Research* **146**, 201–208.

Heresco-Levy U, Ermilov M, Lichtenberg P, Bar G, Javitt DC (2004). High-dose glycine added to olanzapine and risperidone for the treatment of schizophrenia. *Biological Psychiatry* **55**, 165–171.

Heresco-Levy U, Javitt DC, Ebstein R, Vass A, Lichtenberg P, Bar G, Catinari S, Ermilov M (2005). D-serine efficacy as add-on pharmacotherapy to risperidone and olanzapine for treatment-refractory schizophrenia. *Biological Psychiatry* **57**, 577–585.

Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Horowitz a, Kelly D (1996). Double-blind, placebo-controlled, crossover trial of glycine adjuvant therapy for treatment-resistant schizophrenia. *The British journal of psychiatry : the journal of mental science* **169**, 610–617.

Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Silipo G, Lichtenstein M (1999). Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. *Archives of General Psychiatry* **56**, 29–36.

Holmes MK, Erickson K, Luckenbaugh DA, Drevets WC, Bain EE, Cannon DM, Snow J, Sahakian BJ, Manji HK, Zarate CA (2008). A comparison of cognitive functioning in medicated and unmedicated subjects with bipolar depression. *Bipolar Disorders* **10**, 806–815.

International Schizophrenia Consortium, Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, Sklar P (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **460**, 748–52.

Jamrozinski K, Gruber O, Kemmer C, Falkai P, Scherk H (2009). Neurocognitive functions in euthymic bipolar patients. *Acta Psychiatrica Scandinavica* **119**, 365–374.

Javitt DC (1999). Treatment of negative and cognitive symptoms. *Current psychiatry reports* **1**, 25–30.

Javitt DC (2007). Glutamate and Schizophrenia: Phencyclidine, N-Methyl-d-Aspartate Receptors, and Dopamine-Glutamate Interactions. *International Review of Neurobiology* **78**, 69–108.

Javitt DC, Buchanan RW, Keefe RSE, Kern R, McMahon RP, Green MF, Lieberman J, Goff DC, Csernansky JG, McEvoy JP, Jarskog F, Seidman LJ, Gold JM, Kimhy D, Nolan KS, Barch DS, Ball MP, Robinson J, Marder SR (2012). Effect of the neuroprotective peptide davunetide (AL-108) on cognition and functional capacity in schizophrenia. *Schizophrenia Research* **136**, 25–31.

Javitt DC, Zukin SR (1991). Recent advances in the phencyclidine model of schizophrenia. *American Journal of Psychiatry* **148**, 1301–1308.

Javitt DC, Zylberman I, Zukin SR, Heresco-Levy U, Lindenmayer JP (1994). Amelioration of negative symptoms in schizophrenia by glycine. *American Journal of Psychiatry* **151**, 1234–1236.

Joffe RT, MacDonald C, Kutcher SP (1988). Lack of differential cognitive effects of lithium and carbamazepine in bipolar affective disorder. *Journal of clinical psychopharmacology* **8**, 425–428.

Jubelt LE, Barr RS, Goff DC, Logvinenko T, Weiss AP, Evins AE (2008). Effects of transdermal nicotine on episodic memory in non-smokers with and without schizophrenia. *Psychopharmacology* **199**, 89–98.

Kahn RS, Keefe RSE (2013). Schizophrenia is a cognitive illness: Time for a change in focus. *JAMA Psychiatry* **70**, 1107–1112.

Kahn RS, Sommer IE (2014). The neurobiology and treatment of first-episode schizophrenia. *Molecular Psychiatry* **20**, 84–97.

Kane JM, D'Souza DC, Patkar AA, Youakim JM, Tiller JM, Yang R, Keefe RSE (2010). Armodafinil as adjunctive therapy in adults with cognitive deficits associated with schizophrenia: A 4-week, double-blind, placebo-controlled study. *Journal of Clinical Psychiatry* **71**, 1475–1481.

Kantrowitz JT, Malhotra AK, Cornblatt B, Silipo G, Balla A, Suckow RF, D'Souza C, Saksa J, Woods SW, Javitt DC (2010). High dose D-serine in the treatment of schizophrenia. *Schizophrenia Research* **121**, 125–130.

Kaye NS, Graham J, Roberts J, Thompson T, Nanry K (2007). Effect of open-label lamotrigine as monotherapy and adjunctive therapy on the self-assessed cognitive function scores of patients with bipolar I disorder. *Journal of Clinical Psychopharmacology* **27**, 387–391.

Keefe RSE, Bilder RM, Davis SM, Harvey PD, Palmer BW, Gold JM, Meltzer HY, Green MF, Capuano G, Stroup TS, McEvoy JP, Swartz MS, Rosenheck R a, Perkins DO, Davis CE, Hsiao JK, Lieberman J a (2007). Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE Trial. *Archives of general psychiatry* **64**, 633–47.

Keefe RSE, Malhotra AK, Meltzer HY, Kane JM, Buchanan RW, Murthy A, Sovel M, Li C, Goldman R (2008). Efficacy and safety of donepezil in patients with schizophrenia or schizoaffective disorder: significant placebo/practice effects in a 12-week, randomized, double-blind, placebo-controlled trial. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **33**, 1217–28.

Kelly DL, Buchanan RW, Boggs DL, McMahon RP, Dickinson D, Nelson M, Gold JM, Ball MP, Feldman S, Liu F, Conley RR (2009). A randomized double-blind trial of atomoxetine for cognitive impairments in 32 people with schizophrenia. *Journal of Clinical Psychiatry* **70**, 518–525.

Khan A, Ginsberg LD, Asnis GM, Goodwin FK, Davis KH, Krishnan AA, Adams BE (2004). Effect of lamotrigine on cognitive complaints in patients with bipolar I disorder. *Journal of Clinical Psychiatry* **65**, 1483–1490.

Kreinin A, Bawakny N, Ritsner M (2014). Adjunctive pregnenolone ameliorates the cognitive deficits in recent-onset schizophrenia. *Clinical Schizophrenia and Related Psychoses*, 1–31.

Krivoy A, Weizman A, Laor L, Hellinger N, Zemishlany Z, Fischel T (2008). Addition of memantine to antipsychotic treatment in schizophrenia inpatients with residual symptoms: A preliminary study. *European Neuropsychopharmacology* **18**, 117–121.

Kuman V, Postma P (2005). Nicotine use in schizophrenia: The self medication hypotheses. *Neuroscience and Biobehavioral Reviews* **29**, 1021–1034.

Laan W, Grobbee DE, Selten JP, Heijnen CJ, Kahn RS, Burger H (2010). Adjuvant aspirin therapy reduces symptoms of schizophrenia spectrum disorders: results from a randomized, double-blind, placebo-controlled trial. *The Journal of clinical psychiatry* **71**, 520–527.

Lane HY, Chang YC, Liu YC, Chiu CC, Tsai GE (2005). Sarcosine or D-Serine Add-on Treatment for Acute Exacerbation of Schizophrenia: a Randomized, Double-blind, Placebo-Controlled Study. *Archives of General Psychiatry* **62**, 1196–1204.

Lane HY, Lin CH, Green MF, Hellemann G, Huang CC, Chen PW, Tun R, Chang YC, Tsai GE (2013). Add-on treatment of benzoate for schizophrenia: a randomized, double-blind, placebo-controlled trial of D-amino acid oxidase inhibitor. *JAMA psychiatry* **70**, 1267–75.

Lane HY, Lin CH, Huang YJ, Liao CH, Chang YC, Tsai GE (2010). A randomized, double-blind, placebo-controlled comparison study of sarcosine (N-methylglycine) and d-serine add-on treatment for schizophrenia. *The International Journal of Neuropsychopharmacology* **13**, 451.

Large CH, Webster EL, Goff DC (2005). *The potential role of lamotrigine in schizophrenia*. *Psychopharmacology* **181**, 415–436.

Lawrence NS, Ross TJ, Stein EA (2002). Cognitive mechanisms of nicotine on visual attention. *Neuron* **36**, 539–548.

Lee JG, Lee SW, Lee BJ, Park SW, Kim GM, Kim YH (2012). Adjunctive memantine therapy for cognitive impairment in chronic schizophrenia: a placebo-controlled pilot study. *Psychiatry Investig* **9**, 166–173.

Lee SW, Lee JG, Lee BJ, Kim YH (2007). A 12-week, double-blind, placebo-controlled trial of galantamine adjunctive treatment to conventional antipsychotics for the cognitive impairments in chronic schizophrenia. *International Clinical Psychopharmacology* **22**, 63–68.

Leucht S, Cipriani A, Spineli L, Mavridis D, Örey D, Richter F, Samara M, Barbui C, Engel RR, Geddes JR, Kissling W, Stapf MP, Lässig B, Salanti G, Davis JM (2013). Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: A multiple-treatments meta-analysis. *The Lancet* **382**, 951–962.

Levin ED, McClernon FJ, Rezvani AH (2006). Nicotinic effects on cognitive function: Behavioral characterization, pharmacological specification, and anatomic localization. In *Psychopharmacology* vol 184, pp523–539.

Lieberman JA Csernansky J, Litman R, Volavka J, Jia XD, Gage A, MEM-MD-29 Study Group PK, Lieberman JA, Papadakis K, Csernansky J, Litman R, Volavka J, Jia XD, Gage A, Group M-M-29 S (2009). A randomized, placebo-controlled study of memantine as adjunctive treatment in patients with schizophrenia. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **34**, 1322.

Lieberman JA, Dunbar G, Segreti AC, Girgis RR, Seoane F, Beaver JS, Duan N, Hosford DA (2013). A randomized exploratory trial of an alpha-7 nicotinic receptor agonist (TC-5619) for cognitive enhancement in schizophrenia. *Neuropsychopharmacology* **38**, 968–975.

Lindenmayer JP, Khan A (2011). Galantamine augmentation of long-acting injectable risperidone for cognitive impairments in chronic schizophrenia. *Schizophrenia Research* **125**, 267–277.

Liu F, Guo X, Wu R, Ou J, Zheng Y, Zhang B, Xie L, Zhang L, Yang L, Yang S, Yang J, Ruan Y, Zeng Y, Xu X, Zhao J (2014). Minocycline supplementation for treatment of negative symptoms in early-phase schizophrenia: a double blind, randomized, controlled trial. *Schizophrenia Research* **153**, 169–176.

López-Jaramillo C, Lopera-Vásquez J, Ospina-Duque J, García J, Gallo A, Cortez V, Palacio C, Torrent C, Martínez-Arán A, Vieta E (2010). Lithium treatment effects on the neuropsychological functioning of patients with bipolar I disorder. *The Journal of clinical psychiatry* **71**, 1055–1060.

MacCabe JH, Lambe MP, Cnattingius S, Sham PC, David AS, Reichenberg A, Murray RM, Hultman CM (2010). Excellent school performance at age 16 and risk of adult bipolar disorder: national cohort study. *The British journal of psychiatry : the journal of mental science* **196**, 109–15.

MacCabe JH, Lambe MP, Cnattingius S, Torrång A, Björk C, Sham PC, David AS, Murray RM, Hultman CM (2008). Scholastic achievement at age 16 and risk of schizophrenia and other psychoses: A national cohort study. *Psychological Medicine* **38**, 1133–1140.

Madeira C, Freitas ME, Vargas-Lopes C, Wolosker H, Panizzutti R (2008). Increased brain d-amino acid oxidase (DAAO) activity in schizophrenia. *Schizophrenia Research* **101**, 76–83.

Martínez-Arán A, Vieta E, Colom F, Torrent C, Sánchez-Moreno J, Reinares M, Benabarre A, Goikole JM, Brugue E, Daban C, Salamero M (2004a). Cognitive impairment in euthymic bipolar patients: Implications for clinical and functional outcome. *Bipolar Disorders* **6**, 224–232.

Martínez-Arán A, Vieta E, Reinares M, Colom F, Torrent C, Sanchez-Mprena J, Benabarre A, Goikolea JM, Comes M, Salamero M (2004b). Cognitive function across manic or hypomanic, depresses, and euthymic states in bipolar disorder. *American Journal of Psychiatry* **161**, 262-270.

Marx CE, Keefe RSE, Buchanan RW, Hamer RM, Kilts JD, Bradford DW, Strauss JL, Naylor JC, Payne VM, Lieberman JA, Savitz AJ, Leimone LA, Dunn L, Porcu P, Morrow AL, Shampine LJ (2009). Proof-of-concept trial with the neurosteroid pregnenolone targeting cognitive and negative symptoms in schizophrenia. *Neuropsychopharmacology* **34**, 1885–1903.

Marx CE, Lee J, Subramaniam M, Rapisarda A, Bautista DCT, Chan E, Kilts JD, Buchanan RW, Wai EP, Verma S, Sim K, Hariram J, Jacob R, Keefe RSE, Chong SA (2014). Proof-of-concept randomized controlled trial of pregnenolone in schizophrenia. *Psychopharmacology* **231**, 3647–3662.

McIntosh AM, Harrison LK, Forrester K, Lawrie SM, Johnstone EC (2005). Neuropsychological impairments in people with schizophrenia or bipolar disorder and their unaffected relatives. *British Journal of Psychiatry* **186**, 378–385.

Melancon BJ, Tarr JC, Panarese JD, Wood MR, Lindsley CW (2013). Allosteric modulation of the M1 muscarinic acetylcholine receptor: improving cognition and a potential treatment for schizophrenia and Alzheimer's disease. *Drug discovery today* **18**, 1185–99.

Minzenberg MJ, Carter CS (2008). Modafinil: A review of neurochemical actions and effects on cognition. *Neuropsychopharmacology* **33**, 1477–1502.

Mishara AL, Goldberg TE (2004). *A meta-analysis and critical review of the effects of conventional neuroleptic treatment on cognition in schizophrenia: Opening a closed book*. *Biological Psychiatry* **55**, 1013–1022.

Nunes P V, Forlenza O V, Gattaz WF (2007). Lithium and risk for Alzheimer's disease in elderly patients with bipolar disorder. *The British Journal of Psychiatry* **190**, 359–360.

Okuma T, Inanaga K, Otsuki S, Sarai K, Takahashi R, Hazama H, Mori A, Watanabe S (1981). A preliminary double-blind study on the efficacy of carbamazepine in prophylaxis of manic-depressive illness. *Psychopharmacology* **73**, 95–96.

Olincy A, Harris JG, Johnson LL, Pender V, Kongs S, Allensworth D, Ellis J, Zerbe GO, Leonard S, Stevens KE, Stevens JO, Martin L, Adler LE, Soti F, Kem WR, Freedman R (2006). Proof-of-concept trial of an α7 nicotinic agonist in schizophrenia. *Archives of General Psychiatry* **63**, 630–638.

Olincy A, Young DA, Freedman R (1997). Increased levels of the nicotine metabolite cotinine in schizophrenic smokers compared to other. *Biological Psychiatry* **42**, 1–5.

Owen MJ, Craddock N (2009). Diagnosis of functional psychoses: time to face the future. *The Lancet* **373**, 190–191.

Pachet AK, Wisniewski AM (2003). The effects of lithium on cognition: An updated review. *Psychopharmacology* **170**, 225–234.

Pietrzak RH, Snyder PJ, Maruff P (2010). Use of an acute challenge with d-amphetamine to model cognitive improvement in chronic schizophrenia. *Human Psychopharmacology* **25**, 353–358.

Poyurovsky M, Koren D, Gonopolsky I, Schneidman M, Fuchs C, Weizman A, Weizman R (2003). Effect of the 5-HT2 antagonist mianserin on cognitive dysfunction in chronic schizophrenia patients: An add-on, double-blind placebo-controlled study. *European Neuropsychopharmacology* **13**, 123–128.

Rakofsky JJ, Dunlop BW, Beyer JL, Oliver AM, Mansson EE, Sancheti MT, Harvey PD (2014). *Cognitive effects of quetiapine XR in patients with euthymic bipolar disorder*. *Journal of Clinical Psychopharmacology* **34**, 383–385.

Reichenberg A, Caspi A, Harrington H, Houts R, Keefe RSE, Murray RM, Poulton R, Moffitt TE (2010). Static and dynamic cognitive deficits in childhood preceding adult schizophrenia: A 30-year study. *American Journal of Psychiatry* **167**, 160–169.

Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421–427.

Rosell DR, Zaluda LC, McClure MM, Perez-Rodriguez MM, Strike KS, Barch DM, Harvey PD, Girgis RR, Hazlett E a, Mailman RB, Abi-Dargham A, Lieberman J a, Siever LJ (2014). Effects of the D1 Dopamine Receptor Agonist Dihydrexidine (DAR-0100A) on Working Memory in Schizotypal Personality Disorder. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 1–8.

Rybakowski JK, Permoda-Osip A, Borkowska A (2009). Response to prophylactic lithium in bipolar disorder may be associated with a preservation of executive cognitive functions. *European Neuropsychopharmacology* **19**, 791–795.

Rybakowski JK, Suwalska A (2010). Excellent lithium responders have normal cognitive functions and plasma BDNF levels. *International Journal of Neuropsychopharmacology* **13**, 617–622.

Sacco KA, Termine A, Seyal A, Dudas MM, Vessicchio JC, Krishnan-Sarin S, Jatlow PI, Wexler BE, George TP (2005). Effects of cigarette smoking on spatial working memory and attentional deficits in schizophrenia involvement of nicotinic receptor mechanisms. *Archives of General Psychiatry* **62**, 649–659.

Savitz JB, van der Merwe L, Stein DJ, Solms M, Ramesar RS (2008). Neuropsychological task performance in bipolar spectrum illness: Genetics, alcohol abuse, medication and childhood trauma. *Bipolar Disorders* **10**, 479–494.

Schouws SNTM, Stek ML, Comijs HC, Beekman ATF (2010). Risk factors for cognitive impairment in elderly bipolar patients. *Journal of affective disorders* **125**, 330–5.

Schubert MH, Young KA, Hicks PB (2006). Galantamine Improves Cognition in Schizophrenic Patients Stabilized on Risperidone. *Biological Psychiatry* **60**, 530–533.

Scoriels L, Barnett JH, Soma PK, Sahakian BJ, Jones PB (2012). Effects of modafinil on cognitive functions in first episode psychosis. *Psychopharmacology* **220**, 249–258.

Senturk V, Goker C, Bilgic A, Olmez S, Tugcu H, Oncu B, Cem Atbasoglu E (2007). Impaired verbal memory and otherwise spared cognition in remitted bipolar patients on monotherapy with lithium or valproate. *Bipolar Disorders, Supplement* **9**, 136–144.

Sevy S, Rosenthal MH, Alvir J, Meyer S, Visweswaraiah H, Gunduz-Bruce H, Schooler NR (2005). Double-blind, placebo-controlled study of modafinil for fatigue and cognition in schizophrenia patients treated with psychotropic medications. *Journal of Clinical Psychiatry* **66**, 839–843.

Sharma T, Reed C, Aasen I, Kumari V (2006). Cognitive effects of adjunctive 24-weeks Rivastigmine treatment to antipsychotics in schizophrenia: A randomized, placebo-controlled, double-blind investigation. *Schizophrenia Research* **85**, 73–83.

Shekhar A, Potter WZ, Lightfoot J, Lienemann J, Dubé S, Mallinckrodt C, Bymaster FP, McKinzie DL, Felder CC (2008). Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *American Journal of Psychiatry* **165**, 1033–1039.

Shi LZ, Schuh LM, Trzepacz PT, Huang LX, Namjoshi MA, Tohen M (2004). Improvement of positive and negative syndrome scale cognitive score associated with olanzapine treatment of acute mania. *Current Medical Research and Opinion* **20**, 1371–1376.

Shiina A, Shirayama Y, Niitsu T, Hashimoto T, Yoshida T, Hasegawa T, Haraguchi T, Kanahara N, Shiraishi T, Fujisaki M, Fukami G, Nakazato M, Iyo M, Hashimoto K (2010). A randomised,

double-blind, placebo-controlled trial of tropisetron in patients with schizophrenia. *Annals of General Psychiatry* **9**, 27.

Smigan L, Perris C (1983). Memory functions and prophylactic treatment with lithium. *Psychological Medicine* **13**, 529-536.

Smith RC, Warner-Cohen J, Matute M, Butler E, Kelly E, Vaidhyanathaswamy S, Khan A (2006). Effects of nicotine nasal spray on cognitive function in schizophrenia. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **31**, 637–643.

Sommer IE, Van Westrhenen R, Begemann MJH, De Witte LD, Leucht S, Kahn RS (2014). Efficacy of anti-inflammatory agents to improve symptoms in patients with schizophrenia: an update. *Schizophrenia Bulletin* **40**, 181–191.

Stokes PE, Shamoian CA, Stoll PM, Patton MJ (1971). Efficacy of lithium as acute treatment of manic-depresieve illness. *The Lancet* **297**, 1319–1325.

Sumiyoshi T, Matsui M, Nohara S, Yamashita I, Kurachi M, Sumiyoshi C, Jayathilake K, Meltzer HY (2001). Enhancement of cognitive performance in schizophrenia by addition of tandospirone to neuroleptic treatment. *American Journal of Psychiatry* **158**, 1722–1725.

Sumiyoshi T, Park S, Jayathilake K, Roy A, Ertugrul A, Meltzer HY (2007). Effect of buspirone, a serotonin1A partial agonist, on cognitive function in schizophrenia: A randomized, double-blind, placebo-controlled study. *Schizophrenia Research* **95**, 158–168.

Torrent C, Martinez-Aran A, Daban C, Amann B, Balanza-Martinez V, del Mar Bonnin C, Cruz N, Franco C, Tabares-Seisdedos R, Vieta E (2011). Effects of atypical antipsychotics on neurocognition in euthymic bipolar patients. *Comprehensive psychiatry* **52**, 613–622.

Toulopoulou T, Quraishi S, McDonald C, Murray RM (2006). The Maudsley Family study: Premorbid and current general intellectual function levels in familial bipolar I disorder and schizophrenia. *Journal of Clinical and Experimental Neuropsychology* **28**, 243–259.

Trotta A, Murray RM, MacCabe JH (2014). Do premorbid and post-onset cognitive functioning differ between schizophrenia and bipolar disorder? A systematic review and meta-analysis. *Psychological Medicine* **755**

Tsai G, Lane HY, Yang P, Chong M, Lange N (2004). Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. *Biological Psychiatry* **55**, 452–456.

Tsai GE, Yang P, Chang YC, Chong MY (2006). D-alanine added to antipsychotics for the treatment of schizophrenia. *Biological Psychiatry* **59**, 230–234.

Tugal O, Yazici KM, Yagcioglu AE, Gogus A (2004). A double-blind, placebo controlled, cross-over trial of adjunctive donepezil for cognitive impairment in schizophrenia. *Int J Neuropsychopharmacol* **7**, 117–123.

Tuominen HJ, Tiihonen J, Wahlbeck K (2005). Glutamatergic drugs for schizophrenia: A systematic review and meta-analysis. *Schizophrenia Research* **72**, 225–234.

Turner DC, Clark L, Pomarol-Clotet E, McKenna P, Robbins TW, Sahakian BJ (2004). Modafinil improves cognition and attentional set shifting in patients with chronic schizophrenia. *Neuropsychopharmacology* **29**, 1363–1373.

Turner DC, Robbins TW, Clark L, Aron AR, Dowson J, Sahakian BJ (2003). Cognitive enhancing effects of modafinil in healthy volunteers. *Psychopharmacology* **165**, 260–269.

Umbricht D, Alberati D, Martin-Facklam M, Borroni E, Youssef EA, Ostland M, Wallace TL, Knoflach F, Dorflinger E, Wettstein JG, Bausch A, Garibaldi G, Santarelli L (2014a). Effect of bitopertin, a glycine reuptake inhibitor, on negative symptoms of schizophrenia: A randomized, double-blind, proof-of-concept study. *JAMA Psychiatry* **71**, 637–646.

Umbricht D, Keefe RS, Murray S, Lowe DA, Porter R, Garibaldi G, Santarelli L (2014b). A randomized, placebo-controlled study investigating the nicotinic alpha7 agonist, RG3487, for cognitive deficits in schizophrenia. *Neuropsychopharmacology* **39**, 1568–1577.

Van Oel CJ, Sitskoorn MM, Cremer MPM, Kahn RS (2002). School performance as a premorbid marker for schizophrenia: A twin study. *Schizophrenia Bulletin* **28**, 401–414.

Verrall L, Burnet PWJ, Betts JF, Harrison PJ (2010). The neurobiology of D-amino acid oxidase and its involvement in schizophrenia. *Molecular Psychiatry* **15**, 122–137.

Wallace TL, Bertrand D (2013). *Importance of the nicotinic acetylcholine receptor system in the prefrontal cortex*. *Biochemical Pharmacology* **85**, 1713–1720.

Weiser M, Heresco-Levy U, Davidson M, Javitt DC, Werbeloff N, Gershon AA, Abramovich Y, Amital D, Doron A, Konas S, Levkovitz Y, Liba D, Teitelbaum A, Mashiach M, Zimmerman Y (2012). A multicenter, add-on randomized controlled trial of low-dose D-serine for negative and cognitive symptoms of schizophrenia. *Journal of Clinical Psychiatry* **73**

Wingo AP, Wingo TS, Harvey PD, Baldessarini RJ (2009). Effects of lithium on cognitive performance: A meta-analysis. *Journal of Clinical Psychiatry* **70**, 1–3.

Woodward ND, Purdon SE, Meltzer HY, Zald DH (2005). A meta-analysis of neuropsychological change to clozapine, olanzapine, quetiapine, and risperidone in schizophrenia. *International Journal of Neuropsychopharmacology* **8**, 457–472.

Wu FS, Gibbs TT, Farb DH (1991). Pregnenolone sulfate: a positive allosteric modulator at the N-methyl-D- aspartate receptor. *Molecular Pharmacology* **40**, 333–336.

