The influence of genes and environment on the development of bipolar disorder

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The Influence of Genes and Environment on the Development of Bipolar Disorder

A Twin Study

Ronald Vonk

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A Twin Study

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Chapter

1

Introduction

Introduction

Bipolar disorder

Bipolar disorder is a severe, chronic and often life-threatening complex disease in which the core feature is a pathological disturbance in mood ranging from episodes with extreme elation or irritability to severe depressive episodes. In many patients mood episodes can also be accompanied by abnormalities in thinking and behavior, which may include psychotic symptoms. Typically, it is an episodic illness, with (almost) complete recovery of mood and psychotic symptoms in-between episodes (Craddock and Jones 1999).

In this thesis we use the US diagnostic classification system DSM-IV to define diagnoses (American Psychiatric Association 1984). A manic episode is characterized by an elevated, expansive or irritable mood, with the possible following symptoms: inflated self-esteem, sleeplessness, talkativeness, racing thoughts, distractibility, hyperactivity and reckless behaviour. A depressive episode is characterized by a depressed mood together with the possible following symptoms: sleep disturbances, psychomotor retardation or agitation, fatigue, feelings of worthlessness or guilt, impaired thinking or concentration, change of appetite or weight and suicidal thoughts (Goodwin and Jamison 2007). Both manic and depressive episodes can include psychotic symptoms, which complicate the differential diagnosis with psychotic disorders, e.g. schizophrenia.

Bipolar I disorder is the most severe and most typical form of the illness, characterized by manic or mixed episodes (in which standards are met for both depression and mania), alternated by depressive episodes. Patients with bipolar II disorder have one or more major depressive episodes accompanied by at least one hypomanic episode (an episode with manic symptoms, but without associated functional impairment). The third form is called cyclothymic disorder characterized by more or less chronic alternating hypomanic and minor depressive episodes. The mood cycles between high and low, but it will never reach the full criteria of mania or major depression. When patients clearly have certain symptoms of bipolar disorder but do not meet the criteria for one of the subtypes described above, the diagnosis bipolar disorder Not Otherwise Specified (NOS) is used (Goodwin and Jamison 2007).

The lifetime prevalence of bipolar disorder (bipolar I together with bipolar II disorder) according to DSM-IV criteria is 1-2%, whereas the lifetime prevalence of the broader bipolar spectrum, including cyclothymia and subtreshold bipolar disorder, is about 4-5% (Ten Have et al. 2002, Regeer et al. 2004). Bipolar disorder together with unipolar depression belongs to the ten leading conditions of disability worldwide (Kupfer 2005) with a high comorbidity (Soreca et al. 2009, Perron et al. 2009) and mortality (McIntyre et al. 2008, Roshanaei-Moghaddam et al. 2009).

For the treatment of bipolar disorder it is essential to accomplish stabilisation of the mood episodes. Pharmacotherapy and psychoeducation, often combined with more intensive psychological training or support or even formal psychotherapy, can achieve a stable mood. After stabilisation of mood, social and psychological support is needed to repair the functional impairment in social functioning in the family, work, finance and daily routine. (Goodwin and Jamison 2007, Kupka et al. 2008).

Genes and environment

Family and twin studies in bipolar disorder have established the importance of genetic factors in its etiology, with heritability estimates in the range of 60–85% (Craddock and Jones 2001, Smoller and Finn 2003, McGuffin et al. 2003). Environmental factors are likely to be involved as well, given that the concordance rate for monozygotic (MZ) twins is not 100% but only 40%–70% (Craddock and Jones 2001). Recently, Lichtenstein et al. (2009) estimated heritability to be 59% and influences of common environment 3.4% in a sample of over 40,000 first-degree relatives of patients with bipolar disorder. However, the exact size of the independent and combined effects of relevant risk factors is not known (Craddock and Jones 2001).

Neurodevelopmental aspects of bipolar disorder

The last two decades there is an ongoing dispute whether bipolar disorder and schizophrenia are separate entities or different manifestations of a single underlying pathological process (Demjaha et al. 2012). Some have argued that - just like schizophrenia - bipolar disorder is a neurodevelopmental illness (Blumberg et al. 2004, van Os et al. 1997). Indeed, an

accumulating body of evidence established that structural brain abnormalities are related to both schizophrenia (Wright et al. 2000, Shenton et al. 2001, Pantelis et al. 2005) and bipolar disorder (McDonald et al. 2004, Kempton et al. 2008, Hallahan et al. 2011), with structural brain abnormalities as the result of an interaction between genetic risk and obstetric complications. Obstetric complications, including indicators of fetal growth, have been associated with an increased risk for some psychiatric disorders including schizophrenia and autism. It has been suggested that obstetric complications might be one of the environmental factors in the etiology of bipolar disorder (Ogendahl et al. 2006), although a systematic review did not show robust evidence that exposure to obstetric complications increases the risk of developing bipolar disorder (Scott et al. 2006). So, recent studies support a model whereby, on a background of some shared genetic liability for both schizophrenia and bipolar disorder, only patients with schizophrenia have been subject to additional genetic and/or environmental factors that impair neurodevelopment (Demjaha et al. 2012).

Genetic risk markers (endophenotypes)

There still are a lot of unresolved issues associated with the etiology of bipolar disorder and the endophenotype concept might help to resolve these questions (Hasler et al. 2005). Endophenotypes are traits that are associated with the expression of an illness and are believed to represent the genetic liability of the disorder (Leboyer 1998). Endophenotypes represent more elementary phenomena as opposed to the behavioural features/criteria used in classification systems such as DSM-IV. The endophenotype concept assumes that the number of genes involved in the variations of endophenotypes is less than the number involved in producing the full disease (Gottesman and Gould 2003, Hasler et al. 2005). Neurophysiologic, biochemical, endocrinologic, neuroanatomic, and cognitive abnormalities often accompany bipolar disorder and may thus be candidates to serve as endophenotypes (Lenox et al. 2002). Five criteria have been described that need to be met for a marker to serve as an endophenotype. The marker 1) is associated with the illness in the population, 2) is heritable, 3) is state-independent (i.e., is manifested in an individual whether or not the illness is active), 4) cosegregates with the illness within families, and 5) is found in nonaffected family members at a higher rate than in the general population (Gershon and

Goldin 1986, Gottesman and Gould 2003, Hasler et al. 2006, Leboyer et al. 1998, Lenox et al. 2002).

Twin studies make it possible to examine the relationship of a possible biological marker with the disease or with the (genetic or environmental) vulnerability for the disease.

Risk factors

Structural brain abnormalities

An accumulating body of evidence suggests that structural brain abnormalities are related to bipolar disorder (Jeste et al. 1988, Elkis et al. 1995, McDonald et al. 2004). To date the most consistent findings are increases in white matter hyperintensities and ventricular enlargement (McDonald et al. 2004, van der Schot 2009). Brain regions that have been found to be involved in bipolar disorder include the (pre)frontal and temporal lobes, the basal ganglia and parts of the limbic system (e.g. hippocampus, amygdala, insula and anterior cingulate cortex. (Jeste et al. 1988, Elkis et al. 1995, Videbech 1997, McDonald et al. 2004, van der Schot 2009). However, other studies failed to find changes in total brain, white and gray matter volume (Savitz et al. 2009, van der Schot 2009). These inconsistent results could partly be explained by medication, especially lithium (Sassi et al. 2002, Moore et al. 2000, Moore et al. 2009, Yucell et al. 2008), disease severity and sample heterogeneity (Van der Schot 2009). Moreover, whether these abnormalities are related to the genetic risk or whether they are secondary to environmental effects or the disease process itself is unclear. The brain changes are present once the illness is established (Elkis et al.1995, McDonald et al. 2004; Vita et al. 2009), but it is unknown when these brain changes develop since prospective studies prior to illness onset (in high risk subjects) are lacking.

Obstetric Complications

Obstetric complications have been associated with an increased risk for several psychiatric disorders including schizophrenia for more than 50 years (Cannon et al. 2002a). Cannon et al. (2002a) subdivided obstetrical complications into three groups that were all significantly associated with schizophrenia: complications of pregnancy (e.g. bleeding, diabetes or preeclamsia); abnormal fetal growth and development (e.g. low birth weight, congenital

malformations or reduced head circumference); and complications of delivery (e.g. uterine atony, asphyxia or emergency Cesarean section).

The precise nature of environmental factors in the etiology of bipolar disorder is unclear, but several studies (Dalen 1965, Kinney et al. 1993, Buka et al. 1993, Kinney et al. 1998, Buka and Fan 1999) have suggested that also in this illness obstetric complications, that impair prenatal or perinatal environment, play a role. This provides also support for the hypothesis of a neurodevelopmental disorder for both bipolar disorder and schizophrenia (Murray and Lewis 1987, Blumberg et al. 2004, van Os et al. 1997).

The first study of obstetric complications in bipolar disorder by Dalen (1965) showed significant more obstetric complications in bipolar patients without a positive family history of bipolar disorder, compared with bipolar patients with a positive family history of bipolar disorder. Probands with bipolar disorder had more obstetric complications than their unaffected siblings, which strongly suggests that obstetric complications increase the risk for bipolar disorder (Kinney et al. 1993, Kinney et al. 1998). In the excess of obstetric complications among bipolar patients, perinatal complications seem to be more important than the prenatal complications (Kinney et al. 1993). However, these findings that patients with bipolar disorder have greater exposure to prenatal and perinatal obstetric complications compared to their healthy siblings or normal controls are inconsistent with two studies that found no association between bipolar disorder and obstetric complications (Stober et al 1997, Ogendahl et al 2006). In addition, in a meta-analysis performed by Scott et al. individuals who developed bipolar disorder were no more likely to have been exposed to any one or a range of non-specific obstetric complications than healthy controls (Scott et al. 2006). One study compared the presence of obstetric complications in schizophrenic patients, bipolar patients and controls. Information about pregnancy and perinatal complications were obtained from the mother with a semi-structured interview. Pregnancy complications were more frequent and birth complications more frequent and more severe in female schizophrenic than in female bipolar patients or in normal controls. For male patients there was no difference between the schizophrenic and bipolar patients and controls. (Verdoux and Bourgeois 1993).

In schizophrenia the twin-design with respect to obstetric complications has been used widely and has found significant more obstetric complications among the affected twin within monozygotic twin pairs discordant for schizophrenia (McNeil et al. 1994), except one

study, that did not found a difference in birth weight and obstetric complications between concordant and discordant monozygotic twins (Onstad et al. 1992). Perinatal complications seemed to be especially associated with the development of schizophrenia in discordant twins (McNeil et al. 1994). For schizophrenia it has been suggested, that environmental stressors, such as obstetric complications may be more etiologically influential in pairs where only one twin is affected, while schizophrenia in concordant pairs might well represent a more hereditary variant (McNeil et al. 1994). To our knowledge no study examined the influence of obstetric complications in bipolar twins.

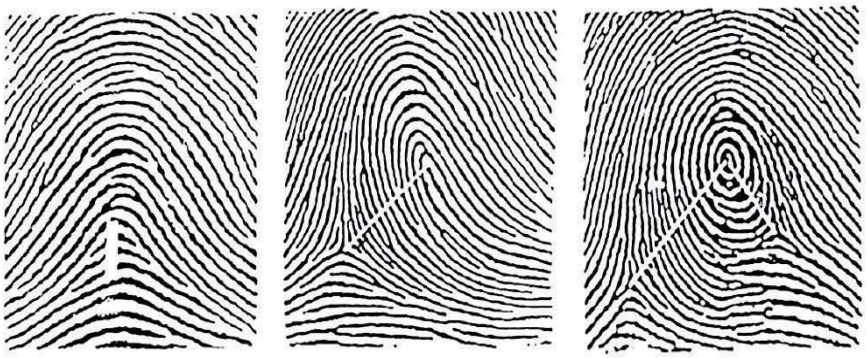
Till now, the repeated association between obstetric complications and the risk for bipolar disorder or schizophrenia is not fully explained. Possible there is an "interaction" between genes and environment, which means that genes influence the individual's sensitivity to the environmental risk factor. There is also the possibility of "correlation" between genotype and environment, which implies genetic control of exposure to the environment. Interaction and correlation are independent and may be operating at the same time. One recent study examined the possibility of a genotype – environment correlation. Patients with psychotic symptoms (schizophrenic, schizo-affective and bipolar patients) had significant more obstetric complication compared with controls, when they had a positive family history of affective disorder. So the factors that contribute to familial aggregation of affective symptoms in psychotic patients also influence the likelihood to experience obstetric complications (Marcelis et al. 1998).

Dermatoglyphics

The patterned traceries of fine ridges on fingers and palms are called dermatoglyphics. Palmar and finger dermatoglyphics are formed early during intrauterine life in a restricted period of time between the 10th and the 17th weeks of gestation (Babler 1991, van Oel et al. 2001, Fatjó-Vilas et al. 2008). Once their formation is complete, dermatoglyphics remain unchanged over lifetime, except for an increase in size correlated with general growth (Fatjó-Vilas et al. 2008). Their morphology can be influenced by genetic or environmental factors interfering with normal intrauterine development, such as maternal exposure to rubella, cytomegalovirus, or alcohol (Schaumann and Alter 1976, van Os et al. 1997). Both the skin and the brain develop from the same embryonal ectoderm and massive neural cell migration takes place in the brain at the same time as dermatoglyphics are established.

Therefore, dermatoglyphics are considered a fossilized evidence of a specific period of prenatal neurodevelopment (Fatjó-Vilas et al. 2008) and are informative for early abnormal developmental processes to later psychiatric illnesses like schizophrenia or bipolar disorder (Van Os et al. 1997).

The most commonly reported dermatoglyphic markers investigated in psychiatric disorders are pattern frequency, ridge simplification (a decreased number of total finger ridge count (TFRC) or palmar a-b ridge count (ABRC)), directional asymmetry (differences between right and left, consistently in the same direction) and fluctuating asymmetry (random differences between right and left (FA)(Figure 1 and 2) (Gutiérrez et al. 1998, van Oel et al. 2001).



Arch (No Triradius)	Loop (One Triradius)	Whorl (Two Triradii)
Finger Ridge Count 0	Finger Ridge Count 13	Finger Ridge Count 17 (on left) 8 (on right)

Figure 1: Types of finger patterns

Where tree ridge systems meet, they form a triradius. The straight lines crossing the ridges in the loop and in the whorl are the lines used to determine the finger ridge counts (modified from Holt 1968).

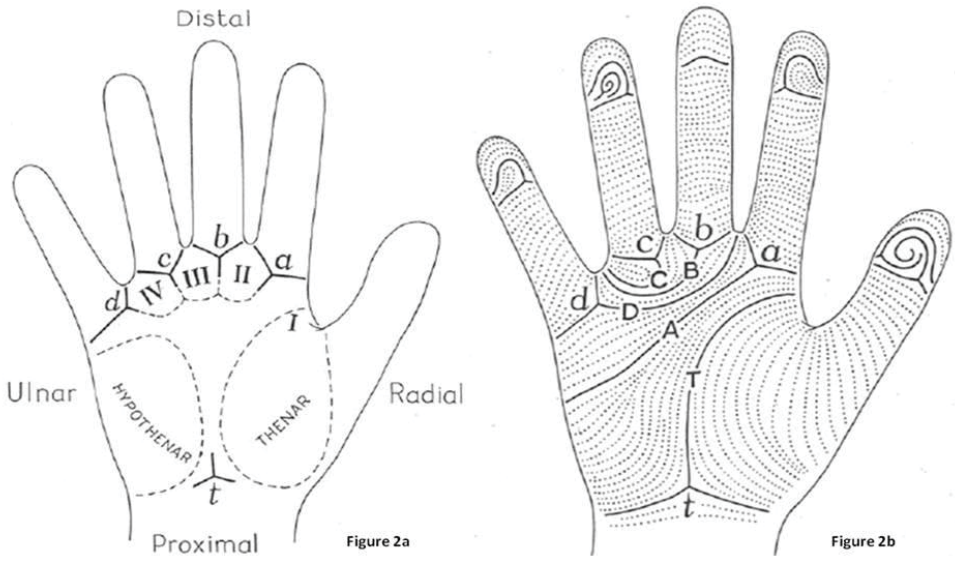


Figure 2: Example of configuration of the digital and palmar dermatoglyphics

In Figure 2a the dermatoglyphic palmar areas can be seen. In the second palmar area (II), the straight line (Figure 2b) crossing the ridges between triradius *a* and *b* is the line used for determining the *a*-*b* ridge count.

In Figure 2b the finger patterns are represented; the thumb presents a whorl, the index a radial loop, the middle finger an arch, the ring finger a whorl and the little finger an ulnar loop (modified from Holt 1968).

Throughout the past century dermatoglyphic studies in mental illness have been carried out and schizophrenia has been subjected to this form of investigation for more than seventy years (Duis 1937, Balgir et al. 1980). Although to a far lesser extent, studies have also been conducted in patients with bipolar disorder. Some studies have reported a different frequency of finger patterns (Srinivasa Murthy et al. 1974, Balgir et al. 1982, Chakraborty et al. 2001), lower TFRC (Balgir et al. 1982, Jelovac et al. 1999) and ABRC (Balgir et al. 1982, Jelovac et al. 1999) or more fluctuating asymmetry (Yousefi-Nooraie et al. 2007) in patients with bipolar disorder compared with controls, while others failed to find differences (Markow et al. 1986, Gutierrez et al. 1998, Saha et al. 2003). Thus, the small amount of dermatoglyphic studies in bipolar disorder revealed heterogeneous results. The lack of consistency may be due to the differences in sample characteristics (i.e. different diagnostic DSM criteria), methodology (i.e. small groups with lack of power) or analytical

On the other hand, in schizophrenia dermatoglyphic alterations are markers of disrupted early development and contributed support to the neurodevelopmental model of schizophrenia (Murray and Lewis 1987, Rosa et al. 2002, Bramon et al. 2005). In their meta-analysis of eight studies Bramon et al. (2005) confirmed the presence of small but significant reductions in palmar a-b ridge counts (ABRC) in schizophrenia. Remarkable, in one study this was especially marked in those without a psychiatric family history (Faňanás et al. 1990, 1996) while in another study only those patients with obstetric complications had significantly reduced ABRC compared to controls, suggesting that the subtle a-b ridge count reductions could be related to early environmental factors like obstetric complications, which – as discussed before - are a well-known risk factor for schizophrenia (Cannon et al. 2002, McGrath & Murray 2003, Bramon et al. 2005). In contrast however, the most recent study in schizophrenia did not find differences in ABRC between patients and controls, although among the patients those with a low birth weight or absence of psychiatric family history showed lower ABRC than the others (Fatjo-Vilas et al. 2008).
Interestingly and supporting the view that the cerebral structural abnormalities found in schizophrenia are the result of an early pathological process affecting the development of fetal ectodermal structures, van Os et al. (2000) found larger associations between dermatoglyphic features (TABRC) and frontal CSF and fourth ventricular volume in 28 male patients with schizophrenia compared with 19 male controls. However, a replication study failed to find a positive association between a-b ridge count and cerebral volumes (Rosa et al. 2003).

Parameters of Thyroid Pathology

Several studies have underlined the high prevalence of psychiatric symptoms and disorders in thyroid diseases. Patients with different thyroid diseases during their lifetimes showed higher rates of panic disorder, simple phobia, obsessive-compulsive disorder, major depressive disorder, bipolar disorder and cyclothymia than the general population. These findings suggest that the co-occurence of psychiatric and thyroid diseases may be the result of common biochemical abnormalities (Placidi et al. 1999).
Abnormal thyroid functioning can affect mood and influence the course of unipolar and bipolar disorder. Even mild thyroid dysfunction has been associated with changes in mood and cognitive functioning. Also thyroid hormone suppletion may have role in the treatment

of certain mood disorders, particularly rapid-cycling bipolar disorder (Hendrick et al. 1998). Autoimmune thyroiditis is a major cause of hypothyroidism and is marked by the presence of thyroid antibodies. Asymptomatic autoimmune thyroiditis with a euthyroid state and a mildly increased level of circulating antibodies is not uncommon, occurring in 5% to 15% of the normal population, predominantly women, and increasing with age (Rapoport and McLachlan 1996, Kupka et al. 2002)

The association of autoimmune thyroiditis, using TPO-Abs as a marker, with bipolar disorder in the population, was demonstrated by Kupka et al. (2002). They found an elevated prevalence of TPO-Abs (28%) in a large sample (n = 226) of outpatients with bipolar disorder compared with a control group from the general population (n= 252) and compared with psychiatric inpatients with any diagnosis (n=3190; 3%–18%. The presence of TPO-Abs in bipolar patients was associated with thyroid failure, but not with age, gender, mood state, rapid cycling, or lithium exposure. Thus, thyroid autoimmunity was highly prevalent in this sample of outpatients with bipolar disorder and not associated with lithium treatment. These variables appear to be independent risk factors for the development of hypothyroidism, especially in women with bipolar disorder (Kupka et al. 2002). Two other studies reported an association between increased antibody titers and depressed or mixed features (Haggerty et al 1990, 1997) or a history of rapid cycling (Oomen et al 1996). There were no differences in TPO-Abs between bipolar patients in various mood states, including euthymia. So, the presence of TPO-Abs is more likely a trait marker of bipolar disorder than a state marker of an episode of the disorder (Kupka et al. 2002). Whether autoimmune thyroiditis and bipolar disorder cosegregate within families and whether autoimmune thyroiditis is found in nonbipolar relatives at a higher rate than in the general population is unknown. If so, TPO-Abs would serve as an appropriate endophenotype.

Underperformance at school

Although the genetic risk to develop bipolar disorder is obviously present from conception, the first frank symptoms of the illness generally become evident in late adolescence or early adulthood (Kennedy et al. 2005, Goodwin and Jamison 2007, Hillegers 2007). However, when the first signs of (the risk for) the illness in adults become apparent is still unclear. Some argue that bipolar disorder is a neurodevelopmental illness (Blumberg et al. 2004, van Os et al. 1997). Were this to be the case one would expect the first signs of the illness to

manifest themselves early in development and before the onset of the first (hypo)manic or depressive episode. To capture these first signs in subjects who will later go on to develop bipolar disorder, large prospective studies in subjects at risk are generally needed since retrospective studies are usually hampered by informant recall-bias. However, studies in twins of whom at least one has developed bipolar disorder may also clarify the possible neurodevelopmental nature of the disorder, particularly when information is used that does not rely on recall but uses objective archival data, since the co-twin of the future patient is an ideal genetic and environmental control (Van Oel et al. 2002).

For schizophrenia evidence is accumulating, that developmental abnormalities, poor neuromotor function, and increased problem behaviour precede the manifestation of the illness (Van Oel et al. 2002). A motor coordination deficit in children aged 7 to 11 years, but not a poor academic performance in elementary school, was a risk factor for schizophrenia (Cannon et al. 1999). Teachers rated pre-schizophrenic children aged 7 (males) or 11 (females) as manifesting more social maladjustment than children, who did not develop schizophrenia (Done et al. 1994).