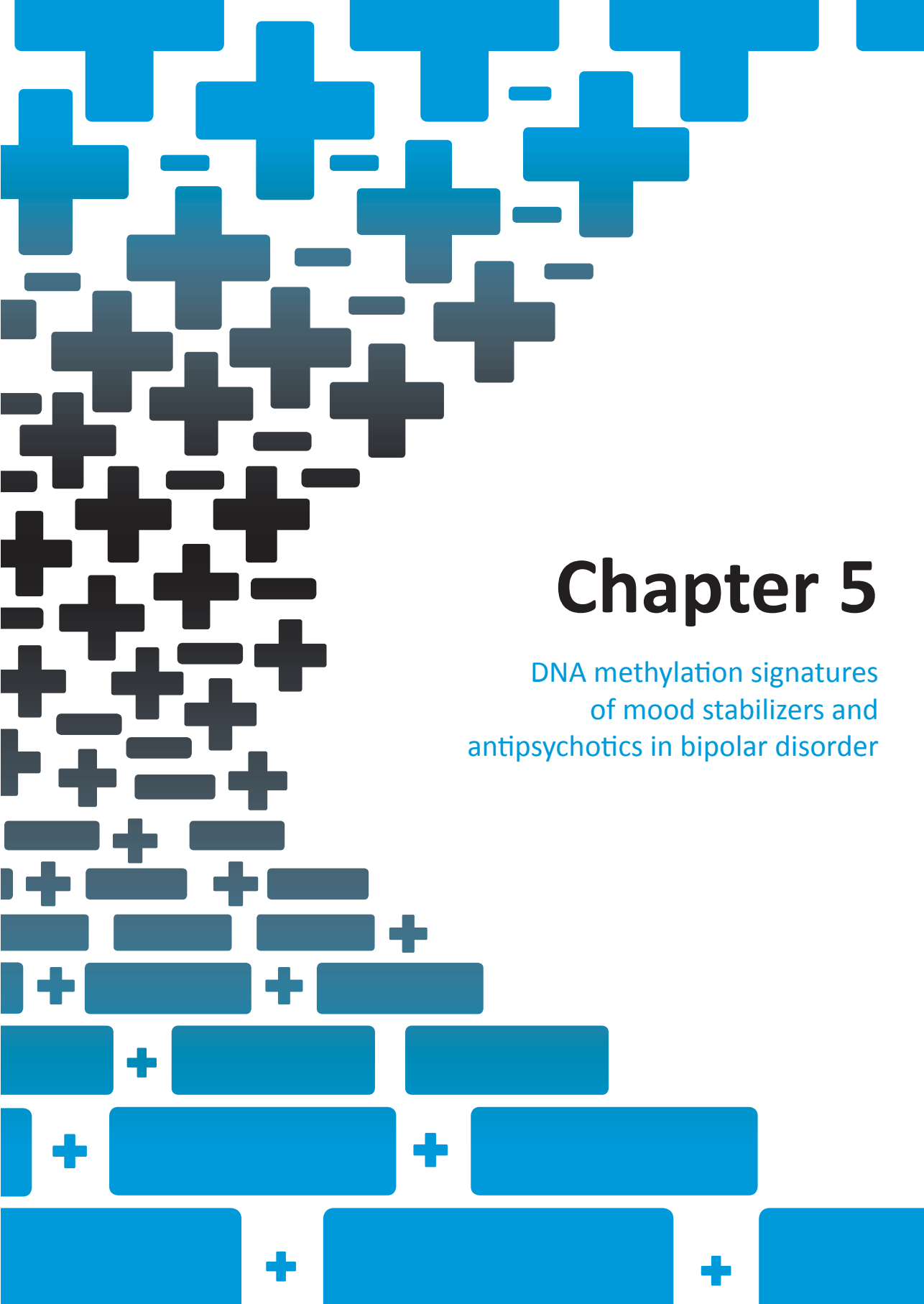
Young AH, Hammond JM (2007). Lithium in mood disorders: Increasing evidence base, declining use? *British Journal of Psychiatry* **191**, 474–476.

Zammit S, Allebeck P, David AS, Dalman C, Hemmingsson T, Lundberg I, Lewis G (2004). A Longitudinal Study of Premorbid IQ Score and Risk of Developing Schizophrenia, Bipolar Disorder, Severe Depression, and Other Nonaffective Psychoses. *Archives of General Psychiatry* **61**, 354–360.

Annet H. van Bergen
Lotte C. Houtepen
Christiaan H. Vinkers
Marco P.M. Boks

*equal contribution

Epigenomics 2016; 8(2): 197-208



Chapter 5

DNA methylation signatures of mood stabilizers and antipsychotics in bipolar disorder

Abstract

Aim:
In view of the potential effects of psychiatric drugs on DNA methylation, we investigated whether medication use in bipolar disorder is associated with DNA methylation signatures.

Patients and methods:
Blood-based DNA methylation patterns of six frequently used psychotropic drugs were examined in 172 bipolar disorder patients. After adjustment for cell type composition, we investigated gene networks, principal components, hypothesis-driven genes and epigenome-wide individual loci.

Results:
Valproic acid and quetiapine were significantly associated with altered methylation signatures after adjustment for drug-related changes on cell type composition.

Conclusions:
Psychiatric drugs influence DNA methylation patterns over and above cell type composition in bipolar disorder. Drug-related changes in DNA methylation are therefore not only an important confounder in psychiatric epigenetics but may also inform on the biological mechanisms underlying drug efficacy.

Keywords:
DNA methylation, bipolar, antipsychotics, mood stabilizer, antidepressant, medication, cell type.

Introduction

Epigenetic mechanisms are important in the development of the central nervous system and brain plasticity throughout life by influencing gene expression (Flavell et al. 2008; Tsankova et al. 2007). A growing body of evidence suggests that the epigenome also contributes to the pathogenesis of several psychiatric disorders (Mill et al. 2008; Dempster et al. 2011; Wong et al. 2014). Among numerous epigenetic mechanisms, DNA methylation is the most frequently studied epigenetic mark (Reik 2007; Suzuki & Bird 2008). In this process a methyl group is attached to 5'-cytosine residues at cytosine-guanine sequences (CpG) in the DNA (Bird 1986). The majority of the CpG sequences are heavily methylated, resulting in a relatively stable repression of gene activity (Klose & Bird 2006). However, the CpG sequences that cluster at promoter regions of genes, called CpG islands, generally display relatively low levels of DNA methylation (Bird 2002). Although a large proportion of DNA methylation programming is stable and genetically regulated (Boks et al. 2009; van Eijk et al. 2012; Schübeler 2015; Kim et al. 2009), environmental factors such as nutrition and medication can influence this process (Kofink et al. 2013; Rutten & Mill 2009). For instance, prenatal exposure to famine is associated with hypo-methylation at the Insulin-like Growth Factor 2 (*IGF-2*) gene in humans (Heijmans et al. 2008). Such changes in methylation status of promoter CpGs can occur across the life span in a small but significant part of the genome (Rutten & Mill 2009; Bjornsson et al. 2008). In cancer the possibility to influence DNA methylation has already lead to therapeutic pharmaceutical applications (Minucci & Pelicci 2006). With regard to psychotropic medication, compelling evidence emerges from several preclinical and *in vitro* studies indicating that a variety of psychotropic medication show epigenetic effects as well including alterations in DNA methylation (For review see Boks et al. (2012)). Psychotropic medication can affect DNA methylation by altering activity of DNA methyltransferases (DNMTs) that are essential in initiating and maintaining DNA methylation (Bird 2002; Grayson & Guidotti 2013) during development and in adulthood (Roth & Sweatt 2009).

In order to investigate the epigenetic effects of psychotropic medication, Bipolar Disorder (BD) patients are of particular interest because the treatment of both mood and psychotic symptoms require a wide variety of pharmaceutical compounds, including anti-psychotics and mood stabilizers (Kowatch et al. 2005; Goodwin 2009). This in contrast to schizophrenia patients who are generally all on the same class of drugs. An example of a drug that we are able to study in BD patients is valproic acid; a mood stabilizer that acts as an histone-deacetylase (HDAC) inhibitor (Gottlicher 2004) and indirectly counteracts hypermethylation of GABA promoters by inhibiting DNMT1 in prenatal stressed mice (Tremolizzo et al. 2005; Matrisciano et al. 2013). In human studies valproic acid use is associated with altered DNMT1 expression in the frontal cortex of patients with Schizophrenia (SCZ) and Bipolar Disorder with psychosis (Guidotti et al. 2009; Veldic et al. 2007). There are many more examples of psychotropic drugs that alter epigenetic marks in candidate gene studies in mice as well as

post-mortem brains of schizophrenia patients (Li et al. 2004; Matrisciano et al. 2011; Dong et al. 2009; Dong et al. 2008; Yasuda et al. 2009) (For review see Boks et al. (2012)).

Even though several preclinical and in vitro studies have reported medication-related changes in DNA methylation, it is unknown whether such changes are truly present in psychiatric patients. Therefore, we examined the DNA methylation signatures of psychotropic medication in the blood of 172 bipolar disorder patients. These patients used a variety of mood stabilizing and antipsychotic drugs (Kowatch et al. 2005; Goodwin 2009) that allowed the study of DNA methylation signatures of the mood stabilizers lithium, valproic acid, carbamazepine and lamotrigine, as well as the antipsychotics olanzapine and quetiapine. Because of the known association between cell type composition and medication, this study carefully addressed possible confounding effects of cell type composition (Sun et al. 2010; Lam et al. 2012).

Experimental procedures

Participants

Participants were eligible for participation if they had three or more Dutch grandparents and met criteria for diagnosis of BD. Data was collected in two waves at the Utrecht Medical Center; the first wave from 2009 to 2011 included 122 participants whereas in the second wave 50 participants were included between December 2011 and May 2013. The study was approved by the Utrecht Medical Center ethical review board and performed according to the ICH guidelines for Good Clinical Practice and the latest amendments of the Declaration of Helsinki. All participants gave their written informed consent prior to their inclusion in the study and were financially compensated.

Procedures:

General

Participants were invited to the UMC Utrecht for the assessment that included a blood draw and interview. The interview was conducted by at least one well-trained independent rater. Clinical characteristics including mood and psychotic symptoms, comorbid psychiatric diagnosis, number of manic and depressive episodes, and age of disease onset were established with the Comprehensive Assessment of Symptoms and History (CASH) (Andreasen et al. 1992). Participants of the second wave were assessed with the Structured Clinical Interview for DSM-IV (SCID) (First et al. 2002). Current use of psychoactive substances was determined with the CASH in the first wave and a self-report questionnaire in the second wave. All participants reporting psychiatric medication use (antidepressants, benzodiazepines, anticonvulsants and antipsychotics), were on a stable (at least one month) dosing schedule. If participants smoked daily, they were defined as a smoker.

DNA methylation analyses

Whole blood DNA was extracted using Gentra Puregene Kit (Qiagen, Valencia, CA, USA). DNA concentration was assessed using riboGreen, and integrity using BioAnalyser. Bisulphite conversion was conducted using Zimo kits (ZYMO Research, Orange, CA, USA) using standard procedures. Genome-wide DNA methylation levels were assessed using Illumina Infinium HumanMethylation27K BeadChip (Illumina) arrays in the first wave (n=122) and Illumina Infinium HumanMethylation450K BeadChip (Illumina) arrays in the second wave (n=50). Samples were equally distributed over the 24 arrays balancing gender and age on each of them to reduce any batch effects to the minimum. Intensity read outs, quality control parameters and methylation measures were obtained from the genome studio software. DNA methylation measures were excluded based on a detection p-value larger than 0.001. Probes with failed detection in more than 1% of the participants or less than 5 beads in 5 percent of samples were excluded as were samples with more than 1% of probes failed (Schalkwyk et al. 2013). X chromosome, y chromosome or non-specific probes were removed (Chen et al. 2013). Probes with SNPs of Minor Allele Frequency larger than 5 percent within 1 base pairs of the primer were also removed after constructing ancestry estimates based on their principal components as proposed by Barfield et al (Barfield et al. 2014). After this step the 27k and 450k data were combined, selecting the probes surviving quality control and present on both arrays (22988 probes). The combined set was subsequently quantile normalized using the lumi package to remove technical variation between individuals.

Batch effects were analyzed by investigating the association of the principal component of the methylation levels with plate, sentrix array and position using correlation and visual inspection of heat maps (see supplemental material 1 figure 1). The Combat procedure as implemented in the sva package was used to remove batch effects for sentrix array. In this way we also remove any differences that may have occurred by using the two types of arrays in different experiments. After this procedure no batches for array, plate or sentrix were apparent (Johnson et al. 2007) (see supplemental material 1 figure 2). Finally cell type composition was calculated based on the methylation values for the 27k data using the Houseman algorithm (Houseman et al. 2012), while the calculation for the 450k data was based on relating the methylation values to data derived from FACS sorted methylation data using the Houseman algorithm as implemented in a minfi based procedure (Aryee et al. 2014). In short, we used DNA methylation data from the arrays to analyze several differentially methylated regions (DMRs) that are markers of immune cell identity. To estimate sample-specific cell proportion in our whole blood samples, we applied a statistical algorithm (Houseman et al. 2012) based on cell specific methylation profiles of an independent reference dataset of flow sorted cell types (Monocytes, CD4+ and CD8+ T cells, Granulocytes, B cells, natural killer cells) (Reinius et al. 2012). Cell type composition was investigated as a potential confounder (see figure 1, supplemental material 1 figure 2 and the results section cell type composition).

Statistical analyses

All statistical analyses were carried out using R version 3.1.2 (R Core Team & R Foundation For Statistical Computing 2014). For regression modeling, the Limma package was used (Smyth 2004). Outliers were detected using Cook's Distance with a cut-off value of 1. If more than ten participants reported taking a specific type of medication, this medication type was added as a covariate to the linear model (see table 1 and supplemental material 1 table 1). Following this criterion olanzapine, quetiapine, lithium, carbamazepine, valproic acid and lamotrigine were included as specific medication types in all models. For methylation, beta values were used for graphical display, but analyses were carried out using M-values (log2 ratio of methylation probe intensity) which has better statistical validity (Du et al. 2010). Because methylation may vary with age, sex and smoking (Boks et al. 2009), these were included as covariates in all analyses. To investigate population stratification, ancestry was estimated from methylation-based principal components as proposed in the Barfield study (Barfield et al. 2014). Population stratification did not play a role (see supplemental material 1 figure 1-3) and methylation-based population principal components were not included in the models. First, the potential confounding effects of cell type composition was investigated by analyzing the association of medication with cell type composition. To account for confounding due to cell type composition, the cell-count variances were regressed out while protecting for the association between medication and methylation all other analyses (see supplemental material 1 figure 3) as implemented in the sva package (Johnson et al. 2007). Finally, in accordance with WGCNA default pre-processing steps (Langfelder & Horvath 2008), we checked for any obvious outliers in our sample with an average linkage hierarchical cluster analysis of the DNA methylation levels as implemented in the hclust function of the stat package in R (R Core Team & R Foundation For Statistical Computing 2014; Langfelder & Horvath 2012). No outliers were identified and all analyses were performed on 172 subjects.

Cell type composition of whole blood

We investigated whether the differences in DNA methylation between medication groups were due to differences in cell type composition of the samples (i.e. whether changes in cell counts were a mediator of the relationship between medication, global methylation levels and blood cell counts). First, to determine for which medication types there was an association with cell type composition a multivariate analysis of variance was performed with the five cell types (natural killer (NK), Bcell, CD8T, CD4T and monocytes) as outcome and the six medication types (olanzapine, quetiapine, lithium, carbamazepine, valproic acid and lamotrigine), sex, age and smoking status as determinants. Then principal components for methylation were calculated as a measure for global methylation and mediator analyses were performed with the mediation package in R (Tingley et al. 2014). In the mediator analyses the first five principal components were the outcome measures, the biggest cell fraction the possible mediator and the dependent measure was the medication type that

had the highest correlation with the selected cell type in the multivariate analysis. The other cell types, sex, age and smoking were added to the model as covariates.

Network analysis

Weighted gene co-expression network analysis was performed with the WGCNA package in R to identify and characterize methylation clusters (Langfelder & Horvath 2012; Langfelder & Horvath 2008) based on their relationship with medication, the principal components and biological processes (using GO-term analysis). The association of the medication types with the identified methylation clusters was investigated using in a linear model including age, sex and smoking status as covariates. Results were reported only for models with a good fit (p value < 0.05).

The principal components were calculated for all 22988 loci and based on the screeplot we used the first five principal components (PC) for analyses (see supplementary info 1 figure 10, proportion explained variance per principal component was: PC1=0.055; PC2=0.046; PC3=0.028; PC4=0.024; PC5=0.018, Cumulative proportion=0.17). First, the association between each principal component and the selected medication types was tested in a linear regression model with smoking status, age and sex as covariates. Second, the correlation between the identified methylation clusters and principal components was explored by correlating the WGCNA methylation cluster module score to the principal component scores. Finally, to investigate enrichment for biological processes with the GOstat package (Falcon & Gentleman 2007), we tested the GO-terms of the probes in the identified methylation clusters against all GO-terms of the probes surviving quality control. We only reported biological enrichment if the GO-term is significant ($p<0.05$) after applying bonferroni correction for all GO-terms tested.

Epigenome-wide association study

The association between all 22988 loci and the six selected medication types was tested in one overall linear model with age, sex and smoking status as covariates. From this model coefficients per medication type (adjusted for the other medication types) were extracted and the distribution of p-values was investigated by QQ-plotting and calculation of the genomic inflation factor. Only if the genomic inflation factor and visual inspection of the QQ plot indicated an acceptable distribution of p values (see supplemental material 1 Figure 4-9), did we include the analysis results for the top 1000 probes in supplemental material 2. Epigenome-wide significant results were loci with a p-value lower than 0.05 after applying false discovery rate (FDR) correction.

Detailed analysis of candidate genes

Based on previous DNA methylation studies we selected the following candidate genes: *RELN* (Matrisciano et al. 2011; Mitchell et al. 2005; Dong et al. 2007), *SLC1A2* (Perisic et al. 2010), *MTNR1A* (Kim et al. 2008), *IGF2* (Popkie et al. 2010; Leng et al. 2008), *H19* (Popkie et al. 2010; Leng et al. 2008), *BDNF* (Yasuda et al. 2009; Fukuchi et al. 2009), *SLC6A4* (Perisic et al. 2010) and *GAD1* (Matrisciano et al. 2011). We interrogated all the probes on these selected candidate genes for their association with our six selected medication types in one overall linear model with age, sex and smoking status as covariates. Per medication type the p-values were adjusted for multiple testing by applying false discovery rate (FDR) correction (alpha=0.05).

Results

Baseline characteristics

A summary of the sample characteristics can be found in table 1. Six medication types were used by more than 10 patients, in order of number of users lithium (65%), followed by valproic acid (19%), quetiapine (17%), olanzapine (16%), carbamazepine (9%) and lamotrigine (8%). All other medication types were randomly distributed over these six main medication types (see supplemental material 1 table 1). Diagnoses were: 169 patients with Bipolar type I disorder and 3 patients with Bipolar type II disorder.

Table 1 Sample characteristics (n=172).

Variabele	n(%) or mean (range)
Age, yrs (mean,range)	43 (19-77)
Female sex (%))	94 (55%)
Smoking (%)	74 (43%)
Age at onset, yrs (mean, range)	26 (7-60)
Number of episodes (mean, range)	9.3 (1-27)
Lithium	112 (65%)
Olanzapine	27 (16%)
Quetiapine	29 (17%)
Valproic acid	33 (19%)
Carbamazepine	15 (9%)
Lamotrigine	14 (8%)

Association between cell type composition and medication

There was a significant association between the five cell types and quetiapine (see figure 1 *Pillai's trace=0.13, F(5,158)=4.9, p=0.0003*) and valproic acid (*Pillai's trace =0.07, F(5,158)=2.4, p=0.04*), but not for lamotrigine (*Pillai's trace =0.06, F(5,158)= 2.3, p=0.05*),

olanzapine (*Pillai's trace =0.05, F(5,158)=1.9, p=0.10*), lithium (*Pillai's trace =0.03, F(5,158)=1.0, p=0.41*) and carbamazepine (*Pillai's trace =0.02, F(5,158)=0.9, p=0.50*) (also see figure 1 for correlation plot). The biggest cell fraction in our sample is CD4T and in the follow up ANOVA the strongest association with CD4T was present in quetiapine users *(F(1,162)=16.7, p=6.7^{e-05})*. However, we found no evidence that the effect of quetiapine on global methylation, expressed as principal components (pc) one till five, was mediated by CD4T *(Proportion mediated for pc1 0.007,p=0.78; pc2 0.003,p=0.85; pc3 -0.008,p=0.89 ;pc4 -0.009,p=0.91; pc5 0.004,p=0.81)*. To correct for any possible confounding due to cell type composition all other analyses were performed on methylation data with the cell type composition effects regressed out while conserving the association with DNA methylation.

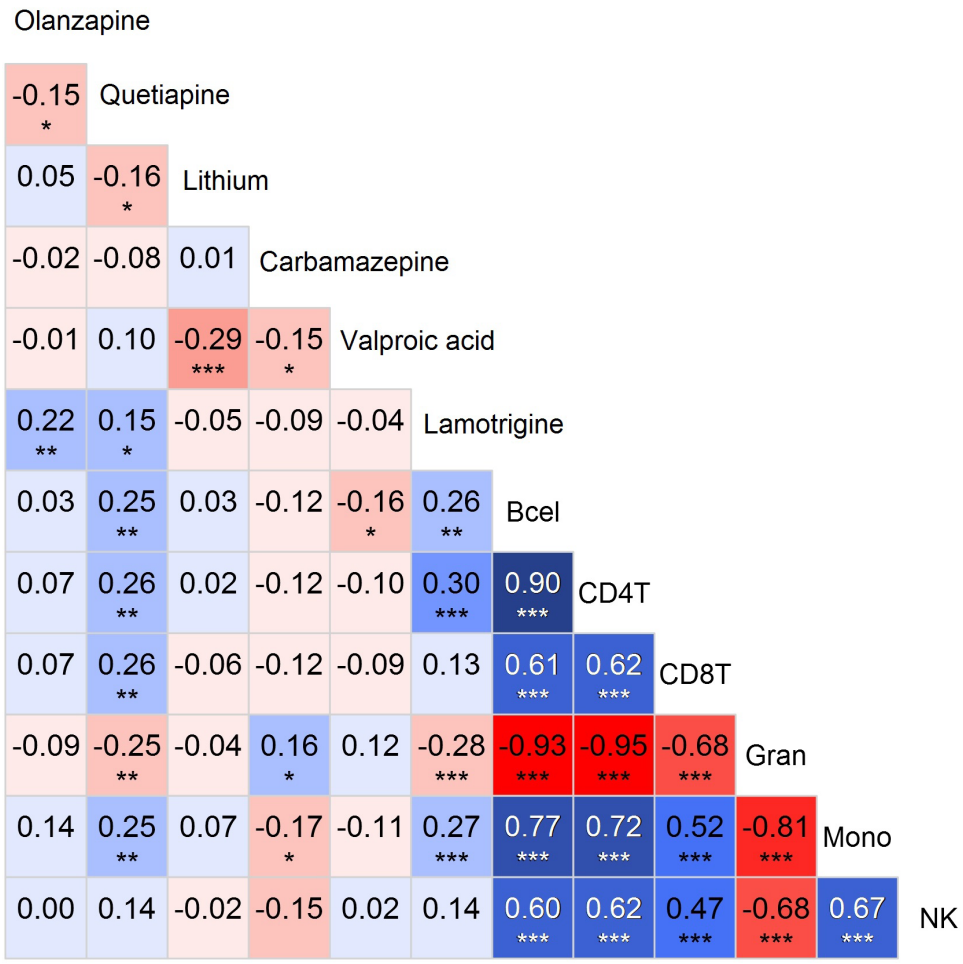


Figure 1 Heatmap depicting the correlation between medication and cellcounts.

Significant values are denoted by * p<0.05, ** p<0.001, ***p<0.0001. Abbreviations: CD8T= CD8 T, CD4T=CD4 T cell, cell NK=Natural Killer, Mono=Monocytes, Gran=Granulocytes.

Association between medication and network analysis of the methylation levels

We investigated DNA methylation levels represented in WGCNA modules and principal components. We derived 7 modules based on the intercorrelation patterns among probes of which the blue (*F(9,162)=2.74, p=0.005)*, the red *(F(9,162)=3.06, p=0.002)*, the yellow *(F(9,162)=3.56, p<0.001)* and, the green (*F(9,162)=5.97, p<0.001*) modules showed a good (significant) fit. The grey module contained 14,208 remaining probes that were not correlated to any of the 6 modules. Figure 2 shows that several of the medication types were related to a WGCNA module. The strongest finding is the association of the blue module (containing 2103 probes) with valproic acid use *(B=0.040, p=0.009)*, this module was related to the response to wounding GO-term GO:0009611 (see supplemental material 3). In the yellow module (1450 probes, enriched for stimulus and detection-related GO-terms see supplemental material 3) with valproic acid *(B=-0.032, p=0.028)* and lamotrigine *(B=0.045, p=0.038)*. In the red module (254 probes, enriched for immune-related GO-terms see supplemental material 3) with quetiapine *(B=0.033, p=0.040)* and valproic acid *(B=-0.053, p=0.0005)*. Finally the green module (974 probes, enriched for neurogenesis, embryonic and regulatory GO-terms see supplemental material 3) was associated with quetiapine *(B=-0.031, p=0.036).* Supplemental material 3 shows the full results of the modules and their enrichment.

Valproic acid was also significantly related to higher values of the second principal component of methylation levels, while olanzapine and lithium were associated with lower values on this principal component (see figure 3 and correlation plot in supplemental material 1 figure 3) *(model fit: F(9,162)=2.28, p=0.02 , olanzapine B=-0.43 , t=-2.0, p=0.05, lithium B=-0.33 , t=-2.0, p=0.05,; valproic acid B=0.47, t=2.3, p=0.02)*. Consistently; the blue module (related to valproic acid use), was highly correlated to pc2 (*Blue: r= -0.81, p =3.1x10* $^{e\ -42}$). Furthermore, lamotrigine was related to lower values of the fourth principal component (*model fit: F(9,162)=7.83, p<0.001 , lamotrigine B=-0.67 , t=-2.6, p=0.009*). Finally, lamotrigine users scored higher whereas quetiapine users scored lower on the fifth principal component (*model fit: F(9,162)=2.08, p=0.03 , lamotrigine B=-0.43 , t=-2.0, p=0.05, quetiapine B=-0.43 , t=-2.0, p=0.04*).

Genome wide association between medication and DNA methylation levels

The distribution of p values was acceptable for olanzapine, lithium and carbamazepine (respective genomic inflation factors: 1.011, 1.075 and 0.974, see supplemental material 1 figure 5-10 for qqplots and supplemental material 2 for the top 1000 probes). None of the associations between probes and these three medication types were significant after FDR correction, but for carbamazepine the highest ranking probe (cg24523000) is located on the *GABRA1* gene *(logFC=0.18, p=0.205)*.

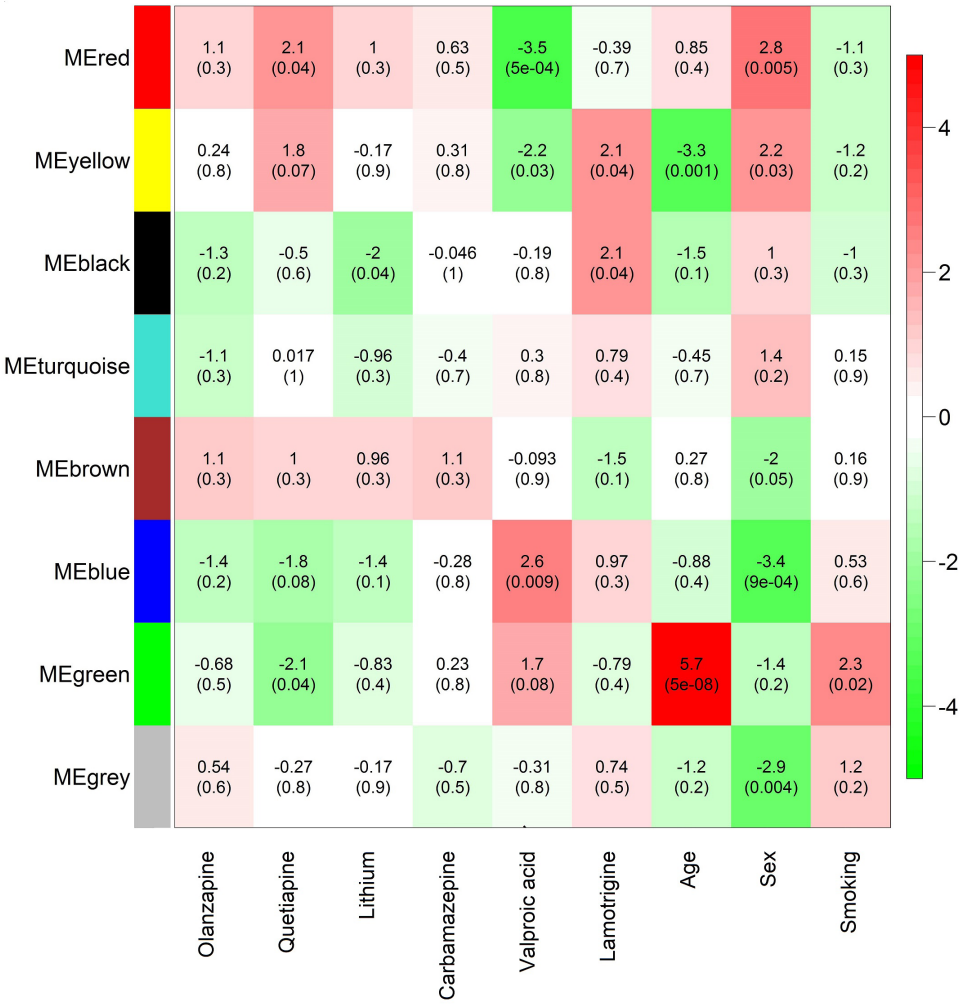


Figure 2 Heatmap for the relationship between the different WGCNA modules and the six selected medication types.

In each cell the top value corresponds to the model t value, whereas the bottom value between brackets denotes the p value for this particular covariate.

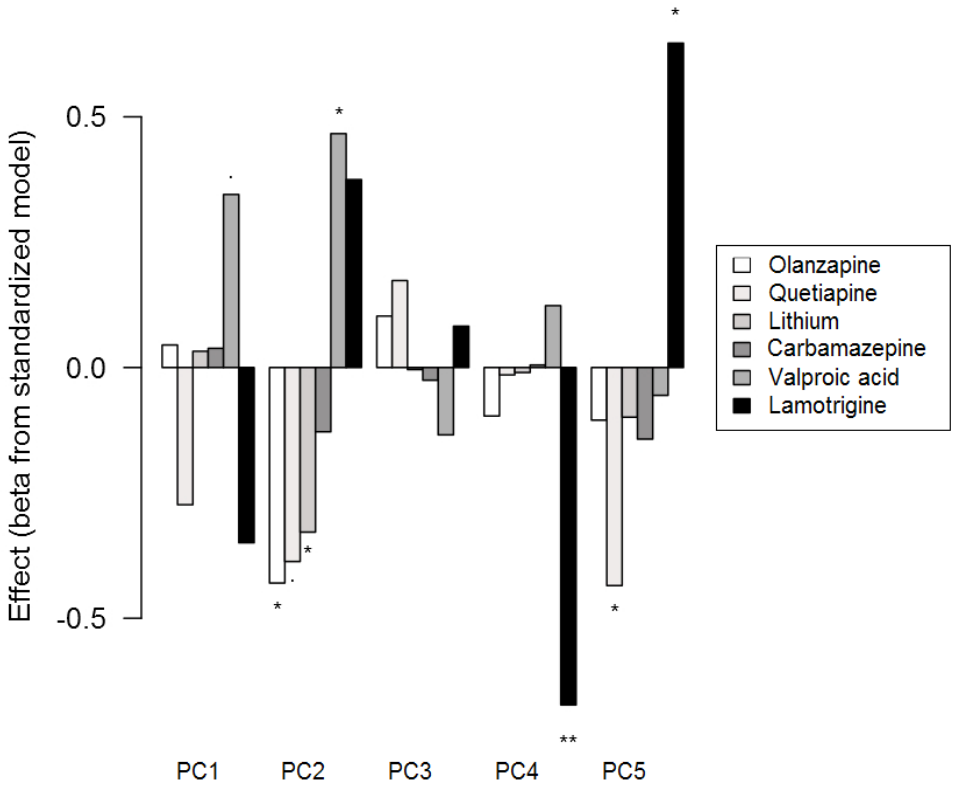


Figure 3 Barplot depicting the association between the six selected medication types and global DNA methylation measures principal component (pc) one till five.

To enable comparison of the impact of the association between the five principal components and the six selected medication types the beta from a standardized model are used in this graph •: p<0.10; *:p<0.05; **:p<0.01;***p<0.001.

Association between medication and methylation on candidate genes

The results for the methylation probes for specific candidate genes (*RELN*, *SLC1A2*, *MTNR1A*, *IGF2*, *H19*, *BDNF*, *SLC6A4* and *GAD1*) are presented in supplemental material 1 table 2, but overall the association between specific medication types and methylation status of the loci did not provide any replication for these candidate genes after FDR correction.

Discussion

This study explored the influence of six psychotropic drugs on blood-based DNA methylation levels by analyzing networks, principal components, hypothesis driven candidate genes and epigenome-wide association in bipolar disorder patients. The network and principal

components analyses study global DNA methylation changes, whereas the candidate gene and epigenome-wide techniques evaluate individual methylation sites. The main findings of this study suggest that, after adjustment for cell type composition in whole blood, psychotropic medication use remains associated with alterations in DNA methylation levels at least in methylation networks and potentially at individual loci. Our study shows that DNA methylation based co-expression networks and principal components are linked to several medication types. The network modules indicate that immune and neurogenesis-related processes are involved. In the candidate- and epigenome-wide analysis no specific differentially methylated CpG site survived multiple testing correction, but qq plot analysis and trend level results suggest that this is most likely the result of limited power. Overall both network and single locus analyses implicate biologically plausible mechanisms for future epigenetic studies of psychotropic drug action. However, the cross-sectional design of the current study prevents a direct causal inference from the methylation differences and should be interpreted with caution.

The gene weighted correlation network analysis is an unbiased and data driven method which has a high stability across tissue (van Eijk et al. 2012). Four methylation networks were related to the use of valproic acid, quetiapine or lamotrigine. Valproic acid showed the strongest associations and was linked to three different co-expression modules, consistent with valproic acid's documented relation with DNA methylation and neurotrophic actions such as promoting neurite growth and cell survival enhancing neuronal function (Yuan et al. 2001; Hao 2004). In terms of methylation co-expression networks, valproic acid as well as lamotrigine use were associated to a network characterized by the go term detection stimuli, but the strongest connection between any medication type and a methylation network was for valproic acid and an immune-related methylation network. Altered immune system responses and increased inflammation are frequently linked to psychiatric disorders (for review see Réus et al. (2015)). Interestingly valproic acid can reduce immune cell signaling by inactivating several enzymes involved in inflammation (Watkins et al. 2014). Quetiapine use was also related to the same immune-related methylation network and anti-inflammatory properties (Bian et al. 2008; Jaehne et al. 2015). Thus, the immune-related methylation network could reflect the inflammation-reducing properties of valproic acid and quetiapine (Watkins et al. 2014; Bian et al. 2008; Jaehne et al. 2015). More importantly quetiapine use was linked to a methylation network with the highest enrichment for neurogenesis, which could correspond with the neurogenesis enhancing properties of quetiapine (Luo et al. 2005). Reinstating adult neurogenesis is another potential treatment target for psychiatric disorders (Borsini et al. 2015; Miller & Hen 2015) and the current findings could provide new leads to study the mechanism of action of psychotropic drugs such as quetiapine.
Another reflection of the relevance of medication use for DNA methylation is the association of the principal components of methylation with medication. Particularly, the second principal component of DNA methylation was associated with several medication types

including valproic acid, olanzapine and lithium use (see figure 3). Although the explained variance of this second principal component was modest (4.6 percent), it could indicate that different types of medication may affect similar methylation in a similar way.
One approach to explore DNA methylation levels per individual locus was to perform a genome wide association study of all six medication types. Although after adjustment for multiple testing the associations rendered non-significant, the highest ranking probe for carbamazepine was on the *GABRA1* gene, which encodes for one of the subunits of GABA-A receptor in the GABA neurotransmitter system. Interestingly, the *GABRA1* gene has been proposed in the literature as a possible candidate gene for BD (Serretti & Mandelli 2008; Horiuchi et al. 2004). These findings suggest potential local effects on methylation of specific genes by psychotropic medications. Although the shapes of the QQ plots suggest a signal, limited sample size may have led to insufficient power to provide evidence. Limited power may also explain the inability to replicate several hypothesis driven analyses of previously associated candidate genes.

All analyses performed, were adjusted for whole blood cell type composition (for review see Houseman et al. (2015)). That such adjustments are important is underscored by the profound influence of psychotropic medication on cell count of a variety of cell types reported here. Psychotropic medication in almost all classes has been reported to cause changes in cell type composition. Mechanisms include direct toxic effects upon the bone marrow, the formation of antibodies against haematopoietic precursors or involve peripheral destruction of cells (Flanagan & Dunk 2008; O. et al. 1999; Vasudev et al. 2010; Shankar 2007; Huynh et al. 2005). Valproic acid may exhibit cell type composition alterations through immunosuppressive effects by activating apoptosis of activated lymphocytes and by weakening the cytotoxic effects of NK cells as well as the function of macrophages and monocytes but the underlying mechanisms need further investigation (Chen et al. 2011). In our data particularly quetiapine and valproic acid use exerted a notable influence on cell type composition reaffirming this known effect of medication on cell type composition and underscore the need to adjust for this confounding effect in studies of whole blood. However, the reported DNA methylation differences were not mediated by the cell type differences and DNA methylation differences remained after elaborate adjustment for cell type and using network analysis that are more robust to tissue type influences.

Caution is required when interpreting results of this explorative cross-sectional DNA methylation study. The main limitations lie in the cross-sectional observational study design. In absence of randomization, blinding, placebo control groups and a longitudinal set up, there remains a risk of selection bias, confounding by indication and the inability to infer causality. Inherent to the study design is the presence of potential residual confounding, such as genotype, nutrition, other medical conditions or concomitant non-psychotropic medication use. Finally, since participants often use several medication types at the same

time, it is not possible to fully disentangle selective effects of each medication type. Regarding the effects of polypharmacy (i.e. patients taking other types of medications), in our population the use of other psychotropic medication is low and randomly distributed across the six main medication types. Even though we cannot completely exclude the influence of other medication types on our results, this suggests that psychiatric polypharmacy is probably not of large influence. The use of blood also poses a limitation considering that most effects of psychotropic medication are in the brain. Several studies have now pointed out that although there are vast differences between tissue types, particularly blood and brain (Davies et al. 2012; Walton et al. 2016), the differences between exposed and non-exposed individuals are often reflected in multiple tissues, with larger effect sizes for the differences between individuals than for differences between tissues (Davies et al. 2012; Illingworth et al. 2015). Moreover, because blood cells are also exposed to these drugs and many of the lymphocytes, such as B-, T- and NK-cells, express similar receptors (e.g. BDNF, dopamine, GABA) as neuronal cells (Gladkevich et al. 2004) the results are likely to be of use. For instance haloperidol administration in mice is associated with correlated changes in blood and brain methylation in more than 65% of the affected methylation sites (Aberg et al. 2013).

Overall the current study found a profound influence of psychotropic medication on cell-counts, but also presents evidence for an association between psychotropic medication and DNA methylation levels over and above altered cell type composition. Nevertheless, the precise nature of this association remains to be established in longitudinal studies.

Future Perspective:

Our understanding of the interaction between environmental exposure, such as psychotropic medication, and DNA methylation is in its early stages. Studies in cancer have succeeded in developing compounds that are essentially epigenetic drugs.
Considering the importance of epigenetic mechanisms in brain development and plasticity, manipulation of these epigenetic mechanisms may be a new target for treatment of psychiatric disorders. Indeed our study underscores the potential of psychiatric drugs to alter DNA methylation signatures and therefore highlights the need to further investigate and develop epigenetic treatments of psychiatric disorders. A challenge remains to extend the current study of the epigenome by including other relevant epigenetic mechanisms. Subsequently the molecular relevance of such epigenetic changes needs to be established. Ultimately the goal should be to establish clinical epigenetic therapy for psychiatric disorders in the future.

Executive summary

Aims & methods:

- Cross sectional observational study of methylation signatures of psychotropic medication in whole blood DNA of 172 patients with Bipolar Disorder focusing on:
  - → Weighted gene co-expression networks
  - → Principal component analysis
  - → Epigenome-wide association analysis (EWAS)
  - → Hypothesis-driven gene analysis

Conclusions:

- Psychotropic medication has a profound influence on blood cell type composition.
- Over and above altered cell type composition this study provides evidence that psychotropic medication exerts an effect on DNA methylation levels of individual loci and networks.

Recommendations:

- The influence of psychotropic medication is currently underestimated in epigenetic research and should be taken into account as an important confounder.
- Further exploration of the epigenetic effects of psychotropic medication can inform about potential drug mechanisms and facilitate the development of epigenetic drugs for psychiatric disorders.

Financial & competing interests disclosure:

This study was made possible by a NARSAD Young Investigator Grant to MPM Boks. The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.
No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

Aberg KA, Xie LY, McClay JL, Nerella S, Vunck S, Snider S, Beardsley PM, Van Den Oord EJ (2013). Testing two models describing how methylome-wide studies in blood are informative for psychiatric conditions. *Epigenomics* **5**, 367–377.

Andreasen NC, Flaum M, Arndt S (1992). The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnosis and psychopathology. *Archives of general psychiatry* **49**, 615–23.

Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, Irizarry RA (2014). Minfi: A flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics* **30**, 1363–1369.

Barfield RT, Almli LM, Kilaru V, Smith AK, Mercer KB, Duncan R, Klengel T, Mehta D, Binder EB, Epstein MP, Ressler KJ, Conneely KN (2014). Accounting for population stratification in DNA methylation studies. *Genetic Epidemiology* **38**, 231–241.

Bian Q, Kato T, Monji A, Hashioka S, Mizoguchi Y, Horikawa H, Kanba S (2008). The effect of atypical antipsychotics, perospirone, ziprasidone and quetiapine on microglial activation induced by interferon-γ. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **32**, 42–48.

Bird A (2002). DNA methylation patterns and epigenetic memory. *Genes & Development* **16**, 6–21.

Bird AP (1986). CpG-Rich islands and the function of DNA methylation. *Nature* **321**, 209–213.

Bjornsson HT, Sigurdsson MI, Fallin MD, Irizarry RA, Aspelund T, Cui H, Yu W, Rongione MA, Ekström TJ, Harris TB, Launer LJ, Eiriksdottir G, Leppert MF, Sapienza C, Gudnason V, Feinberg AP (2008). Intra-individual change over time in DNA methylation with familial clustering. *JAMA - Journal of the American Medical Association* **299**, 2877–2883.

Boks MP, Derks EM, Weisenberger DJ, Strengman E, Janson E, Sommer IE, Kahn RS, Ophoff RA (2009). The relationship of DNA methylation with age, gender and genotype in twins and healthy controls. *PLoS ONE* **4**

Boks MP, de Jong NM, Kas MJH, Vinkers CH, Fernandes C, Kahn RS, Mill J, Ophoff R a (2012). Current status and future prospects for epigenetic psychopharmacology. *Epigenetics : official journal of the DNA Methylation Society* **7**, 20–8.

Borsini A, Zunszain PA, Thuret S, Pariante CM (2015). *The role of inflammatory cytokines as key modulators of neurogenesis*. *Trends in Neurosciences* **38**, 145–157.

Chen Q, Ouyang D, Geng M, Xu L, Zhang Y, Wang F, He X (2011). Valproic acid exhibits biphasic effects on apoptotic cell death of activated lymphocytes through differential modulation of multiple signaling pathways. *Journal of immunotoxicology* **8**, 210–218.

Chen YA, Lemire M, Choufani S, Butcher DT, Grafodatskaya D, Zanke BW, Gallinger S, Hudson TJ, Weksberg R (2013). Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics* **8**, 203–209.

Davies MN, Volta M, Pidsley R, Lunnon K, Dixit A, Lovestone S, Coarfa C, Harris RA, Milosavljevic A, Troakes C, Al-Sarraj S, Dobson R, Schalkwyk LC, Mill J (2012). Functional annotation of the

human brain methylome identifies tissue-specific epigenetic variation across brain and blood. *Genome Biology* **13**, R43.

Dempster EL, Pidsley R, Schalkwyk LC, Owens S, Georgiades A, Kane F, Kalidindi S, Picchioni M, Kravariti E, Toulopoulou T, Murray RM, Mill J (2011). Disease-associated epigenetic changes in monozygotic twins discordant for schizophrenia and bipolar disorder. *Human Molecular Genetics.* **20**, 4786–4796.

Dong E, Grayson DR, Guidotti A, Costa E (2009). Antipsychotic subtypes can be characterized by differences in their ability to modify GABAergic promoter methylation. *Epigenomics* **1**, 201–211.

Dong E, Guidotti A, Grayson DR, Costa E (2007). Histone hyperacetylation induces demethylation of reelin and 67-kDa glutamic acid decarboxylase promoters. *Proceedings of the National Academy of Sciences* **104**, 4676–4681.

Dong E, Nelson M, Grayson DR, Costa E, Guidotti A (2008). Clozapine and sulpiride but not haloperidol or olanzapine activate brain DNA demethylation. *Proceedings of the National Academy of Sciences* **105**, 13614–13619.

Du P, Zhang X, Huang CC, Jafari N, Kibbe WA, Hou L, Lin SM (2010). Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. *BMC Bioinformatics* **11**

van Eijk KR, de Jong S, Boks MPM, Langeveld T, Colas F, Veldink JH, de Kovel CGF, Janson E, Strengman E, Langfelder P, Kahn RS, van den Berg LH, Horvath S, Ophoff RA (2012). Genetic analysis of DNA methylation and gene expression levels in whole blood of healthy human subjects. *BMC genomics* **13**, 636.

Falcon S, Gentleman R (2007). Using GOstats to test gene lists for GO term association. *Bioinformatics* **23**, 257–258.

First MB, Spitzer RL, Gibbon M, Williams JBW (2002). *Structured Clinical Interview for DSM-IV Axis I Disorders, Research Version, patient edition with psychotic screen (SCID-I/ PW/ PSY SCREEN).*

Flanagan RJ, Dunk L (2008). Haematological toxicity of drugs used in psychiatry. *Human Psychopharmacology* **23**, 27–41.

Flavell SW, Greenberg ME, Gene A (2008). Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. *Annual review of neuroscience* **31**, 563–90.

Fukuchi M, Nii T, Ishimaru N, Minamino A, Hara D, Takasaki I, Tabuchi A, Tsuda M (2009). Valproic acid induces up- or down-regulation of gene expression responsible for the neuronal excitation and inhibition in rat cortical neurons through its epigenetic actions. *Neuroscience Research* **65**, 35–43.

Gladkevich A, Kauffman HF, Korf J (2004). Lymphocytes as a neural probe: Potential for studying psychiatric disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **28**, 559–576.

Goodwin GM (2009). Evidence-based guidelines for treating bipolar disorder: Revised second edition-recommendations from the British association for psychopharmacology. *Journal of Psychopharmacology* **23**, 346–388.

Gottlicher M (2004). Valproic acid: an old drug newly discovered as inhibitor of histone deacetylases. *Annal Hematology* **83 Suppl 1**, S91-2.

Grayson DR, Guidotti A (2013). The dynamics of DNA methylation in schizophrenia and related psychiatric disorders. *Neuropsychopharmacology* **38**, 138–66.

Guidotti A, Dong E, Kundakovic M, Satta R, Grayson DR, Costa E (2009). Characterization of the action of antipsychotic subtypes on valproate-induced chromatin remodeling. *Trends in Pharmacological Sciences* **30**, 55–60.

Hao Y (2004). Mood Stabilizer Valproate Promotes ERK Pathway-Dependent Cortical Neuronal Growth and Neurogenesis. *Journal of Neuroscience* **24**, 6590–6599.

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey LH (2008). Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proceedings of the National Academy of Sciences* **105**, 17046–17049.

Horiuchi Y, Nakayama J, Ishiguro H, Ohtsuki T, Detera-Wadleigh SD, Toyota T, Yamada K, Nankai M, Shibuya H, Yoshikawa T, Arinami T (2004). Possible association between a haplotype of the GABA-A receptor alpha 1 subunit gene (GABRA1) and mood disorders. *Biological Psychiatry* **55**, 40–45.

Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, Wiencke JK, Kelsey KT (2012). DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics* **13**

Houseman EA, Kim S, Kelsey KT, Wiencke JK (2015). DNA Methylation in Whole Blood: Uses and Challenges. *Current environmental health reports* **2**, 145–154.

Huynh M, Chee K, Lau DHM (2005). Thrombotic thrombocytopenic purpura associated with quetiapine. *Annals of Pharmacotherapy* **39**, 1346–1348.

Illingworth RS, Gruenewald-Schneider U, De Sousa D, Webb S, Merusi C, Kerr ARW, James KD, Smith C, Walker R, Andrews R, Bird AP (2015). Inter-individual variability contrasts with regional homogeneity in the human brain DNA methylome. *Nucleic Acids Research* **43**, 732–744.

Jaehne EJ, Corrigan F, Toben C, Jawahar MC, Baune BT (2015). The effect of the antipsychotic drug quetiapine and its metabolite norquetiapine on acute inflammation, memory and anhedonia. *Pharmacology Biochemistry and Behavior* **135**, 136–144.

Johnson WE, Li C, Rabinovic A (2007). Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* **8**, 118–127.

Kim B, Rincón Castro LM, Jawed S, Niles LP (2008). Clinically relevant concentrations of valproic acid modulate melatonin MT1 receptor, HDAC and MeCP2 mRNA expression in C6 glioma cells. *European Journal of Pharmacology* **589**, 45–48.

Kim JK, Samaranayake M, Pradhan S (2009). Epigenetic mechanisms in mammals. *Cellular and Molecular Life Sciences* **66**, 596–612.

Klose RJ, Bird AP (2006). Genomic DNA methylation: The mark and its mediators. *Trends in Biochemical Sciences* **31**, 89–97.

Kofink D, Boks MPM, Timmers HTM, Kas MJ (2013). Epigenetic dynamics in psychiatric disorders: Environmental programming of neurodevelopmental processes. *Neuroscience and Biobehavioral Reviews* **37**, 831–845.

Kowatch RA, Fristad M, Birmaher B, Wagner KD, Findling RL, Hellander M (2005). Treatment guidelines for children and adolescents with bipolar disorder. *Journal of the American Academy of Child and Adolescent Psychiatry* **44**, 213–235.

Lam LL, Emberly E, Fraser HB, Neumann SM, Chen E, Miller GE, Kobor MS (2012). Factors underlying variable DNA methylation in a human community cohort. *Proc Natl Acad Sci U S A* **109 Suppl**, 17253–17260.

Langfelder P, Horvath S (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics* **9**

Langfelder P, Horvath S (2012). Fast *R* Functions for Robust Correlations and Hierarchical Clustering. *Journal of Statistical Software* **46**

Leng Y, Liang M-H, Ren M, Marinova Z, Leeds P, Chuang D-M (2008). Synergistic Neuroprotective Effects of Lithium and Valproic Acid or Other Histone Deacetylase Inhibitors in Neurons: Roles of Glycogen Synthase Kinase-3 Inhibition. *Journal of Neuroscience* **28**, 2576–2588.