Earlier, we examined school performance in elementary and secondary school as an objective measure for general functioning in twins concordant and discordant for schizophrenia (Van Oel et al. 2002). School performance, we argued, especially in the highly regulated Dutch schooling system, may be a rather objective measure of the development of general cognitive functioning. In both monozygotic and dizygotic twin pairs with schizophrenia, divergence in school performance occurred about 7,5 years earlier than it did in control twins, at 12 years of age, preceding the onset of psychosis by 10 years. In 90% of the twin pairs discordant for schizophrenia, the twin who underperformed at school was the one who later developed schizophrenia. These findings confirmed the decline in premorbid IQ in schizophrenia compared to controls reported in other studies (Murray et al. 2004) and suggest that the first signs of schizophrenia may manifest as cognitive dysfunction many years prior to the onset of the first psychosis.

In contrast with schizophrenia the premorbid adjustment of patients who develop bipolar disorder has been studied less frequently. Cannon et al. (1997) examined social functioning in childhood and adolescence among patients with schizophrenia, bipolar disorder and controls. The patients with bipolar disorder exhibited poorer social impairment in adolescence than controls, though a lesser degree than the schizophrenic subjects, but

were not different from controls on premorbid IQ or school performance. In a birth cohort study, 20 children who later fulfilled diagnostic criteria for mania exhibited difficulties in social, behavioural and emotional development, but not in motor performance, language or cognition (Cannon et al. 2002b). This normal premorbid intellectual function in subjects who later go on to develop bipolar disorder has also been reported in population-based studies of Israeli (Reichenberg et al. 2002) and Swedish conscripts (Zammit et al. 2004) and in a study of 39 patients with bipolar disorder from multiply affected families (Toulopoulou et al. 2006). So, several studies suggest that bipolar disorder is not preceded by a decline in school performance and Maccabe et al. (2010) indicated that even the opposite may be true. In a retrospective cohort study using national Swedish population registers excellent school performance (based on school records) at sixteen years preceded the development of bipolar disorder. Also a population-based study of Finnish conscripts found an association between increased risk of bipolar disorder and premorbid high arithmetic intellectual performance (Tiihonen et al. 2005).

Twin design

History of twin studies in bipolar disorder

For more than a century, twins have been employed as a natural experiment for addressing the relative influences of nature and nurture on behavior (Hall et al. 2003). The important first goal of twin studies in psychiatry in the past century was to establish the role of genetic factors and environmental factors in the etiology of the disorder and to estimate heritability – defined by Kendler (2001) as the proportion of individual differences in risk in a population at a given time that are due to genetic differences among individuals.

Although many twin studies of mood disorder have been undertaken in the past century after the first twin study by Luxenburger in 1928, it is only since the 1970s that researchers have used the current concept of bipolar disorder (Craddock and Jones 2001). Early studies, summarized in Table 1a, did not distinguish between bipolar and unipolar illness and reported a proband-wise concordance rate of 78% for monozygotic (MZ) twin pairs and of 29% for dizygotic (DZ) pairs with a summary estimate of heritability of 63%, supporting

Table 1a: Twin (pair) studies in bipolar disorder

Study	Sample size (twin pairs)	Measurement	Findings	
Luxenburger 1928 / 1930	4 MZ / 13 DZ	Probandwise Concordance	Heritability 0.80	Concordance MZ 0.80 / DZ 0.00
Rosanoff, et al1935	23 MZ / 67 DZ	Probandwise Concordance	Heritability 0.77	Concordance MZ 0.68-0.82 / DZ 0.17-0.37
Slater 1953	7 MZ / 32 DZ	Probandwise Concordance	Heritability 0.60	Concordance MZ 0.57-0.73 / DZ 0.19-0.32
Kallmann 1953 / 1954	27 MZ / 55 DZ	Probandwise Concordance	Heritability 1.00	Concordance MZ 1.00 / DZ 0.26
Da Fonseca 1959 (Portugal)	21 MZ / 39 DZ	Probandwise Concordance	Heritability 0.42	Concordance MZ 0.60 / DZ 0.31
Kringlen 1967 (Norway)	3 MZ / 0 DZ	Probandwise Concordance	Heritability 0.80	Concordance MZ 0.67-0.80
Pollin et al 1969 (USA)	24 MZ / 58 DZ	Probandwise Concordance	Heritability 0.08	Concordance MZ 0.04-0.08
Allen et al 1969 (USA)	5 MZ / 15 DZ	Probandwise Concordance	Heritability 0.33	Concordance MZ 0.20-0.33 / DZ 0.00
Bertelsen et al 1977 (Denmark)	27 MZ / 36 DZ	Probandwise Concordance	Heritability 0.59	Concordance MZ 0.62 / DZ 0.08
Torgersen et al 1986 (Norway)	4 MZ / 6 DZ	Probandwise Concordance		Concordance MZ 1.00 / DZ 0.00
Modified from Tsunag and Faraone 1990				
Kendler et al 1993 (Sweden)	13 MZ / 22 DZ	Probandwise Concordance	Heritability 0.79	Concordance MZ 0.39 / DZ 0.05
Cardno et al 1999 (London) Maudsley Twin Register	22 MZ / 27 DZ	Probandwise Concordance	Heritability 0.84	Concordance MZ 0.36 / DZ 0.07
Cardno et al 2002 (London) Maudsley Twin Register	77 MZ / 89 DZ	RDC criteria	If diagnostic hierarchies are relaxed, there is a degree of overlap in the genes contributing to RDC schizophrenic, schizoaffective, and manic syndromes.	
McGuffin 2003 (London) (Maudsley Twin Register	30 MZ / 37 DZ	DSM-IV Diagnosis	Substantial genetic and nonshared environmental correlations between mania and depression were found, but most of the genetic variance in liability to mania is specific to the manic syndrome.	
Kieseppa et al 2004 (Finland) (Population Based Sample)	7 MZ / 19 DZ	Probandwise Concordance	Concordance MZ 0.43 (95% CI=0.10 to 0.82) / DZ 0.06 (95% CI=0.00 to 0.27)	
Cardno et al 2012 (London) (Maudsley Twin Register	77 MZ / 89 DZ	RDC criteria	Schizoaffective-mania was due to co-occurring elevated liability to schizophrenia, mania, and depression; and schizoaffective-depression as due to co-occurring elevated liability to schizophrenia and depression, but with less elevation of liability to mania.	
Vonk et al 2012 (The Netherlands)	24 MZ / 29 DZ	Probandwise Concordance	Concordance MZ 0.55 / DZ 0.24	

Abbreviations: MZ, monozygotic;, DZ, Dizygotic; RDC, Research Diagnostic Criteria; CI, Confidence Interval;

the involvement of genes in *broadly defined mood disorders* (Tsuang and Faraone 1990, Craddock and Jones 1999, Smoller and Finn 2003) (Table 1a).

Six twin studies (Kringlen 1967, Allen et al. 1974, Bertelsen et al. 1977, Torgersen 1986, Kendler et al. 1993, Cardno et al. 1999) (Table 1a) have used the current concept of bipolar disorder and all showed an increased probandwise concordance rate in monozygotic (MZ) twins (20-75%) when compared with dizygotic (DZ) twins (0-19%) (Craddock and Jones 1999). Pooling the data from these studies provides an estimate of probandwise MZ concordance for *narrowly defined bipolar disorder* of 50% (95% confidence intervals 40%-60%). In a more recent, representative and well-defined nationwide population-based Finnish twin sample assessed with structured personal interviews Kieseppa et al. (2004) found a probandwise concordance rates for Bipolar I disorder of 0.43 (95% CI=0.10 to 0.82) for monozygotic twins and 0.06 (95% CI=0.00 to 0.27) for dizygotic twins, demonstrating the high heritability of bipolar disorder.

Despite differences in ascertainment, assessment, and diagnostic methods, twin studies of bipolar disorder are in general consistent in observing greater concordance among MZ twins than DZ twins (Smoller and Finn 2003). In this way, twin studies together with family and adoption studies, provided an impressive and consistent body of evidence supporting the

role of genes indetermining predisposition to bipolar disorder (Craddock and Jones 1999, Craddock and Jones 2001). In fact, the results of biometrical modelling suggest that familial aggregation in bipolar disorder is predominantly due to genetic factors, with heritability estimates in the range of 60–85% while there is little evidence that shared family environment plays a major role (Smoller and Finn 2003). However, the fact that not all monozygotic twin pairs are concordant proves that environmental factors also play a role in the development of bipolar disorder. Moreover, the precise interaction between the genetic vulnerability to develop bipolar disorder and environmental factors is not clear, nor is the exact size of the independent and combined effects of relevant risk factors known (Craddock and Jones 2001).

Another focus of twin studies in psychiatry in general and bipolar disorder is the interest in the relationship between different psychiatric disorders and particularly between psychotic disorders and mood disorders. An understanding of the extent to which these disorders share genetic and environmental risk factors is important for the classification of these disorders as well as for attempts to locate and identify genes that contribute to these disorders (Cardno et al. 2002).

Monozygotic twin pairs in which one twin has schizophrenia and the other bipolar disorder would be more consistent with the continuum hypothesis between these two disorders (O'Reilly et al. 2013). However, reports of such twin pairs in the literature (summarized in Table 1b) are rare, suggesting that an identical set of genes does not confer liability for both illnesses (O'Reilly et al. 2013). However, although rare, schizophrenia and bipolar disorder can occur in identical co-twins suggesting a putative role of de novo mutations and epigenetic mechanisms (McGuffin et al. 1982, Dalby et al. 1986, Lohr and Bracha 1992, O'Reilly et al. 2013) (Table 1b).

Table 1b: Twinpair casestudies in bipolar disorder

Study	Case	Measurement	Findings
McGuffin et al 1982	Identical Triplet	Diagnosis	Two with schizophrenia, one with manic-depressive illness.
Dalby et al 1982	Monozygotic Twinpair	Diagnosis	One with schizophrenia, one with bipolar disorder.
Lohr and Bracha 1992	Monozygotic Twinpair	Diagnosis	One with schizophrenia, one with schizo-affective disorder.
O'Reilly et al 2013	Monozygotic Twinpair	Diagnosis	One with early-onset schizophrenia, one with late-onset bipolar disorder.
Patel et al 2004	MZ Concordant Twinpair	Diagnosis	Folie a deux occurring in the context of Bipolar Affective Disorder

Abbreviations: MZ, monozygotic

More systematically, the occurrence of mood disorders and schizoaffective disorders in co-twins of schizophrenic probands was investigated in the first half of the Maudsley twin study in London (Farmer et al. 1987). When the full range of DSM-III diagnoses were considered, both affective disorder (according DSM-III, and called mood disorders in DSM-IV) and schizophrenia were found in genetically identical individuals (Farmer et al. 1987). Subsequently, schizo-affective disorder in relation to schizophrenia and mania was investigated in a series of 77 monozygotic and 89 same-sex dizygotic twin pairs in which the proband met the Research Diagnostic Criteria (RDC) for lifetime-ever schizophrenic, schizoaffective, or manic syndrome (i.e. bipolar disorder). Diagnostic hierarchies were relaxed, showing a degree of overlap in the genes contributing to RDC schizophrenic, schizoaffective, and mania/bipolar (Cardno et al. 2002) (Table 1a).
In the same way McGuffin et al. (2003) examined 67 bipolar twin pairs of the Maudsley Twin Register to explore the etiological overlap with unipolar depression. Substantial genetic and nonshared environmental correlations between mania/bipolar and depression were found, but approximately 71% of the genetic variance of mania was not shared with depression, so most of the genetic variance in liability to mania/bipolar is specific to the manic syndrome (McGuffin et al. 2003) (Table 1a).

During the last two decades twin studies in bipolar disorder moved their focus from concordance rates and heritability estimates to the investigation of traits related to bipolar disorder, such as structural brain abnormalities and neurocognitive impairments, summarized in Table 1c. To study a sample of monozygotic twins discordant for bipolar disorder is attractive because gender and age effects are minimized, as well as genetic variation, compared with singletons or dizygotic twins (Noga et al. 2001). This offers possibilities to identify a variable in one twin only of a discordant pair, implicating predominantly environmental factors or in both twins of the twin pair, raising the possibility of heritable factors predisposing to bipolar disorder. In other words, is there an association of the study parameter, for example structural brain abnormalities, with either the presence of disease, i.e. present in only the affected twin, or risk factors, i.e. present in both twins? Six monozygotic (MZ) twin pairs discordant for bipolar disorder were compared with normal MZ twins with magnetic resonance imaging (MRI) on volumes of basal ganglia, amygdala-hippocampus and cerebral hemisphere (Noga et al. 2001)(Table 1c). Caudate nuclei were larger in both affected and unaffected bipolar twins than in normal MZ twins, suggesting a

genetic contribution. However, the right hippocampus was smaller and less asymmetric in the affected bipolar twins than in the well cotwins and the normal MZ twins. These anatomical structures continue to be of interest in bipolar disorder research (Noga et al. 2001).

In Finland Kieseppa et al. (2003) (Table 1c) also examined structural brain abnormalities in bipolar disorder and performed MRI brain scans on a nationwide (population based) sample of 24 twins with BPI, as well as on 15 healthy co-twins and a demographically balanced sample of 27 control twin subjects, to detect any structural alterations related to the disorder and to the increased genetic risk. The established high heritability of bipolar disorder made it reasonable to postulate that subjects at high genetic risk also showed detectable biological signs of increased liability to the disorder. They hypothesized that any brain structural alterations detected in bipolar twins would also occur in healthy co-twins but to a les ser extent. Patients and co-twins showed a significant decrease in left hemispheric white matter volume compared with control twin subjects. No gray matter decrease was seen in patients or co-twins. Alterations of the left hemisphere white matter in BPI seemed to reflect genetic factors predisposing to the disorder. In this same Finnish twin sample bipolar (BPI) probands, but not their co-twins, showed significant callosal thinning and area reduction, most pronounced in the genu and splenium, relative to healthy twins. So, these findings of callosal thinning appear to be disease related, rather than reflecting genetic vulnerability to bipolar illness (Bearden et al. 2011) (Table 1c). Furthermore, the influence of lithium on brain structures was shown in this twin sample, as lithium-treated BPI patients had significantly larger global hippocampal volume compared to both healthy controls and non-bipolar co-twins, and trend-level larger volumes relative to non-lithium-treated BPI patients (Van Erp et al. 2012) (Table 1c).

In contrast, hippocampal volumes in non-lithium-treated BPI patients did not differ from those of non-bipolar co-twins and control twins. Thus, regionally thickened hippocampi in bipolar I disorder may be partly due to familial factors and partly due to lithium-induced neurotrophy, neurogenesis, or neuroprotection (Van Erp et al. 2012).

Table 1c: Recent twin studies in bipolar disorder

Study	Sample size (Proband / Cotwin / Healthy Control Twin)	Measurement	Findings
Noga et al 2001	6 MZ Discordant /11 MZ HCT	MRI scans brain	Caudate nuclei were larger in both affected and unaffected bipolar twins than in normal MZ twins, suggesting a genetic contribution. The right hippocampus was smaller in the sick bipolar twins vs. well cotwins. The hippocampus was also less asymmetric in the affected bipolar twins than in the well cotwins and the normal MZ twins.
Kieseppa et al 2003 (Finnish Population Based Sample)	24 BT/15 HCO/27 HCT	MRI scans brain	Alterations of the left hemisphere white matter in BPI may reflect genetic factors predisposing to the disorder.
Bearden et al 2011 (Finnish Population Based Sample)	21 BT 19 Non-Bipolar Cotwins 34 HCT	MRI scans brain	Bipolar (BPI) probands, but not their co-twins, showed significant callosal thinning and area reduction, most pronounced in the genu and splenium, relative to healthy twins.
Erp et al 2012 (Finnish Population Based Sample)	18 BT (10 lithium treatment) 14 Non-Bipolar Cotwins 32 HCT	MRI scans brain	Regionally thickened hippocampi in bipolar I disorder may be partly due to familial factors and partly due to lithiuminduced neurotrophy, neurogenesis, or neuroprotection. Hippocampal alterations in co-twins of bipolar I disorder probands are likely to manifest as subtle volume excess rather than deficit, perhaps indicating protective rather than risk effects.

Abbreviations: MZ, monozygotic;, DZ, Dizygotic; HCT, Healthy Control Twin; BT, Bipolar Twin;

In summary, twin studies in psychiatry and similarly in bipolar disorder have played an important role in advancing our insight in the etiology of this illness. Initially, in the past century the main aim was to determine the role of genetic factors in the etiology of the disorder and to estimate the heritability by comparison of the concordance rates of monozygotic and dizygotic twin pairs. Next, case-reports as well as twin studies contributed to explore the (genetic) relationship between psychiatric disorders and more specifically contributed to unpick the ongoing discussion whether schizophrenia and bipolar disorder are (genetically) distinct or shared disorders. Subsequently, in the current century twin studies moved their focus on the investigation of specific traits related to bipolar disorder and the question whether these traits are related to the disease process itself or to the genetic risk to develop bipolar disorder. In this way, twin studies appeared highly accurate for the search of putative endophenotypes for bipolar disorder. In the last decade with the more

sophisticated statistical analyse methods available, twin studies are capable of establishing the exact influence of additive genetic, common, and unique environmental factors on specific traits related to bipolar disorder and the extent of genetic overlap between two traits, such as the disease and a putative endophenotype. Most recently, the study of monozygotic (discordant) twin pairs confirm an ideal design for investigating the contribution of epigenetic factors to disease etiology.

Methodology of twin studies

Classical design

Twin studies depend on the notion that monozygotic, identical twins come from one fertilized egg (zygote), and thus share 100% of their genes. Dizygotic, fraternal twins share only half of their genetic material on average (two separately fertilized eggs). Thus, differences (discordance) between MZ twins were attributed to environmental factors, whereas dizygotic twins would be expected to be different on the basis of both genetic and environmental factors (Hall et al. 2003), while both types of twins are usually reared together and consequently share their family environment, usually named common environment (Van der Schot 2009).

Twin studies typically compare the concordance rates of a disorder between monozygotic (MZ) twins (who are essentially genetically identical) and dizygotic (DZ) twins (who share half of their genes). Assuming that shared environmental influences (common environment) on MZ twins are not different from environmental influences on DZ twins (the equal environments assumption), significantly higher concordance rates in MZ twins reflect the action of genes. Nevertheless, an MZ concordance rate that is less than 100% means that environmental factors influence the phenotype (Smoller and Finn 2003).

Twin studies are particularly informative to examine the relative contribution of genetic and environmental risk factors in the parameter under study reported in bipolar disorder. The values of interest under study and variables (age and gender) are pairwise analyzed using repeated-measures analysis of covariance (ANCOVA) with Twin (bipolar patient vs. cotwin, control twin 1 vs. control twin 2) as the within-subjects variable, Group (discordant, healthy) and Zyg (monozygotic, dizygotic) as the between-subjects variables.

The following main and interaction effects were tested: Zyg, Group, Twin, Group X Zyg, Twin X Group, Twin X Zyg, and Zyg X Twin X Group. In this way we analysed the absolute TPO-Abs values of the MZ and DZ discordant twin pairs and the MZ and DZ healthy control twin pairs (Chapter 5).

Twin studies can also be used to estimate the contribution of genetic and environmental factors to the variance in liability to the disorder (Kendler 2001). These are often partitioned into three components: 1) additive genetic influences, 2) shared familial environment (e.g., social class during childhood, parents' rearing style), and 3) individual-specific environment (e.g., stressful life events). The heritability of the disorder is an estimate of the proportion of phenotypic variance that can be attributed to genetic influences. Heritability refers to the strength of genetic influences in a population — not a particular individual — and heritability estimates may differ depending on the population studied (Smoller and Finn 2003). Because a heritability estimate is a population and time specific "snapshot", it is important to know how stable such estimates are across space and time (Kendler 2001).

In the classical twin design data from MZ and DZ twins are used to decompose the variation of a trait into genetic (additive, A, or dominant genetic, D) and environmental contributions (shared/common within a twin pair (C) or unique for each twin (E)) by comparing within pair resemblance for both types of twins. The twin-pair correlations (rMZ and rDZ), representing the resemblance of the twin pairs, offers an estimate of the relative influence to which genes or shared/unique environment determine phenotypic variation of that trait. To study the genetic contribution of variance in a certain phenotype, the trait needs to be heritable. A first estimate of the heritability of a phenotype, denoted in the literature as (h2), or, alternatively as (a2), for the additive part of heritability (narrow heritability), is obtained as: a2=2 (rMZ-rDZ). The expectation of the correlation in MZ equals: rMZ=a2+c2, where c2 represents the proportion of the variance attributable to common environment). The influence of common environmental factors is suggested when correlations in DZ twins are larger than half the MZ correlations and can be calculated as 2(rDZ) – (rMZ). Finally, the part of the variance that MZ twins do not resemble each other is attributable to unique environmental factors (e2), like life events, diseases, employment and peers not shared with their co-twins (1-rMZ) (Falconer 1989, van der Schot 2009).

By extending the univariate twin study to multivariate designs, in which more than one phenotype per person is analysed, the causes of associations between traits can be investigated (Boomsma et al. 2002). A multivariate twin design makes it possible to study if there is an association between bipolar disorder and the parameter on study (phenotypic correlation). Furthermore it can be studied whether such association is due to a common set of genes, common environmental and/or unique environmental factors. To answer this question 'cross-trait-cross-twin correlations' in MZ and DZ twin pairs were calculated. The 'cross-trait–cross-twin' correlation is the correlation between a trait (ie, bipolar disorder) of twin 1 with another trait of twin 2 (parameter on study), where twin 1 and twin 2 represent a twin pair. If the absolute value of the correlation between brain volume of twin1 and bipolar disorder liability of twin 2 is larger in MZ twins than in DZ twins, this indicates that genes influencing the parameter on study (partly) overlap with genes that influence bipolar disorder. The extent of the overlap is reflected by the magnitude of the genetic correlation.

Genetic model fitting

Model fitting approaches involve constructing a model that best describes the observed data. In quantitative genetic studies, the observed data to be modelled are the variance-covariance matrices including the various parameters (A,D,C,E). Structural equation modelling (SEM) is a statistical technique which tries to fit observed data to models of genetic and environmental effects. It is suitable to test whether a genetic or environmental contribution significantly contributes in explaining the (co)variance within or between traits (Van der Schot 2009). SEM involves path analysis, that as defined by Ullmann (1996): 'allows examination of a set of relationships between one or more independent variables, either continuous or discrete, and one or more dependent variables, either continuous or discrete'. Path analysis only deals with measured variables, while SEM deals with measured and latent variables. A measured variable is a variable that can be observed directly and is measurable (e.g. brain volume). A latent variable is a variable that cannot be observed directly and must be inferred from measured variables. These latent variables are implied by the covariances among two or more observed variables (also known as factors). Visualisation of the correlational and causal relationships between variables is possible in a path diagram (see figure 3). By using path analysis, specific hypothesis about relationships between the variables are quantified by parameter estimates or path coefficients. In this figure the overall

phenotypic variance is explained using three factors: Additive genetic variance (A), common or shared environment (C) and unique environmental variation (E). A, C, E are latent (unobserved) variables, the factor loadings a,c,e are the parameter estimates that represent the variances due to those factors: a^2,c^2 and e^2. Parameters can be removed from the full ACE-model. For example an ACE model is compared to an AE model. In this case the influence of common environment is excluded. The CE model excluded additive genetic influence and the E model excludes all familial resemblance. The aim is to find the most parsimonious model that most accurately describes the observed data. This can be tested via likelihood ratio tests (LRT). This LRT statistic follows a chi-square distribution. A Chi-square larger than 3.84 (1 df) indicates a significant difference at alpha=0.05, and indicates that the discarded effect (e.g. effect of C on brain volume) cannot be left out of the model without seriously deteriorating the goodness of fit.