Li J, Guo Y, Schroeder FA, Youngs RM, Schmidt TW, Ferris C, Konradi C, Akbarian S (2004). Dopamine D2-like antagonists induce chromatin remodeling in striatal neurons through cyclic AMP-protein kinase A and NMDA receptor signaling. *Journal of Neurochemistry* **90**, 1117–1131.

Luo C, Xu H, Li XM (2005). Quetiapine reverses the suppression of hippocampal neurogenesis caused by repeated restraint stress. *Brain Research* **1063**, 32–39.

Matrisciano F, Dong E, Gavin DP, Nicoletti F, Guidotti A (2011). Activation of group II metabotropic glutamate receptors promotes DNA demethylation in the mouse brain. *Molecular pharmacology* **80**, 174–82.

Matrisciano F, Tueting P, Dalal I, Kadriu B, Grayson DR, Davis JM, Nicoletti F, Guidotti A (2013). Epigenetic modifications of GABAergic interneurons are associated with the schizophrenia-like phenotype induced by prenatal stress in mice. *Neuropharmacology* **68**, 184–194.

Mill J, Tang T, Kaminsky Z, Khare T, Yazdanpanah S, Bouchard L, Jia P, Assadzadeh A, Flanagan J, Schumacher A, Wang SC, Petronis A (2008). Epigenomic Profiling Reveals DNA-Methylation Changes Associated with Major Psychosis. *American Journal of Human Genetics* **82**, 696–711.

Miller BR, Hen R (2015). The current state of the neurogenic theory of depression and anxiety. *Current Opinion in Neurobiology* **30**, 51–58.

Minucci S, Pelicci PG (2006). Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nature Reviews Cancer* **6**, 38–51.

Mitchell CP, Chen Y, Kundakovic M, Costa E, Grayson DR (2005). Histone deacetylase inhibitors decrease reelin promoter methylation in vitro. *Journal of Neurochemistry* **93**, 483–492.

Oyesanmi O, Kunkel EJ, Monti DA, Field HL (1999). Hematologic side effects of psychotropics. *Psychosomatics* **40**, 414–421.

Perisic T, Zimmermann N, Kirmeier T, Asmus M, Tuorto F, Uhr M, Holsboer F, Rein T, Zschocke J (2010). Valproate and amitriptyline exert common and divergent influences on global and gene

promoter-specific chromatin modifications in rat primary astrocytes. *Neuropsychopharmacology* **35**, 792–805.

Popkie AP, Zeidner LC, Albrecht AM, D'Ippolito A, Eckardt S, Newsom DE, Groden J, Doble BW, Aronow B, McLaughlin KJ, White P, Phiel CJ (2010). Phosphatidylinositol 3-kinase (PI3K) signaling via glycogen synthase kinase-3 (Gsk-3) regulates DNA methylation of imprinted loci. *Journal of Biological Chemistry* **285**, 41337–41347.

R Core Team, R Foundation For Statistical Computing (2014). R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing* **1**, 2673.

Reik W (2007). Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* **447**, 425–432.

Reinius LE, Acevedo N, Joerink M, Pershagen G, Dahlén SE, Greco D, Söderhäll C, Scheynius A, Kere J (2012). Differential DNA methylation in purified human blood cells: Implications for cell lineage and studies on disease susceptibility. *PLoS ONE* **7**

Réus GZ, Fries GR, Stertz L, Badawy M, Passos IC, Barichello T, Kapczinski F, Quevedo J (2015). The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. *Neuroscience* **300**, 141–154.

Roth TL, Sweatt JD (2009). Regulation of chromatin structure in memory formation. *Current Opinion in Neurobiology* **19**, 336–342.

Rutten BPF, Mill J (2009). Epigenetic mediation of environmental influences in major psychotic disorders. *Schizophrenia Bulletin* **35**, 1045–1056.

Schalkwyk LC, Pidsley R, Wong CCY (2013). WateRmelon: Illumina 450 methylation array normalization and metrics. *R package version 1.2.2*

Schübeler D (2015). Function and information content of DNA methylation. *Nature* **517**, 321–326.

Serretti A, Mandelli L (2008). The genetics of bipolar disorder: Genome 'hot regions,' genes, new potential candidates and future directions. *Molecular Psychiatry* **13**, 742–771.

Shankar BR (2007). Quetiapine-induced leucopenia and thrombocytopenia. *Psychosomatics* **48**, 530–1.

Smyth GK (2004). Linear Models and Empirical Bayes Methods for Assessing Differential Expression in Microarray Experiments Linear Models and Empirical Bayes Methods for Assessing Differential Expression in Microarray Experiments. *Statistical Applications in Genetics and Molecular Biology* **3**, 1–26.

Sun Y V., Turner ST, Smith JA, Hammond PI, Lazarus A, Van De Rostyne JL, Cunningham JM, Kardia SLR (2010). Comparison of the DNA methylation profiles of human peripheral blood cells and transformed B-lymphocytes. *Human Genetics* **127**, 651–658.

Suzuki MM, Bird A (2008). DNA methylation landscapes: Provocative insights from epigenomics. *Nature Reviews Genetics* **9**, 465–476.

Tingley D, Yamamoto T, Hirose K, Keele L, Imai K (2014). Mediation: R Package for Causal Mediation Analysis. *Journal of Statistical Software* **59**, 1–38.

Tremolizzo L, Doueiri MS, Dong E, Grayson DR, Davis J, Pinna G, Tueting P, Rodriguez-Menendez V, Costa E, Guidotti A (2005). Valproate corrects the schizophrenia-like epigenetic behavioral modifications induced by methionine in mice. *Biological Psychiatry* **57**, 500–509.

Tsankova N, Renthal W, Kumar A, Nestler EJ (2007). Epigenetic regulation in psychiatric disorders. *Nature Reviews Neuroscience* **8**, 355–367.

Vasudev K, Keown P, Gibb I, McAllister-Williams RH (2010). Hematological effects of valproate in psychiatric patients: What are the risk factors? *Journal of Clinical Psychopharmacology* **30**, 282–285.

Veldic M, Kadriu B, Maloku E, Agis-Balboa RC, Guidotti A, Davis JM, Costa E (2007). Epigenetic mechanisms expressed in basal ganglia GABAergic neurons differentiate schizophrenia from bipolar disorder. *Schizophrenia Research* **91**, 51–61.

Walton E, Hass J, Liu J, Roffman JL, Bernardoni F, Roessner V, Kirsch M, Schackert G, Calhoun V, Ehrlich S (2016). Correspondence of DNA methylation between blood and brain tissue and its application to schizophrenia research. *Schizophrenia Bulletin* **42**, 406–414.

Watkins CC, Sawa A, Pomper MG (2014). Glia and immune cell signaling in bipolar disorder: Insights from neuropharmacology and molecular imaging to clinical application. *Translational Psychiatry* **4**

Wong CCY, Meaburn EL, Ronald A, Price TS, Jeffries AR, Schalkwyk LC, Plomin R, Mill J (2014). Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. *Molecular Psychiatry* **19**, 495–503.

Yasuda S, Liang MH, Marinova Z, Yahyavi A, Chuang DM (2009). The mood stabilizers lithium and valproate selectively activate the promoter IV of brain-derived neurotrophic factor in neurons. *Molecular Psychiatry* **14**, 51–59.

Yuan PX, Huang LD, Jiang YM, Gutkind JS, Manji HK, Chen G (2001). The mood stabilizer valproic acid activates mitogen-activated protein kinases and promotes neurite growth. *The Journal of biological chemistry* **276**, 31674–83.

Supplemental information

Supplemental material 3.1

Extra graphics and tables for quality control and sample description

Quality control

Below are correlation plots for medication, methylation potential confounders for three stages of quality control. First before any batch correction was applied, second after correction for sentrix array and position on the sentrix array and third after also regressing out the effects of cellcount while protecting for possible medication effects.

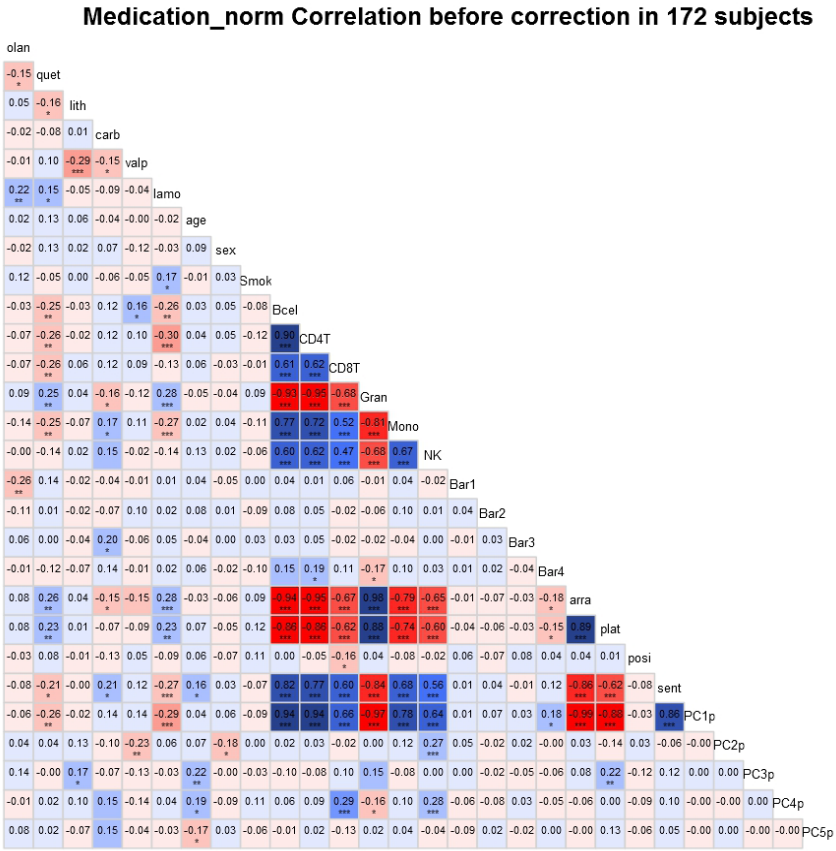


Figure S3.1 Correlation between medication, methylation and potential confounders before batch correction.

Significant values are denoted by * p<0.05, ** p<0.001, ***p<0.0001. Abbreviations: olan=olanzapine, quet= quetiapine, lith=lithium, carb=carbamazepine, depa=depakine, lamo=lamotrigine, Smok=smoking, CD8T= CD8 T, CD4T=CD4 T cell, cell NK=Natural Killer, Mono=Monocytes,Gran=Granulocytes, Bar= ancestry estimates calculated according to Barfield et al, arra=27k array, plat=plate,posi=position on sentrix, sent=sentrix, PC= principal component.

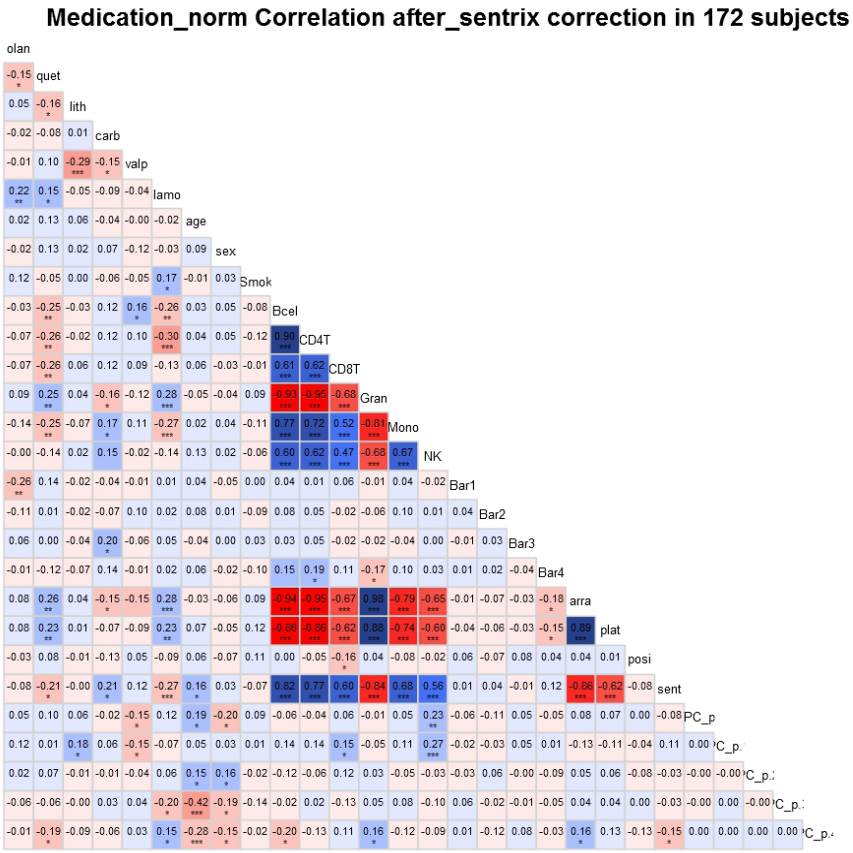
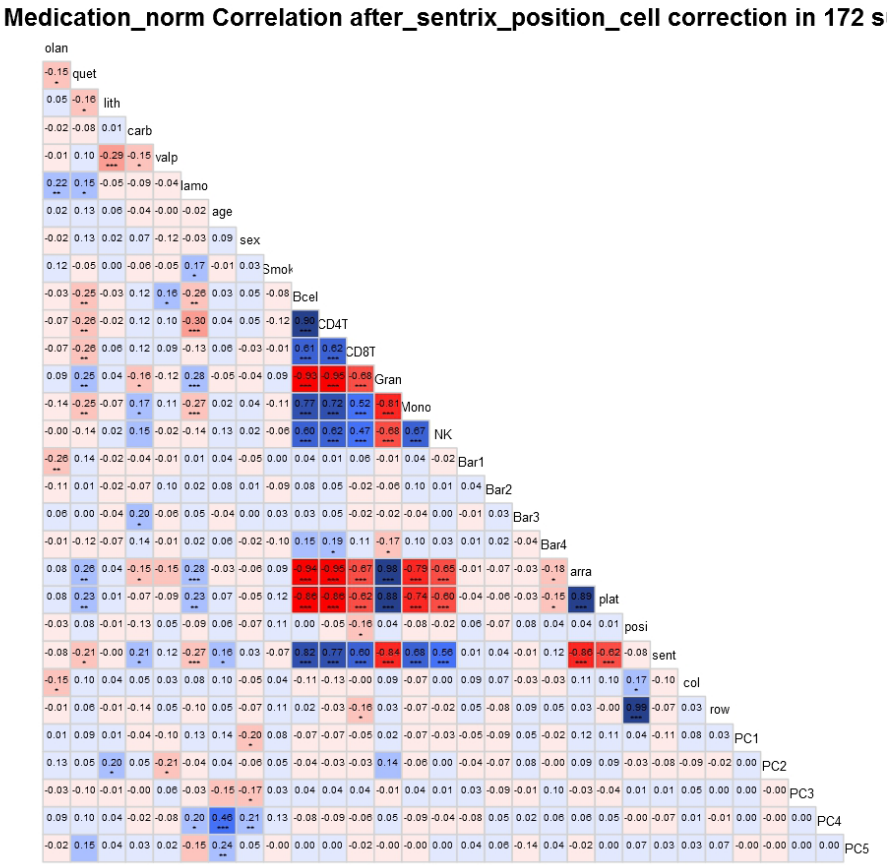


Figure S3.2 Correlation between medication, methylation and potential confounders after batch correction for sentrix and position.

Significant values are denoted by * p<0.05, ** p<0.001, ***p<0.0001. Abbreviations: olan=olanzapine, quet= quetiapine, lith=lithium, carb=carbamazepine, Valp=valproic acid, lamo=lamotrigine, Smok=smoking, CD8T= CD8 T, CD4T=CD4 T cell, cell NK=Natural Killer, Mono=Monocytes,Gran=Granulocytes, Bar= ancestry estimates calculated according to Barfield et al, arra=27k array, plat=plate,posi=position on sentrix, sent=sentrix, PC= principal component.



172 subjects for 22988 probes 2015-06-29 15:32

Figure S3.3 Correlation between medication, methylation and potential confounders after batch correction for sentrix and position and regressing out cellcount while protecting for possible medication effects.

Significant values are denoted by * p<0.05, ** p<0.001, ***p<0.0001. Abbreviations: olan=olanzapine, quet= quetiapine, lith=lithium, carb=carbamazepine, valp=valproic acid, lamo=lamotrigine, Smok=smoking, CD8T= CD8 T, CD4T=CD4 T cell, cell NK=Natural Killer, Mono=Monocytes,Gran=Granulocytes, Bar= ancestry estimates calculated according to Barfield et al, arra=27k array, plat=plate,posi=position on sentrix, sent=sentrix, PC= principal component.

Sample description

Table S3.1 Frequencies for all medication types in the sample.

Medication group	Medication type	Entire sample (n=172)	Olanzapine users (n=27)	Quetiapine users (n=29)	Lithium users (n=112)	Carbamazepine users (n=15)	Valproic acid users (n=33)	Lamotrigine users (n=14)
Antipsychotic	Olanzapine	27	-	1	19	2	5	6
	Quetiapine	29	1	-	14	1	8	5
	Haloperidol	4	0	0	2	1	0	1
	Cisordinal	8	1	0	5	2	0	0
	Risperidon	6	0	0	3	0	3	0
	Clozapine	4	0	0	2	0	0	0
	Broomperidol	1	0	0	1	0	0	0
	Pimozide	2	0	1	1	0	0	0
	Pipamperon	1	0	0	1	0	0	1
Mood stabiliziers	Lithium	112	19	14	-	10	12	8
	Carbamazepine	15	2	1	10	-	0	0
	Valproic acid	33	5	8	12	0	-	2
	Lamotrigine	14	6	5	8	0	2	-
	Gabapentine	1	0	1	1	0	0	0
	Topiramaat	1	0	0	1	0	0	0
Antidepressant	Tranylcypromine	1	0	0	1	0	0	0
	Clomipramine	1	0	0	1	0	0	1
	Venlafaxine	9	1	4	6	1	2	0
	Paroxetine	3	0	0	2	1	0	0
	Sertraline	4	0	2	0	1	3	0
	Citalopram	5	0	0	5	0	0	0
	Escitalopram	2	1	1	1	0	0	1
	Fluvoxamine	1	0	0	1	0	0	0
	Mirtazapine	1	0	0	1	0	0	0
	Fluoxetine	1	0	1	0	0	0	1
	Trazodon	1	0	0	0	0	1	0
ADHD	Methylfenidaat	2	1	1	1	0	0	0
Anxiolytic	Zolpidem	2	0	1	0	0	0	1
	Zopiclon	4	2	0	4	0	0	2
	Buspiron	1	0	0	1	0	0	0
	Lorazepam	2	1	0	2	0	0	0
	Oxazepam	5	1	3	4	0	0	2
	Temazepam	4	1	0	3	0	1	1

QQ plots for the genome-wide association analysis

Model:

Methylation probe ~ Olanzapine + Quetiapine + Lithium + Carbamazepine + Valproic acid + Lamotrigine + age + sex + Smoking status

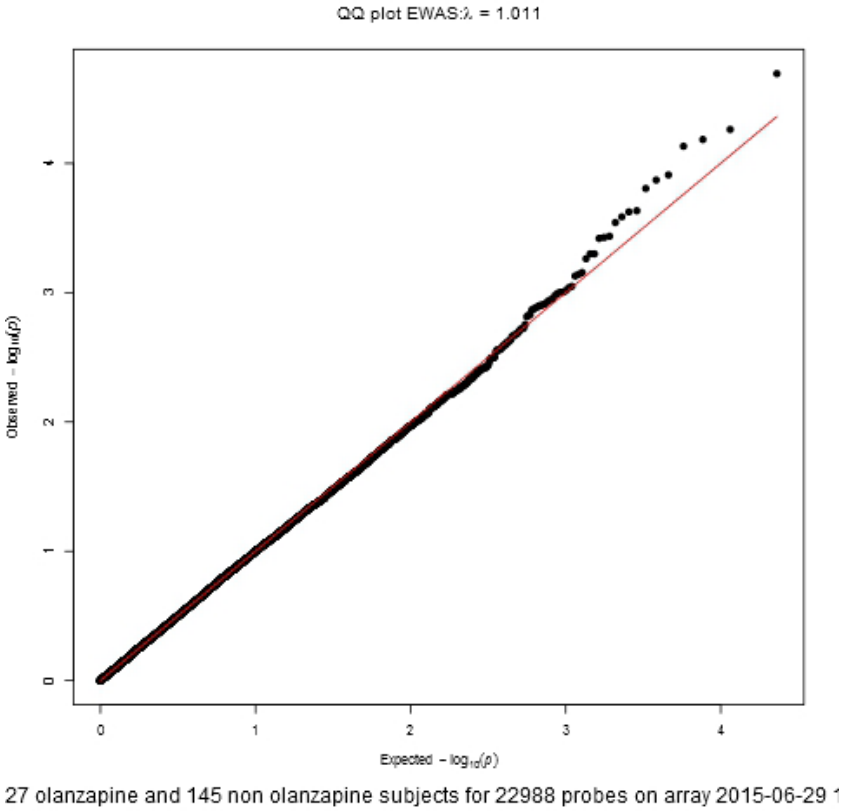


Figure S3.4 QQplot displaying the distribution of actual p values compared to the expected distribution (red line) for olanzapine.

The genomic inflation factor lambda is calculated and displayed above the graph.

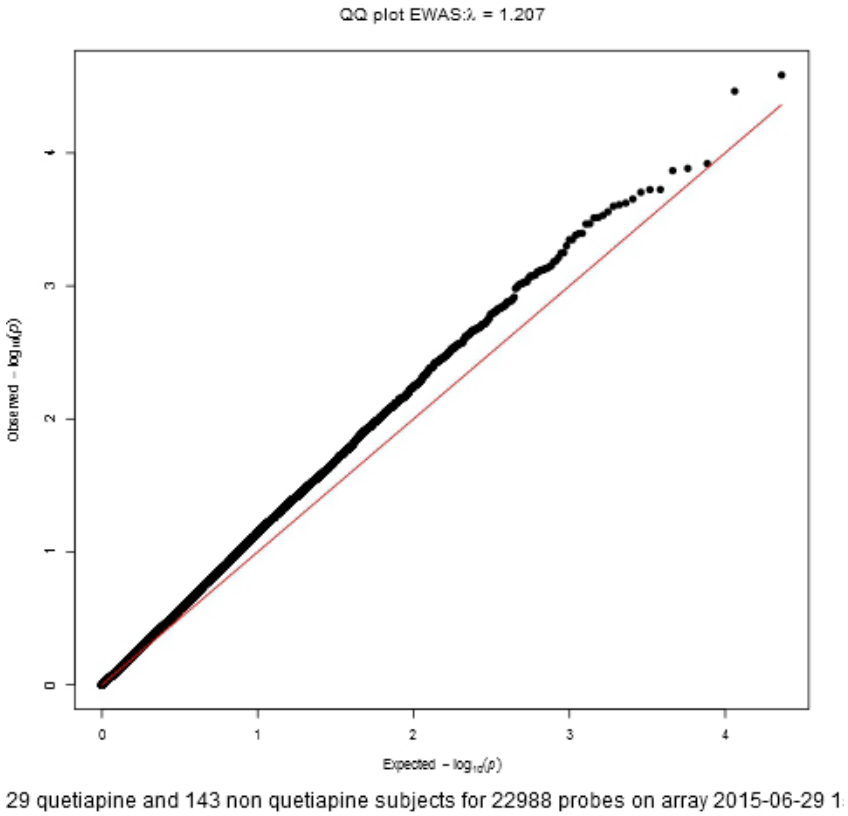


Figure S3.5 QQplot displaying the distribution of actual p values compared to the expected distribution (red line) for quetiapine.

The genomic inflation factor lambda is calculated and displayed above the graph.

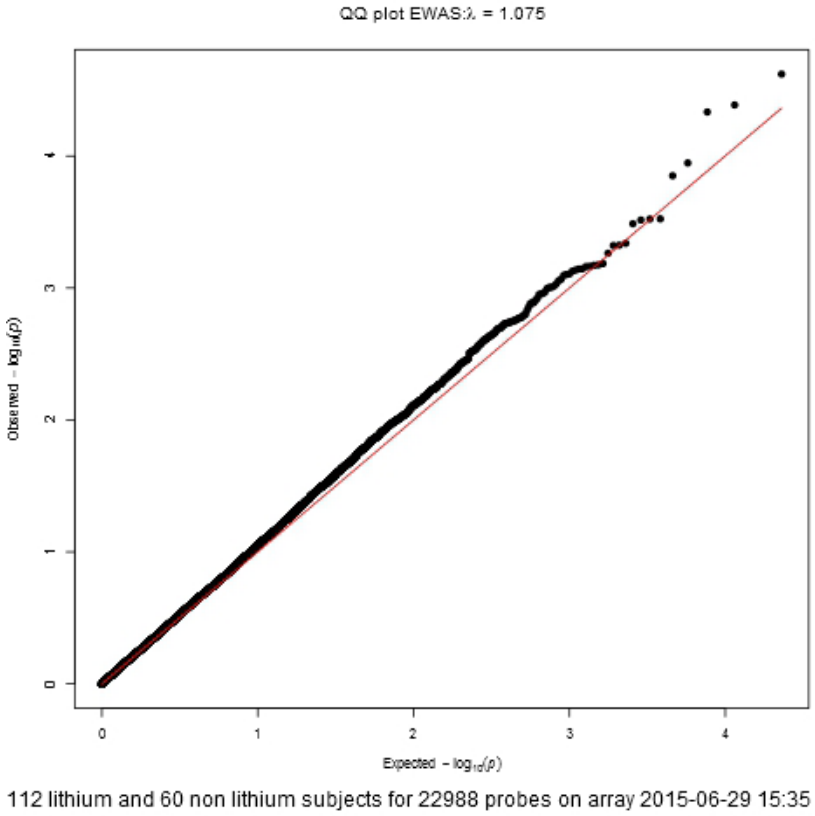


Figure S3.6 QQplot displaying the distribution of actual p values compared to the expected distribution (red line) for lithium.

The genomic inflation factor lambda is calculated and displayed above the graph.

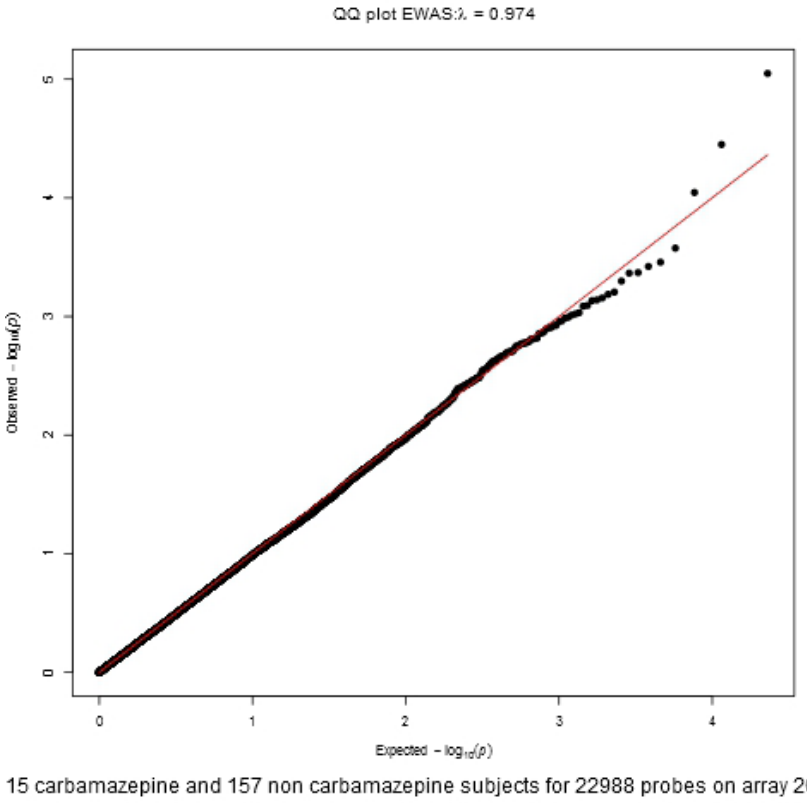


Figure S3.7 QQplot displaying the distribution of actual p values compared to the expected distribution (red line) for carbamazepine.

The genomic inflation factor lambda is calculated and displayed above the graph.

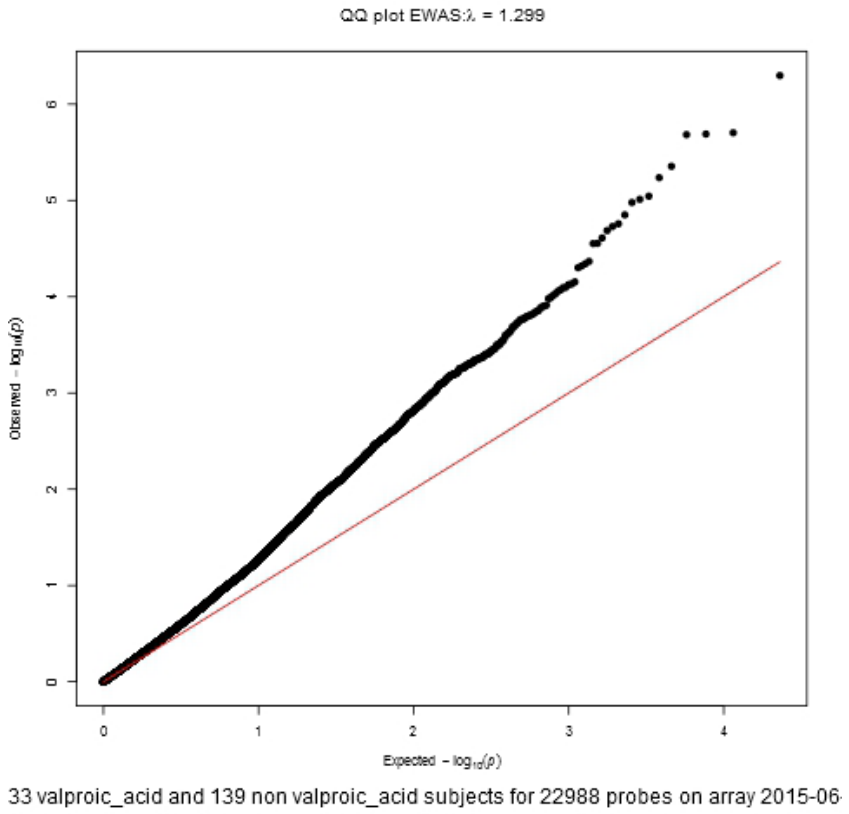


Figure S3.8 QQplot displaying the distribution of actual p values compared to the expected distribution (red line) for valproic acid.

The genomic inflation factor lambda is calculated and displayed above the graph.

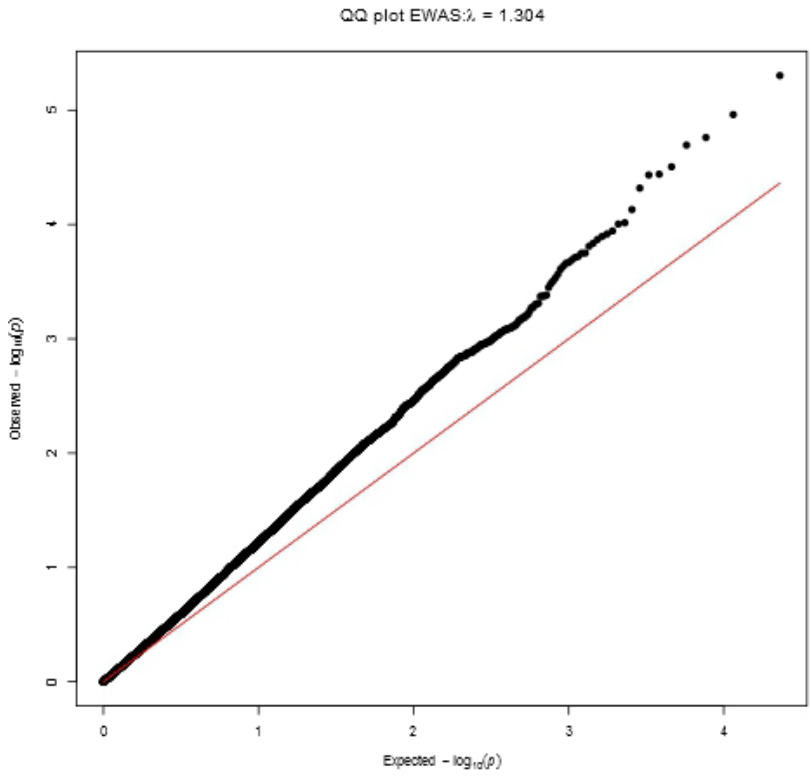


Figure S3.9 QQplot displaying the distribution of actual p values compared to the expected distribution (red line) for lamotrigine.

The genomic inflation factor lambda is calculated and displayed above the graph.

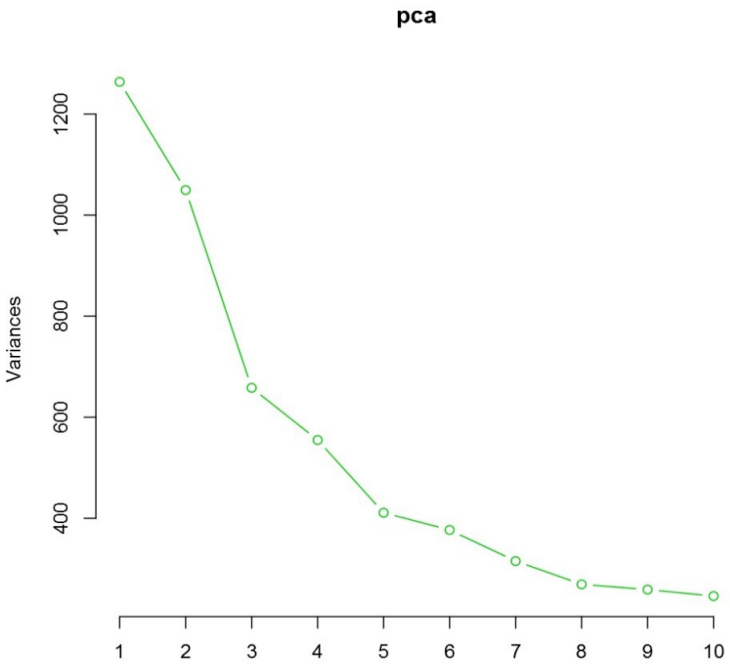


Figure S3.10 Screeplot depicting the proportion explained variance per principal component.

The numbers on the x axis correspond to the number of each principal components.

Per candidate gene description

Table S3.2 Beta and nominal significant p value per medication type for all probes per gene.

If methylation levels are significantly higher(B is positive and p<0.05) in medication users the cell is red, if methylation levels are significantly lower(B is negative and p<0.05) in medication users the cell is green. If a nominal significant association between methylation and medication is in line with literature the values are in italic and bold.

Gene	Expectation based on literature	Probes	Olanzapine	Quetiapine	Lithium	Carbamazepine	Valproic_acid	Lamotrigine	Rsquared
BDNF	Hypomethylation promotor lithium	cg27351358	B=-0.024, p=0.626, FDR p=0.776	B=-0.019, p=0.694, FDR p=0.897	B=0.031, p=0.417, FDR p=0.901	B=0.084, p=0.178, FDR p=0.989	B=0.034, p=0.461, FDR p=0.688	B=-0.049, p=0.464, FDR p=0.803	0.065
GAD1	Hypomethylation promotor valproic acid	cg00915206	B=-0.001, p=0.979, FDR p=0.993	B=-0.015, p=0.676, FDR p=0.897	B=-0.001, p=0.983, FDR p=0.983	B=0.01, p=0.824, FDR p=0.989	B=0.015, p=0.661, FDR p=0.853	B=-0.017, p=0.731, FDR p=0.965	0.0547
		cg11582100	B=0.036, p=0.329, FDR p=0.776	B=-0.017, p=0.641, FDR p=0.897	B=0.011, p=0.688, FDR p=0.901	B=0.023, p=0.623, FDR p=0.989	B=-0.012, p=0.723, FDR p=0.863	B=-0.034, p=0.497, FDR p=0.803	0.0262
H19	Hypomethylation lithium	cg11492040	B=-0.022, p=0.596, FDR p=0.776	B=-0.026, p=0.529, FDR p=0.897	B=-0.02, p=0.534, FDR p=0.901	B=0.033, p=0.534, FDR p=0.989	B=-0.046, p=0.245, FDR p=0.648	B=0.033, p=0.562, FDR p=0.803	0.0198
		cg06197492	B=0.012, p=0.763, FDR p=0.882	B=-0.038, p=0.341, FDR p=0.789	B=-0.021, p=0.491, FDR p=0.901	B=0.039, p=0.437, FDR p=0.989	B=0.002, p=0.965, FDR p=0.965	B=-0.008, p=0.888, FDR p=0.965	0.0239
		cg10602543	B=0.018, p=0.629, FDR p=0.776	B=-0.022, p=0.556, FDR p=0.897	B=-0.043, p=0.138, FDR p=0.901	B=0.001, p=0.989, FDR p=0.989	B=-0.077, p=0.031, FDR p=0.312	B=0.082, p=0.108, FDR p=0.635	0.0941
		cg23977670	B=-0.032, p=0.278, FDR p=0.776	B=-0.024, p=0.419, FDR p=0.897	B=-0.015, p=0.511, FDR p=0.901	B=-0.098, p=0.01, FDR p=0.183	B=0.026, p=0.352, FDR p=0.688	B=0.037, p=0.353, FDR p=0.726	0.0836
		cg11716026	B=-0.064, p=0.11, FDR p=0.776	B=-0.012, p=0.769, FDR p=0.925	B=-0.046, p=0.141, FDR p=0.901	B=-0.049, p=0.334, FDR p=0.989	B=-0.071, p=0.064, FDR p=0.345	B=0.075, p=0.171, FDR p=0.635	0.0588
		cg22172494	B=-0.077, p=0.351, FDR p=0.776	B=-0.016, p=0.845, FDR p=0.925	B=0.057, p=0.369, FDR p=0.901	B=-0.083, p=0.424, FDR p=0.989	B=0.119, p=0.129, FDR p=0.473	B=0.024, p=0.827, FDR p=0.965	0.0398

		cg26808784	B=-0.075, p=0.046, FDR p=0.776	B=-0.044, p=0.232, FDR p=0.789	B=-0.028, p=0.328, FDR p=0.901	B=-0.009, p=0.851, FDR p=0.989	B=-0.007, p=0.853, FDR p=0.915	B=0.068, p=0.177, FDR p=0.635	0.054
		cg25852472	B=0.028, p=0.585, FDR p=0.776	B=0.008, p=0.866, FDR p=0.925	B=0.028, p=0.475, FDR p=0.901	B=-0.002, p=0.974, FDR p=0.989	B=0.036, p=0.445, FDR p=0.688	B=0.077, p=0.258, FDR p=0.714	0.0953
		cg15269875	B=0.023, p=0.537, FDR p=0.776	B=0.056, p=0.132, FDR p=0.789	B=-0.008, p=0.791, FDR p=0.944	B=-0.048, p=0.308, FDR p=0.989	B=-0.051, p=0.152, FDR p=0.473	B=-0.079, p=0.121, FDR p=0.635	0.0591
		cg15317267	B=0.037, p=0.237, FDR p=0.776	B=0.044, p=0.156, FDR p=0.789	B=0.011, p=0.654, FDR p=0.901	B=0.12, p=0.003, FDR p=0.101	B=0.04, p=0.18, FDR p=0.512	B=0.002, p=0.968, FDR p=0.971	0.0993
		cg17769238	B=-0.039, p=0.123, FDR p=0.776	B=-0.006, p=0.808, FDR p=0.925	***B=-0.053, p=0.008, FDR p=0.307***	B=0.012, p=0.716, FDR p=0.989	B=0.003, p=0.913, FDR p=0.939	B=0.045, p=0.189, FDR p=0.635	0.108
		cg02657360	B=0.043, p=0.427, FDR p=0.776	B=0.099, p=0.066, FDR p=0.49	B=0.038, p=0.361, FDR p=0.901	B=0.033, p=0.628, FDR p=0.989	B=-0.009, p=0.865, FDR p=0.915	B=-0.046, p=0.526, FDR p=0.803	0.0334
IGF2	Hypomethylation lithium	cg02807948	B=-0.086, p=0.133, FDR p=0.776	B=-0.028, p=0.624, FDR p=0.897	***B=-0.092, p=0.04, FDR p=0.534***	B=0.058, p=0.424, FDR p=0.989	B=0.036, p=0.502, FDR p=0.688	B=0.111, p=0.155, FDR p=0.635	0.0713
		cg22956483	B=-0.014, p=0.75, FDR p=0.882	B=0.007, p=0.875, FDR p=0.925	B=0.016, p=0.645, FDR p=0.901	B=-0.006, p=0.916, FDR p=0.989	B=0.039, p=0.348, FDR p=0.688	B=-0.064, p=0.285, FDR p=0.714	0.028
		cg02166532	B=0, p=0.993, FDR p=0.993	B=-0.025, p=0.456, FDR p=0.897	B=0.011, p=0.686, FDR p=0.901	B=0.043, p=0.315, FDR p=0.989	B=0.029, p=0.367, FDR p=0.688	B=-0.033, p=0.474, FDR p=0.803	0.078
		cg20339650	B=-0.037, p=0.335, FDR p=0.776	B=-0.09, p=0.018, FDR p=0.49	B=-0.004, p=0.899, FDR p=0.978	B=0.06, p=0.215, FDR p=0.989	B=0.067, p=0.065, FDR p=0.345	B=0.075, p=0.149, FDR p=0.635	0.119
		cg13756879	B=0.038, p=0.406, FDR p=0.776	B=-0.051, p=0.257, FDR p=0.789	B=0.013, p=0.718, FDR p=0.901	B=0.005, p=0.926, FDR p=0.989	B=0.046, p=0.277, FDR p=0.654	B=-0.012, p=0.844, FDR p=0.965	0.0343
		cg25163476	B=-0.004, p=0.899, FDR p=0.979	B=-0.069, p=0.028, FDR p=0.49	B=0.023, p=0.339, FDR p=0.901	B=-0.01, p=0.81, FDR p=0.989	B=0.062, p=0.037, FDR p=0.312	B=0.025, p=0.564, FDR p=0.803	0.0927
		cg13791131	B=-0.007, p=0.872, FDR p=0.978	B=-0.047, p=0.308, FDR p=0.789	B=-0.02, p=0.582, FDR p=0.901	B=0.012, p=0.834, FDR p=0.989	B=0.065, p=0.136, FDR p=0.473	B=-0.015, p=0.806, FDR p=0.965	0.0525

		cg25574024	B=-0.052, p=0.296, FDR p=0.776	B=-0.092, p=0.062, FDR p=0.49	B=-0.026, p=0.506, FDR p=0.901	B=-0.022, p=0.73, FDR p=0.989	B=0.068, p=0.148, FDR p=0.473	B=-0.092, p=0.174, FDR p=0.635	0.124
		cg21237591	B=-0.027, p=0.446, FDR p=0.776	B=0.013, p=0.703, FDR p=0.897	B=0.009, p=0.731, FDR p=0.901	B=-0.02, p=0.65, FDR p=0.989	B=-0.023, p=0.484, FDR p=0.688	B=0.049, p=0.301, FDR p=0.714	0.0339
		cg10501065	B=0.044, p=0.242, FDR p=0.776	B=0.019, p=0.607, FDR p=0.897	B=0.001, p=0.982, FDR p=0.983	B=-0.022, p=0.637, FDR p=0.989	B=-0.029, p=0.418, FDR p=0.688	B=0.006, p=0.913, FDR p=0.965	0.0407
		cg04112019	B=-0.046, p=0.2, FDR p=0.776	B=-0.008, p=0.829, FDR p=0.925	B=-0.006, p=0.826, FDR p=0.955	B=-0.025, p=0.575, FDR p=0.989	B=-0.008, p=0.822, FDR p=0.915	B=0.051, p=0.296, FDR p=0.714	0.0507
		cg20792294	B=-0.028, p=0.514, FDR p=0.776	B=-0.042, p=0.316, FDR p=0.789	B=0.032, p=0.332, FDR p=0.901	B=0.03, p=0.58, FDR p=0.989	B=0.082, p=0.042, FDR p=0.312	B=-0.125, p=0.032, FDR p=0.635	0.124
		cg12322132	B=0.016, p=0.606, FDR p=0.776	B=-0.03, p=0.326, FDR p=0.789	B=0.014, p=0.552, FDR p=0.901	B=-0.038, p=0.319, FDR p=0.989	B=-0.022, p=0.449, FDR p=0.688	B=0.027, p=0.516, FDR p=0.803	0.0487
		cg16817891	B=0.003, p=0.948, FDR p=0.993	B=0.022, p=0.565, FDR p=0.897	B=-0.024, p=0.434, FDR p=0.901	B=0.004, p=0.931, FDR p=0.989	B=-0.097, p=0.009, FDR p=0.312	B=-0.015, p=0.77, FDR p=0.965	0.0629
		cg11005826	B=-0.029, p=0.474, FDR p=0.776	B=-0.022, p=0.582, FDR p=0.897	B=0.002, p=0.952, FDR p=0.983	B=0.001, p=0.982, FDR p=0.989	B=0.027, p=0.478, FDR p=0.688	B=0.097, p=0.073, FDR p=0.635	0.0266
RELN	Hypomethylation promotor valproic acid	cg17923358	B=0.049, p=0.197, FDR p=0.776	B=-0.04, p=0.281, FDR p=0.789	B=0.01, p=0.726, FDR p=0.901	B=0.063, p=0.184, FDR p=0.989	B=0.079, p=0.028, FDR p=0.312	B=0.037, p=0.473, FDR p=0.803	0.0775
SLC1A2	Hypomethylation promotor valproic acid	cg09017174	B=0.022, p=0.573, FDR p=0.776	B=-0.003, p=0.931, FDR p=0.931	B=-0.034, p=0.26, FDR p=0.901	B=0.011, p=0.813, FDR p=0.989	B=-0.015, p=0.679, FDR p=0.853	B=0.002, p=0.971, FDR p=0.971	0.0143
		cg08258650	B=0.024, p=0.515, FDR p=0.776	B=-0.035, p=0.334, FDR p=0.789	B=0.004, p=0.884, FDR p=0.978	B=-0.03, p=0.518, FDR p=0.989	B=-0.027, p=0.428, FDR p=0.688	B=-0.049, p=0.319, FDR p=0.714	0.0382
SLC6A4	Hypomethylation mood stabilizers	cg22584138	B=-0.112, p=0.068, FDR p=0.776	B=-0.036, p=0.555, FDR p=0.897	B=0.096, p=0.043, FDR p=0.534	B=-0.052, p=0.497, FDR p=0.989	B=-0.018, p=0.76, FDR p=0.879	B=0.163, p=0.049, FDR p=0.635	0.262
		cg05016953	B=0.029, p=0.471, FDR p=0.776	B=-0.004, p=0.917, FDR p=0.931	B=0.031, p=0.321, FDR p=0.901	B=-0.049, p=0.331, FDR p=0.989	B=-0.041, p=0.283, FDR p=0.654	B=-0.028, p=0.603, FDR p=0.827	0.0413

Supplemental material 3.2

Due to the length of Supplemental material 3.2, only the first page of the top 1000 probes for carbamazepine is displayed here. The complete list is available online as a supplement to the article and can be obtained from the author on request.

Probe	MAPINFO	CHR	logFC	P.Value	adj.P.Val
cg26039806	71639257	11	0.18266910	8.934441e-06	0.2053849
cg24523000	161273839	5	0.31284492	3.549399e-05	0.8159003
cg03776060	133972575	9	0.26183637	9.010746e-05	1.0000000
cg17818900	105941190	14	-0.15041581	2.653458e-04	1.0000000
cg12766348	178054039	5	0.15647049	3.485552e-04	1.0000000
cg01261503	62493599	17	0.15652095	3.785217e-04	1.0000000
cg02260587	140474248	5	-0.22313579	4.262647e-04	1.0000000
cg09447105	15126020	12	0.12991776	4.316616e-04	1.0000000
cg13425637	61788328	14	0.15849650	5.030361e-04	1.0000000
cg26767897	31637348	2	-0.15540333	6.219644e-04	1.0000000
cg27003827	120906953	12	0.12736193	6.509288e-04	1.0000000
cg16864658	42306150	3	-0.21599284	6.977283e-04	1.0000000
cg22960185	16772516	19	-0.15428807	7.257049e-04	1.0000000
cg18250832	232395463	2	0.17612798	7.348156e-04	1.0000000
cg13986130	186649330	1	-0.18423438	8.079960e-04	1.0000000
cg03627896	30934334	16	0.15464420	8.189945e-04	1.0000000
cg23613177	124739793	10	0.14991828	9.306037e-04	1.0000000
cg05417615	147443478	4	0.22844968	9.527989e-04	1.0000000
cg17404605	7968429	19	-0.17314762	9.728408e-04	1.0000000
cg12194493	493061	4	0.13963742	1.015792e-03	1.0000000
cg21406461	158978957	1	-0.21079107	1.028362e-03	1.0000000
cg20141013	9186050	16	-0.13988631	1.087482e-03	1.0000000
cg07595943	84224901	16	-0.23965676	1.103408e-03	1.0000000
cg02686769	106695932	12	-0.15537774	1.188572e-03	1.0000000
cg22496683	155702610	4	-0.52175756	1.190690e-03	1.0000000
cg26029248	48594205	3	-0.12666289	1.241657e-03	1.0000000
cg21529807	42134478	19	-0.21256782	1.248559e-03	1.0000000

Supplemental material 3.3

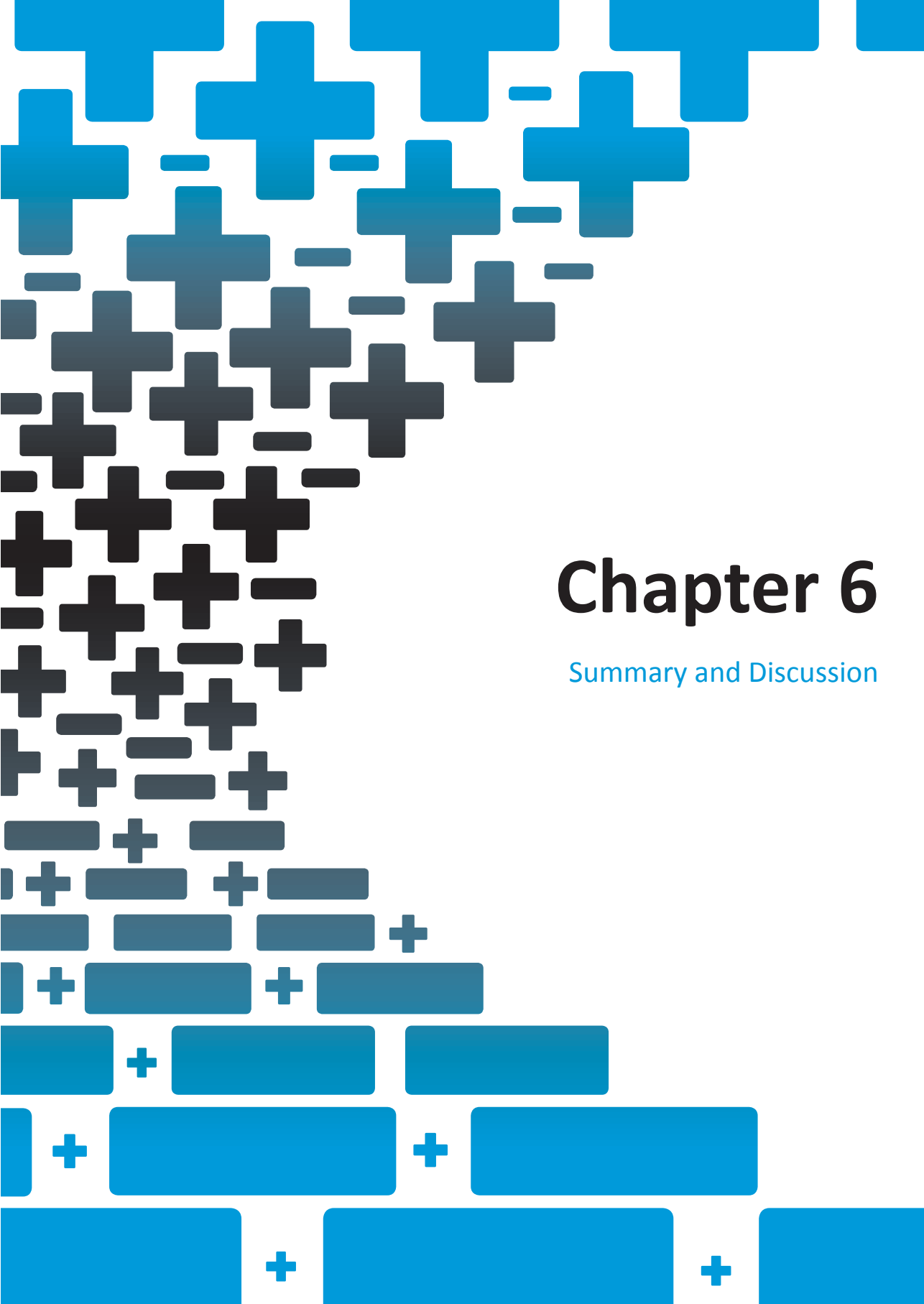
Per WGCNA module, overview of the significantly enriched GO terms for that specific module.