The liability threshold model for a disorder (e.g. bipolar disorder) holds that for binary traits (presence or absence of the disorder) influenced by multiple factors of small effect, an underlying liability exist, with a threshold that divides the population into two categories for the trait. Liability is a hypothetical continuous variable that determines whether an individual will develop the disorder (Rijsdijk et al. 2002). A continuum of risk is assumed that is normally distributed within the disorder occurring only when a certain threshold of liability is exceeded. A person with a high value on the liability scale crossing a certain threshold would be scored 'patient' on our dichotomous variable, and in all other cases considered to be healthy (discordant co-twin of patient of healthy comparison twin pairs. The critical threshold and heritability for the underlying bipolar disorder liability was not based on our sample. Since twin pairs are selected for bipolar disorder, this would result in an overestimation for the prevalence of bipolar disorder. Therefore we fixed prevalence to 1% and heritability of bipolar disorder to 85% (Ten Have et al. 2002, McGuffin et al. 2003, Regeer et al. 2004, van der Schot 2009).

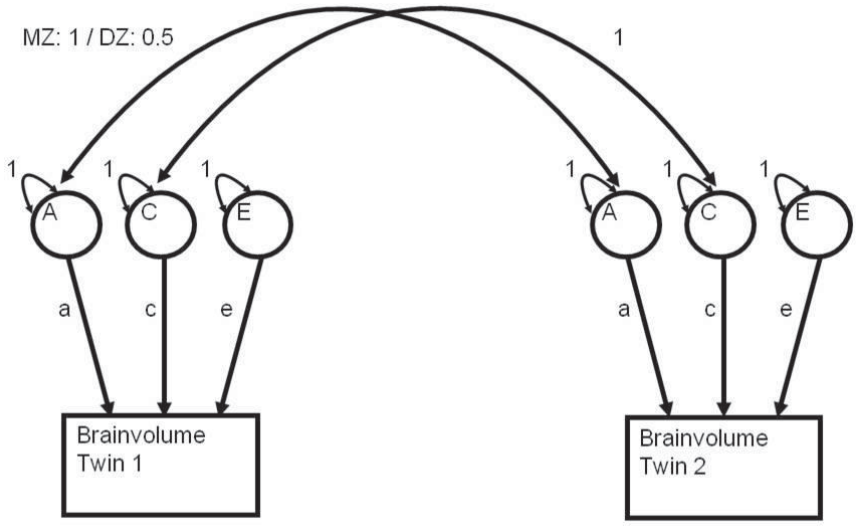


Figure 3. Phenotypes of twin 1 and twin 2 are influenced by additive genetic (A), common environmental (C) and unique environmental influence (E).The correlation between C of twin 1 and C of twin 2 is 1 and the correlation between A of twin 1 and A of twin 2 is 1 for MZ twins (genetically identical) and ½ for DZ twins (share on average 50% of their segregating genes.

In summary, the twin design augmented by the sophisticated structural equation modelling techniques, is able to examine the extent of genetic overlap between two traits, such as a disease and a putative endophenotype (Boomsma et al. 2002, Hall et al. 2007). Understanding the extent of genetic overlap may be crucial, because a significant genetic association validates the proposed phenotypic measure as an endophenotype for the disorder (Lenox et al. 2002, Hall et al. 2007).

In this study with Structural Equation Modelling (SEM) we analysed the influence of additive genetic, common, and unique environmental factors on brain volume in bipolar disorder (Chapter 2), on grey and white matter brain densities (Chapter 3), on dermatoglyphics (Chapter 4) and the association between dermatoglyphics and brain volumes in bipolar disorder (Chapter 4) and finally on the age of first underperformance at school (Chapter 6).

Aim of the study

The general aim of this twin study was to investigate presumed relevant risk factors for bipolar disorder or for the vulnerability (the risk) for bipolar disorder in relation to genotype and environment. Presumed relevant risk factors were psychiatric illness of relatives, structural brain abnormalities, autoimmune thyroiditis with levels of thyroperoxidase antibodies (TPOAbs), (under)performance at school, obstetric complications, dermatoglyphic alterations and life-events (with social rhythm disruption).

More specific, firstly the aims were to investigate whether these risk factors were related to the disease process itself or to the vulnerability (the risk) for the disease, and to which degree the vulnerability (the risk) was under genetic or environmental control. Secondly, to investigate which risk factors were related to possible structural brain abnormalities in bipolar disorder.

Furthermore a secondary aim of this study was to compare the presumed relevant risk factors for the disorder or for the vulnerability (the risk) for the disorder in bipolar disorder and in schizophrenia, by comparing the results of this twin study in bipolar disorder with the results of the similar twin study in schizophrenia at the University Medical Centre Utrecht.

Description of the sample

Subjects

Subjects were twin-pairs, aged 18 to 60 years, with at least one twin suffering from bipolar I or bipolar II disorder according to DSM-IV criteria. The twin pairs were monozygotic concordant, monozygotic discordant as well as dizygotic concordant and dizygotic discordant.

Clinical diagnosis for axis I psychiatric disorders was confirmed with the Structured Clinical Interview for DSM-IV (SCID) (First et al. 1996), for axis II personality disorders using the Structured Interview For DSM-IV Personality (SIDP) (Pfohl et al. 1997) and for both also using available medical records. The twins had no history of drug or alcohol dependency for the last half year, no history of cognitive disorder and no severe medical illness (e.g. no pacemaker), verified by a medical history inventory. Patients were also interviewed on their

medication history and their use of medication on the day of the MRI scan. In order to avoid state effects, the bipolar twins were examined when they were euthymic i.e. the absence of a acute (hypo)manic or major depressive episode, which was defined with the symptom rating scales for mania and depression, respectively the Young Mania Rating Scale (YMRS) (Young et al. 1978) and the Inventory for Depressive Symptomatology (IDS) (Rush et al. 1986). Euthymia was defined as a YMRS score of 4 or less and IDS score of 12 or less.
The bipolar twins were compared to healthy control twins matched on zygosity, gender, age and parental education. The healthy control twins had no history of axis I psychiatric disorder or axis II personality disorder according to DSM-IV criteria, confirmed with a SCID and SIDP interview respectively and no history of a severe medical illness. Furthermore, they had no first degree relative with a history of a major axis I psychiatric disorder (DSM-IV) such as schizophrenia, psychotic disorder, mood disorder, anxiety disorder or substance related disorder. Family history of both affected and control twins was obtained using the Family Interview Genetic Studies (Nurnberger et al. 1994), by interviewing separately and independently the index twin and the cotwin.
The parents of the bipolar and healthy control twin pairs, more preferably the mothers, were asked to give complete information about the prenatal and obstetric complications of the twins and possibly about the psychiatric illness of relatives.
All subjects included in the study were asked to be interviewed with the Life Events and Difficulties Schedule (LEDS) (Brown and Harris 1978).
Zygosity was determined by DNA fingerprinting using high polymorphic microsatellite markers 9 to 11 in the laboratory of the Division Biomedical Genetics, University Medical Center Utrecht.
The study was approved by the Medical Ethical Review Board of the UMC Utrecht and all participants gave written informed consent after full explanation of the study aims and procedures.

Ultimately, a total of 53 affected twin pairs (9 monozygotic (MZ) concordant, 15 MZ discordant, 4 Dizygotic (DZ) concordant and 25 DZ discordant pairs) took part in the study as well as 51 (32 MZ and 19 DZ) healthy control twin pairs. Except for one control twin pair (which was separated at age 12, when both parents died), all twins were reared together. The bipolar twin pairs as well as the control twin pairs were living in all the different provinces of the Netherlands. Although a few co-twins stayed in a foreign country (Belgium,

Germany and Australia), they were willing to participate in the study during a visit to the Netherlands.

The diagnostic characteristics of the bipolar twin pairs are presented in Table 2. Forty-six bipolar patients (index twins and concordant cotwins) met DSM-IV criteria for bipolar I, and 18 for bipolar II disorder. Bipolar disorder Not Otherwise Specified (NOS) was diagnosed in 2 bipolar cotwins, one of them also suffering from schizophrenia, paranoid type. Thirty-six bipolar patients (55%) also had psychotic symptoms. Fifty-four bipolar patients had no lifetime comorbid diagnosis and in 12 bipolar patients one or more comorbid diagnoses was present.

Table 2. Lifetime Psychiatric Diagnosis of the Affected Twin pairs (n = 53)

	Index Twin (n = 53)	Cotwin (n = 53)	
		Concordant (n = 13)	Discordant (n = 40)
Diagnosis			
Bipolar I disorder	38	8	
Bipolar II disorder	15	3	
Bipolar disorder NOS		1	
Bipolar disorder NOS and schizophrenia, paranoid type		1	
Major depressive disorder			4
Depressive disorder NOS			4
Schizophrenia, paranoid type			3
Dissociative disorder NOS			1
Comorbid diagnosis			
Depressive disorder NOS			3
Mood disorder due to hyperthyroidism			1
Psychotic disorder NOS	1		
Psychotic disorder due to cannabis	1		
Agoraphobia without history of panic disorder	2		
Panic disorder without agoraphobia			1
Post traumatic stress disorder	1		
Obsessive-compulsive disorder in full remission			1
Alcohol use disorder in full remission	2		1
Cannabis use disorder in full remission	1		
Sedative use disorder	1		
Sedative use disorder in full remission		1	
Anorexia nervosa	1		
Borderline personality disorder	5		1
Obsessive-compulsive personality disorder	1		
Dependant personality disorder	1		
Personality disorder NOS		1	1
No diagnosis			28

Abbreviation: NOS, not otherwise specified

Of the (discordant) non-bipolar cotwins (n=40), 8 were diagnosed with another mood disorder (4 major depressive disorder and 4 depressive disorder NOS), 3 with schizophrenia, paranoid type (all with a comorbid depressive disorder NOS) and 1 with a dissociative disorder NOS and a borderline personality disorder. Twenty-eight cotwins were healthy with no lifetime psychiatric diagnosis. There were no differences in symptom severity between recruitment sources.

At the time of the study, none of the patients were in a depressive, manic, or hypomanic episode, i.e. they were euthymic or in an episode in partial remission with a YMRS score of 4 or less and an IDS score of 12 or less, except for 4 patients, who met criteria for a depressive episode (IDS scores, 15, 20, 29 and 38, respectively).

The demographic and clinical characteristics of all twin pairs are presented in Table 3. Lithium use on the day of the MRI scan could be ascertained most reliably and was quantified as on/off on that day and implemented as such.

Table 3. Demographic Data of the Affected Twin Pairs and Control Twin Pairs

	Affected Twin Pairs (n=53)		Control Twin Pairs (n=51)	
	MZ [a] (n=24)	DZ [b] (n=29)	MZ (n=32)	DZ (n=19)
Female, N (%)	34 (71)	38 (66)	40 (63)	25 (66)
Mean age, yrs (SD)	37.8 (10.6)	44.3 (8.5)	40.3 (11.5)	42.0 (7.4)
Mean education father, yrs, (SD)	10.6 (3.8)	10.6 (4.4)	10.6 (3.8)	9.7 (4.2)
Mean education mother, yrs (SD)	9.7 (3.2)	9.0 (2.5)	8.8 (2.7)	9.9 (3.0)
Mean education, yrs (SD)	12.4 (2.0)	13.3 (2.6)	13.6 (2.7)	13.0 (2.4)
First-degree relative, N (%)				
bipolar disorder	8 (33)	5 (17)		
depression	4 (17)	11 (38)		
mood disorder	11 (46)	15 (52)		

	Bipolar patients (n=66)	Non-bipolar cotwins (n=40)	Control twins (n=102)
Female, N (%)	45 (68)	27 (68)	65 (64)
Mean age, yrs (SD)	40.8 (10.1)	42.4 (9.9)	40.9 (10.1)
Mean education, yrs (SD)	13.1 (2.2)	12.5 (2.6)	13.4 (2.6)
Mean age of onset, yrs (SD)	28.3 (9.7) [14-59]		
Psychotic symptoms, N (%)	35 (53%)		

Abbreviations: DZ, dizygotic; MZ, monozygotic [a] Concordant, 9; discordant, 15 [b] Concordant, 4; discordant, 25

The mean age of onset of bipolar disorder in this sample was 28.3 (9.7) [14–59] years (Table 3). In four patients the age of onset was before 18 years (14, 15, 16 and 17 years, respectively).

Recruitment

The twin pairs were recruited throughout the Netherlands in the period from 2000 to 2006. The recruitment of the bipolar twin probands started by the Dutch Patients Association for Manic Depressives and Relatives (Vereniging voor Manisch-Depressieven en Betrokkenen (VMDB)[1], a well organized association with about 3000 members for patients, partners and family members. Right from the start in 2000 the VMDB fully supported this twin study with several evocations to participate to all of their twin members at the national meetings or by articles in their own magazine Plus Minus[2]. The same is true for the collaborating group of psychiatrists working on bipolar disorder in the Netherlands, previously called the Lithium-Plus Working Group, later combined with the patients association into the Kenniscentrum voor Bipolaire Stoornissen (KenBis)[3] , meanwhile the official Dutch chapter of the International Society of Bipolar Disorders (ISBD). Several presentations were done at the 3 monthly meetings with evocations to all members to screen their bipolar patients on twin membership.

Psychiatrists working in several Dutch psychiatric institutes received posters and flyers[4] for their waiting room, meant to achieve as many bipolar patients, who were part of a twin pair. Bipolar twin pairs also were approached directly via the Dutch media (articles or advertisements in national and regional newspapers, radio).

Most of the necessary healthy control twins were already available from the ongoing twin study on schizophrenia of the UMC Utrecht (Van Oel et al. 2001). Additionally, in collaboration with the Netherlands Twin Register (NTR) at the VU University in Amsterdam, healthy control twins of their register were approached by their newsletter “Twinfo”[5] and also participated in our twin study on bipolar disorder. Just as for the recruitment of the bipolar twin pairs, several articles and advertisements were placed in local and national newspapers and radio action was organized. Furthermore, during the course of this study, family, friends, colleagues and acquaintances of the researchers continuously were pressed hard whether they were acquainted with twin pairs in their social environment.

Ultimately, the 53 twin pairs concordant and discordant for bipolar disorder were recruited via the VMDB (n=16 twin pairs), via the Lithium-Plus Working Group (n=11 twin pairs), via referral by psychiatrists working in several Dutch psychiatric institutes (n=10 twin pairs) and via articles or advertisements in national and regional newspapers (n=16 twin pairs).
The 51 control twin pairs were recruited from the ongoing twin study on schizophrenia of the UMC Utrecht (N=20 twin pairs), via the NTR (N=15 twin pairs), via articles or advertisements in national and regional newspapers (N=8 twin pairs) and via friends and acquaintances of the researchers (N=8 twin pairs).

[1] http://www.vmdb.nl

[2] Vonk R., Oproep onderzoek tweelingen met MDS. Plusminus 1998, 2, 17.

Vonk R., Oproep onderzoek tweelingen met MDS. Plusminus 2000, 1, 24.

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De Graaf R., Onderzoek onder de loep: Tweelingonderzoek: een tipje van de bipolaire sluier opgelicht? Plusminus 2008, 1, 30-33.

[3] http://www.kenniscentrumbipolairestoornissen.nl

[4] Flyer; Tweelingonderzoek

[5] Astrid van der Schot, Tweelingonderzoek in de psychiatrie. Twinfo, 2002, 15, 5-6.

Outline of the thesis

Research questions:

Do genes and environment have an influence on the global and on focal brain structures in bipolar disorder?

In Chapter 2 the results of the investigation of the contribution of genetic and environmental factors on brain volume in bipolar disorder are presented.

Structural neuroimaging studies suggest the presence of subtle abnormalities in the brains of patients with bipolar disorder. The influence of genetic and/or environmental factors on these brain abnormalities is unknown. Magnetic resonance imaging (1.5 T) brain scans of 50 bipolar twin pairs and 67 healthy twin pairs were conducted to investigate the associations between the brain structure (volumes of the intracranium, total brain, lateral and third ventricle, cerebellum and gray and white matter of the cerebrum and lobar volumes (prefrontal, parietal, occipital and temporal lobe) and bipolar disorder. By using genetic model fitting the extent to which genetic and environmental factors significantly contribute to these associations was quantified.

Chapter 3 focuses on focal brain structures (gray and white matter density) by using voxelbased morphometry for the investigation of the contribution of genetic and environmental factors on grey and white matter brain densities in bipolar disorder. Fifty bipolar twin pairs were scanned using 1.5 Tesla magnetic resonance imaging and compared with 67 healthy twin pairs. Because a possible neurotrophic or neuroprotective effect of lithium was suggested, on both global and focal brain structures this effect of lithium was controlled for.

Are dermatoglyphic alterations related to structural brain abnormalities in bipolar disorder and have they a genetic or environmental origin?
If so, are dermatoglyphic alterations a time-linked (i.e. between the 10th and the 17th weeks of gestation) genetic or environmental marker for an abnormal neurodevelopmental origin of bipolar disorder?

Chapter 4 was designed to examine whether dermatoglyphic alterations are related to structural brain abnormalities in bipolar disorder and whether they are of a genetic or environmental origin. Palmar and finger dermatoglyphics are formed between the 10th and the 17th weeks of gestation and their morphology can be influenced by genetic or environmental factors interfering with normal intrauterine development. As both the skin and the brain develop from the same embryonal ectoderm, dermatoglyphic alterations may be informative for early abnormal neurodevelopmental processes in the brain. Brain abnormalities have been found in bipolar disorder, it is unclear when these originate. Dermatoglyphics and volumetric data from structural MRI were obtained in 53 bipolar twin pairs and 51 healthy matched control twin pairs. To estimate the relative contributions of additive genetic (A), common environmental (C), and unique environmental (E) factors on individual differences in dermatoglyphics and the relationship with bipolar disorder and brain volumes, structural equation modelling was used.

Is autoimmune thyroiditis related to the disease itself or to the (genetic) vulnerability to develop bipolar disorder?
If so, is autoimmune thyroiditis a possible endophenotype for bipolar disorder?

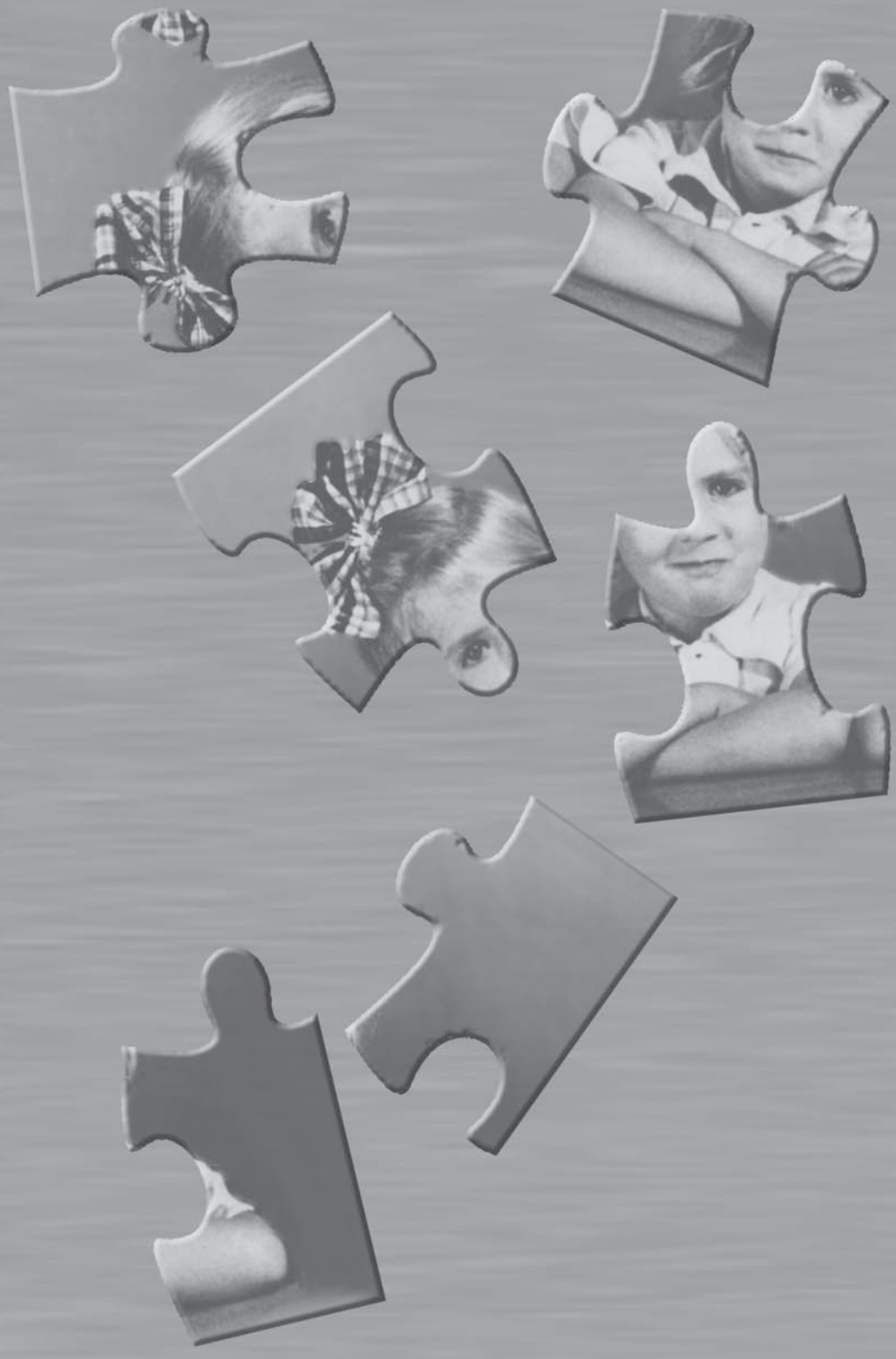
Chapter 5 was designed to examine whether autoimmune thyroiditis is related to the disease itself, to the (genetic) vulnerability to develop bipolar disorder, or both.