Module	GOterm ID biological pathway	p value	OddsRatio	Expected Count	Count	Size	Term	p value after bonferroni correction
Blue								
Blue	GO:0009611	6.88E-06	2.189866	26.895	50	198	response to wounding	0.037273
Green								
Green	GO:0022008	8.84E-26	3.725049	39.38722	113	674	neurogenesis	3.49E-22
Green	GO:0007268	3.41E-17	3.696516	23.83689	71	392	synaptic transmission	1.35E-13
Green	GO:0007275	1.47E-15	2.328924	85.8398	156	1704	multicellular organismal development	5.8E-12
Green	GO:0009792	2.51E-15	3.071022	31.83755	81	509	embryo development ending in birth or egg hatching	9.91E-12
Green	GO:0006928	5.06E-13	2.552699	43.10932	93	718	movement of cell or subcellular component	2E-09
Green	GO:0040011	2.67E-12	2.490364	42.93896	91	712	locomotion	1.05E-08
Green	GO:0051674	6.33E-12	2.213825	60.03097	114	961	localization of cell	2.5E-08
Green	GO:0001763	1.55E-11	4.227287	11.15519	38	179	morphogenesis of a branching structure	6.11E-08
Green	GO:0045944	1.5E-10	2.285376	45.37052	90	729	positive regulation of transcription from RNA polymerase II promoter	5.93E-07
Green	GO:1902680	7.89E-10	2.020278	64.05039	113	1024	positive regulation of RNA biosynthetic process	3.12E-06
Green	GO:0007422	8.37E-09	10.17263	2.189035	14	35	peripheral nervous system development	3.31E-05
Green	GO:0006935	9.39E-09	2.720024	21.15648	50	344	chemotaxis	3.71E-05
Green	GO:0009605	1.04E-08	2.508893	26.53023	58	436	response to external stimulus	4.11E-05
Green	GO:0048636	1.04E-08	8.81044	2.564518	15	41	positive regulation of muscle organ development	4.12E-05
Green	GO:1901863	1.53E-08	8.483333	2.627067	15	42	positive regulation of muscle tissue development	6.04E-05
Green	GO:0045666	1.98E-08	6.343612	3.834203	18	62	positive regulation of neuron differentiation	7.84E-05
Green	GO:0021872	2.22E-08	7.410066	3.064911	16	49	forebrain generation of neurons	8.78E-05
Green	GO:0009187	3.48E-08	6.534375	3.548971	17	57	cyclic nucleotide metabolic process	0.000138
Green	GO:0007423	3.82E-08	4.208001	7.237659	25	122	sensory organ development	0.000151

Green	GO:0045935	3.85E-08	1.810281	77.37337	124	1237	positive regulation of nucleobase-containing compound metabolic process	0.000152
Green	GO:0051270	4.22E-08	2.523633	23.4603	52	379	regulation of cellular component movement	0.000167
Green	GO:0072163	7.26E-08	4.809433	5.50433	21	88	mesonephric epithelium development	0.000287
Green	GO:0001764	7.9E-08	5.02574	5.054007	20	82	neuron migration	0.000312
Green	GO:0051962	8.57E-08	2.888756	15.55926	39	250	positive regulation of nervous system development	0.000339
Green	GO:0051241	1.28E-07	2.644934	18.9586	44	314	negative regulation of multicellular organismal process	0.000505
Green	GO:0009888	1.34E-07	2.607068	19.63522	45	347	tissue development	0.000529
Green	GO:0010720	1.48E-07	3.139248	12.22936	33	198	positive regulation of cell development	0.000585
Green	GO:0007156	1.55E-07	4.789568	5.254133	20	84	homophilic cell adhesion via plasma membrane adhesion molecules	0.000611
Green	GO:0003148	2.19E-07	24.23989	0.81314	8	13	outflow tract septum morphogenesis	0.000866
Green	GO:0080090	2.37E-07	1.622835	129.5059	181	2062	regulation of primary metabolic process	0.000936
Green	GO:0055021	2.68E-07	10.47605	1.684842	11	27	regulation of cardiac muscle tissue growth	0.00106
Green	GO:0042472	2.86E-07	5.857761	3.604979	16	58	inner ear morphogenesis	0.001131
Green	GO:0016202	3.63E-07	10.00828	1.723228	11	28	regulation of striated muscle tissue development	0.001434
Green	GO:0051252	3.77E-07	1.549773	154.9945	210	2471	regulation of RNA metabolic process	0.00149
Green	GO:0021510	5.25E-07	9.502511	1.775874	11	29	spinal cord development	0.002076
Green	GO:0045168	7.18E-07	7.935927	2.189222	12	35	cell-cell signaling involved in cell fate commitment	0.002835
Green	GO:0023052	7.24E-07	1.705893	91.44528	134	1757	signaling	0.002861
Green	GO:0072175	7.29E-07	4.07429	6.254921	21	100	epithelial tube formation	0.002882
Green	GO:0023019	7.29E-07	13.64788	1.188435	9	19	signal transduction involved in regulation of gene expression	0.002882
Green	GO:0060421	7.57E-07	10.84296	1.501181	10	24	positive regulation of heart growth	0.002989
Green	GO:0021514	9.79E-07	17.31097	0.938238	8	15	ventral spinal cord interneuron differentiation	0.003867
Green	GO:0086091	1.19E-06	10.11915	1.56373	10	25	regulation of heart rate by cardiac conduction	0.004705
Green	GO:0007409	1.43E-06	6.440161	2.694823	13	46	axonogenesis	0.005656
Green	GO:0009954	1.81E-06	9.498402	1.624289	10	26	proximal/distal pattern formation	0.007139
Green	GO:0001657	2.18E-06	4.315607	5.110197	18	82	ureteric bud development	0.008605

Green	GO:0032970	2.27E-06	6.182654	2.815504	13	45	regulation of actin filament-based process	0.008983
Green	GO:0007411	3.75E-06	2.45924	17.31739	38	283	axon guidance	0.014803
Green	GO:0048856	4.12E-06	2.970402	10.34453	27	237	anatomical structure development	0.016277
Green	GO:0060341	4.13E-06	2.216796	23.59805	47	375	regulation of cellular localization	0.016312
Green	GO:0016331	4.35E-06	3.713781	6.399702	20	103	morphogenesis of embryonic epithelium	0.017175
Green	GO:0060581	4.48E-06	17.64831	0.81314	7	13	cell fate commitment involved in pattern specification	0.017694
Green	GO:0007610	5.11E-06	3.182442	8.698777	24	147	behavior	0.020207
Green	GO:0008015	5.37E-06	3.276596	8.156164	23	133	blood circulation	0.021202
Green	GO:0001759	5.57E-06	12.12918	1.124606	8	18	organ induction	0.022022
Green	GO:0030326	6.77E-06	7.767153	1.836034	10	30	embryonic limb morphogenesis	0.026748
Green	GO:0048663	7.21E-06	15.52907	0.854558	7	14	neuron fate commitment	0.028468
Green	GO:0001755	7.94E-06	7.618234	1.869093	10	30	neural crest cell migration	0.031354
Green	GO:0098609	8.76E-06	3.271074	7.818651	22	125	cell-cell adhesion	0.034624
Green	GO:0060045	9.18E-06	11.01196	1.188435	8	19	positive regulation of cardiac muscle cell proliferation	0.036266
Green	GO:0009953	9.97E-06	8.686141	1.536939	9	25	dorsal/ventral pattern formation	0.039386
Red								
Red	GO:0071593	6.94E-14	6.765183	5.333042	29	326	lymphocyte aggregation	1.25E-10
Red	GO:0007159	8.15E-13	6.070311	5.87289	29	359	leukocyte cell-cell adhesion	1.47E-09
Red	GO:0034109	5.33E-12	5.581182	6.330942	29	387	homotypic cell-cell adhesion	9.59E-09
Red	GO:0002694	5.42E-11	7.119338	3.559347	21	223	regulation of leukocyte activation	9.76E-08
Red	GO:0098602	5.29E-09	3.841761	9.55367	31	584	single organism cell adhesion	9.53E-06
Red	GO:0031295	7.33E-09	15.52384	0.829994	10	51	T cell costimulation	1.32E-05
Red	GO:0050867	1.46E-08	5.941904	3.533549	18	216	positive regulation of cell activation	2.63E-05
Red	GO:0006968	1.54E-08	17.71559	0.67072	9	41	cellular defense response	2.78E-05
Red	GO:0022409	2.21E-08	6.607517	2.830111	16	173	positive regulation of cell-cell adhesion	3.98E-05
Red	GO:0050852	1.83E-07	10.52289	1.144559	10	71	T cell receptor signaling pathway	0.000329

Red	GO:0045058	4.15E-07	18.97271	0.490771	7	30	T cell selection	0.000747
Red	GO:0050778	7.22E-07	5.005192	3.643321	16	230	positive regulation of immune response	0.0013
Red	GO:0006955	7.81E-07	6.237061	2.401601	13	168	immune response	0.001406
Red	GO:0048535	1.29E-06	38.58516	0.212667	5	13	lymph node development	0.002313
Red	GO:0023052	6.41E-06	1.984816	57.86624	86	3605	signaling	0.011536
Red	GO:0001775	7.6E-06	4.353614	3.875547	15	259	cell activation	0.01368
Red	GO:0033077	2.22E-05	9.512995	0.863071	7	53	T cell differentiation in thymus	0.039969
Yellow								
Yellow	GO:0050906	6.66E-09	4.286384	9.000525	29	95	detection of stimulus involved in sensory perception	2.84E-05
Yellow	GO:0009593	1.47E-07	4.860586	5.959758	21	63	detection of chemical stimulus	0.000628
Yellow	GO:0031424	1.18E-06	7.389534	2.840143	13	30	keratinization	0.005025
Yellow	GO:0045087	1.95E-06	2.019539	36.21137	65	386	innate immune response	0.008328
Yellow	GO:0072376	5.42E-06	6.826399	2.741319	12	29	protein activation cascade	0.023081
Yellow	GO:0050911	9.34E-06	5.702549	3.315983	13	35	detection of chemical stimulus involved in sensory perception of smell	0.0398



Chapter 6

Summary and Discussion

The aim of the first part of this thesis was to define a psychotic subtype within the bipolar spectrum by investigating psychotic symptoms in relation to clinical, demographic and neuropsychological characteristics to improve psychiatric diagnostics. The second part explores three aspects of pharmacological treatment in bipolar disorder: 1) the effectiveness of lithium after a period of discontinuation, 2) the cognitive effects of medication and 3) the involvement of epigenetic mechanisms.
The current chapter summarizes the main findings of the studies and discusses the implications of the findings.

Summary part I: Psychotic symptoms in bipolar disorder

Diagnostic heterogeneity within psychiatric disorders is substantial, but the underlying reason for this heterogeneity is not fully understood (Cuthbert 2016). The overlap of psychotic symptomatology in schizophrenia and bipolar disorder may point to the presence of a diagnostic continuum with shared etiology (Van Os & Reininghaus 2016). This raises the question whether bipolar patients with a history of psychotic symptoms display similar types of psychotic symptoms as observed in schizophrenia patients and whether risk and outcome factors for these symptoms show a resemblance as well. **Chapter 2** reports on a large comprehensively characterized sample of 1,342 bipolar disorder type I patients and shows a high frequency of lifetime psychotic symptoms (73.8%) including delusions (68.9%), hallucinations (42.7%), mood incongruent symptoms (30.1%), Schneiderian symptoms (21.2%) and formal thought disorder (59.7%). Psychotic symptoms were associated with a more severe illness course, an earlier onset of disease and more frequent hospitalizations for a manic episode.
The characteristics of patients with different types of psychotic symptoms showed considerable overlap, but were significantly different for the level of childhood maltreatment. Auditory hallucinations stood out as the psychotic feature that was associated with higher levels of childhood maltreatment. The results underscore the high frequency of psychotic symptoms in bipolar disorder type I, which are associated with a more severe disease course consisting of an earlier onset of disease and more frequent hospitalizations for a manic episode. In addition, the results emphasize the strength of the relationship between childhood maltreatment and hallucinations. The results did not distinguish a clear categorical psychotic subtype, but do support a differentiation in severity within BDI based on psychosis vulnerability. Interestingly, data from a recent genetic study, which this study contributed to, showed that bipolar patients with either psychotic symptoms, an earlier onset of disease or more frequent hospitalizations showed a greater genetic overlap with schizophrenia patients compared to patients without these features (Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018). Above all, a large heterogeneity of psychotic symptoms was reported in patients with bipolar disorder

type I. The role of distinct risk factors such as trauma in relation to specific psychotic symptoms provide an important lead in further unravelling the etiology of psychosis across psychiatric disorders. It is known that the presence of childhood trauma in schizophrenia patients is associated with hallucinations in later life as well (Varese et al. 2012). Trauma is therefore a prime example of a consistent relationship between a risk factor (trauma) and symptoms (psychosis) across diagnostic boundaries. Therefore this relationship is of interest to study the etiology of hallucinations independent from diagnosis.

Discussion on psychosis subtypes in bipolar disorder (Part I):

This study shows the potential of investigating specific symptoms within disease categories to unravel heterogeneity within psychiatric diagnostics. Bipolar patients with a history of psychotic symptoms had a more severe disease course including an earlier age of onset and more hospitalizations for a manic episode as compared to bipolar patients without a history of psychosis. This bipolar cohort contributed to a genome wide association study (GWAS) of over 100,000 bipolar and schizophrenia patients conducted by the Cross-Disorder Group of the Psychiatric Genomics Consortium (PGC) of which findings were recently published (Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018). The study confirmed an extensive degree of genetic sharing between bipolar disorder and schizophrenia. Despite the degree of sharing, several loci significantly differentiated both disorders. Interestingly, the results of GWAS demonstrated that bipolar patients with psychotic features have significantly higher schizophrenia polygenic risk scores than bipolar patients without psychotic features. Additional evidence showed that significantly higher polygenic risk scores for schizophrenia in bipolar patients is associated with a more severe illness course reflected by more frequent hospitalizations and an earlier onset of the disease (Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018). This is in line with our findings showing that bipolar disorder patients with a history of psychotic symptoms have an earlier disease onset and more hospitalizations for a manic episode versus patients without psychotic symptoms. Together, this suggests a differentiation within the bipolar spectrum that is clinically expressed with psychotic features and a more severe disease course and genetically shows a higher overlap with schizophrenia. The Cross-Disorder Group of the PGC demonstrated that psychotic features within bipolar disorder is an heritable trait. The Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium (2018) suggests that further investigation of psychosis across diagnostic boundaries can facilitate the search for genetic variants that contribute to specific symptom dimensions. Dissecting symptom heterogeneity among related disorders suggests that further work could aid in characterizing patients for more personalized treatment. A potential diagnostic model could consist of several symptom dimensions (i.e. manic, psychotic, cognitive etc.) across current diagnostic boundaries with

an overlapping genetic background which characterizes individual patient's level and level of dysfunction. This can be used to inform disease course and optimal treatment of each individual patient.

Whereas the majority of studies of psychosis in bipolar disorder focuses on psychosis as a binary trait, the objective of the study of this thesis was to disentangle the psychosis spectrum within bipolar disorder, by studying hallucinations, delusions, mood incongruent symptoms, Schneiderian symptoms and formal thought disorder as separate psychotic symptom groups. The findings show that bipolar type I patients suffering from these specific types of psychotic symptom groups showed some interesting differences with regards to demographic characteristics, childhood trauma and illness course, but overall reported a large overlap in all the other characteristics that were investigated. A history of psychotic symptoms was associated with differences in illness course consisting of an earlier disease onset and a manic disease profile (characterized by more manic hospitalizations). Interestingly, the subgroups of psychotic symptoms such as a history of delusions, hallucinations and disorganized speech were also associated with a more manic disease profile, whereas patients with mood incongruent and Schneiderian symptoms did not show differences in manic versus depressive profile. Against expectations with regard to the potential existence of a psychosis continuum, none of the five psychotic symptoms were associated with features that represented a more severe disease course or a lower level of functioning as well as cognitive functioning. The presence of a history of hallucinations stood out by the significant association with a higher level of childhood treatment. The relationship of childhood trauma and psychosis is reported in schizophrenia and psychosis in general as well (Read et al. 2005; Varese et al. 2012), suggesting the relationship exists across diagnostic boundaries. This study emphasizes the strength of the relationship between childhood maltreatment and hallucinations which may be of great importance in further investigating the pathophysiology of psychosis.

Future studies investigating childhood maltreatment and psychosis would benefit from including other risk factors as well such as genetic risk and substance abuse, and investigate a potential dose-response relation of trauma and psychosis. Besides investigation of genetic risk factors, epigenetic mechanisms are of great interest, because they may play an essential role in the link between exposure to trauma in early developmental stages of life and the increased risk for psychosis in later life. Moreover, the selection of patients across diagnostic boundaries with common symptom dimensions will greatly facilitate understanding the diagnostic heterogeneity within psychiatry.

In summary, the stated hypothesis of this study that patients with a history of psychotic symptoms have a more severe illness course, lower level of global functioning, lower level of cognitive functioning and higher levels of childhood maltreatment compared to patients without the presence of a history of psychotic symptoms was not confirmed. The results do not point to a clear categorical distinct psychotic subtype but do support a differentiation

in severity within BDI based on psychosis vulnerability. The findings in this thesis show that the role of distinct risk factors such as trauma in relation to specific psychotic symptoms, combined with recent genetic insight, may provide progress in further unravelling the etiology of psychosis across disorders.

Summary Part II: Pharmacological treatment in bipolar disorder

Pharmacological treatment is available for bipolar disorder. However still more than a third of the bipolar patients do not or only partly respond to pharmacological treatment (Perlis & Ostacher 2006; Geddes & Miklowitz 2013). The development of new effective treatments is hampered by the limited knowledge of disease etiology and the mechanisms of action of current available psychotropic medication. The second part of this thesis focuses on lithium, the current most often used mood stabilizer, and developments in the field of cognitive and epigenetic effects of mood stabilizers and antipsychotics.

The findings of the conducted review and meta-analysis of the four available studies to date in **chapter 3** do not show convincing evidence for a decreased treatment effect of lithium after a period of discontinuation compared to continuous lithium treatment. In clinical practice there is no reason to assume that the effects of discontinuation are smaller. This sheds new light on a recurrent myth that lithium treatment is less effective after an episode of non-treatment. This is important from a clinical perspective, since discontinuation of lithium was suggested to have long-term effects on the subsequent course of illness. Nevertheless, this study does not rule out the possibility of the existence of discontinuation refractoriness in selected subgroups and it may be of interest to further investigate the characteristics of these subgroups.

Chapter 4 presents a review of studies on; 1) the effects of cognitive enhancing agents in schizophrenia patients and 2) the cognitive effects of psychotropic medication in bipolar patients.

In conclusion, cognitive enhancing agents for schizophrenia have not yet been developed with a clinical relevant effect and have hardly been studied in bipolar disorder. In bipolar disorder, findings on cognitive effects of medication must be interpreted with caution, due to relatively small sample sizes and mainly cross-sectional and natural designs. Nevertheless results point to a negative cognitive effect of lithium, anticonvulsants and antipsychotics. Prospective randomized studies are needed to increase the understanding of the effects of different types of medication on cognitive function in bipolar disorder.

To develop new treatment options in the future it is essential to search for new pharmacological targets. Epigenetic mechanisms are of great interest as they may play an important role in the pathophysiology of psychiatric disorders (Mill et al. 2008; Dempster et al. 2011). There are several studies showing that environmental factors, like stress, result in etiological changes in DNA methylation (Labonté et al. 2012; Vinkers et al. 2015; Jaffe et

al. 2016; Melka et al. 2014; Mill et al. 2008; Pidsley et al. 2014). The results described in **chapter 5** show that use of psychotropic medication has a profound influence on cell-count and affects global blood DNA methylation patters in bipolar disorder patients. Across all six medication types examined (lithium, valproic acid, carbamazepine, lamotrigine, olanzapine and quetiapine), quetiapine and valproic acid were consistently associated with global changes in DNA methylation. These DNA methylation alterations were not only related to quetiapine and valproic acid use, but were also associated with an immune related methylation network, indicating inflammation reducing effects. This study underscores the importance to include the use of medication as a confounder in future epigenetic research. In addition, it can also increase the understanding of the underlying biological mechanisms of current medication. For the future, it is important to conduct longitudinal studies to distinguish pharmacological treatment effects from disease-related differences in DNA methylation and to further investigate other epigenetic underlying mechanisms.

Discussion pharmacological treatment in bipolar patients (Part II):

The main findings of the second part of this thesis indicate that currently available psychopharmacological treatment of bipolar patients has cognitive and epigenetic effects. The three investigated topics are separately discussed below, starting with lithium, followed by cognitive and epigenetic effects of currently available psychopharmacological treatment and new developments in this field.

1. Lithium treatment

In **chapter 3** the effect of lithium after a period of discontinuation was investigated, showing no evidence for the existence of lithium-discontinuation-induced-refractoriness, i.e. reduced effectiveness of lithium after discontinuation, in an unselected bipolar population. The reduced effect was thought to be driven by the neuroprotective and neurotrophic effects of lithium (Post 2012). The findings did not rule out the possibility of the existence of discontinuation refractoriness in selected subgroups, like excellent/complete lithium responders. The conducted meta-analysis did not provide data to investigate potential subgroups.

Since the publication of the meta-analysis of **chapter 3** in 2013, a new study investigating lithium-induced refractoriness was published by Cakir et al. in 2017. This study consisted of retrospective life chart data of 65 bipolar patients and showed that more than a quarter of patients had a poorer or deficient response to lithium in the second treatment phase following discontinuation compared to the first phase (Cakir et al. 2017). Against expectations, the acquired non-responsiveness was more often seen in those who initially showed a partial rather than excellent lithium response. This contradicts earlier findings that suggested a decreased response, when lithium was reinstituted in a subgroup of

patients with an excellent lithium response initially (Maj et al. 1995). Important to consider, the likelihood of finding an increase in reduced efficacy of reinstituted lithium treatment is present when a selection towards initial lithium responders is made. Due to the extreme first outcome, regression to the mean is likely to occur on the second measurement.
In addition, the results of the study by Cakir et al. suggested that longer duration of lithium discontinuation was associated with decreased responsiveness to re-treatment (Cakir et al. 2017). This observation is in line with earlier findings showing a negative effect of a longer period of discontinuation (Maj et al. 1995; Post et al. 1992). However, the studies of Maj et al. (1995) and Post et al. (1992) consisted of relatively small samples (respectively N= 54, N= 4). Above all, the meta-analysis is by far the largest study published on this topic (N= 212) and did not select bipolar patients on initial lithium response. These factors are important when findings are translated to an advice in general clinical practice. To correctly inform patients and to make evidence based recommendations about the duration of lithium prophylaxis larger prospective studies are needed to finalize the debate on the phenomenon of lithium-induced refractoriness.

In summary, the hypothesis of this study was confirmed; when looking at an unselected group of bipolar patients, lithium-discontinuation-induced refractoriness does not exist which is consistent with general clinical experience where a high frequency of discontinuation and a successful reinstitution of lithium is general practice.

2. Cognitive dysfunction and pharmacological treatment

The cognitive effects of pharmacological agents (lithium, anticonvulsants and antipsychotics) in bipolar disorder, investigated in **chapter 4**, are inconsistent and point to mainly neuropsychological negative side effect. For instance lithium is associated with both neurotoxic and neuroprotective effects. It was suggested that negative cognitive effects of lithium are a function of duration of treatment and appear to be minor. At least in patients who do not have an optimal lithium response (Wingo et al. 2009; Pachet & Wisniewski 2003), because excellent lithium responders may be a subgroup in which this cognitive decline is not present (Rybakowski & Suwalska 2010). Therefore it is important to characterize neurocognitive subgroups as not all bipolar patients suffer from (the same level of) cognitive impairment. New cognitive enhancing agents currently investigated in bipolar patients are N-acetyl cysteine (NAC) and lurasidone which have shown promising results. A subgroup of psychotic bipolar patients together with schizophrenia patients treated with NAC as an add-on treatment for 6 months showed a significantly improved working memory performance (Rapado-Castro et al. 2017). This study emphasizes the relevance of research on cognitive heterogeneity to explore and obtain more valid and homogeneous neurocognitive phenotypes to make progress in developing cognitive enhancing agents. Another potential cognitive enhancing agent in bipolar disorder is lurasidone. Add-on treatment of Lurasidone in euthymic bipolar patients in an open-

label pilot trial showed a significant improvement in global cognition score compared to treatment as usual (Yatham et al. 2017). The underlying mechanisms which causes the cognitive effects remains unclear, but might be due to the high affinity for 5-HT_7 receptors. Despite the extensive efforts to develop cognitive enhancing drugs for schizophrenia patients, to date no medication with such properties have become available. The review in **chapter 4** suggests mild cognitive effects of antipsychotics (especially in first episode patients), dopamine agonists, glutamergic and cholinergic agents, but none of the cognitive effects are yet clinically relevant. Recently a review on the D1 receptor agonist, dihydrexidine, was published by Arnsten et al. arguing promising effects for cognitive enhancement (Arnsten et al. 2017). The revised dopamine hypothesis suggests that decreased dopamine D(1) activity in the prefrontal cortex – clinically expressed as negative symptoms and cognitive dysfunction – leads to increased activity of dopamine at D2 receptors in the mesolimbic system – clinically expressed as psychosis (Davis et al. 1991). Indeed, decreased D1 receptor signaling in the prefrontal cortex has been linked to cognitive deficits in schizophrenia (Goldman-Rakic et al. 2004). Results of D1 agonists are encouraging but studies are still limited by the pharmacokinetics of the drug. The development of drugs with a more selective pharmacokinetic mechanism, i.e. functionally selective D1 ligands, are needed to enable translation to clinical practice (Arnsten et al. 2017). Most important, future research should focus on patients in an earlier phase of the illness, preferably when the first cognitive effects appear as these symptoms appear most frequent before the first psychotic episode.

In summary, the hypothesis of this study was confirmed; currently there is medication with cognitive enhancing effects in schizophrenia, but not yet with clinical relevant results. Development of cognitive enhancing medication for bipolar disorder patients is still in the starting phase.

3. Epigenetic effects of pharmacological treatment

The findings in **chapter 5** provide evidence that psychopharmacological medication have epigenetic effects. The data show an immune-related genetic network based on DNA methylation differences that may well reflect the inflammation-reducing properties of valproic acid and quetiapine. Altered immune system responses and increased inflammation are frequently linked to psychiatric disorders (Réus et al. 2015). Valproic acid and quetiapine have indeed been related to anti-inflammatory properties (Watkins et al. 2014; Jaehne et al. 2015; Bian et al. 2008). The immune-related network identified in this study, could reflect these inflammation-reducing properties. Whether psychopharmacological medication can exert some of their therapeutic effects by altering DNA methylation in patients with bipolar disorder remains unknown. However, a study conducted by Dong et al. (2016) in prenatally stressed mice investigating schizophrenia-like behavioral phenotypes and brain derived neurotrophic factor (BDNF) transcript levels provided evidence for a therapeutic effect. The first main finding of their study showed a significant correlation between altered behavioral

phenotypes and BDNF transcript levels. It suggests that DNA methylation alterations underlie the schizophrenia like behavioral endophenotypic profile in these mice. Secondly, clozapine treatment in these mice reduced hypermethylation at the promotor region of the BDNF gene and enhanced transcription (Dong et al. 2016). The results reported in chapter 5 add to the growing evidence that psychotropic medication exert an effect on DNA methylation. It shows the importance of including medication as a confounder in epigenetic research. Further research is essential to learn more about potential drug mechanisms of the current available psychotropic medication, which will hopefully facilitate the development of epigenetic drugs for psychiatric disorders.

In summary, the hypothesis of this study stating that psychopharmacological agents cause alterations in DNA methylation signatures is supported by the findings, which confirm that psychiatric drugs influence DNA methylation patterns. Whether psychiatric drugs exert some of their therapeutic effects by altering DNA methylation remains the question for further research.

Methodological considerations:

There are limitations that need to be considered when interpreting the results described in this thesis.

The strength of the study investigating psychotic symptoms in bipolar disorder type I (**chapter 2**) lies in the very comprehensive assessment in a large sample of bipolar disorder I patients. The most important limitation is the cross-sectional design. Moreover, the retrospective data collection poses an inherent limitation and can induce recall bias. Furthermore, despite multivariate analysis residual confounding may remain as adjustments for several unmeasured potentially confounding factors was omitted, such as the number of psychotic episodes, the age of onset of psychosis and comorbid disorders other than anxiety disorders. Also, whereas the current selection of clinical characteristics is comprehensive and constitutes the most relevant items, it is by no means exhaustive and other measures may have additional value for identifying distinct subgroups of patients. Finally, despite the large sample of 1,342 bipolar patients we cannot be sure that our population is representative. Although, there is also no reason to assume bias, particularly considering the predominantly non-clinical recruitment.

The meta-analysis, described in **chapter 3,** included four studies investigating lithium-induced-refractoriness and used a crude measure to establish the effect of discontinuation. In this way the duration of treatment and discontinuation periods are not taken into account. In addition, blood levels of lithium were not available and information on concomitant medication was not known in the selected studies. This complicates the assessment and the

basis for firm conclusions whether lithium discontinuation-induced refractoriness does or does not exist, especially in subgroups of patients.
The limitation of the review of cognitive enhancing medication (**chapter 4**) lies specifically in the paucity of studies of a prospective randomized controlled design in bipolar disorder. This applies especially to studies that investigated cognitive effects of antipsychotics and anticonvulsants in bipolar patients which makes it impossible to conclude if these drugs have a negative or positive influence on cognitive functioning.
Caution is required when interpreting the results of the explorative cross-sectional DNA methylation study (**chapter 5**). The main limitations lie in the cross-sectional observational study design. In the absence of randomization, blinding, placebo control groups and a longitudinal set up, there remains a risk of selection bias, confounded by indication and the inability to infer causality. Residual confounding consists potentially of factors as genotype, nutrition, other medical conditions or concomitant use of non-psychotropic medication, that was not taken into account. In addition, participants in the study used several medication types at the same time. Therefore, it is impossible to fully disentangle selective effects of each medication type. Regarding the effects of polypharmacy (i.e. patients taking other types of medications) in this study population the use of other psychotropic medication is low and randomly distributed across the six main medication types. Even though we cannot completely exclude the influence of other medication types on our results, this suggests that psychiatric polypharmacy is probably not of significant influence. Another factor to consider in DNA methylation studies is tissue specificity, which particularly applies to DNA methylation differences between blood and brain tissue (Davies et al. 2012; Walton et al. 2016). Several studies have pointed out that although there are vast differences between DNA from blood and brain (Davies et al. 2012; Walton et al. 2016), the differences between exposed and non-exposed individuals are often reflected in multiple tissues with larger effect sizes for the differences between individuals than for differences between tissues (Davies et al. 2012; Illingworth et al. 2015). Moreover, the results are relevant, because blood cells are also exposed to these drugs and many of the lymphocytes, such as B-, T- and NK-cells, express similar receptors (e.g. BDNF, dopamine, GABA) as neuronal cells (Gladkevich et al. 2004) the results are likely to be of use.

Future directions:

The findings described in this thesis underscore the relevance of investigating heterogeneity within psychiatric categories. Defining symptom groups with different risk factors beyond the boundaries of current diagnostic categories is essential in future research. This facilitates the detection of underlying biological mechanisms of these symptom groups. A new model in psychiatric diagnostics may consist of symptom dimensions across the current diagnostic boundaries like psychosis, cognition, manic or depressive profile that predict disease course

and outcomes of individual patients. To reach the goal of defining symptom groups across psychiatric disorders, it is essential to gather more detailed information on risk factors, disease course and outcomes of psychiatric patients. This thesis contributes to this goal by investigating psychosis as a symptom dimension within the bipolar spectrum. Initiatives such as the Research Domain criteria (RDoC) of the US National Institute of Mental health (Cuthbert 2016) are the result of a wider felt need for transdiagnostic research approaches and revisitation of the current diagnostic criteria.

Defining symptom groups with common underlying biological mechanisms could also help in developing new targets for pharmacological treatment, i.e. targeting cognitive functioning or development of better antipsychotics or mood stabilizers. This thesis suggests DNA methylation as a potential new target for treatment. The challenge for the future is to extend knowledge of DNA methylation and include other epigenetic mechanisms in research as these mechanisms as a whole can provide insight into the impact of environmental exposures on psychiatric disorders. These epigenetic mechanisms may play an essential role in the pathogenesis of psychiatric disorders.

Conclusion:

In this thesis the characteristics of psychosis in bipolar disorder were investigated. The results provide evidence that psychotic symptoms within bipolar disorder type I constitute a dimension of severity echoing recent genome wide association studies (Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018). Furthermore, it demonstrates that bipolar type I patients suffering from specific types of psychotic symptoms showed some interesting differences with regards to demographic characteristics, childhood trauma and illness course, but overall reported a large overlap in the investigated characteristics. Hallucinations stood out in the relation to childhood trauma and provides a lead for further research unravelling the etiology of psychosis across psychiatric disorders.

With respect to pharmacological treatment the results of this thesis show that cognitive enhancing drugs for schizophrenia have not yet been developed with clinical relevant effects. Development of cognitive enhancing medication for bipolar disorder patients is still in the starting phase. In addition, evidence is presented for an association between psychopharmacological treatment and DNA methylation levels. The precise nature of this association and whether psychiatric drugs exert some of their therapeutic effects by altering DNA methylation remains to be investigated in future longitudinal studies.

Overall these studies contribute to the understanding of bipolar disorder and its complex heterogeneous phenotype and attempt to open new avenues for studying the role of psychosis across diagnostic boundaries.

References

Arnsten AFT, Girgis RR, Gray DL, Mailman RB (2017). Novel Dopamine Therapeutics for Cognitive Deficits in Schizophrenia. *Biological Psychiatry* **81**, 67–77.

Bian Q, Kato T, Monji A, Hashioka S, Mizoguchi Y, Horikawa H, Kanba S (2008). The effect of atypical antipsychotics, perospirone, ziprasidone and quetiapine on microglial activation induced by interferon-γ. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **32**, 42–48.

Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium(2018). Genomic Dissection of Bipolar Disorder and Schizophrenia, Including 28 Subphenotypes. *Cell* **173**, 1705–1715.

Cakir S, Yazıcı O, Post RM (2017). Decreased responsiveness following lithium discontinuation in bipolar disorder: A naturalistic observation study. *Psychiatry Research* **247**, 305–309.

Cuthbert BN (2016). The NIMH Research Domain Criteria Project: Toward an Integrated Neuroscience of Mental Disorders. *Genomics, Circuits, and Pathways in Clinical Neuropsychiatry*, 397–409.

Davies MN, Volta M, Pidsley R, Lunnon K, Dixit A, Lovestone S, Coarfa C, Harris RA, Milosavljevic A, Troakes C, Al-Sarraj S, Dobson R, Schalkwyk LC, Mill J (2012). Functional annotation of the human brain methylome identifies tissue-specific epigenetic variation across brain and blood. *Genome Biology* **13**, R43.

Davis KL, Kahn RS, Ko G, Davidson M (1991). Dopamine in schizophrenia: A review and reconceptualization. *American Journal of Psychiatry* **148**, 1474–1486.

Dempster EL, Pidsley R, Schalkwyk LC, Owens S, Georgiades A, Kane F, Kalidindi S, Picchioni M, Kravariti E, Toulopoulou T, Murray RM, Mill J (2011). Disease-associated epigenetic changes in monozygotic twins discordant for schizophrenia and bipolar disorder. *Human Molecular Genetics* **20**, 4786–4796.

Dong E, Tueting P, Matrisciano F, Grayson DR, Guidotti A (2016). Behavioral and molecular neuroepigenetic alterations in prenatally stressed mice: relevance for the study of chromatin remodeling properties of antipsychotic drugs. *Translational psychiatry* **6**, e711.

Geddes JR, Miklowitz DJ (2013). Treatment of bipolar disorder. *The Lancet* **381**, 1672–1682.

Gladkevich A, Kauffman HF, Korf J (2004). Lymphocytes as a neural probe: Potential for studying psychiatric disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **28**, 559–576.

Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams G V, Castner PSGSA, Svensson TH, Siever LJ, Williams G V, Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams G V, Castner PSGSA, Svensson TH, Siever LJ, Williams G V, Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams G V (2004). Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. *Psychopharmacology* **174**, 3–16.

Illingworth RS, Gruenewald-Schneider U, De Sousa D, Webb S, Merusi C, Kerr ARW, James KD, Smith C, Walker R, Andrews R, Bird AP (2015). Inter-individual variability contrasts with regional homogeneity in the human brain DNA methylome. *Nucleic Acids Research* **43**, 732–744.

Jaehne EJ, Corrigan F, Toben C, Jawahar MC, Baune BT (2015). The effect of the antipsychotic drug quetiapine and its metabolite norquetiapine on acute inflammation, memory and anhedonia. *Pharmacology Biochemistry and Behavior* **135**, 136–144.

Jaffe AE, Gao Y, Deep-Soboslay A, Tao R, Hyde TM, Weinberger DR, Kleinman JE (2016). Mapping DNA methylation across development, genotype, and schizophrenia in the human frontal cortex HHS Public Access. *Nature Neuroscience* **19**, 40–47.

Labonté B, Suderman M, Maussion G, Navaro L, Yerko V, Mahar I, Bureau A, Mechawar N, Szyf M, Meaney MJ, Turecki G (2012). Genome-wide epigenetic regulation by early-life trauma. *Archives of General Psychiatry* **69**, 722–731.

Maj M, Pirozzi R, Magliano L (1995). Nonresponse to reinstituted lithium prophylaxis in previously responsive bipolar patients: Prevalence and predictors. *American Journal of Psychiatry* **152**, 1810–1811.

Melka MG, Laufer BI, McDonald P, Castellani CA, Rajakumar N, O'Reilly R, Singh SM (2014). The effects of olanzapine on genome-wide DNA methylation in the hippocampus and cerebellum. *Clinical Epigenetics* **6**

Mill J, Tang T, Kaminsky Z, Khare T, Yazdanpanah S, Bouchard L, Jia P, Assadzadeh A, Flanagan J, Schumacher A, Wang SC, Petronis A (2008). Epigenomic Profiling Reveals DNA-Methylation Changes Associated with Major Psychosis. *American Journal of Human Genetics* **82**, 696–711.

Van Os J, Reininghaus U (2016). Psychosis as a transdiagnostic and extended phenotype in the general population. *World Psychiatry* **15**, 118–124.

Pachet AK, Wisniewski AM (2003). *The effects of lithium on cognition: An updated review*. *Psychopharmacology* **170**, 225–234.

Perlis R, Ostacher M (2006). Predictors of recurrence in bipolar disorder: primary outcomes from the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD). *American Journal of Psychiatry* **163**, 217–224.

Pidsley R, Viana J, Hannon E, Spiers H, Troakes C, Al-saraj S, Mechawar N, Turecki G, Schalkwyk LC, Bray NJ, Mill J (2014). Methylomic profiling of human brain tissue supports a neurodevelopmental origin for schizophrenia. *Genome Biology* **15**

Post RM (2012). Acquired lithium resistance revisited: Discontinuation-induced refractoriness versus tolerance. *Journal of Affective Disorders* **140**, 6–13.

Post RM, Leverich GS, Altshuler L, Mikalauskas K (1992). Lithium-discontinuation-induced refractoriness: Preliminary observations. *American Journal of Psychiatry* **149**, 1727–1729.

Rapado-Castro M, Dodd S, Bush AI, Malhi GS, Skvarc DR, On ZX, Berk M, Dean OM (2017). Cognitive effects of adjunctive N-acetyl cysteine in psychosis. *Psychological Medicine* **47**, 866–876.

Read J, van Os J, Morrison AP, Ross CA (2005). Childhood trauma, psychosis and schizophrenia: a literature review with theoretical and clinical implications. *Acta psychiatrica Scandinavica* **112**, 330–50.

Réus GZ, Fries GR, Stertz L, Badawy M, Passos IC, Barichello T, Kapczinski F, Quevedo J (2015). The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. *Neuroscience* **300**, 141–154.

Rybakowski JK, Suwalska A (2010). Excellent lithium responders have normal cognitive functions and plasma BDNF levels. *International Journal of Neuropsychopharmacology* **13**, 617–622.

Varese F, Smeets F, Drukker M, Lieverse R, Lataster T, Viechtbauer W, Read J, Van Os J, Bentall RP (2012). Childhood adversities increase the risk of psychosis: A meta-analysis of patient-control, prospective-and cross-sectional cohort studies. *Schizophrenia Bulletin* **38**, 661–671.

Vinkers CH, Kalafateli AL, Rutten BPF, Kas MJ, Kaminsky Z, Turner JD, Boks MPM (2015). Traumatic stress and human DNA methylation: a critical review. *Epigenomics* **7**, 593–608.

Walton E, Hass J, Liu J, Roffman JL, Bernardoni F, Roessner V, Kirsch M, Schackert G, Calhoun V, Ehrlich S (2016). Correspondence of DNA methylation between blood and brain tissue and its application to schizophrenia research. *Schizophrenia Bulletin* **42**, 406–414.

Watkins CC, Sawa A, Pomper MG (2014). Glia and immune cell signaling in bipolar disorder: Insights from neuropharmacology and molecular imaging to clinical application. *Translational Psychiatry* **4**, e350

Wingo AP, Wingo TS, Harvey PD, Baldessarini RJ (2009). Effects of lithium on cognitive performance: A meta-analysis. *Journal of Clinical Psychiatry* **70**, 1–3.

Yatham LN, Mackala S, Basivireddy J, Ahn S, Walji N, Hu C, Lam RW, Torres IJ (2017). Lurasidone versus treatment as usual for cognitive impairment in euthymic patients with bipolar I disorder: a randomised, open-label, pilot study. *The Lancet Psychiatry* **4**, 208–217.

Nederlandse Samenvatting

Dit proefschrift getiteld 'Pharmacological treatment and determinants of psychosis in Bipolar Disorder' bevat een aantal onderzoeken naar de medicamenteuze behandeling van bipolaire stoornis en verschillende determinanten van psychotische symptomen bij deze stoornis.
Het eerste gedeelte van het proefschrift beschrijft de resultaten van onderzoek naar demografische, klinische en neurocognitieve determinanten in relatie tot het voorkomen van psychotische symptomen bij bipolaire patiënten (Deel I, Hoofdstuk 2). Het tweede deel van dit proefschrift beschrijft de resultaten van drie onderzoeken naar medicamenteuze behandeling van bipolaire stoornis; 1. De effectiviteit van lithium na een onderbreking van de behandeling (Hoofdstuk 3); 2. Cognitieve effecten van medicamenteuze behandeling (Hoofdstuk 4); 3. Epigenetische effecten van medicamenteuze behandeling (Hoofdstuk 5).

Hoofdstuk 1 geeft een algemene introductie van bipolaire stoornis. De symptomen van bipolaire stoornis en de huidig beschikbare medicamenteuze behandelingen worden beschreven.
Bipolaire stoornis, ook wel manisch-depressieve stoornis genoemd, is één van de grote psychiatrische ziektebeelden en kent een recidiverend beloop met episodes van depressie, hypomanie en manie, afgewisseld door kortere of langere symptoomvrije perioden. Binnen het bipolaire spectrum worden verschillende syndromen onderscheiden. In dit proefschrift wordt bipolaire stoornis type I onderzocht. De depressieve episoden van de bipolaire I stoornis gaan gepaard met de volgende symptomen: somberheid, anhedonie, slaapstoornissen, moeheid, verminderde concentratie, besluiteloosheid, gevoelens van waardeloosheid en suïcide gedachten. Bij de bipolaire I stoornis treden er naast depressieve episoden, ook manische episoden op, welke gepaard gaan met de volgende symptomen: voortdurend eufore stemming, toegenomen energie, expansieve en/of prikkelbare stemming en slapeloosheid. De bipolaire stoornis wordt geclassificeerd als een stemmingsstoornis, maar naast stemmingssymptomen zijn er ook frequent psychotische symptomen aanwezig. Psychotische symptomen kunnen zowel in een depressieve als manische stemmingsperiode voorkomen en bestaan uit wanen en hallucinaties. Psychotische symptomen treden ook op bij andere psychiatrische ziektebeelden zoals schizofrenie. Schizofrenie is geclassificeerd als een psychotische stoornis en wordt geassocieerd met een algemeen lager functioneren en een ernstiger ziekte beloop met een lager cognitief functioneren in vergelijking met patiënten met een bipolaire stoornis (Bowie et al. 2010; Green 2006). Een belangrijke risicofactor voor het ontstaan van schizofrenie is jeugdtrauma. Jeugdtrauma vergroot de kans op de ontwikkeling van psychotische klachten later in het leven (Varese et al. 2012).
Het huidige diagnostische classificatiesysteem (American Psychiatric Association 2013) maakt een duidelijk onderscheid tussen deze twee stoornissen. Echter symptomen als psychose of depressie kunnen bij beide ziekten voorkomen. Mogelijk lijken de bipolair type

I patiënten waarbij psychotische symptomen voorkomen meer op schizofrenie patiënten dan bipolair type I patiënten waarbij deze symptomen niet voorkomen. De hypothese die in deel I van dit proefschrift wordt onderzocht luidt dan ook als volgt: Bipolaire type I patiënten waarbij psychotische symptomen zijn opgetreden vertonen een ernstiger ziektebeloop, een lager niveau van algemeen en cognitief functioneren en een frequentere historie van jeugdtrauma in vergelijking tot bipolaire type I patiënten, waarbij deze symptomen niet voorkomen.

De eerste studie in deel II van dit proefschrift onderzoekt de stemmingsstabilisator lithium. Lithium is het oudste beschikbare middel voor de behandeling van bipolaire stoornis en is vandaag de dag nog steeds eerste keus. Deel II van deze thesis bevat onderzoeken naar nieuwe ontwikkelingen in de medicamenteuze behandeling van bipolaire stoornis. Er is een breed scala aan medicatie beschikbaar voor de behandeling van bipolaire stoornis bestaande uit stemmingsstabilisatoren, antipsychotica en antidepressiva. Echter bij meer dan een derde deel van de bipolaire patiënten leidt de behandeling met deze middelen niet of onvoldoende tot een verbetering (Perlis & Ostacher 2006; Geddes & Miklowitz 2013). Het exacte werkingsmechanisme van de meeste psychiatrische medicatie is nog grotendeels onbekend en voornamelijk gericht op reductie van stemmings- en psychotische klachten bij bipolaire stoornis, terwijl bij bipolaire patiënten ook cognitieve klachten voorkomen, die het algemeen functioneren kunnen beïnvloeden (Martínez-Arán et al. 2004; Bora et al. 2009; Vreeker et al. 2016). Samenvattend, is er nog veel vooruitgang te boeken op het gebied van medicamenteuze behandeling voor bipolaire stoornis. Dit proefschrift tracht daar aan bij te dragen door de volgende drie onderwerpen te onderzoeken: 1. De effectiviteit van lithium na een onderbreking van de behandeling (Hoofdstuk 3), 2. Cognitieve effecten van huidige en nieuwe ontwikkelingen in medicamenteuze behandeling voor patiënten met bipolaire stoornis en schizofrenie (Hoofdstuk 4), 3. Epigenetische effecten van medicamenteuze behandeling bij bipolaire stoornis (Hoofdstuk 5).

Deel I: Psychotische symptomen bij bipolaire stoornis

Hoofdstuk 2 beschrijft het onderzoek naar de prevalentie van psychotische symptomen in een groot cohort bipolaire type I patiënten. Psychotische symptomen werden tevens onderzocht in relatie tot demografische, klinische en cognitieve karakteristieken met als doel een psychotisch subtype van bipolaire stoornis op te sporen. In deze cross-sectionele studie werden 1,342 bipolaire type I patiënten uitgebreid onderzocht op demografische en klinische factoren middels een SCID-I-interview (Structural Clinical Interview DSM-IV). Daarnaast werd het IQ getest en de prevalentie van jeugdtrauma in kaart gebracht. De relatie tussen psychotische symptomen en al deze karakteristieken werd geanalyseerd door multipele lineaire modellen. Psychotische symptomen waren ooit aanwezig geweest bij 73.8% van alle patiënten. Voor wanen en hallucinaties was dat in respectievelijk 68.9% en 42.6% van de patiënten het geval. Formele denkstoornissen, Schneideriaanse- en stemmingsincongruente symptomen kwamen respectievelijk bij 59.7%, 21.2% en 30.1% van de patiënten voor. Patiënten met psychotische symptomen hadden een ernstiger ziektebeloop, bestaande uit een significant jongere leeftijd waarop de ziekte zich openbaarde en een significant hoger aantal opnames voor een manische episode. Totaal IQ was vergelijkbaar tussen de groepen. Patiënten met hallucinaties hadden significant vaker jeugdtrauma in de voorgeschiedenis.
Het onderzoek naar psychotische symptomen in dit grote bipolaire type I cohort laat zien, dat het merendeel van de patiënten psychotische episoden doormaakt gedurende het ziektebeloop. Bipolaire patiënten met psychotische symptomen hebben een ernstiger ziektebeloop dan bipolaire patiënten zonder psychotische symptomen. Recent is deze associatie bevestigd in genetisch onderzoek. De data van dit onderzoek hebben daaraan bijgedragen. Het genetisch profiel van bipolaire patiënten met psychotische symptomen toonde meer gelijkenis met schizofreniepatiënten dan de bipolaire patiënten zonder psychotische symptomen. Ook hadden de bipolaire patiënten met een ernstiger ziekte beloop meer gelijkenis in genetisch proefiel met schizofreniepatiënten (Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018). Daarnaast benadrukt onze studie de relatie tussen jeugdtrauma en het voorkomen van hallucinaties later in het leven. De resultaten wijzen echter niet op het bestaan van een psychotisch subtype, maar laten juist zien, dat er sprake is van grote heterogeniteit van psychotische symptomen bij bipolaire stoornis.