Both genetic and environmental factors are involved in the etiology of bipolar disorder; however, biological markers for the transmission of the bipolar genotype ("endophenotypes") have not been found. Autoimmune thyroiditis with raised levels of thyroperoxidase antibodies (TPO-Abs) is related to bipolar disorder and may be such an endophenotype. Blood was collected from 22 monozygotic (MZ) and 29 dizygotic (DZ) bipolar twin pairs and 35 healthy matched control twin pairs to determine TPO-Abs.

Does underperformance at school precede the onset of the bipolar disorder? If so, is underperformance at school a genetically related risk marker for developing bipolar?

In Chapter 6 we investigated whether underperformance at school precedes the onset of the illness and is a genetically related risk marker for developing bipolar disorder. Although the genetic risk to develop bipolar disorder is present from conception, the first frank symptoms of the illness generally become evident in late adolescence or early adulthood. However, except for pediatric bipolar disorder (PBD), it is still unclear when the first signs of the illness in adults become apparent and whether these are related to the genetic risk to develop bipolar disorder. Information on school performance was obtained using objective archival data from 53 bipolar twin pairs and 42 healthy matched control twin pairs.

In Chapter 7 the results will be summarized and discussed, while considering the strengths and weaknesses of the study.



Chapter

2

Influence of genes and environment on brain volumes in twin-pairs concordant and discordant for bipolar disorder

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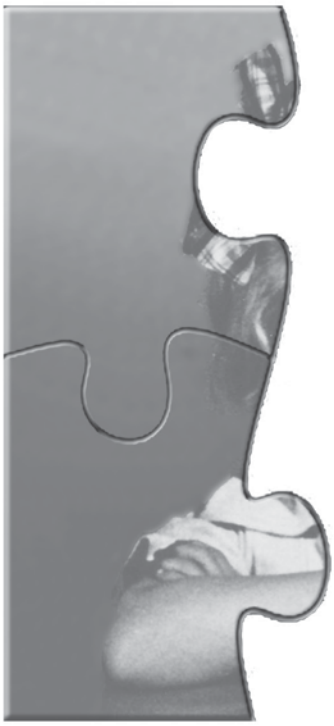


Abstract

Context: Structural neuroimaging studies suggest the presence of subtle abnormalities in the brains of patients with bipolar disorder. The influence of genetic and/or environmental factors on these brain abnormalities is unknown.

Objective: To investigate the contribution of genetic and environmental factors on brain volume in bipolar disorder.

Design: Magnetic Resonance Imaging (1.5 T) brain scans of monozygotic (MZ) or dizygotic (DZ) twins concordant and discordant for bipolar disorder were compared with healthy twin pairs.

Setting: Subjects were recruited from the population, the Netherlands Twin Register, and the twin pair cohort at the University Medical Center Utrecht, Utrecht, The Netherlands.

Participants: A total of 234 subjects including 50 affected twin pairs (9 MZ concordant; 15 MZ discordant; 4 DZ concordant; 22 DZ discordant) and 67 healthy twin pairs (39 MZ and 28 DZ) were included.

Main outcome measures: Volumes of the intracranium, cerebrum, cerebellum, lateral and third ventricle, and gray and white matter from the cerebrum and frontal, parietal, temporal and occipital lobes, both with and without correction for lithium use. To estimate the influence of additive genetic, common and unique environmental factors structural equation modelling was applied.

Results: Bipolar disorder was associated with a decrease in total cortical volume. Decreases in white matter were related to the genetic risk of developing bipolar disorder (bivariate heritability,77%; 95% confidence interval, 38% to 100%). Significant environmental correlations were found for cortical gray matter. These relationships all became more pronounced when data were corrected for lithium use.

Conclusions: Focusing on genes controlling white matter integrity may be a fruitful strategy in the quest to discover genes implicated in bipolar disorder. Elucidating the mechanism by which lithium attenuates brain matter loss may lead to the development of neuroprotective drugs.

Introduction

The high heritability (the percentage of phenotypic variance explained by genetic factors) of bipolar disorder (BD) has been well documented (McGuffin et al, 2003). However, environmental factors are likely to be involved as well, because the concordance rate for monozygotic (MZ) twin pairs is only around 70% (Craddock et al 2001).

The pathophysiology of bipolar disorder remains poorly understood although findings from structural imaging studies suggest the presence of subtle abnormalities in the brains of patients with bipolar disorder. These abnormalities include decreases in cortical volume (DelBello et al 2004, Lopez-Larson et al 2002, Lim et al 1999, Strakowski et al 1993), cerebral white matter (Davis et al 2004), cortical (Lim et al 1999, Davis et al 2004, Farrow et al 2005) and prefrontal gray matter, particularly in the subgenual and dorsolateral prefrontal cortex (Dickstein et al 2005), and increased ventricular volumes (McDonald et al 2004, Strasser et al 2005, Zipursky et al 1997, Strakowski et al 2002, Elkis et al 1995) compared with healthy subjects. However, these findings are not consistent; other studies fail to find changes in total brain(McDonald et al 2004, Strasser et al 2005, Bearden et al 2007) white (Lim et al 1999, Brambilla et al 2001) and gray matter volume (Strakowski et al 1993, Zipursky et al 1997, Brambilla et al 2001, McDonald et al 2005, Kieseppa et al 2003, Schlaepfer et al 1994), while some studies even report increases in gray matter volume in bipolar disorder (Adler et al 2005, Sassi et al 2002, Moore et al 2000). Some of the reported discrepancies may be owing to effects of medication, especially lithium (Moore et al 2000, Yucel et al 2007), or small sample sizes. Despite the pronounced genetic effects on both bipolar disorder (Craddock et al 1999) and brain volume (Baare et al 2001), the question whether the genetic risk of developing bipolar disorder is associated with some of the reported brain abnormalities in this illness has hardly been addressed.

Examination of the unaffected relatives of patients is often used to study the relationship between increased genetic risk and brain abnormalities because these subjects carry the genetic risk for the disease but not the disease itself. However, these studies are limited by the fact that they cannot discriminate genetic from shared environmental influence. To date, one volumetric magnetic resonance imaging (MRI) study compared psychotic bipolar patients (n=38) and their unaffected relatives (n=52) with healthy subjects (n=54). No differences in volumes of the cerebrum, lateral and third ventricle or hippocampus were reported (McDonald et al 2006).

In contrast to studies in unaffected relatives, examining MZ and dizygotic (DZ) twin pairs with at least 1 twin affected by the illness is a powerful approach to determine the relative contribution of genetic and environmental influences. So far only 2 studies of twins have been conducted on bipolar disorder measuring brain volume with MRI. One study included 6 MZ discordant and 6 healthy MZ twin pairs measuring the basal ganglia, amygdala-hippocampus complex, and the cerebral hemispheres. The authors concluded that genetic factors may be associated with an increased left caudate nucleus volume (Noga et al 2001). The second study, examining volumes of (frontal and temporal) gray and white matter and ventricular cerebrospinal fluid in 16 twin subjects with bipolar I disorder, 15 healthy co-twins, and 27 control twins found decreased left white matter volume to be influenced by familiar, possibly genetic, factors (Rijsdijk et al 2002).

In larger twin samples, a genetic model-fitting approach, also called structural equation modeling, enables quantification of the relative contribution of genetic and environmental influences to the possible phenotypic correlation between bipolar disorder and brain volume. With this method, the extent to which common genes or environmental factors influence both bipolar disorder and brain volume can be estimated (bivariate heritability) (Rijsdijk et al 2002).

To quantify the genetic and environmental effects on brain volume in bipolar disorder, we included 50 twin pairs of whom at least 1 had bipolar disorder and 67 healthy twin pairs (n=234), measuring global and regional (gray and white matter) brain volumes. Because several studies have suggested a neurotrophic or neuroprotective effect of lithium (Sassi et al 2002, Moore et al 2000), we also used genetic model fitting to estimate the genetic and environmental associations after controlling for the possible effect of lithium on brain volumes.

Methods

Subjects

A total of 50 twin pairs affected with bipolar disorder (9 MZ concordant; 15 MZ discordant; 4 DZ concordant; 22 DZ discordant) were included and compared with 67 (39 MZ and 28 DZ) healthy control twin pairs.

All twins were raised together, except for one control pair, who were separated at 12 years of age when both parents died. The subjects were between 18 and 60 years of age. Demographic information is presented in Table 1.

Clinical diagnosis for axis I psychiatric disorders was confirmed using the Structured Clinical Interview for DSM-IV (First et al 1996), for axis II personality disorders using the Structured Interview For DSM-IV Personality (Pfohl et al 1997), and for both through available medical records (Table 2). The twin pairs had no history of drug or alcohol dependency for the last 6 months and no severe medical illness, verified with a medical history inventory. Their current mood state was assessed using the Young Mania Rating Scale (Young et al 1978) and the Inventory for Depressive Symptomatology (Beck et al 1961). At the time of the study, all patients were euthymic, i.e., were not in a depressive, manic, or hypomanic episode, or were in an episode in partial remission with a Young Mania Rating Scale score of 4 or less and an Inventory for Depressive Symptomatology score of 12 or less, except for 4 patients, who met criteria for a depressive episode (Inventory for Depressive Symptomatology scores, 15, 20, 29 and 38, respectively).

The healthy control pairs were matched to the bipolar pairs for zygosity, sex, age, parental education, and birth order. Healthy control pairs had no history of axis I psychiatric disorder or axis II personality disorder according to DSM-IV criteria (confirmed with a Structured Clinical Interview for DSM-IV and a Structured Interview For DSM-IV Personality interview, respectively) and no history of severe medical illness. Furthermore, they had no first degree relative with a history of a major axis I psychiatric disorder (DSM-IV) such as schizophrenia, psychotic disorder, mood disorder, anxiety disorder or substance-related disorder. The family histories of both the affected and control twins were obtained via the Family Interview Genetic Studies (Neurnberger et al 1994) performed with both the proband and cotwin. Zygosity was determined by DNA fingerprinting using high polymorphic microsatellite markers 9 to 11 in the laboratory of the Division Biomedical Genetics, University Medical Center Utrecht.

The study was approved by the medical ethics review board of the University Medical Center Utrecht and all participants gave written informed consent after full explanation of the study aims and procedures.

Table 1. Demographic Data

	Bipolar Twin pairs (n=50)				Control Twin pairs (n=67)			
	MZ[a] (n=24)		DZ[b] (n=26)		MZ (n=39)		DZ (n=28)	
Female, No.	34		34		46		31	
Mean (SD) age, y	37.4 (10.6)		43.8 (8.5)		39.0 (9.9)		39.0 (7.5)	
Mean(SD) parental education, y	10.9 (3.5)		11.2 (3.8)		11.3 (3.3)		11.5 (3.5)	
First-degree relative, No. (%)								
bipolar disorder	8 (33)		4 (15)					
depression	4 (17)		9 (35)					
mood disorder	11 (48)		13(50)					
	BP patient	Co-twin	BP patient	Co-twin	Twin 1	Twin 2	Twin 1	Twin 2
Mean (SD) education, y	11.9 (2.0)	12.0 (2.2)	13.6 (2.6)	12.3 (3.1)	13.5 (2.8)	13.8 (2.7)	13.3 (2.5)	12.6 (2.8)
First born, No. (%)	16 (48)		12 (40)					
Handedness (left/right/both), No.	6/24/3	4/10/1	1/25/4	1/20/1	4/34/1	8/30/1	6/21/1	1/26/1
Mean (SD) onset age, y[c]	26.5 (8.9)		31.3 (9.9)					
Lithium/no lithium on day MRI, No.[d]	26/7		20/10					
Psychotic symptoms, No.	15		18					
Mean (SD) IDS score[e]	6.47 (6.7)	2.0 (2.5)	5.8 (8.3)	2.0 (2.5)	2.14 (2.9)	2.44 (2.7)	2.43 (3.9)	2.92 (2.7)
Mean (SD) YMRS score	1.1 (1.5)	.50 (.82)	.48 (.97)	.14 (.65)	.21 (.57)	.13 (.34)	.29 (.82)	.31 (.75)

Abbreviations: BD, bipolar disorder; DZ, dizygotic; IDS, inventory of depressive symptoms (both groups below score for depressive state); MRI, magnetic resonance imaging; MZ, monozygotic; YMRS, young mania rating scale.

aConcordant, 9; discordant, 15.

bConcordant, 4; discordant, 22.

c Significant difference between MZ and DZ ($F1,61=4.03$; $P=.05$).

d Six patients in the bipolar patients who did not take lithium group took lithium in the past, 5 of whom had not taken lithium for at least 2 years (range, 2-8 years). One patient took lithium for 13 years and stopped 1 month before the MRI. Analyses excluding this patient did not change the results.

e Significant difference between patients who were taking lithium and those who were not ($F1,60=6.3$; $P=.01$), but both groups were below the score for a depressive state.

Table 2. Lifetime Psychiatric Diagnosis of the Bipolar Twin Pairs

	No.		
		Co-twins (n=50)	
	Index (n=50)	Concordant (n=13)	Discordant (n=37)
Diagnosis			
Bipolar I disorder	37	8	
Bipolar II disorder	1	3	
Bipolar disorder NOS		1	
Bipolar disorder NOS and schizophrenia, paranoid type		1	
Major depressive disorder			4
Depressive disorder NOS			3
Schizophrenia, paranoid type			3
Dissociative disorder NOS			1
Comorbid diagnosis			
Depressive disorder NOS			3
Mood disorder due to hyperthyroidism			1
Psychotic disorder NOS	1		
Psychotic disorder due to cannabis	1		
Agoraphobia without history of panic disorder	1		
Panic disorder without agoraphobia	1		1
Post traumatic stress disorder	1		
Alcohol use disorder in full remission	2		1
Cannabis use disorder in full remission	1		
Sedative use disorder	1		
Sedative use disorder in full remission		1	
Anorexia nervosa	1		
Borderline personality disorder	5		1
Obsessive-compulsive disorder in full remission	1		
Obsessive-compulsive personality disorder			1
Personality disorder NOS		1	1
No diagnosis			**26**

Abbreviation: NOS, not otherwise specified

MRI acquisition and Image analysis

Image acquisition and data processing have been described in previous studies from this group (Hulshoff et al 2004). Quantitative assessments of the intracranium, cerebrum (total brain excluding cerebellum and stem), gray and white matter of the cerebrum, lateral and third ventricular volume, and cerebellum were performed based on histogram analyses and series of mathematical morphology operators to connect all voxels-of-interest (Schnack et al 2001, Schnack et al 1999). All images were checked after measurement and corrected manually if necessary. To evaluate regional contributions, these gray- and white-matter segments from the individual images were used to identify gray and white matter for each individual lobe (frontal, parietal, temporal and occipital). A fully automated warping technique was used (Figure 1). This technique uses nonlinear transformations to register every brain scan in the study to a model brain. The model brain was selected earlier from 200 brain images of subjects aged between 16 and 70 years (Mandl et al 1999).
Frontal, parietal, temporal, and occipital lobes were manually demarcated on this image. The borders have been described in detail previously (Palmen et al 2004). In short, the cingulate gyrus and the insula were excluded from all cortical segments. The prefrontal segment excluded the precentral gyrus, although a frontal segment including the precentral gyrus was also defined. The parietal segment was separated from the frontal lobe by the central sulcus. The parietooccipital fissure defined the boundary with the occipital lobe. The boundary between the temporal and occipital segments was defined using the temporooccipital notch. Cortical volume is defined as the sum of the gray and white matter of the separate lobes, cortical gray matter as gray matter of all lobes, and lobar white matter as total white matter of all lobes. Brain images were registered to the model brain using the ANIMAL algorithm (Collins et al 1994) to remove global differences in the sizes and shapes of the individual brains. The inverse of the transformation process registered the manual segmentations of the model brain to all subjects' brain images.

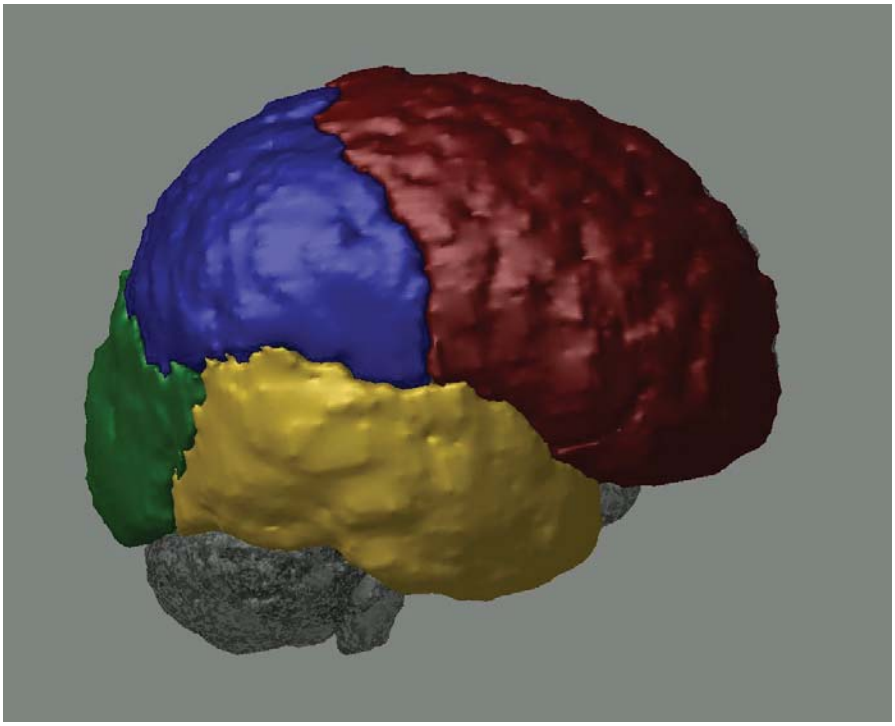


Figure 1.
Segmentation of lobar volumes; frontal lobe (red), parietal lobe (blue), temporal lobe (yellow) and occipital lobe (green) (UMC Utrecht, Dept of Psychiatry, Imaging Lab).

Statistical analysis

The main aim of the bivariate genetic model-fitting analyses was to separate an expected correlation between bipolar disorder and brain volume into genetic and environmental components (Rijsdijk et al 2005, Hall et al 2007).

To estimate the relative contributions of additive genetic (A), common environmental (C), and unique environmental (E) factors on individual differences in brain volume and the relationship with bipolar disorder, structural equation modeling was used. The extent to which A, C, and E explained the variance in brain volumes or covariance between brain volume and bipolar disorder, was expressed as the percentage of the total covariance and variance, resulting in estimates of, respectively, h2 (heritability), c2 (common or shared environmentability), and e2 (unique environmentability). The factor E also included measurement error (Figure 2).

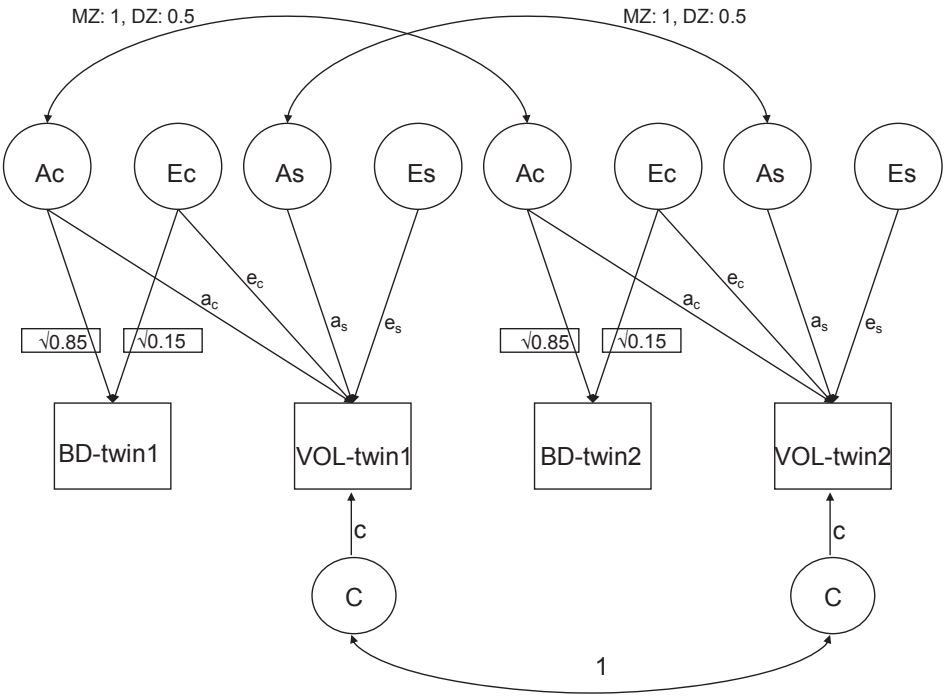


Figure 2.

Example of a path diagram of the ACE genetic model for bipolar disorder (BD) and brain volume (BV). Bivariate twin model: genetic (A) and environmental (C/E) influences on BV and BD. The additive genetic factors (Ac and As) of monozygotic (MZ) twins are perfectly correlated (1.0), whereas those of dizygotic (DZ) twins are correlated at 0.5. Common environmental factors shared by twins from the same family (C) are correlated at 1 for both types of twins (only modeled for BV); the unique environmental influences (Ec and Es) are always uncorrelated between twins. Path coefficients (ac and as) quantify the effects of genetic influences Ac and As on BV, where Ac represents genetic influences that also influence BD and As represents genetic influences that are unique for BV.Similarly, path coefficients ec and es quantify the effect of unique environmental (Ec and Es) influences on BV. Path coefficient c quantifies the effect of common environmental influence on BV. Genetic and environmental variance of bipolar disorder is fixed on 0.85 and 0.15.

Prior to structural equation modelling, phenotypic correlations (r_{ph}) between bipolar disorder and brain volumes and cross-trait-cross-twin correlations were calculated. A phenotypic correlation provides information on whether the specific volume is associated with bipolar disorder. It can result from a common set of genes or common set of environmental factors. Phenotypic correlations between bipolar disorder and brain volume were separated into genetic (r_g) and environmental components (r_e), thus providing information regarding the possible shared genetic and environmental influences of bipolar

liability and brain volumes. Separation of these sources was based on the comparison of so called cross-trait-cross-twin correlations for MZ and DZ twins. The cross-trait-cross-twin correlation is the correlation between a trait (i.e., bipolar disorder) of twin 1 with another trait of twin 2 (brain volume), where twin 1 and twin 2 represent a twin pair.
If the absolute value of the correlation between brain volume of twin 1 and bipolar disorder liability of twin 2 is larger in MZ twins than in DZ twins; this indicates that genes influencing brain volume (partly) overlap with genes that influence bipolar disorder. The extent of the overlap is reflected by the magnitude of the genetic correlation (r_g).
To implement these models, data were examined for outliers, and subsequently the residuals of the brain volumes, after regression on intracranial volume, sex and age (SPSS version 12.0;SPSS Inc, Chicago, Illinois) were used to calculate a 5-category ordinal scale. This allowed for a bivariate (bipolar disorder and brain volume) ordinal genetic data analysis using the statistical package Mx (Neale et al 2003) (www.psy.vu.nl/mxbib). For ordinal genetic model fitting, the dichotomous variable 'bipolar disorder' was assumed to represent an underlying continuous liability with a mean (SD) of 0 (1). A person with a high value on the liability scale crossing a certain threshold would be scored 'patient' on our dichotomous variable, and in all other cases considered to be healthy (discordant co-twin of patient of healthy comparison twin-pairs), thus receiving the alternative score.
The critical threshold and heritability for the underlying bipolar disorder liability was not based on our sample because we included approximately equal numbers of concordant, discordant, and healthy twin pairs. We fixed prevalence and heritability of bipolar disorder to the population values; prevalence was set to 1% (Regeer et al 2004, ten Have et al 2002) and heritability was set to 85% (McGuffin et al 2003).
Effects of genes and familial background (C) were tested by fitting different models to the data. Parameters A, C, or both can be removed from the basic ACE model to generate submodels (i.e., AE, CE and E) that can be tested via likelihood ratio tests. This likelihood ratio test statistic follows a χ^2distribution. A χ^2_1 larger than 3.84 indicates a significant difference at α=0.05, and indicates that the discarded effect (e.g., the effect of C on brain volume) cannot be left out of the model without seriously deteriorating the goodness of fit. The most restrictive model was accepted as the best fitting one in case the difference between two models was not significant (Neale et al 2003). For relevant estimates, 95%

confidence intervals (CI) were obtained (Neale et al 1997). For bipolar disorder, the influence of C was not implemented into the model because the literature showed no evidence for family-related (common) environmental influences on bipolar disorder (McGuffin et al 2003).

The first likelihood ratio test for brain volume was whether the influence of C on brain volumes could be discarded from the model (see results). For the best-fitting model heritabilities and environmentabilities (i.e., percentage of total variance accounted for by unique environmental influences, 1-h^2) of brain volumes, bivariate heritabilities (the percentage of covariance between bipolar disorder and brain volume that is accounted for by a common genetic factor $h^2_{BV/BD} = |cov_A| / (|cov_A| + |cov_E|)$, where BV is brain volume, BD is bipolar disorder, and cov is covariance) and genetic and environmental correlations between bipolar disorder and brain volumes were obtained.

Effects of lithium

Multiple univariate analysis of variance were used to determine if the patients who did not take lithium differ from the patients who did take lithium in age, age at onset, educational level, manic and depressive symptoms, number of depressive and manic episodes, number of hospitalizations, and all brain volumes with age, sex and intracranial volume as covariates. A χ^2 test was used for the difference in sex and zygosity. A Pearson correlation was used for all brain volumes and duration of lithium use.

After regression on intracranial volume, age and sex the differences in means for the separate brain volumes between patients who did not take lithium (L^-; n=17 [for 1 patient no gray/white matter separation was possible]) and the patients who took lithium (L^+; n=46) were calculated (Table 3). This difference was subtracted from the values of the lithium-using patients, resulting in an estimate of their volumes when no lithium would have been used. After this correction, structural equation modelling was performed including all subjects, as described in the previous paragraph. This correction for the effects of lithium is believed to yield better estimates of the true genetic and environmental effects on the relationship between brain volume and bipolar disorder.

Effects of psychotic symptoms

Multiple univariate analysis of variance were used to determine if patients with psychotic symptoms (lifetime) differ from patients without psychotic symptoms in age, age of onset, educational level, manic and depressive symptoms, number of depressive and episodes, or number of hospitalizations. After genetic model fitting was performed, the volumes that were significantly associated with bipolar disorder (R_{ph} see table 4 and 6) were analyzed post hoc using multiple univariate analyses of covariance to determine if these volumes were significantly different in patients with or without psychotic symptoms. Intracranial volume, age and sex were used as covariates manic.

Results

The demographic and clinical characteristics of all twin pairs are presented in Table 1. Bipolar patients who were taking lithium (L^+; n=46) were not significantly different from the bipolar patients who did not take lithium (L^-; n=17) on all clinical parameters (except for current depressive symptoms, ($F_{1,61}$=6.3; P=.01); when correcting for multiple comparisons (Bonferroni correction), this effect was no longer significant. In Table 3, raw mean volumes and differences in brain volumes (in percentages) are presented for bipolar patients, co-twins of patients, and healthy comparison subjects. The differences between the volumes of L^- and L^+ bipolar patients are presented in Table 3. Multiple univariate analyses of covariance showed a significant difference in cerebral ($F_{1,58}$=6.8; P=.01), cortical ($F_{1,58}$=6.8; P=.01), and ventricular volumes ($F_{1,58}$=5.4; P=.02 for lateral ventricle and ($F_{1,58}$=6.8; P=.01 for third ventricle).

There were no significant correlations between brain volume and duration of lithium use (lithium and cerebral volume r= -.04; P=.78; cerebral gray matter r= -.23; P=.10).

Bipolar patients without psychotic symptoms differed from bipolar patients with psychotic symptoms on number of depressive episodes (4.25 vs 3.0 episodes; $F_{1,56}$=5.53; P=.02). All other clinical variables were not significantly different. Brain volumes were not significantly different between patients with and without psychotic symptoms.

Common environmental influence

The influence of common environment was not significant for brain volumes. There was one exception; for third ventricular volume, either a model containing common environmental influences or a model containing genetic influences explained the data best. The effects (common environment/genetic influence) could not be discarded simultaneously from the models without significantly reducing the goodness of fit (χ^2_3=26.514) and there was not enough power to distinguish between them. We therefore report on the AE model for all brain volumes.

Table 3. Brain volumes of bipolar patients, their co-twins and healthy comparison subjects.

	Mean (SD) Volume, mL[a]			Increase, %[b]		
	BP HC (n=63)[c]	Co-twins[d] (n=37)	HC (n=134)	BP vs HC	Co-twins[d] vs HC	Difference Between L^- and L^+, %[e]
Intracranium	1420 (174)	1451 (179)	1428 (129)	+1.18	+ 2.62	+ 2.8
Cerebrum	1063 (128)	1089 (129)	1086 (111)	- 1.37	-0.64	**- 3.30** [f]
Gray matter	607 (69)	615 (71)	619 (70)	-1.13	-0.80	- 3.64
White matter	456 (77)	474 (79)	467 (66)	-1.97	-0.78	- 2.77
Lateral ventricle	17.1 (9.7)	16.6 (8.6)	15.2 (7.7)	+ 8.70	+ 4.48	**+ 12.49** [f]
Third ventricle	.89 (.53)	.84 (.41)	.79 (.41)	+ 7.7	- 2.56	**+ 17.18** [f]
Cerebellum	138 (15)	138 (13)	141 (13)	-0.72	-2.17	- 2.32
Cortical Volume[g]	745 (92)	766 (92)	764 (80)	- .47	- 0.54	**- 3.26** [f]
Cortical gray matter[h]	447 (50)	456 (53)	458 (51)	-1.45	- 0.47	- 3.4
Lobar white matter[i]	297 (52)	310 (55)	306 (39)	- 2.44	- 1.25	- 3.05
Gray Matter						
Prefrontal lobe	153 (18)	155 (18)	156 (18)	- 0.89	-0.58	- 2.80
Temporal lobe	133 (14)	136 (16)	136 (14)	- 1.35	-0.08	- 2.92
Parietal lobe	109 (12)	110 (13)	111 (13)	- 1.51	-1.14	- 3.73
Occipital lobe	53 (8)	55 (8)	55 (8)	- 3.07	-0.14	- 5.60
White Matter						
Prefrontal lobe	108 (18)	112 (19)	110 (14)	- 1.16	-0.23	- 3.08
Temporal lobe	66 (13)	69 (14)	68 (10)	- 2.0	-0.97	- 2.52
Parietal lobe	78 (14)	81 (14)	81 (10)	- 3.25	- 2.56	- 4.89
Occipital lobe	45 (9)	48 (10)	46 (7)	- 2.63	-0.04	- 0.54

Abbreviations: BP, patients with bipolar disorder; HC, healthycontrol subjects (twin and co-twin); L^+, bipolar patients who are taking lithium (n=46); L^-, bipolar patients who did not take lithium (n=17).

[a] Uncorrected for age, sex, or intracranial volume.

[b] Corrected for age, sex, and intracranial volume; based on a mean age of 39.04 years, mean intracranial volume of 1430 mL, and female sex.

[c] Including 26 patients from concordant pairs (9 monozygotic; 4 dizigotic); for one patient, no separation of gray and white matter volume was possible.

[d] Co-twin without bipolar disorder

[e] Difference obtained by subtracting L^+ from L^-.

[f] Values in bold face are significant at α=.05.

[g] Sum of the volumes of the prefrontal, parietal, temporal, and occipital lobes.

[h] Sum of the volumes of the gray matter of separate lobes.

[i] Sum of the volumes of the white matter of separate lobes.

Table 4. Correlations and Sources of Covariance Between Bipolar Disorder and Brain Volume

	Correlation (95% CI)						
		Cross-trait-Cross-twin[a]					
Brain volumes	**r_{ph}[a]**	**MZ**	**DZ**	**r_g**	**r_e**	**$h^2_{BV/BD}$,% (95%CI)[b]**	**$e^2_{BV\ BD}$, % (95%CI)[b]**
Intracranium	0.06 (-0.07 to 0.18)	0.07 (-0.06 to 0.21)	0.04 (-0.12 to 0.19)	0.09 (-0.07 to 0.24)	-0.18 (-0.66 to 0.33)	80	20
Cerebrum	-0.11 (-0.23 to 0.02)	-0.09 (-0.23 to 0.05)	-0.06 (-0.22 to 0.10)	-0.12 (-0.32 to 0.07)	-0.06 (-0.41 to 0.20)	86	14
Cerebral GM	-0.05 (-0.18 to 0.07)	0.03 (-0.12 to 0.17)	-0.06 (-0.22 to 0.10)	0.02 (-0.18 to 0.22)	-0.28 (-0.65 to 0.13)	18	82
Cerebral WM	-0.12 (-0.24 to 0.01)	-0.12 (-0.26 to 0.02)	-0.10 (-0.25 to 0.06)	-0.15 (-0.33 to 0.02)	0.02 (-0.33 to 0.37)	97	3
Lateral ventricle[c]	0.07 (-0.06 tot 0.20)	-0.03 (-0.13 to 0.16)	0.04 (-0.07 to 0.26)	0.02 (-0.17 to 0.22)	0.23 (-0.17 to 0.60)	26	74
Third ventricle[c]	0.07 (-0.06 to 0.19)	-0.03 (-0.18 to 0.11)	0.04 (-0.12 to 0.21)	-0.02 (-0.24 to 0.18)	0.31 (-0.04 to 0.62)	15	85
Cerebellum	-0.02 (-0.15 to 0.10)	0.02 (-0.12 to 0.16)	-0.13 (-0.28 to 0.04)	0.02 (-0.15 to 0.18)	-0.24 (-0.06 to 0.15)	28	72
Total cortical volume[d]	**-0.14** (-0.26 to -0.01)	-0.11 (-0.25 to 0.03)	-0.04 (-0.20 to 0.12)	-0.14 (-0.32 to 0.04)	-0.17 (-0.52 to 0.21)	75	25
Cortical GM[e]	-0.08 (-0.21 to 0.05)	0.02 (-0.12 to 0.16)	-0.09 (-0.25 to 0.07)	0.02 (-0.16 to 0.20)	**-0.46** (-0.81 to -0.02)[f]	13 (0 to 66)	**87** (34 to 100)[f]
Lobar WM	-0.11 (-0.26 to 0.02)	**-0.16** (-0.30 to -0.02)[f]	-0.05 (-0.21 to 0.11)	**-0.20** (-0.37 to -0.02)[f]	0.24 (-0.14 to 0.58)	**77** (38 to 100)[f]	23 (0 to 62)

Abbreviations: BD, bipolar disorder; BV, brain volume; CI, confidence intervals; DZ, dizygotic; $e^2_{BV/BD}$, unique environmental influence on both bipolar disorderand brain volume; $h^2_{BV/BD}$, bivariate heritability (common genetic influence on BV and BD); GM, gray matter; MZ, monozygotic; *r*e, environmental correlation; r_g, genetic correlation; r_{ph}, phenotypic correlations; WM, white matter.

[a]The correlations used point estimates of r_{MZ}=0.85[1] and a prevalence of 1%.[2] A lower prevalence (0.5% instead of 1%) and heritability (75% instead of 85%) did not result in other estimates (other than rounding errors) and resulted in only slight differences in CI.

[b]The 95% CI range 0 to 100 are not presented.

[c]Ventricular volumes were log-transformed.

[d]The sum of the volumes of the prefrontal, parietal, temporal, and occipital lobes.

[e]Only occipital gray matter had a significant phenotypic (r_{ph}=−0.13) and environmental (r_e=−0.38) correlation. The volumes of the other lobes (prefrontal, parietal, and temporal) and GM and WM per lobe were in the same direction as total cortical volume, cortical GM, and WM volume (values are available on request).

[f]Values in boldface are significant at α=.05.

Associations of brain volume with bipolar disorder

Bipolar disorder was significantly and negatively associated with cortical volume (the sum of the gray and white matter of all cortical lobes; phenotypic correlation, $r_{ph=}$ -0.14), indicating a smaller cortical volume in bipolar patients. Adjusted for the effect of prescribed lithium, all brain volumes except for cerebellum and occipital white matter were significantly associated with bipolar disorder; ventricular volumes (lateral ventricle r_{ph} =0.24; third ventricle r_{ph} =0.29) and intracranial volume (r_{ph} =0.15) were positively correlated, indicating an increase in these volumes in bipolar patients while all other volumes were negatively correlated (ranging from r_{ph}= -0.15 for temporal white matter volume to r_{ph} =-0.37 for total cortical volume) suggesting decreases of these volumes in bipolar patients (Table 4).

Common genetic influence on bipolar disorder and brain volume

Irrespective of bipolar disorder, significant moderate to high heritabilities (h^2_{BV}) were found for brain volumes, ranging from 54% for third ventricle volume to 93% for intracranial volume (Table 5).

Table 5. Estimated influences of additive genetic (h^2) and Unique Environmental (e^2) Factors on Brain Volume, Irrespective of Disease[a].

Brain volumes	h^2_{BV}, % (95% CI)	$e^2_{BV,\%}$ (95% CI)	$h^2_{BV\_L,}$[b] % (95%CI)	$e^2_{BV\_L,}$[b] % (95% CI)
Intracranium	93 (87-96)	7 (4-13)	91 (84-95)	9 (5-16)
Cerebrum	65 (46-78)	35 (22-54)	64 (45-77)	36 (23-55)
Cerebral GM[b]	60 (39-75)	40 (25-61)	50 (29-67)	50 (33-71)
Cerebral WMb	74 (58-84)	26 (16-42)	80 (66-88)	20 (22-34)
Lateral ventricle[c]	65 (47-78)	35 (22-53)	65 (47-78)	35 (22-53)
Third ventricle[c]	54 (35-70)	46 (30 –.65)	60 (43-73)	40 (27 -57)
Cerebellum	83 (72-90)	17 (10 –18)	84 (73-90)	26 (10-27)
Total cortical volume[d]	70 (53-82)	30 (18-47)	69 (49-70)	31 (30-51)
Cortical GM	74 (57-84)	26 (16-43)	64 (57-84)	36 (16-43)
Lobar WM	76 (61-85)	24 (15-39)	82 (70-90)	18 (10-30)

Abbreviations: BV, brain volume; CI, confidence interval; e^2, unique environmentability; GM, gray matter; h^2, heritability; WM, white matter.
[a] All analyses were corrected for intracranial volume, age, and sex.
[b] h^2_{BV} and $e^2_{BV\_L}$ are estimates after correction on the values of the patients who were taking lithium.
[c] Ventricular volumes were log-transformed.
[d] The sum of the volumes of the prefrontal, parietal, temporal, and occipital lobes.

Bipolar disorder showed a genetically mediated association with lobar white matter. This was indicated by a significant negative MZ cross-trait-cross-twin correlation with bipolar

disorder (-0.16) and a lower and non-significant DZ cross-trait-cross-twin correlation. Common genes appear to be involved because the genetic correlation with lobar white matter was significant (r_g =-0.20), as evidenced by the bivariate heritability ($h^2_{BV/BD}$). Additive genetic factors (A) were estimated to account for 77% of the covariance (CI, 38% to 100%), indicating that at least 38% (i.e., the lower end of the CI range) of the covariance between lobar white matter and bipolar disorder liability can be explained by common genetic factors.

After correcting for the effects of lithium, the (common) genetic influence on white matter volumes and the risk for bipolar disorder became more pronounced; all white matter volumes showed significant MZ cross-trait-cross-twin correlations (Table 6). Significant genetic correlations reflecting involvement of common genes were found for all white matter volumes (except for the occipital lobe), cerebral and total cortical volume, occipital gray matter, and third ventricular volume (R_g; Table 6). The extent to which additive genetic factors (A) explain the covariance between bipolar disorder and brain volume was 68% for cerebral volume, 99% for lobar white matter and 85% for cerebral white matter. The white matter of the separate cortical lobes contributed in almost equal measure to this high percentage (see $h^2_{BV/BD}$ [CI]; Table 6). Gray matter volumes did not show a significant common genetic influence.

Unique environmental influence on bipolar disorder and brain volume

First it must be noted that because the heritability of bipolar disorder was set to 85%, only 15% of the variance in the underlying liability can be explained by unique environmental factors. Irrespective of disease, brain volumes showed unique environmental influences ranging from 7% for intracranial volume to 46% for third ventricular volume (Table 5). Environmental correlations represent unique environmental factors that are associated with both brain volume and bipolar disorder. For cortical gray matter, a significant environmental correlation was found (r_e = -0.46). The bivariate environmentability (the proportion of the correlation between bipolar disorder and brain volume associated with unique environmental factors) was significant for cortical gray matter; environmental influence explained 87% of the covariance between gray matter volume and bipolar disorder (CI, 34% to 100%).

After correction for the effects of lithium use significant negative environmental correlations were found for all gray matter volumes, cerebral and cortical volume and cerebellar volume. Ventricular volumes were positively correlated with bipolar disorder (r_e; Table 6). The Table 6 ($e^2_{BV/BD}$) shows the extent to which unique environmental factors influence both brain volume and bipolar disorder (ranging from 32% for cerebral volume to 76% for prefrontal gray matter ($e^2_{BV/BD}$ [CI] ; Table 6). White matter volumes in bipolar disorder showed no significant environmental influences.

Comment

We examined the relative contributions of genetic and environmental influences on brain volume in bipolar disorder. Gray and white matter and ventricular volumes were measured in 50 twin pairs with bipolar disorder and 67 healthy control twin pairs. To our knowledge, this is the first MRI study using genetic model fitting in twin pairs discordant and concordant for bipolar disorder. The main finding is that a decrease in white matter is related to the genetic risk of developing bipolar disorder, while unique environmental factors are related to a decrease in (cortical) gray matter volume in patients with bipolar disorder.

By applying structural equation modeling, it was demonstrated that at least 38% of the covariance between white matter volume and bipolar disorder could be explained by genetic factors that influence both the volume of white matter and (the risk for developing) bipolar disorder. This indicates that genes involved in the etiology of bipolar disorder may contribute to the white matter decreases found in bipolar patients and in their cotwins.

In addition, a significant environmental correlation between the bipolar phenotype and cortical gray matter volume was found, with at least 34% of the covariance explained by unique environmental factors that are common to bipolar disorder and cortical gray matter brain volume. This suggests that environmental factors unique for each individual influence both bipolar disorder and gray matter volume, most likely in relation to the effects of the illness itself. Finally, lithium showed considerable effects on the brain changes found in this study, attenuating the decrease in both gray and white matter. As a consequence, findings became more pronounced, but did not change fundamentally, when analyses were corrected for lithium.

Table 6. Correlations and Estimated Influences of Additive Genetic (h2) and Unique (e2) Environmental Factors on Brain Volume of the Patients With Bipolar Disorder, Their Co-twins, and the Healthy Comparison Twin Pairs After Correction on the Values of Patients Who Were Taking Lithium.