Deel II: Medicamenteuze behandeling van bipolaire stoornis

Hoofdstuk 3 beschrijft het onderzoek naar mogelijk verminderde effectiviteit van lithium als het herstart wordt na een onderbreking van de behandeling. Deze literatuurstudie en meta-analyse onderzoekt het risico op toename van terugval in een stemmingsperiode na herstart van lithium na een stop-periode in vergelijking tot continu lithium gebruik. Het literatuuronderzoek werd gedaan in de databases van Pubmed, Embase, Cochrane en PsychINFO. Vijf relevante studies werden gevonden, waarvan drie geschikt waren voor een meta-analyse van 212 patiënten. Twee studies rapporteerden dat lithium minder effectief was na een onderbreking van de behandeling en drie studies vonden geen verschil van effectiviteit. De resultaten van de meta-analyse rapporteerden geen verschil tussen het voorkomen van één of meer stemmingsperiode(n) na onderbreking van lithium behandeling in vergelijking tot continue lithium behandeling. Concluderend is er ondanks het lage aantal beschikbare studies geen evidentie dat lithium behandeling minder effectief is na onderbreking van de behandeling in vergelijking tot continue behandeling.

Hoofdstuk 4 beschrijft een literatuuronderzoek naar 1) de effecten van medicijnen die het cognitief functioneren van schizofreniepatiënten verbeteren en 2) de cognitieve effecten van de huidig beschikbare medicatie voor bipolaire stoornis. Cognitief disfunctioneren is een kernsymptoom van schizofrenie (Kahn & Keefe 2013) en is ook aanwezig bij bipolaire stoornis, maar vaak in een minder ernstige vorm (Martínez-Arán et al. 2004; Vreeker et al. 2016). Bij schizofreniepatiënten is er sprake van een afname van het cognitief functioneren reeds voordat de eerste psychotische symptomen zich openbaren (MacCabe et al. 2008; Reichenberg et al. 2010), terwijl bij bipolaire patiënten het cognitief functioneren pas vermindert na de start van de symptomen (Hedman et al. 2013). Ook al zijn er vele studies naar cognitie verbeterende medicatie bij schizofrenie verricht, een medicijn met een klinisch relevant cognitief verbeterend effect is tot op heden niet gevonden. Het is voor toekomstig onderzoek bij schizofreniepatiënten van belang om de focus te leggen op patiënten in de beginfase van hun ziekte, of zelfs voordat de eerste psychotische symptomen zich openbaren. Bij bipolaire stoornis zijn dergelijke onderzoeken naar cognitie verbeterende medicijnen nog nauwelijks verricht. Toekomstig onderzoek bij bipolaire patiënten zal zich moeten richten op de eerste fase van de ziekte, omdat dan de eerste cognitieve achteruitgang inzet. Mogelijk kan er op deze manier nieuwe effectieve medicatie worden ontwikkeld voor de invaliderende gevolgen van het cognitief disfunctioneren bij deze patiënten.

Hoofdstuk 5 beschrijft een studie naar epigenetische effecten van medicatie voor bipolaire patiënten. Bloedmonsters van 172 bipolaire patiënten werden onderzocht op verschillende DNA methylatie patronen in relatie tot 6 soorten medicatie (lithium, olanzapine, quetiapine, valproïnezuur, carbamazepine en lamotrigine). De resultaten laten zien dat verschillende medicijnen globale DNA methylatie patronen in het bloed beïnvloedden. Dit benadrukt het

belang om in toekomstig DNA methylatie onderzoek bij psychiatrische stoornissen tevens te kijken naar medicatie effecten, omdat blijkt dat dit de resultaten kan beïnvloeden. Daarnaast duiden de medicatie gerelateerde verschillen in DNA methylatie wellicht op de onderliggende werkingsmechanismen van de medicatie. Van alle zes onderzochte medicatie types werden valproïnezuur, een stemmingsstabilisator, en quetiapine, een antipsychoticum, gerelateerd aan grote verschillen in globale DNA methylatie, die ook gelinkt waren aan functies van het immuunsysteem. Afwijkingen in het immuunsysteem en verhoogde ontstekingswaarden zijn vaker geassocieerd met psychotische stoornissen (Réus et al. 2015). Tevens is uit eerder onderzoek gebleken dat quetiapine en valproïnezuur ontstekingsremmend kunnen werken (Watkins et al. 2014). Meer onderzoek is nodig om te begrijpen hoe DNA methylatie patronen worden beïnvloed door deze medicijnen. Dit onderzoek kan verder helpen in het identificeren van onderliggende pathofysiologie van de ziekte en de kennis vergroten van de werking van psychiatrische medicatie. Eveneens kan epigenetisch onderzoek leiden tot een mogelijk nieuwe focus voor medicamenteuze therapie voor psychiatrische stoornissen.

Hoofdstuk 6 geeft een samenvatting en discussie van de bevindingen van het uitgevoerde onderzoek, waarvan hieronder enkele belangrijke uitkomsten worden vermeld.
Het onderzoek naar psychotische symptomen bij bipolaire stoornis (deel I) heeft aangetoond, dat het voorkomen van deze symptomen is geassocieerd met een ernstiger ziektebeloop. Specifieke psychotische symptomen laten interessante verschillen zien in relatie tot levensloop en demografische, klinische en neurocognitieve karakteristieken, waarvan de relatie tussen hallucinaties en jeugdtrauma de meest opmerkelijke is. Mogelijk biedt deze relatie inzichten voor verder onderzoek naar de onderliggende pathofysiologie van psychose, omdat deze relatie tevens gevonden is bij andere psychiatrische stoornissen zoals schizofrenie.
De verrichte studies in deel II laten zien dat psychiatrische medicatie cognitieve en epigenetische effecten heeft. Het blijkt dat er nog geen medicatie is ontwikkeld voor het verbeteren van cognitief disfunctioneren bij schizofrenie met een klinisch relevant effect. Het onderzoek bij bipolaire stoornis op dit terrein is nog maar in de beginfase. Daarnaast laten de resultaten van dit proefschrift zien, dat psychiatrische medicatie geassocieerd is met veranderingen in het DNA methylatie patronen. Hoe deze veranderingen precies tot stand komen is nog onbekend. Verder onderzoek zal moeten uitwijzen of medicatie ook door epigenetische veranderingen zijn klinische effect bewerkstelligt.

Referenties

American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Disorders. *Arlington*

Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium (2018). Genomic Dissection of Bipolar Disorder and Schizophrenia, Including 28 Subphenotypes. *Cell* **173**, 1705–1715.

Bora E, Yucel M, Pantelis C (2009). Cognitive functioning in schizophrenia, schizoaffective disorder and affective psychoses: meta-analytic study. *The British journal of psychiatry : the journal of mental science* **195**, 475–82.

Bowie CR, Depp C, McGrath JA, Wolyniec P, Mausbach BT, Thornquist MH, Luke J, Patterson TL, Harvey PD, Pulver AE (2010). Prediction of real-world functional disability in chronic mental disorders: a comparison of schizophrenia and bipolar disorder. *The American journal of psychiatry* **167**, 1116–24.

Geddes JR, Miklowitz DJ (2013). Treatment of bipolar disorder. *The Lancet* **381**, 1672–1682.

Green MF (2006). Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. *The Journal of clinical psychiatry* **67**, 3–8.

Hedman AM, van Haren NEM, van Baal CGM, Kahn RS, Hulshoff Pol HE (2013). IQ change over time in schizophrenia and healthy individuals: A meta-analysis. *Schizophrenia Research* **146**, 201–208.

Kahn RS, Keefe RSE (2013). Schizophrenia is a cognitive illness: Time for a change in focus. *JAMA Psychiatry* **70**, 1107–1112.

MacCabe JH, Lambe MP, Cnattingius S, Torrång A, Björk C, Sham PC, David AS, Murray RM, Hultman CM (2008). Scholastic achievement at age 16 and risk of schizophrenia and other psychoses: A national cohort study. *Psychological Medicine* **38**, 1133–1140.

Martínez-Arán A, Vieta E, Colom F, Torrent C, Sánchez-Moreno J, Reinares M, Bernabarre A, Goikolea JM, Brugué E, Daban C, Salamero M (2004). Cognitive impairment in euthymic bipolar patients: Implications for clinical and functional outcome. *Bipolar Disorders* **6**, 224–232.

Perlis R, Ostacher M (2006). Predictors of recurrence in bipolar disorder: primary outcomes from the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD). *American Journal of Psychiatry* **163**, 217–224.

Reichenberg A, Caspi A, Harrington H, Houts R, Keefe RSE, Murray RM, Poulton R, Moffitt TE (2010). Static and dynamic cognitive deficits in childhood preceding adult schizophrenia: A 30-year study. *American Journal of Psychiatry* **167**, 160–169.

Réus GZ, Fries GR, Stertz L, Badawy M, Passos IC, Barichello T, Kapczinski F, Quevedo J (2015). The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. *Neuroscience* **300**, 141–154.

Varese F, Smeets F, Drukker M, Lieverse R, Lataster T, Viechtbauer W, Read J, Van Os J, Bentall RP (2012). Childhood adversities increase the risk of psychosis: A meta-analysis

of patient-control, prospective-and cross-sectional cohort studies. *Schizophrenia Bulletin* **38**, 661–671.

Vreeker A, Boks MPM, Abramovic L, Verkooijen S, van Bergen AH, Hillegers MHJ, Spijker AT, Hoencamp E, Regeer EJ, Riemersma-Van der Lek RF, Stevens AWMM, Schulte PFJ, Vonk R, Hoekstra R, van Beveren NJM, Kupka RW, Brouwer RM, Bearden CE, MacCabe JH, Ophoff RA, GROUP Investigators (2016). High educational performance is a distinctive feature of bipolar disorder: a study on cognition in bipolar disorder, schizophrenia patients, relatives and controls. *Psychological Medicine* **46**, 807–818.

Watkins CC, Sawa A, Pomper MG (2014). Glia and immune cell signaling in bipolar disorder: Insights from neuropharmacology and molecular imaging to clinical application. *Translational Psychiatry* **4**, e350.

Publications

White matter disruptions in patients with bipolar disorder.
Abramovic L, Boks MPM, Vreeker A, Verkooijen S, **van Bergen AH**, Ophoff RA, Kahn RS, van Haren NEM.
European Neuropsychopharmacology. 2018 Jun;28(6):743-751.

The relationship between brain volumes and intelligence in bipolar disorder.
Vreeker A, Abramovic L, Boks MPM, Verkooijen S, **van Bergen AH**, Ophoff RA, Kahn RS, van Haren NEM.
Journal of Affective Disorders. 2017 Dec 1; 223:59-64.

An actigraphy study investigating sleep in bipolar I patients, unaffected siblings and controls.
Verkooijen S, **van Bergen AH**, Knapen SE, Vreeker A, Abramovic L, Pagani L, Jung Y, Riemersma-van der Lek R, Schoevers RA, Takahashi JS, Kahn RS, Boks MPM, Ophoff RA.
Journal of Affective Disorders. 2017 Jan 15; 208:248-254.

The association of antipsychotic medication and lithium with brain measures in patients with bipolar disorder.
Abramovic L, Boks MP, Vreeker A, Bouter DC, Kruiper C, Verkooijen S, **van Bergen AH**, Ophoff RA, Kahn RS, van Haren NE.
European Neuropsychopharmacology. 2016 Nov;26(11):1741-1751.

High educational performance is a distinctive feature of bipolar disorder; a study on cognition in 4,888 bipolar disorder or schizophrenia patients, relatives and controls.
Vreeker A, Boks MPM, Abramovic L, Verkooijen S, **van Bergen AH**, Hillegers MHJ, Spijker AT, Hoencamp E, Regeer EJ, Riemersma-van der Lek RF, Stevens AWMM, Schulte PFJ, Vonk R, Hoekstra R, van Beveren NJM, Kupka RW, Brouwer RM, Bearden CE, MacCabe JH, Ophoff RA, Group investigators.
Psychological Medicine. 2016 Mar;46(4):807-18.

DNA methylation signatures of mood stabilizers and antipsychotics in bipolar disorder.
van Bergen AH*, Houtepen LC*, Vinkers CH, Boks MP.
Epigenomics. 2016 Feb;8(2):197-208.

Cognitive enhancing agents in schizophrenia and bipolar disorder.
van Bergen AH*, Vreeker A*, Kahn RS (2015).
European Neuropsychopharmacology. **25**, 969-1002.

The effectiveness of restarted lithium treatment after discontinuation: reviewing the evidence for discontinuation-induced refractoriness.
van Bergen AH*, de Vries C*, Regeer EJ, Benthem E, Kupka RW, Boks MP.
Bipolar Disorders. 2013 Sep;15(6):645-9.

Published abstracts and presentations

Is Clozapine treatment associated with lower mortality rates in schizophrenia? Preliminary evidence from a register based study.
van Bergen AH, Termorshuizen F, Sommer IEC, Kahn RS, Boks MPM.
SIRS, Florence Italy, April 2012

The contribution of medication to DNA methylation levels in whole blood of bipolar disorder patients.
van Bergen AH, Ophoff RA, Kahn RS, Boks MPM.
ISPG, Boston U.S.A., October 2013

The effectiveness of restarted lithium treatment after discontinuation: reviewing the evidence for discontinuation-induced refractoriness.
van Bergen AH, de Vries C, Regeer EJ, Benthem E, Kupka RW, Boks MPM.
APA, New York U.S.A., May 2014

Dankwoord

Graag besluit ik dit proefschrift met het bedanken van iedereen, waarmee ik heb samengewerkt en die mij hebben gesteund om dit proefschrift tot een goed einde te brengen. In mijn dankwoord richt ik mij tot een aantal van hen in het bijzonder.

Allereerst wil ik alle deelnemers aan het onderzoek bedanken, dat in dit proefschrift is beschreven. Dank aan hen voor het geduld bij de lange interviews en het invullen van de vragenlijsten, maar ook voor het enthousiasme en het benadrukken van het belang om dit onderzoek te verrichten naar bipolaire stoornis.

Marco, mijn copromotor, het is af! En daarmee is er een einde gekomen aan onze wekelijkse overleggen gedurende 7 jaar. Eerst gewoon in het UMC en na het afronden van mijn opleiding telefonisch, binnen of buiten kantoortijden. Je was altijd beschikbaar voor overleg, tijdens vakanties en in het weekend voor analyses. Dan volgde opnieuw een revisie met als gevolg nieuwe versies van de tabellen. Gedurende dit promotietraject van bijna acht jaar ben ik psychiater en twee keer moeder geworden. Je begrip en geduld daarvoor heb ik zeer gewaardeerd. Daarnaast je nuchterheid en je relaxte en positieve kijk op elke situatie. Je bent een enthousiaste onderzoeker, waar ik heel veel van heb geleerd. Heel veel dank daarvoor!

Mijn promotoren, prof. dr. R.S. Kahn en prof. dr. R.A. Ophoff. Beste René, veel dank voor de vrijheid en kansen, die ik heb gekregen door dit promotieonderzoek te mogen doen in combinatie met mijn opleiding tot psychiater. Dank voor het waken over mijn promotietraject en het bijsturen op de momenten dat dat nodig was. De maandelijkse gesprekken over de voortgang en de resultaten van het onderzoek werkten motiverend en waren altijd leerzaam en leuk.
Beste Roel, je was letterlijk op grote afstand, maar in de afspraken die we maakten altijd bevlogen en betrokken. Je frisse Amerikaanse benadering werkte altijd motiverend. Helaas kon mijn bezoek aan LA op het laatste moment door omstandigheden niet doorgaan.

Prof. dr. M.L. Bouvy, prof. dr. H.E. Hulshof Pol, prof. dr. J.M.P. Baas, dr. M.P. Creyghton en prof. dr. R. Kupka, dank ik vor het plaatsnemen in de promotie commissie.

Alle onderzoekers van het BIG consortium; Prof. dr. Kupka, prof. dr. Schroevers, prof. dr. Hillegers, dr. Spijker, prof. dr. Hoencamp, dr. Regeer, dr. Riemersma-van der Lek, dr. Schulte, drs. Stevens, drs. Vonk, dr. Hoekstra en dr. van Beveren, dank ik voor de prettige samenwerking bij het werven van de deelnemers aan het onderzoek en het tot stand brengen van het eerste artikel uit dit proefschrift. Ook dank ik de overige coauteurs van de artikelen in dit proefschrift voor de prettige samenwerking en kritische blik. In het bijzonder Lotte Houtepen

en Christine de Vries. Lotte, veel dank voor het mij wegwijs maken in de epigenetica wereld onder het genot van alle soorten (groene) thee, die je maar kan bedenken.

De vereniging voor Manisch Depressieve en Betrokkenen. Enorm veel dank voor jullie betrokkenheid bij het onderzoek. Mede door jullie enthousiasme hebben veel deelnemers zich aangemeld voor dit onderzoek.
Het includeren van alle deelnemers van Bipolar Genetics had nooit kunnen plaatsvinden zonder de hartwerkende stagiaires en onderzoeksassistenten. Heel veel dank voor jullie toewijding en de leuke samenwerking!
Yoon Jung, thank you for the good collaboration during the years and especially for the support in preparing my LA visit.
Dr. J. Wijkstra, beste Jaap, hartelijk dank voor de goede en leerzame begeleiding tijdens mijn opleiding tot psychiater en de flexibiliteit ten aanzien van mijn promotietraject.

Collega arts-assistenten (inmiddels allemaal psychiater), verpleegkundigen, onderzoekers, psychiaters en secretaresses van het UMC Utrecht dank ik voor de geweldige opleidings- en promotietijd.

In het bijzonder gaat mijn speciale dank uit naar Annabel Vreeker, Lucija Abramovic en Sanne Verkooijen. In 2011 begon het avontuur en werd de eerste deelnemer geïncludeerd voor het onderzoek. In de jaren die volgden hebben we lief en (promotie) leed gedeeld op een paar vierkante meter. Na 2016 grotendeels via app en bellen. Ik ben de hekkensluiter van ons vieren. Dank voor de super leuke tijd, de steun en voor de flexibiliteit. Ik was immers het merendeel niet full time aanwezig door mijn opleiding tot psychiater. Ik hoop dat we elkaar nog lang blijven zien. Annabel, nog extra bedankt voor de leuke en goede samenwerking bij het schrijven van de review en voor de hulp in het tot stand komen van dit proefschrift.

Ook mijn opleidingsgenoten Lot de Witte, Melisse Bais, Sabine Lotgering en Kim Maijer wil ik bedanken voor de fantastische tijd, met trips naar Maastricht of New York, vaak koffie in mecaffe, en vooral praten, heel veel praten met elkaar. Bij de volgende trip gaan we Lot opzoeken in New York!

Alle collega's van het Rode Kruis ziekenhuis in Beverwijk dank ik voor hun samenwerking en begrip. Al twee jaar heb ik het er enorm naar mijn zin. Arjen en Thomas, de fijne start die ik bij jullie heb kunnen maken als psychiater en de goede samenwerking die we met z'n drieën hebben, waardeer ik zeer.

En al mijn lieve vriendinnen, sommigen al sinds de kleuterschool, anderen sinds de studie tijd in Amsterdam, allemaal heel erg bedankt voor jullie vriendschap, interesse en nodige afleiding.

Arija Maat, oud-huisgenoot, vanaf mijn start in Utrecht ben jij mijn tutor en ik jouw pupil binnen het vakgebied. Ook buiten het vakgebied is er genoeg om met elkaar over te praten en te klagen, dank daarvoor!

Pinot 2002 ♥, Carlijn en mijn nichtje Karin dank ik voor hun vriendschap.

Mijn vriendinnen van het allereerste begin: Claire, Linde en Rachelle. Ik dank jullie voor de hechte en onvoorwaardelijke vriendschap die we hebben. Vroeger speelden we als kleine kindjes al samen. Nu gaan we (bijna) jaarlijks met onze vier gezinnen op vakantie naar Bono en spelen onze kinderen weer met elkaar. Dit vind ik heel bijzonder. Lieve Rachelle, dank voor je gezelligheid en positieve kijk op het leven. Lieve Claire, je humor, je attentheid, je steun waardeer ik enorm.

Mijn paranimfen, Linde Scholten en Victorien van Verschuer. Lieve Lin, vanaf de peuterspeelzaal zijn wij vriendinnen en nu ook nog samen in het zelfde vakgebied beland. Jij al enkele jaren als gepromoveerd psycholoog. Ik dank je voor je steun die je geeft op de momenten dat het nodig is, ook in dit promotietraject. Als antwoord op jouw dankwoord; ik ga het halen; promoveren voor onze volgende lustrumreis met z'n vieren!
Lieve Vic, de studie adviseur koppelde ons in het eerste jaar en we zijn altijd samen opgetrokken. Samen zijn we gereisd naar China en voor onze coschappen naar Oeganda. Ik dank je voor de steun die je gegeven hebt vanaf eerstejaars student tot aan gepromoveerd specialist. Je avontuurlijkheid en gedrevenheid bewonder ik en dat nu ook in combinatie met het moederschap. We raken nooit uitgepraat, over de perikelen van de wetenschap en klinische praktijk, maar vooral ook over al het andere wat ons bezig houdt.
Dat jullie, beiden doctor in de wetenschap, als paranimfen naast mij staan vind ik bijzonder en geeft mij vertrouwen.

Mijn lieve schoonfamilie, Emilie, Michiel en mijn fantastische drie schoonzussen, dank ik voor de interesse en steun in de afgelopen jaren. Ik voel me bij jullie thuis.

Lieve Marjan en Otto. Ik ben trots op jullie. Als oudere zus en broer zijn jullie voor mij een voorbeeld. Dank lieve Tessa, Matthijs en alle kinderen voor jullie steun, gezelligheid en voor het onderdeel zijn van onze familie.
Lieve pap en mam; dank voor alles wat jullie hebben gedaan, wat er toe heeft geleid dat ik nu promoveer. Van jullie heb ik geleerd om door te zetten. Het gaat nu echt gebeuren, het is af! Jullie hulp door het vele oppassen en de inhoudelijke correcties van pap in de late uurtjes hebben er voor gezorgd dat ik dit proefschrift heb kunnen afmaken. Mam, de interesse voor de medemens en psychiatrie heb ik van jou. De interesse voor de wetenschap heb ik van jou, pap. Veel dank voor de ondersteuning bij het voltooien van mijn proefschrift. Ik ben jullie enorm dankbaar!

Lieve Hanna en Jan; sinds jullie komst stond dit proefschrift op de tweede en daarna derde plek. Dank dat jullie zulke lieve kindjes zijn en dat het toch mogelijk was om het af te maken. Jullie zijn mijn grote geluk.

Lieve Tjerk; je gedrevenheid, je positieve instelling, je liefde en onvoorwaardelijke steun ook in dit traject, waardeer ik enorm. Wat hebben we een goed en gelukkig leven samen met familie en vrienden maar bovenal met onze lieve Hanna en Jan! HVJ

Curriculum vitae

Annet Herma van Bergen werd op 12 augustus 1983 geboren in Bunnik. Zij volgde basisonderwijs aan de Anne Frank school te Bunnik. In 2001 behaalde Annet haar VWO diploma aan het Sint Bonifatius college te Utrecht. Aansluitend studeerde zij een jaar neurowetenschappen aan de University of Sussex te Brighton in Engeland. In 2002 startte zij met haar studie geneeskunde aan de Vrije Universiteit in Amsterdam. Gedurende de doctoraal fase verrichtte zij wetenschappelijk onderzoek bij het Joslin Diabetes Center onder supervisie van Prof. dr. Horton in Boston (USA), dat is geaffilieerd aan Harvard University. In 2009 behaalde zij het doctoraal examen. Zij volgde een keuze coschap tropengeneeskunde in Mateete, Oeganda, en rondde het arts examen af in 2010.
Na het afronden van haar geneeskunde opleiding startte Annet haar opleiding tot psychiater aan het Universitair Medisch Centrum Utrecht met als opleider dr. J Wijkstra. Tegelijkertijd begon zij met haar promotieonderzoek met als promotoren prof. dr. R.S. Kahn en prof. R.A. Ophoff en copromotor M.P.M. Boks.
In 2016 rondde Annet haar opleiding tot psychiater af en is sinds 2017 werkzaam als psychiater in het Rode Kruis Ziekenhuis te Beverwijk.

Annet Herma van Bergen was born in Bunnik, The Netherlands, on August 12, 1983. She followed her primary education at the Anne Frank school in Bunnik. In 2001 she finished high school education at the Bonifatius College in Utrecht. Subsequently she studied for a year neuroscience at the University of Sussex in Brighton, United Kingdom. In 2002 she started studying medicine at the VU University in Amsterdam. During her medical study she completed an academic research internship at the Joslin Diabetes Center under supervision of prof. dr. E.S. Horton in Boston (USA), which is affiliated with Harvard medical School. In 2009 she did an elective clinical rotation in tropical medicine in Mateete, Uganda.
In 2010 Annet completed medical school and began her psychiatry residency at University Medical Center (UMC) at Utrecht with dr. J. Wijkstra as program director. Simultaneously she started as PhD student at the UMC Utrecht with prof. dr. R.S. Kahn and prof. R.A. Ophoff as promotors and M.P.M. Boks as copromotor.
In 2016 Annet completed her residency and is since 2017 working as psychiatrist at the Rode Kruis Ziekenhuis in Beverwijk, The Netherlands.