		Cross-trait-Cross-twin Correlations					
BV After correction for lithium use	**r_{ph}[a]**	**MZ**	**DZ**	**r_g**	**r_e**	**$h^2_{bivariate\ BV/BD}$, % (95% CI)**	**$e^2_{bivariate\ BV/BD}$, % (95% CI)[b]**
Intracranium	0.15 (-0.01 to 0.30)	0.13 (-0.09 to 0.26)	0.05 (-0.11 to 0.20)	0.15 (-0.01 to 0.30)	0.22 (-0.24 to 0.62)	83 (-0.16 to 1)	17
Cerebrum	-0.34 (-0.46 to -0.21)	**-0.24** (-0.37 to -0.09[c])	-0.09 (-0.25 to 0.06)	**-0.31** (-0.51 to -0.12)[c]	**-0.48** (-0.77 to -0.11)[c]	**68** (38 to 92)[c]	**32** (8 to 62)[c]
Cerebral GM	-0.25 (-0.36 to -0.12)	-0.07 (-0.22 to 0.06)	-0.08 (-0.24 to 0.08)	-0.13 (-0.34 to 0.09)	**-0.60** (-0.89 to -0.22)[c]	33 (0 to 74)	**67** (26 to 100)
Cerebral WM	-0.23 (-0.35 to -0.10)	**-0.20** (-0.33 to -0.06)[c]	-0.13 (-0.28 to 0.03)	**-0.24** (-0.36 to -0.07)[c]	-0.18 (-0.53 to 0.20)	**86** (47 to 100)[c]	24 (0 to 53)
Lateral ventricle[d]	0.24 (0.11 to 0.36)	0.12 (-0.02 to 0.26)	0.13 (-0.03 to 0.29)	0.16 (0.08 to 0.84)	**0.51** (0.08 to 0.84)[c]	51 (0 to 90)	**49** (10 to 100)[c]
Third ventricle[d]	0.29 (0.17 to 0.41)	0.07 (-0.06 to 0.17)	0.07 (-0.10 to 0.24)	**0.12** (0.12 to 0.30)[c]	**0.83** (0.49 to 0.99)[c]	30 (0 to 60)	**70** (40 to 100)[c]
Cerebellum	-0.10 (-0.23 to 0.03)	-0.02 (-0.15 to 0.11)	-0.14 (-0.30 to 0.02)	-0.03 (-0.19 to 0.13)	**-0.47** (-0.80 to -0.07)[c]	25 (0 to 88)	**75** (22 to 100)[c]
Total cortical volume	-0.37 (-0.49 to -0.25)	**-0.24** (-0.38 to -0.11)[c]	-0.08 (-0.24 to 0.07)	**-0.32** (-0.50 to -0.14)[c]	**-0.61** (-0.85 to -0.25)[c]	**64** (38 to 92)[c]	**36** (8 to 62)c
Cortical GM	-0.27 (-0.39 to -0.15)	-0.09 (-0.22 to 0.04)	-0.11 (-0.26 to 0.05)	-0.13 (-0.16 to 0.20)	**-0.75** (-0.81 to -0.05)[c]	35 (0 to 67)	**65** (33 to 100)[c]
Lobar WM	-0.22 (-0.33 to -0.08)	**-0.22** (-0.36 to -0.09)[c]	-0.08 (-0.23 to 0.08)	**-0.26** (-0.42 to -0.10)[c]	0.01 (-0.39 to 0.41)	**99** (77 to 100)[c]	1 (0 to 23)
Gray Matter							
Prefrontal lobe[e]	-0.16 (-0.28 to -0.03)	-0.04 (-0.17 to 0.10)	-0.06 (-0.22 to 0.10)	-0.05 (-0.23 to 0.13)	**-0.54** (-0.82 to -0.14)[c]	24 (0 to 79)	76 (21 to 100)[c]
Temporal lobe	-0.25 (-0.36 to -0.12)	-0.12 (-0.26 to 0.02)	-0.05 (-0.20 to 0.11)	-0.15 (-0.32 to 0.03)	**-0.65** (-0.93 to -0.24)[c]	47 (0 to 81)	**53** (19 to 100)[c]
Parietal lobe	-0.23 (-0.35 to -0.11)	-0.07 (-0.21 to 0.06)	-0.14 (-0.29 to 0.02)	-0.12 (-0.31 to 0.07)	**-0.61** (-0.86 to -0.21)[c]	37 (0 to 77)	**63** (23 to 100)[c]
Occipital lobe	-0.30 (-0.41 to -0.17)	**-0.15** (-0.28 to -0.01)[c]	-0.01 (-0.15 to 0.16)	**-0.20** (-0.37 to -0.01)[c]	**-0.65** (-0.87 to -0.32)[c]	**49** (5 to 75)[c]	**51** (25 to 95)[c]
White Matter							
Prefrontal lobe	-0.21 (-0.33 to -0.08)	**-0.17** (-0.31 to -0.03)[c]	-0.05 (-0.20 to 0.11)	**-0.21** (-0.38 to -0.03)[c]	-0.23 (-0.59 to 0.18)	**79** (25 to 100)[c]	21 (0 to 75)
Temporal lobe	-0.15 (-0.28 to -0.02)	**-0.18** (-0.31 to -0.04)[c]	-0.05 (-0.21 to 0.11)	**-0.21** (-0.38 to -0.04)[c]	0.11 (-0.28 to o.48)	**90** (61 to 100)[c]	10 (0 to 39)
Parietal lobe	-0.26 (-0.38 to -0.13)	**-0.20** (-0.33 to -0.06)[c]	-0.10 (-0.25 to 0.05)	**-0.24** (-0.40 to -0.07)[c]	-0.37 (-0.72 to 0.05)	**77** (41 to 100)[c]	23 (0 to 59)
Occipital lobe	-0.09 (-0.21 to 0.04)	-0.11 (-0.25 to 0.04)	0.02 (-0.14 to 0.18)	-0.13 (-0.33 to 0.07)	0.04 (-0.35 to 0.43)	91 (-1 to 1)	9

Abbreviations: BD, bipolar disorder; BV, brain volume; CI, confidence intervals; DZ, dizygotic; e2 BV/BD, unique environmental influence on both bipolar disorderand brain volume; h2 BV/BD, bivariate heritability (common genetic influence on BV and BD); GM, gray matter; MZ, monozygotic; *r*e, environmental correlation; *r*g, genetic correlation; *r*ph, phenotypic correlation of brain volume with bipolar disorder; WM, white matter.

[a] All volumes were significantly associated with bipolar disorder except for intracranium, cerebellar volume, and occipital WM.

[b] AE model; bivariate E=1−bivariate h2 BV/BD. The 95% CI range 0 to 100 is not presented.

[c] Values in boldface are significant at _=.05.

[d] Ventricular volumes were log-transformed.

[e] Results of the prefrontal lobe were comparable with the frontal lobe (including the precentral gyrus); only the prefrontal lobe results were reported

Our finding that a decrease in white matter is related to the genetic risk of developing bipolar disorder is consistent with that of a previous twin study in 16 bipolar twin pairs and 15 healthy cotwins. Those results provided evidence suggesting that the white matter decrease in bipolar disorder is genetically mediated, but owing to the smaller number of subjects and participation of incomplete twin pairs, familial and genetic effects could not be separated in that study (Kieseppa et al 2003).

Our results build on several other lines of evidence, such as those derived from gene expression and genetic association studies, suggesting involvement of white matter pathology in bipolar illness. For instance, reductions in the number, size, and density of glial cells (Carter 2007, Uranova et al 2004, Öngür et al 1998, Rajkowska 2002) as well as downregulation of key oligodendrocyte and myelination genes (including transcription factors that regulate these genes) have been reported in post-mortem studies in bipolar disorder (Tkachev et al 2003, Sequeira et al 2006). White matter abnormalities in the risk of developing bipolar disorder, as found in our study, are also consistent with several of the genetic association studies reporting changes in oligodendroglia-related genes in patients with bipolar disorder (Carter 2007, Sokolov 2007).

Interestingly, white matter pathology has also been suggested to be central to the genetic risk to developing schizophrenia (Davis et al 2003). Indeed, in an earlier MRI study in twins discordant for schizophrenia, we reported that white matter decrease was associated with the increased genetic risk to develop schizophrenia (Hulshoff et al 2004). Similarly, a voxel-based morphometry study that included patients with schizophrenia, bipolar patients, and their unaffected relatives found the genetic risk for both disorders to be associated with a white matter decrease in the left frontal and temporoparietal regions (McDonald et al 2004). Results from brain imaging studies of schizophrenia are consistent with those from post-mortem studies reporting reductions in number, size, and density of glial cells and downregulation of oligodendrocyte and myelination genes in schizophrenia (Uranova et al 2004, Carter 2007, Hakak et al 2001, Vostrikov et al 2007, Segal et al 2007).

Taken together, white matter pathology may constitute a common genetic risk factor for bipolar disorder and schizophrenia. Indeed, findings from genetic association studies suggest considerable overlap in risk genes for bipolar disorder and schizophrenia, particularly regarding oligodendrocyte- and myelin-related genes (Tkachev et al 2003, Sokolov 2007, Carter 2007).

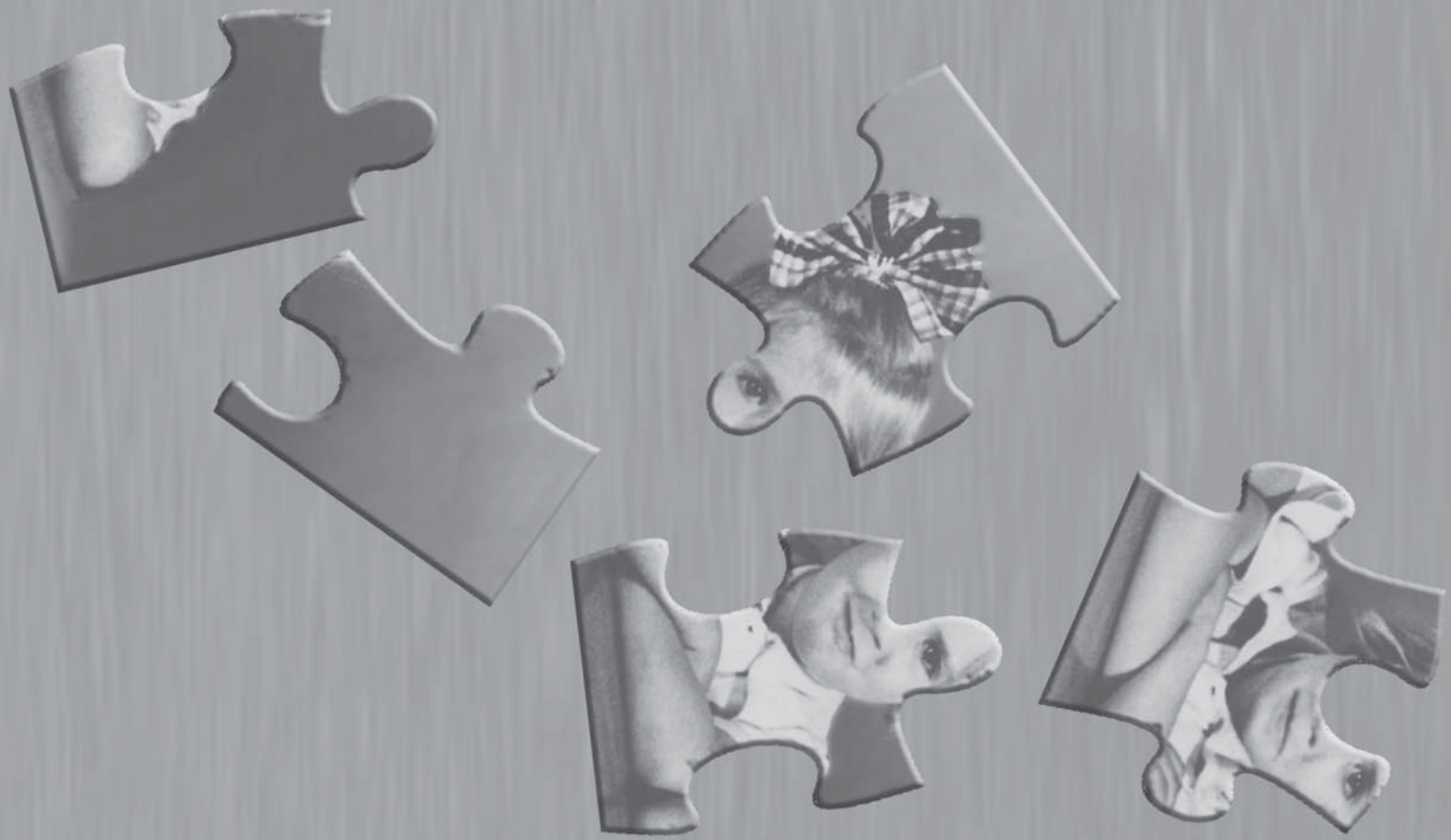
Our study also revealed a significant environmental correlation between bipolar disorder itself and decrease in cortical gray matter volume. A decrease in gray matter in bipolar disorder has been reported earlier (Lim et al 1999, Davis et al 2004, Haznedar et al 2005), but findings have been inconsistent (Kieseppa et al 2003, McDonald et al 2006). In a recent meta-analysis of volumetric studies, a relative preservation of almost all volumes in bipolar disorder was reported; only the right lateral ventricle was found to be enlarged (McDonald et al 2004). Strong heterogeneity for several brain regions, heterogeneous samples, different imaging methods, and medication use were mentioned as possible explanatory factors for the variable results. Indeed, the fact that gray matter decrease in bipolar disorder is an inconsistent finding may be owing to the use of lithium in these patients. Several reports have suggested a neurotrophic and neuroprotective effect of lithium; its use in bipolar patients has been associated with increases in cortical gray matter (Sassi et al 2002, Moore et al 2000) and hippocampal volume (Yucel et al 2007). Interestingly, after chronic administration of lithium a doubling of Bcl-2 (one of the major neuroprotective proteins) levels in cortical layers II and III of the prefrontal cortex was demonstrated in rats (Chen et al 1999). It is these layers that have also been reported to show changes in neuronal and glial cells in post-mortem studies in bipolar disorder. In vivo magnetic spectroscopy studies also support a neurotrophic effect of lithium, finding increases of N-acetyl-aspartate levels, a putative marker for neuronal viability and function, in all brain regions investigated (Moore et al 2000). Furthermore, a striking 0.97 correlation between lithium-induced N-acetyl-aspartate increases and regional voxel gray matter content was observed in bipolar patients after 4 weeks of lithium use (Moore et al 2000). The increases in both Bcl-2 and N-acetyl-aspartate in bipolar disorder have been interpreted as neuroprotective effects of lithium in response to malfunctioning frontal neurons.

Our findings must be viewed in light of several methodological limitations. Genetic models as used in this study cannot determine if these findings were present premorbidly or acquired with the passage of time. Although we realise that in bipolar disorder there is some evidence for progressive brain changes (Koo et al 2008, Nakamura et al 2007, Moorhead et al 2007), this cross-sectional study is not designed to address this question. Also, this twin sample is not a population-based sample but a selected subgroup of bipolar twins and healthy control twins in The Netherlands. Nevertheless, the whole sample of affected twins can be considered representative with probandwise concordance rates for bipolar disorder

of 54% for MZ twins and 26% for DZ twins (McGuffin et al 2003). This study found no significant shared environment contribution to liability to the disorder (McGuffin et al 2003). By constraining the familial environmental effect to zero, it is not possible to estimate a shared environmental correlation. It could be argued that our results may have optimized the genetic correlation. However, the MZ cross-trait-cross-twin correlations were more than twice as large as those for DZ twins in this study, suggesting that shared environmental effects are unlikely to contribute to the phenotypic correlations. Because two-thirds of our bipolar patients used lithium, it can not be ruled out that this is a good compliance sample having structural brain correlates of their own.

Finally, this study only analysed global brain structures in twin pairs with bipolar disorder. Future studies should examine focal brain structures.

In conclusion, we found that white matter volume decrease is related to the genetic risk of developing bipolar disorder, while environmental factors, including the effects of illness, lead to decreased cortical gray matter volume. These findings mirror those reported in studies of schizophrenia and support the notion that the disorders share pathophysiological processes as well as vulnerability genes that are related to deficient myelination or abnormal white matter integrity. Interestingly, lithium greatly attenuated the brain volume changes, suggesting that its use may, in fact, obscure similarities in brain pathology between bipolar disorder and schizophrenia. Our results suggest that focusing on genes controlling white matter integrity and function may be a fruitful strategy in the quest to discover vulnerability genes for bipolar disorder. Elucidating the mechanisms by which lithium attenuates brain matter loss may lead to new drugs with neuroprotective properties



Chapter

3

Genetic and environmental influences on focal brain density in bipolar disorder

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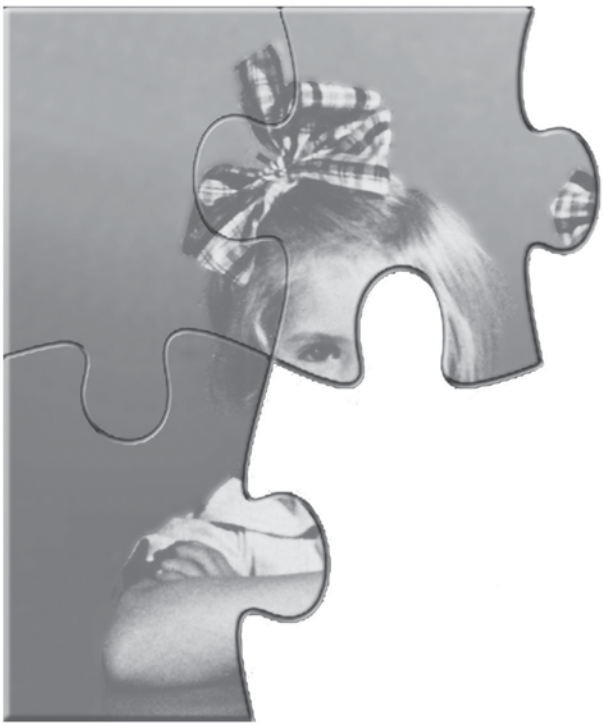


Abstract

Structural neuroimaging studies suggest the presence of subtle abnormalities in the brains of patients with bipolar disorder. The influence of genetic and/or environmental factors on these brain abnormalities is unknown. To investigate the contribution of genetic and environmental factors on grey and white matter brain densities in bipolar disorder, monozygotic and dizygotic twins concordant and discordant for bipolar disorder were scanned using 1.5 Tesla magnetic resonance imaging and compared with healthy twin pairs. A total of 232 subjects: 49 affected twin pairs (8 monozygotic concordant, 15 monozygotic discordant, 4 dizygotic concordant, 22 dizygotic discordant) and 67 healthy twin pairs (39 monozygotic and 28 dizygotic) were included. After correcting for the effect of lithium, the liability for bipolar disorder was associated with decreased grey matter density in widespread areas of the brain, but most prominent in frontal and limbic regions, and with decreased white matter density in (frontal parts of) the superior longitudinal fasciculi. The genetic risk to develop bipolar disorder was related to decreased grey matter density in the right medial frontal gyrus, precentral gyrus and insula and with decreased white matter density in the superior longitudinal fasciculi bilaterally. In conclusion, pathology in the frontal lobe, especially in parts of the superior longitudinal fasciculus, may be central to the genetic risk to develop bipolar disorder, while widespread grey matter abnormalities appear related to the illness itself.

Introduction

Bipolar disorder is a common, severe, chronic and often life-threatening disease with a lifetime prevalence of at least 1% (Regeer et al., 2004). The high heritability (the percentage of phenotypic variance explained by genetic factors) of this illness has been well documented (McGuffin et al., 2003). However, since the concordance rate in monozygotic bipolar twins is only 40–70%, the influence of the environment (subject specificity and/or disease relation) remains an important factor (Craddock et al., 2001).

Although the pathophysiology of bipolar disorder remains poorly understood, findings from structural imaging studies suggest the presence of subtle brain abnormalities in patients with bipolar disorder. These include decreases in cortical volume, cerebral white matter and cortical and particularly prefrontal grey matter (Drevets et al., 1997; Dickstein et al., 2005) compared with healthy subjects (Kempton et al., 2008; Arnone et al., 2009). However, findings have not been consistent; some studies fail to find volume changes, while others report increases in global brain volumes (Kempton et al., 2008). Disease severity, medication, especially lithium use (Moore et al., 2000, 2009; Yucel et al., 2007a,b), and sample heterogeneity might partly explain these discrepancies (Kempton et al., 2008).

Despite the pronounced genetic effects on both bipolar disorder (Craddock et al., 2001) and brain volume (Baare et al., 2001), the question of whether the genetic risk for developing bipolar disorder is associated with some of the reported brain abnormalities in this illness has received limited attention. Recently, in a twin study including 50 affected bipolar and 67 healthy twin pairs, we found an association between decreased total cortical volume and the liability for bipolar disorder, while the genetic risk to develop the disorder was related to decreases in white matter volume. However, it has not been investigated whether the effects of illness and genetic risk are global or whether these effects are restricted to specific areas of the brain. This question can be addressed using voxel-based morphometry, applying voxel- wise comparisons throughout the brain to detect differences in grey or white matter concentration ('density') or volume (optimized voxel-based morphometry) in different groups, providing objective and operator-independent results (Ashburner et al., 2000; Good et al., 2001; Chen et al., 2007). Evidence from previous voxel-based morphometry studies comparing bipolar patients with healthy subjects has revealed a diffuse pattern of focal grey matter decreases as well as increases in

bipolar disorder (Doris et al., 2004; Lochhead et al., 2004; Lyoo et al., 2004; McIntosh et al., 2004; Wilke et al., 2004; Adler et al., 2005, 2007; Dickstein et al., 2005; Farrow et al., 2005; Kubicki et al., 2005; Soares et al., 2005; Bearden et al., 2007; Gogtay et al., 2007; Yatham et al., 2007; Almeida et al., 2009). Focal white matter changes have not been studied extensively. Some studies report no changes in white matter density (McIntosh et al., 2006; Moorhead et al., 2007; Scherk et al., 2008), others find reductions in the anterior limb of the internal capsule (McIntosh et al., 2005) or frontal and temporoparietal regions (Bruno et al., 2004; McDonald et al., 2004).

To date, data on the influence of genes on focal brain abnormalities in bipolar disorder is lacking. Although studies in high risk subjects and in family members of bipolar patients have been conducted, these designs are less well suited to separate the effect of genetic (heritable) and environmental (subject-specific and/or disease-related) factors on brain abnormalities (Martin et al., 1997; Smoller et al., 2003). To disentangle genetic from environmental influences, twin studies are more appropriate (Boomsma et al., 2002). Here we report the genetic and environmental influences on grey and white matter density in bipolar disorder. Since several studies have suggested a neurotrophic or neuroprotective effect of lithium (Moore et al., 2000, 2009; Sassi et al., 2002; Yucel et al., 2007a,b), we controlled for the possible effect of lithium on brain density within the bipolar patients.

Materials and methods

Subjects

This study included 49 twin pairs of whom at least one twin had been diagnosed with bipolar disorder and 67 healthy twin pairs. Global grey and white matter brain volumes of these twins were reported earlier (van der Schot et al., 2009), including detailed description of their demo- graphic and clinical data. Some of the demographic data are presented in Table 1. Briefly, the subjects were between 18 and 60 years of age, had no history of drug or alcohol dependency for the past 6 months and no severe medical illness. Diagnoses were based on the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV; First et al., 1996), and the Structured Interview

Table 1. Demographic Data

	Bipolar Twin pairs (n=50)				Control Twin pairs (n=67)			
	MZ[a] (n=24)		DZ[b] (n=26)		MZ (n=39)		DZ (n=28)	
Female, No.	34		34		46		31	
Mean (SD) age, y	36.9 (10.5)		43.8 (8.5)		39.0 (9.9)		39.0 (7.5)	
Mean(SD) parental education, y	10.9 (3.5)		11.2 (3.8)		11.3 (3.3)		11.5 (3.5)	
First-degree relative, No. (%)								
bipolar disorder	7 (30)		4 (15)					
depression	4 (17)		9 (35)					
mood disorder	10 (44)		13(50)					
	BP patient	**Co-twin**	**BP patient**	**Co-twin**	**Twin 1**	**Twin 2**	**Twin 1**	**Twin 2**
Mean (SD) education, y	12.0 (2.0)	12.2 (2.3)	13.6 (2.6)	12.3 (3.1)	13.5 (2.8)	13.8 (2.7)	13.3 (2.5)	12.6 (2.8)
First born, No. (%)	15 (47)		12 (40)					
Handedness (left/right/both), No.	6/23/3	4/9/1	1/25/4	1/20/1	4/34/1	8/30/1	6/21/1	1/26/1
Mean (SD) onset age, y[c]	26.3 (8.9)		31.3 (9.9)					
Lithium/no lithium on day MRI, No.[d]	26/6		20/10					
Psychotic symptoms, No.	14		18					
Mean (SD) IDS score[e]	6.68 (6.7)	1.7 (2.3)	5.8 (8.3)	2.0 (2.5)	2.14 (2.9)	2.44 (2.7)	2.43 (3.9)	2.92 (2.7)
Mean (SD) YMRS score	1.1 (1.5)	.38 (.71)	.48 (.97)	.14 (.65)	.21 (.57)	.13 (.34)	.29 (.82)	.31 (.75)

Abbreviations: BD, bipolar disorder; DZ, dizygotic; IDS, inventory of depressive symptoms (both groups score below for depressive state); MRI, magnetic resonance imaging, MZ, monozygotic; YMRS, young mania rating scale. [a]Concordant,9; discordant,14. [b]Concordant,4; discordant,22. [c]Age of onset: significant difference between MZ and DZ($F_{1,60} = 4.42$, p=.040). [d]There were six patients in the L^- group that used lithium in the past, five of whom were off lithium for at least 2 years (range 2 – 8 years). One patient used lithium for 13 years and stopped using lithium one month before the MRI. Analyses excluding this patient did not change the results. [e]Significant difference between MZ and DZ ($F_{1,60}$=7.9, p=.01), but both groups below score for depressive state.

for DSM-IV-Personality Disorders (Pfohl et al., 1997). Current mood state was assessed by the Young Mania Rating Scale (Young et al., 1978) and the Inventory for Depressive Symptomatology (Beck et al., 1961). At the time of the study, four patients met criteria for a depressive episode. The other patients were euthymic. Healthy control twin pairs had no history of axis I psychiatric disorder or axis II personality disorder and had no first-degree relative with a history of a major axis I psychiatric disorder (DSM-IV). Family histories of both affected and control twins were obtained via the Family Interview Genetic Studies (Nurnberger et al., 1994) performed with both the proband and cotwin. Zygosity was determined by DNA fingerprinting, using 9–11 high polymorphic microsatellite markers in the laboratory of the Division Biomedical Genetics, University Medical Centre Utrecht. The study was approved by the Medical Ethical Review Board of the University Medical Centre Utrecht, and all participants gave written informed consent after full explanation of the study aims and procedures.

Magnetic resonance imaging acquisition and image analysis

Magnetic resonance images were acquired on a 1.5 Tesla scanner (Philips, the Netherlands). T1-weighted 3D fast field echo scans with 160–180 contiguous coronal slices (echo time = 4.6 ms, repetition time = 30 ms, flip angle = 300 , 1 x 1 x 1.2 mm3 voxels) and T2-weighted dual-echo turbo-spin-echo scans with 120 contiguous coronal slices (echo time1 = 14 ms, echo time2 = 80 ms, repetition time = 6350 ms, flip angle = 900 ,1 x 1 x 1.6 mm3 voxels) were acquired. Quantitative assessments of the intracranial, total brain and grey and white matter of the cerebrum were performed based on histogram analyses and series of mathematical morphological operators to connect all voxels of interest. Grey and white matter segmentation procedures have been validated previously (Schnack et al., 2001).Regional measures of grey and white matter concentration ('density') were generated using voxel-based morphometry in a similar manner as previously described (Hulshoff Pol et al., 2006a,b). Voxel-based morphometry included the following steps: first all individual brain images were registered to a model brain (Hulshof Poll et al., 2006a). The cerebral grey and white matter volumes from this sample (van der Schot et al., 2009) were used to create binary grey matter and white matter masks, which were blurred by a 3D Gaussian kernel [full width at half

maximum (FWHM) = 8 mm], in order to gain statistical power. The voxel values of these blurred grey and white matter segments (between 0 and 1) reflect the local presence, or density, of grey or white matter, respectively. These images are referred to as 'density maps'. To compare brain tissue at the same anatomical location in all subjects, the grey and white matter segments were transformed into a standardized coordinate system (the model space). These transformations were calculated in two steps. First, the T_1-weighted images were linearly transformed to the model brain. In this linear step, a joint entropy mutual information metric was optimized (Maes et al., 1997). In the second step, non- linear (elastic) transformations were calculated to register the linearly transformed images to the model brain up to a scale of 4 mm (FWHM), thus removing global shape differences between the brains, but retaining local differences. For this step the ANIMAL algorithm (Collins et al., 1994) was used. The grey and white matter density maps were now transformed to the model space by applying the concatenated linear and nonlinear transformations. Finally, the maps were resampled to voxels of size $2 \times 2 \times 2.4$ mm^3. Voxels with an average grey matter density below 0.1 were excluded from the grey matter density voxel-based analysis. Similarly, voxels with an average white matter density below 0.1 were excluded from the white matter density voxel-based analysis (Hulshoff Pol et al., 2006a).

Statistical Analysis

Bivariate genetic model fitting

Phenotypic correlations (r_{ph}) between grey or white matter density and the liability for bipolar disorder were calculated with maximum likelihood using the structural equation software package Mx (Neale et al., 2003). To decompose this correlation into genetic and environmental components bivariate genetic model-fitting analyses were performed (Hall et al., 2007; van der Schot et al., 2009), once with and once without correction for lithium. Decomposition was based on the comparison of so-called cross-trait/cross-twin correlations for monozygotic and dizygotic twins. For example, if the cross-correlation between a trait (bipolar disorder) of Twin 1 with another trait of Twin 2 (grey matter/white matter density) is larger in monozygotic twins

than in dizygotic twins, this indicates that a common genetic factor (partly) influences both phenotypes. The extent of the overlap is reflected by the magnitude of the genetic correlation (r_g). Prior to these calculations, the residuals of the brain densities, after regressing out the effects of age, sex and handedness (and lithium use in the second analysis within the bipolar patients) were used to construct a five category ordinal scale. This allowed for a bivariate (bipolar disorder and brain density) ordinal genetic data analysis. For ordinal genetic model fitting, the dichotomous variable 'bipolar disorder' was assumed to represent an underlying continuous liability with mean 0 and variance 1. A person with a high value on the liability scale crossing a certain threshold would be scored 'patient' on our dichotomous variable, and considered to be healthy in all other cases (discordant co-twin of patient or healthy comparison twin-pairs), thus receiving the alternative score. The critical threshold and heritability for the underlying bipolar disorder liability was not based on our sample because we included approximately equal numbers of concordant, discordant and healthy twin pairs. We fixed prevalence and heritability of bipolar disorder to the population values: prevalence was set to 1% (ten Have et al., 2002; Regeer et al., 2004) and heritability was set to 85% (McGuffin et al., 2003). The extent to which genetic and environmental factors explained the variance in brain density or covariance between brain density and liability for bipolar disorder was expressed as percentage of the total (co-)variance, resulting in estimates of h^2 (heritability), e^2 (unique environmentability), bivariate h^2 and bivariate e^2, respectively. The rph-a and rph-e represent the part of the phenotypic correlation that is due to genetic or environmental factors when taking into account both the univariate h^2 and e^2 of each trait and the information from r_g and r_e (Toulopoulou et al., 2007).
No evidence for family-related (common) environmental influences on bipolar disorder have been found previously (McGuffin et al., 2003) and similarly we found no effect of common environment on brain volumes in this sample (van der Schot et al., 2009). Common environmental factors were therefore not implemented in the model Parameters can be removed from the full model to generate submodels (e.g. dropping r_g or r_e). This was tested via likelihood ratio tests.

The likelihood ratio test statistic follows a x^2 distribution. A x^2 larger than 3.84 (1 df, alpha = 0.05, uncorrected for multiple comparisons) indicates that the discarded effect (e.g. r_g: the

effect of genetic factors on the association between brain grey matter density and liability for bipolar disorder) cannot be left out of the model without seriously deteriorating the goodness of fit. Correcting for multiple comparisons, the critical threshold x2 values are x2> 25.3 (df = 1) and x2> 27.7 (df = 2), given the number of subjects, data resolution, voxel size and volume of the search region according to random field theory (Worsley et al., 1996; Ashburner et al., 2000).

Linear regression analysis

The influence of lifetime presence of psychotic symptoms, number of depressive episodes and duration of illness on grey and white matter density in bipolar patients was analysed in a regression analysis. These regression analyses were uncorrected for dependency of the twin data. Age, gender, handedness and lithium use were included as covariates. Correcting for multiple comparisons, the critical threshold t-value is t > 5.5 given the number of subjects, data resolution, voxel size and volume of the search region (random field theory).

Results

The demographic and clinical characteristics of all twin pairs are presented in Table 1. Bipolar patients on lithium (L+, n = 46) were not significantly different from the bipolar patients who did not use lithium (L-, n = 16) on all clinical parameters [except for cur- rent depressive symptoms [F(1,59) = 7.9; P= 0.007)]. Bipolar patients without psychotic symptoms differed from bipolar patients with psychotic symptoms on number of previous depressive episodes {4.2 versus 3.0 episodes [F(1,54) = 6.5; P= 0.013]}. All other clinical variables were not significantly different between the patient groups.

Table 2. Phenotypic correlations between bipolar disorder and gray matter density; genetic and environmental contributions (r_g ,r_e, $h^2_{GMD/BD}$,$e^2_{GMD/BD}$)

Focal brain regions (gyri, peak values) [a]	h^2(95% CI)	BA	R_{ph} chi^2	r_{ph}(95% CI)	r_g(95% CI)	r_e(95% CI)	$h^2_{GMD/BD}$ (95% CI)	$e^2_{GMD/BD}$ (95% CI)
Frontal lobe								
Superior frontal /precentral ,R	30 (1 to 57)	4, 8	**49**	**-0.36** (-0.45 to -0.26)	-0.17 (-0.88 to 0.06)	**-0.85** (-0.99 to -0.49)	24 (0 to 53)	**76** (47 to 100)
Precentral R	71 (56 to 80)	6	**51**	**-0.31** (-0.41 to -0.21)	-0.14 (-0.26 to 0.00)	**-0.98** (-1 to -0.74)	**35** (8 to 55)	**65** (45 to 92)
Medial / dorsolateral R	61 (38 to 75)	9, 46	**44**	**-0.33** (-0.42 to -0.22)	**-0.18** (-0.33 to -0.03)	**-0.81** (-0.97 to -0.47)	**39** (9 to 65)	**60** (35 to 91)
Medial / dorsolateral L	40 (17 to 59)	6, 46	**59**	**-0.25** (-0.36 to -0.15)	0.08 (-0.11 to 0.31)	**-0.98** (-1 to -0.98)	14 (0 to 33)	**86** (67 to 100)
Superior frontal R	32 (6 to 52)	6	**37**	**-0.32** (-0.41 to -0.22)	-0.16 (-0.44 to - 0.06)	**-0.74** (-0.95 to -0.37)	26 (0 to 60)	**74** (40 to 100)
Superior frontal L	65 (46 to 78)	8	**31**	**-0.20** (-0.30 to -0.09)	0.03 (-0.12 to 0.06)	**-0.95** (-0.99 to -0.75)	8 (0 to 36)	**91** (64 to 100)
Inferior frontal R	23 (0.2 to 65)	10,47	**40**	**-0.33** (-0.42 to -0.23)	-0.24 (-1 to 0.00)	**-0.66** (-0.89 to -0.31)	32 (0 to 65)	**67** (35 to 100)
Inferior frontal L	26 (0.9 to 47)	47	**47**	**-0.34** (-0.42 to -0.24)	-0.08 (-1 to 0.19)	**-0.90** (-0.99 to -0.61)	11 (0 to 40)	**89** (60 to 100)
Medial orbital R	38 (16 to 56)	11	**32**	**-0.17** (-0.26 to -0.06)	**0.22** (0.04 to -0.52)	**-0.95** (- 1 to -0.73)	**30** (8 to 44)	**70** (66 to 92)
Orbitofrontal **L**/R	43 (19 to 61)	10	**34**	**-0.29** (-0.38 to -0.18)	-0.10 (-1 to 0.09)	**-0.77** (-0.94 to -0.44)	22 (0 to 54)	**78** (46 to 100)
Cingulate L/**R**[b]	47 (24 to 65)	6,9,32	**33**	**-0.29** (-0.29 to -0.18)	-0.14 (-0.32 to 0.04)	**-0.71** (-0.93 to -0.36)	30 (0 to 62)	**69** (38 to 100)
Temporal lobe								
Inferior/Medial R	34 (9 to 47)	38	**32**	**-0.25** (-0.34 to -0.15)	0.03 (-0.17 to 0.30)	**-0.83** (-0.97 to -0.55)	6 (0 to 30)	**94** (70 to 100)
Inferior temporal L	50 (27 to 66)	38	**43**	**-0.33** (-0.43 to -0.23)	-0.14 (-0.31 to 0.02)	**-0.89** (-0.98 to -0.53)	27 (0 to 55)	**73** (45 to 100)
Superior temporal L	39 (11 to 60)	21	**34**	**-0.23** (-0.33 to -0.13)	0.01 (-0.17 to 0.26)	**-0.80** (-0.95 to -0.50)	3 (0 to 31)	**97** (69 to 100)
Parietal lobe								
Postcentral R	59 (40 to 72)	2	**33**	**-0.26** (-0.36 to -0.17)	-0.04 (-0.20 to 0.11)	**-0.91** (-1 to -0.57)	13 (0 to 46)	**87** (54 to 100)
Postcentral Lc	32 (7 to 52)	40	**36**	**-0.29** (-0.38 to -0.27)	-0.05 (-0.28 to 0.19)	**-0.83** (-0.99 to -0.48)	9 (0 to 45)	**91** (55 to 100)
Inferior parietal R	26 (2 to 49)	7	**33**	**-0.26** (-0.35 to -0.16)	-0.02 (-0.99 to 0.98)	**-0.74** (-0.92 to -0.43)	4 (0 to 43)	**96** (57 to 100)
Angular L	46 (22 to 66)	39	**56**	**-0.25** (-0.35 to -0.15)	0.06 (-0.10 to 0.03)	**-0.99** (-1 to -0.77)	11 (0 to 33)	**89** (67 to 100)
Supramarginal/Intraparietal L	35 (11 to 54)	40	**36**	**-0.30** (-0.40 to -0.20)	-0.08 (-0.31 to 0.14)	**-0.84** (-0.99 to -0.46)	14 (0 to 50)	**86** (50 to 100)
Occipital lobe								
Lingual R	63 (46 to 75)	18	**48**	**-0.28** (-0.37 to -0.24)	-0.08 (-0.21 to 0.04)	**-0.97**(-1 to -0.76)	20 (0 to 45)	**80** (56 to 100)
Lingual L	60 (40 to 73)	18	**27**	**-0.26** (-0.35 to -0.15)	-0.14 (-0.30 to 0.02)	**-0.63** (-0.86 to -0.27)	40 (0 to 73)	**60** (27 to 100)
Parieto-occipital fissure L	53 (32 to 68)	18	**28**	**-0.21** (-0.31 to -0.11)	-0.02 (-0.18 to 0.15)	**-0.75** (-0.92 to -0.45)	7 (0 to 7)	**93** (93 to 100)
Limbic Lobe								
Insula R	55 (34 to 70)	13	**44**	**-0.33** (-0.42 to -0.23)	**-0.17** (-0.32 to - -0.01)	**-0.83** (-0.98 to -0.50)	**35** (3 to 60)	**65** (40 to 97)
Insula L	56 (35 to 71)	13	**52**	**-0.33** (-0.35 to -0.23)	-0.13 (-0.27 to - 0.03)	**-0.96** (-0.99 to -0.71)	26 (0 to 48)	**74** (52 to 100)
Parahippocampal/ Pulvinar R	58 (39 to 71)	27	**36**	**-0.29** (-0.39 to -0.19)	-0.13 (-0.29 to - 0.03)	**-0.78** (-0.96 to -0.46)	32 (0 to 60)	**68** (40 to 100)
(Pre)cuneus R	52 (31 to 68)	7	**37**	**-0.25** (-0.35 to -0.15)	0.01 (-0.13 to - 0.11)	**-0.96** (-1 to -0.71)	3 (0 to 30)	**96** (70 to 100)
Thalamus **R**/L	55 (35 to 69)		**33**	**-0.29** (-0.39 to -0.19)	-0.15 (-0.32 to - 0.01)	**-0.72** (-0.94 to -0.35)	35 (0 to 67)	**64** (33 to 100)

Abbreviations: BA, Brodmann area. BD, bipolar disorder. CI, confidence intervals. $e^2_{BD/GMD}$, bivariate environmentability (unique environmental influence on both bipolar disorder and gray matter density, 1-$h^2_{GMD/BD}$); GM,gray matter. h^2 , heritability (estimated influence of additive genetic influence on gray matter density irrespective of disease). $h^2_{GMD/BD}$, bivariate heritability (common genetic influence on BD and GM density (GMD); L,left; R,right. *re*, environmental correlation; r_g, genetic correlation; r_{ph}, phenotypic correlations. [a]If de peak is lying in a bilateral region the peak (**R**/L (=peak right) is given in bold.
[b] Cingulate gyrus: bilateral region, extending into posterior cingulate gyrus R. [c] parieto-occipital region .
The fixed genetic model for bipolar disorder used 85% for an estimate of the heritability of bipolar disorder., 15% the variance in the underlying liability can be explained by unique

Table 3. Phenotypic correlations between bipolar disorder and white matter density, genetic and environmental contributions (r_g r_e, $h^2_{WMD/BD}$,$e^2_{WMD/BD}$)

Focal brain regions (gyri, peak values)[a]	h^2	R_{ph} chi^2	r_{ph}	r_g	r_e	$h^2_{WMD/BD}$	$e^2_{WMD/BD}$ (=1- $h^2_{GMD/BD}$)
Superior frontal gyrus /Superiorlongitudinal fasciculus (SLF) I* R	22 (0 to 45)	31	**-0.28** (-0.37 to -0.24)	-0.11 (-1 to 1)	**-0.69** (-0.91 to -0.35)	16 (0 to 54)	**84** (46 to 100)
Superior frontal / precentral gyrus/ SLF I/II R	**73** (57 to 84)	48	**-0.33** (-0.43 to -0.23)	**-0.19** (-0.32 to -0.05)	**-0.91** (-1 to -0.60)	**45** (16 to 65)	**55** (35 to 84)
(pre)central gyrus, SLF II ▲ L	18 (0 to 44)	30	**-0.27** (-0.37 to -0.18)	-0.04 (-1 to 1)	**-0.75** (-0.93 to -0.41)	5 (0 to 31)	**95** (69 to 100)
Postcentral gyrus, SLF I▲ L	**49** (28 to 65)	32	**-0.30** (-0.40 to -0.20)	**-0.18** (-0.37 to -0.01)	**-0.66** (-0.89 to -0.29)	**40** (3 to 71)	**60** (29 to 97)
Postcentral gyrus, corticospinal tract L	**54** (34 to 69)	40	**-0.28** (-0.37 to -0.18)	-0.03 (-0.18 to 0.12)	**-0.97** (-1 to -0.72)	8 (0 to 36)	**92** (64 to 100)
Postcentral/ supramarginal gyrus, SLF III▲ L.	11 (0 to 36)	32	**-0.30** (-0.39 to -0.20)	-0.21 (-1 to 1)	**-0.65** (-0.91 to -0.26)	21 (0 to 36)	**79** (64 to 100)
Inferior frontal gyrus L	**60** (41 to 73)	28	**-0.24** (-0.34 to -0.14)	-0.06 (-0.22 to 0.10)	**-0.81** (-1 to -0.44)	18 (0 to 55)	**82** (45 to 100)
Inferior frontal gyrus R	**45** (21 to 63)	29	**0.24** (0.14 to 0.34)	0.02 (-0.17 to 0.20)	**0.79** (0.46 to 0.96)	6 (0 to 44)	**94** (56 to 100)
Superior occipital gyrus /Optic radiation R	24 (0 to 45)	28	**-0.26** (-0.35 to -0.16)	0.04 (-0.23 to 1)	**-0.81** (-0.97 to -0.47)	6 (0 to 29)	**94** (71 to 100)

Abbreviations: BD, bipolar disorder; CI, confidence intervals; $e^2_{BD/WMD}$, unique environmental influence on both bipolar disorder and white matter density; h^2 , heritability (estimated influence of additive genetic influence on white matter density irrespective of disease). $h^2_{WMD/BD}$, bivariate heritability (common genetic influence on BD and white matter density (WMD); L,left; R,right. *r*e, environmental correlation; r_g, genetic correlation; r_{ph}, phenotypic correlations; SLF, superior longitudinal fasciculus,WMD, white matter density.

[a]The WM peaks were overlaid onto post-mortem histological probability maps (Zilles et al 2002) .

▲SLF I: SLF I is located in the white matter of the superior parietal and superior frontal lobes and extends to the dorsal premotor and dorsolateral prefrontal regions. SLF II: occupies the central core of the white matter above the insula. It extends from the cingular gyrus to the caudal-lateral prefrontal regions. SLF III is situated in the white matter of the parietal and frontal opercula and extends from the supramarginal gyrus to the ventral premotor and prefrontal regions (Makris et al 2005, Burgul et al 2006, Schmahmann 2007).

Model fitting results

Phenotypic associations

The associations between liability for bipolar disorder and grey and white matter density (phenotypic correlations, r_{ph}) are presented in Tables 2 and 3, respectively. Bipolar disorder was significantly associated with decreased grey matter density in several areas throughout the brain, including the inferior/medial-dorsolateral/ superior frontal gyri, the anterior cingulate, precentral, inferior temporal gyrus and lingual gyri and several subcortical regions, including the bilateral insula and thalamus [highest peaks in all clusters (r_{ph}), ranging from -0.17 in the right orbitofrontal gyrus up to -0.36 in the right superior precentral gyrus]. These results were attenuated by the effect of lithium, since changes in these areas were significant only after correction for lithium. Without this correction the same effects were found, but with smaller x^2 values (up to 16.4).

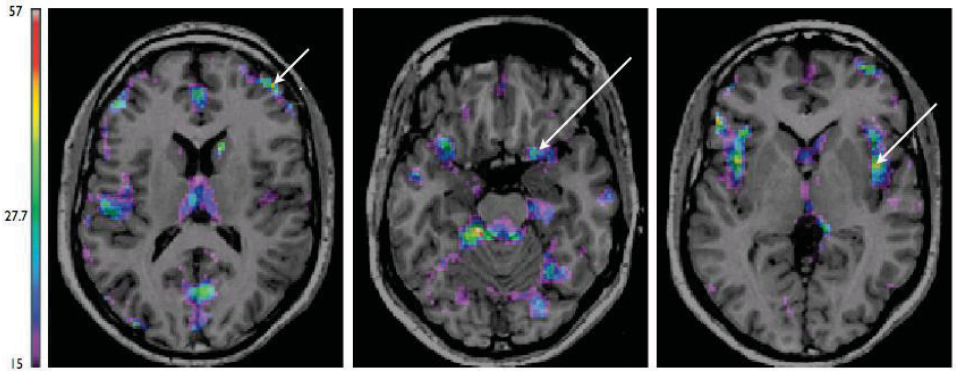
Significant associations between decreased white matter density and bipolar disorder were found in parts of the superior longitudinal fasciculus (I, II and III), the left inferior frontal and postcentral gyrus and the right optic radiation. Increased white matter density associated with bipolar disorder was found in the right inferior frontal gyrus (r_{ph} around 0.24).). All phenotypic correlations were around −0.30.

Genetic influences

Grey matter

As shown in Fig. 1, significant contributions of genetic factors to the association between density and bipolar disorder were found in the right medial frontal gyrus [r_g = −0.18, confidence interval (CI) −0.33 to −0.03] and the right insula (r_g = −0.17 CI −0.32 to −0.01). In the right orbitofrontal gyrus a positive genetic correlation was found, indicating that genetic factors simultaneously increase grey matter density and liability for bipolar disorder (r_g = 0.22 CI 0.04–0.52).

Figure 1. Negative phenotypic associations between liability for bipolar disorder and gray matter density (r_{ph}).

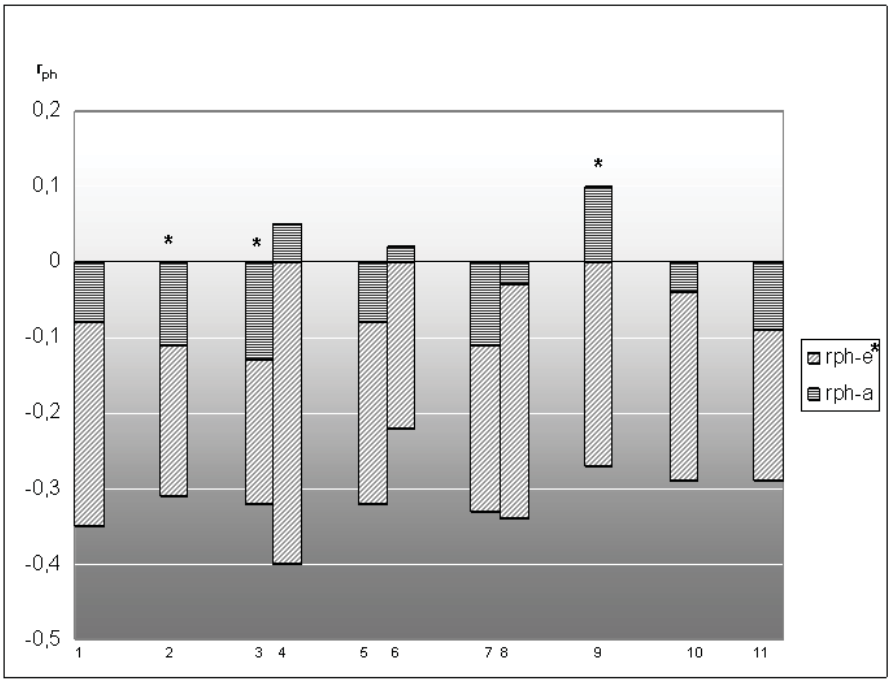


Phenotypic correlations between liability for bipolar disorder and gray matter density. Areas with a significant genetic contribution are indicated by an arrow. For visualisation purposes, $\chi 2$ values in this picture range from 15-57 (significant $\chi 2 > 27.7$)

Left: right medial/dorsolateral prefrontal gyrus. Brodmann area (BA) 9,46. Peakvalue $\chi 2$=44, r_{ph}=-0.33, r_g=-0.18, r_e=-0.81. Middle: right medial orbital gyrus. BA 11. Peak value $\chi 2$=32, r_{ph}=-0.17,r_g=0.22,r_e=-0.95.
Right: right insula, BA 13. Peak value $\chi 2$=44, r_{ph}=-0.33, r_g=-0.17, r_e=-0.83.

Taking into account the univariate heritabilities of both traits(density and bipolar disorder; bivariate h^2), additive genetic factors were estimated to account for 39% (right medial frontal gyrus CI 9–65%), 35% (right precentral gyrus CI 8–55%), 35% (right insula, CI 3–60%) and 30% (right medial orbital gyrus CI 8–44%) of the covariance between bipolar disorder and grey matter density in these regions. Figure 2 shows the part of the phenotypic correlations within the frontal lobe that can be attributed to genetic (rph-a) and unique environmental (rph-e) factors, combining the genetic and environmental correlations and the heritability (h^2) and environmentability (e^2) of both traits (for all regions in grey matter density, see Fig. 1).

Figure 2.



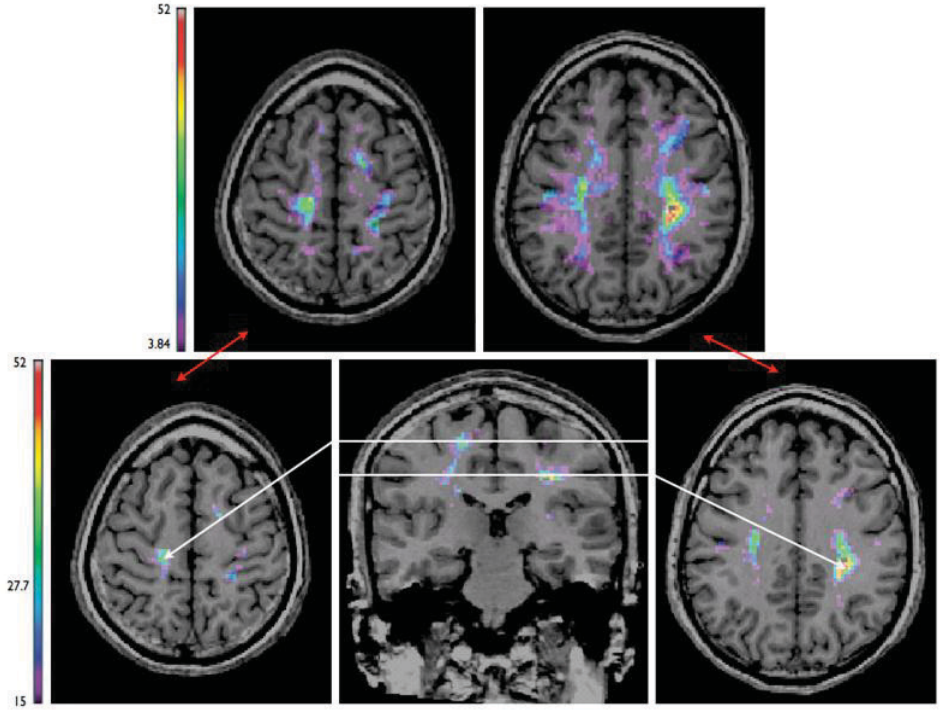
* significant bivariate heritability indicating common genetic influence to both bipolar disorder and decreased or increased gray matter density in frontal lobe regions.
All regions also showed a significant bivariate environmentability (Table1).

1.Superior frontal ,precentral R. 2.Precentral R. 3.Medial/dorsolateral R.
4.Medial/ Dorsolateral L. 5.Superior frontal R. 6.Superior frontal L.
7.Inferior frontal R. 8.Inferior frontal L. 9.Medial Orbital R. 10.Orbitofrontal L/R.
11.Cingulate L/R

White matter

Genetic factors also contributed significantly to the association between white matter density and liability for bipolar disorder in parts of the superior longitudinal fasciculus. As shown in Fig. 3, this was found around the superior frontal/precentral gyrus, part II of the superior longitudinal fasciculus (rg = −0.19) and around the postcentral gyrus, part I of the superior longitudinal fasciculus (rg = −0.18). Bivariate heritabilities showed that 40% (left CI 16–65%) and 45% (right CI 3–71%) of the covariances between decreased

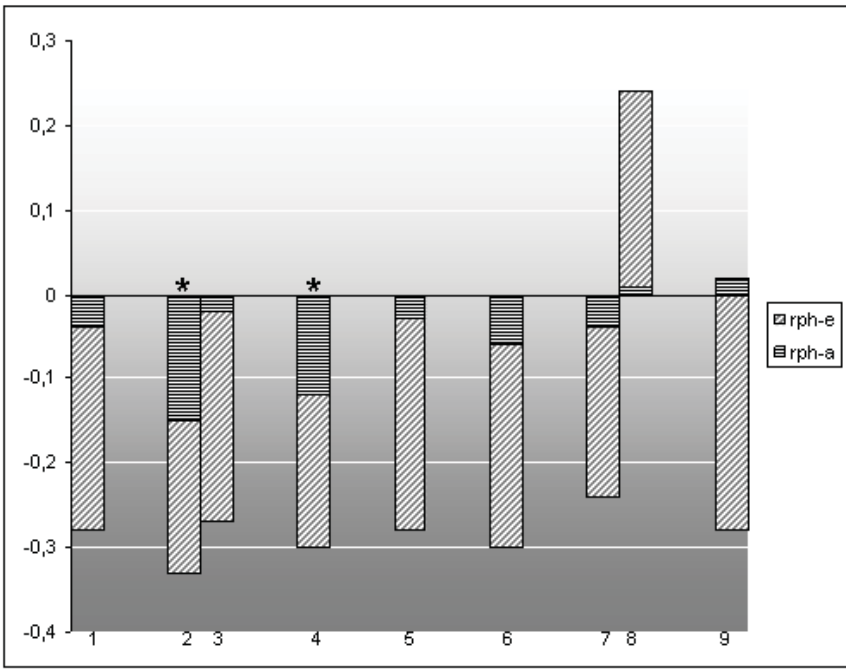
Figure 3. Negative phenotypic correlations between liability for bipolar disorder and white matter density.



The top two pictures show the SLF at uncorrected level ($\chi 2>3.84$). The bottom show the two regions within the SLF where a significant contribution of genetic factors was found.

Left: postcentral gyrus, SLF I. MNI coordinates 51,49,23. peakvalue $\chi 2=32$, $r_{ph}=-0.30$, rg=-0.18, re=-0.66
Right: superior frontal, precentral gyrus, SLF I/II. MNI coordinates 27,51,32, peakvalue $\chi 2=48$, $r_{ph}=-0.33$, $r_g=-0.19$, $r_e=-0.91$.

Figure 4. Phenotypic correlations between bipolar disorder and white matter density, rph-a and rph-e.



Combining the information from the rg and re with the h2 and e2 the phenotypic correlation (rph) can be decomposed into a genetic contribution (rph-a) and an environmental contribution (rph-e). * significant bivariate heritability indicating common genetic influence to both bipolar disorder and decreased or increased white matter density. All regions also showed a significant bivariate environmentability (Table 2).

1. Superior frontal gyrus, superior longitudinal fasciculus (SLF) I, R. 2.Superior frontal gyrus, precentral gyrus, SLF II, R. 3.(pre)central gyrus, SLF II ,L. 4.Postcentral gyrus, SLF I,L. 5. Postcentral gyrus,corticospinal tract,L. 6.Postcentral/supramarginal gyrus, SLF II,L. 7.Inferior frontal gyrus,L. 8.Inferior frontal gyrus,R. 9.Superior occipital gyrus, Optic Radiation,R.

white matter density in these regions and bipolar dis- order were accounted for by common genetic factors. Figure 4 shows the part of the phenotypic correlations in white matter density that can be attributed to genetic (rph-a) and unique environmental (rph-e) factors, combining the genetic and environmental correlations and the heritability (h^2) and environmentability (e^2) of both traits.

Environmental influences

In addition to genetic influences, environmental factors were significant in all grey and white matter regions where an association between these regions and the liability for bipolar disorder was found. The environmental correlations (re) and bivariate are presented in Table 2 for grey matter and in Table 3 for white matter. Environmental correlations ranged from re = -0.63 in the left lingual gyrus (CI -0.86 to -0.27) for grey matter density up to re = -0.97 (CI -1 to -0.6) in the left postcentral gyrus for white matter. This influence was positive for white matter in only the right inferior frontal gyrus [representing increased white matter density in bipolar patients due to environ- mental factors, re = 0.24 (CI 0.14–0.34)].

Regression Analysis

Psychotic symptoms

There were no significant differences in grey and white matter density between patients who had experienced psychotic symptoms at some point during their illness and those without psychotic symptoms according to the significance level of the random field theory.

Number of depressive episodes

There were no significant relationships between the number of depressive episodes and grey or white matter density.

Duration of illness

There was no significant influence of duration of illness on grey and white matter density in bipolar patients. One cluster encompassing the left fusiform, lingual and inferior occipital gyrus in the white matter, showing an increase in white matter, was just below the level of significance (t = 5.0).

Discussion

We examined the relative contributions of genetic and environ- mental influences on focal brain density in bipolar disorder. Grey and white matter density was measured in 116 twin

pairs: 49 with bipolar disorder and 67 healthy control twin pairs. We found that density decreases in widespread areas of grey matter are predominantly associated with unique environmental factors related to bipolar disorder, with most prominent decreases in the frontal areas. In contrast, the brain abnormalities associated with the genetic risk for developing bipolar disorder were much more circumscribed and limited to white matter decreases bilaterally in the superior longitudinal fasciculi and grey matter loss in the right medial frontal gyrus, precentral gyrus and insula. The genetic risk to develop bipolar disorder was also related to increased grey matter density in the right medial orbital gyrus. Our data are the first to suggest that the risk for developing bipolar disorder is related to (white matter) pathology in the frontal lobe, with up to 45% of this relationship explained by common genetic factors. The widespread reductions in grey matter density found here in bipolar patients is consistent with some of the earlier studies comparing bipolar patients with healthy controls [Almeida et al., 2009 (medial prefrontal network); Ha et al., 2009; see Savitz et al., 2009 for a review], although others failed to find differences between patients and controls or even reported increases in volume or density in patients (Bruno et al., 2004; McIntosh et al., 2006; Bearden et al., 2007; Yatham et al., 2007; Kempton et al., 2008; Scherk et al., 2008).

Apart from the effects of lithium, which are large and widespread in the brain, as we and others have demonstrated previously (Moore et al., 2000, 2009; Sassi et al., 2002; Yucel et al., 2007a, b; van der Schot et al., 2009), the reported discrepancies may be due to small sample sizes, type of analysis (region of interest or whole brain) and heterogeneous samples, such as age differences and varying composition of bipolar I and II in the sample (Brooks et al., 2009; Ha et al., 2009; Savitz et al., 2009). Our finding that most prominent grey matter density decreases in bipolar disorder are found in the frontal lobes is both consistent with results from previous studies and with the psychopathology of this illness. First, various studies have reported decreases in several parts of the frontal lobe (dorsolateral (Dickstein et al., 2005; Soares et al., 2005) (paediatric), medial (Lyoo et al., 2004; Janssen et al., 2008), inferior/orbito frontal (Lyoo et al., 2004; McIntosh et al., 2004; Wilke et al., 2004; Almeida et al., 2009), precentral (Lyoo et al., 2004) and anterior cingulate (Doris et al., 2004; Lyoo et al., 2004, McDonald et al., 2004, Haznedar et al., 2005) and widespread frontolimbic regions (Wilke et al., 2004) (cf. Adler et al., 2005, 2007; Bearden et al., 2007)]. In fact, many of the (cognitive) symptoms experienced by

patients with bipolar disorder have been suggested to be associated with dysfunction of the frontal lobe (Drevets et al., 1997; Phillips et al., 2003b; Anderson et al., 2006; Barbas, 2007). Results of our study build on several other lines of evidence such as those from post-mortem studies reporting neuronal size reduction in the inferior/orbito-frontal cortex, reductions in the number, size and density of glial cells (Ongur et al., 1998; Rajkowska, 2002; Uranova et al., 2004; Carter, 2007a) in the subgenual prefrontal and anterior cingulate cortex (Cotter et al., 2005; Kato, 2008) as well as oligodendrocyte density abnormalities in the prefrontal cortex (Uranova et al., 2004).

In contrast to the findings in grey matter, white matter abnormalities related to bipolar disorder were much more circumscribed. Specifically, we found density decreases in the superior longitudinal fasciculus to be related to the liability to develop bipolar illness. The superior longitudinal fasciculus is a large white matter tract consisting of multiple bundles of axons that connect parietal, occipital and temporal regions with regions of the frontal cortex (Makris et al., 2005). This network has been suggested to play a key role in regulating attention and language, motor behaviour and somatosensory information (Stuss et al., 2002). Abnormalities in these tracts may therefore be related to some of the attentional and cognitive deficits found in bipolar disorder (Martinez-Ara´n et al., 2004; Goldberg et al., 2009; Wingo et al., 2009). The white matter decreases in the (frontal part) of the superior longitudinal fasciculus dovetail with the density decreases in grey matter in various areas of the frontal lobe that we found to be related to bipolar illness.

To date, only a few voxel-based morphometry studies have focused on white matter in bipolar disorder. Bruno et al. (2004) reported a significant bilateral reduction in white matter density in prefrontal areas encompassing frontostriatal connections. Others reported abnormalities in parts of the brainstem, prefrontal, temporal and parietal lobes that overlap with the long white matter tracts of the superior longitudinal fasciculus and occipitofrontal fasciculus bilaterally, as well as anterior and posterior parts of the corpus callosum (McDonald et al., 2005). Both studies are consistent with our finding of white matter density reductions in the frontal lobe involving parts of the superior longitudinal fasciculus. Other studies reported reductions in the anterior limb of the internal capsule (McIntosh et al., 2005) or failed to find changes in white matter density (Moorhead et al.,

2007; Scherk et al., 2008). Several diffusion tensor imaging studies also provide evidence for abnormalities in white matter in frontal regions (McIntosh et al., 2005, 2008; Chaddock et al., 2009). Finally the corpus callosum, the largest white matter tract, was found to be mainly related to disease expression in two independent studies (Walterfang et al., 2009a, b).

We found the genetic risk to develop bipolar disorder to be mainly associated with circumscribed abnormalities in the frontal lobe. Specifically we found density decreases in the superior longitudinal fasciculus, in addition to its association with the liability to develop the disorder itself, to also be related to the increased genetic risk to develop bipolar disorder. This finding is consistent with some of the cognitive (Zalla et al., 2004; Bora et al., 2008) and emotional (Kruger et al., 2006) abnormalities related to frontal lobe dysfunction found in relatives of bipolar patients. Interestingly, a study in patients with bipolar disorder (all with psychotic symptoms) and schizophrenia, and unaffected relatives, reported white matter decreases in tracts that connect the left prefrontal and temporoparietal cortices in relation with an increased familiar loading for both disorders (McDonald et al., 2004; Chaddock et al., 2009). Finally, our data are in agreement with a diffusion tensor imaging study including both children with bipolar disorder and children at risk for developing the illness (defined as having a first-degree relative with the disorder) showing reduced fractional anisotropy bilaterally in the superior frontal tracts, including the superior longitudinal fasciculus, in the subjects at increased risk (Frazier et al., 2007). The finding that white matter changes are related to the genetic risk to develop bipolar disorder is supported by several genetic association studies showing a role for oligodendrocyte- and myelin-related genes in the risk to develop this illness (Tkachev et al., 2003; Carter, 2007b; Sokolov, 2007).

Apart from white matter tracts connecting to frontal areas, the genetic risk for developing bipolar disorder was also related to decreases in grey matter density in the medial frontal gyrus and right anterior insula and increases in the right medial orbital gyrus. These results are consistent with those of McDonald et al. (2004), who found decreased grey matter in the right medial frontal gyrus in families with bipolar disorder (including both patients and relatives). However, others did not find evidence for genetic liability to be related to frontal areas (McIntosh et al., 2004, 2006; Kempton et al., 2009) in family-based studies.

Interestingly, the insula plays a key role in the perception and regulation of emotions (Phillips et al., 2003a,b), processes disturbed in bipolar disorder. Indeed, decreased volume in the right insula has been reported previously in patients with a history of both bipolar disorder and schizophrenia (McIntosh et al., 2004), although increased volume of the left insula was associated with the genetic predisposition to develop bipolar disorder but not with the disease itself (Kempton et al., 2009). Furthermore, another study, using PET in bipolar patients and their unaffected relatives, found increases in regional blood flow in the insula and medial frontal cortex (Kruger et al., 2006).

Although genetic factors play an important aetiological role in bipolar disorder, the importance of environmental variables should not be discounted (Savitz et al., 2009). In both grey and white matter density a predominant influence of unique environmental factors was found, most likely reflecting disease-related factors. There are a number of environmental factors that are associated with bipolar disorder, such as stressful life events, virus infections and disruptions in the day–night cycle (Hillegers et al., 2004; McClung, 2007; Beyer et al., 2008; Harvey, 2008; Vieta et al., 2009). Specific pathways, through which such remote risk- conferring and potentially causative factors can exert their influence on brain structure in bipolar disorder, are unknown (Glahn et al., 2008). One example is the relation of stressful life events that can influence the brain indirectly through changes in e.g. the hypothalamic–pituitary–adrenal axis (Feder et al., 2009; Pruessner et al., 2010). Another example is underlying shared, possibly genetic, factors that influence both environment and outcome, also known as gene–environment correlation. This might be alcohol dependence or drug abuse in relation to genetic liability and brain density (Regier et al., 1990; Kendler et al., 1998; Tsuang et al., 2004; Barnett et al., 2009).

Finally, evidence is emerging that non-inherited changes in DNA, also called de novo copy number variations (Bruder et al., 2008), might lead to bipolar disorder (Zhang et al., 2009). In addition, epigenetic factors (e.g. DNA methylation) may influence the emergence of the disease (Machin, 2009). Both of these effects will be modelled in the E (unique, subject-specific) component, because they lead to variability within subjects but not to resemblance between family members. It is therefore important to realize that A (additive genetic) in our models refers to inherited genetic influences, and E to subject-specific influences that mostly, but not exclusively, are environmental.

Our findings must be viewed in light of several methodological limitations. Voxel-based morphometry analysis on anatomical MRIs does not allow for white matter tract tracing, such as is possible with diffusion tensor images. However, the majority of the white matter voxels that we found to be associated with bipolar disorder did overlap with and follow the anatomical pathway of the superior longitudinal fasciculus, as obtained by its superpositioning onto post-mortem histological probability maps (Zilles, 2002; Burgel et al., 2006).

Another issue is that the correlation between bipolar disorder and brain densities can be explained in three ways: (i) bipolar disorder itself induces the decreased (or increased) density in grey and/or white matter; (ii) the changed density causes bipolar disorder; and (iii) there could be a separate underlying factor that influences bipolar disorder as well as brain density independently from each other. Genetic models, as used in this study, cannot answer this question and cannot determine if these findings were present premorbidly or acquired with the passage of time. Although some evidence for progressive brain changes has been reported (Moorhead et al., 2007; Koo et al., 2008), others failed to find age-related reduction in grey matter volume in bipolar disorder (Sarnicola et al., 2009). Also, this twin sample is not a population-based sample but a selected subgroup of bipolar twins and healthy control twins in the Netherlands. Nevertheless, the whole sample of affected twins can be considered representative, with proband-wise concordance rates for bipolar disorder of 54% for monozygotic twins and 26% for dizygotic twins (McGuffin et al., 2003). This study did not model possible shared environment contribution to liability to the disorder. By constraining the familial environmental effect to zero, it is not possible to estimate a shared environmental correlation. However, given the high heritability of both traits, it is not likely that their association is due to shared environmental factors.

In conclusion, we found bipolar disorder to be associated with widespread decreases in grey matter density, most prominent in frontal and limbic regions, while white matter abnormalities were mostly limited to decreases in density in the superior longitudinal fasciculi. Density loss in the latter was also related to the increased genetic risk to develop the illness, as were grey matter density decreases in small areas of the frontal lobe. Our data therefore suggest that (white matter) pathology in the frontal lobe may be central to the genetic risk to develop bipolar disorder, while most of the widespread grey matter abnormalities may be related to environmental effects and the illness itself.

Studying the (genetic and environmental influences on the) development of the frontal lobe, including its connections with limbic areas may be a fruitful strategy to shed light on the pathogenesis of this illness.

Funding

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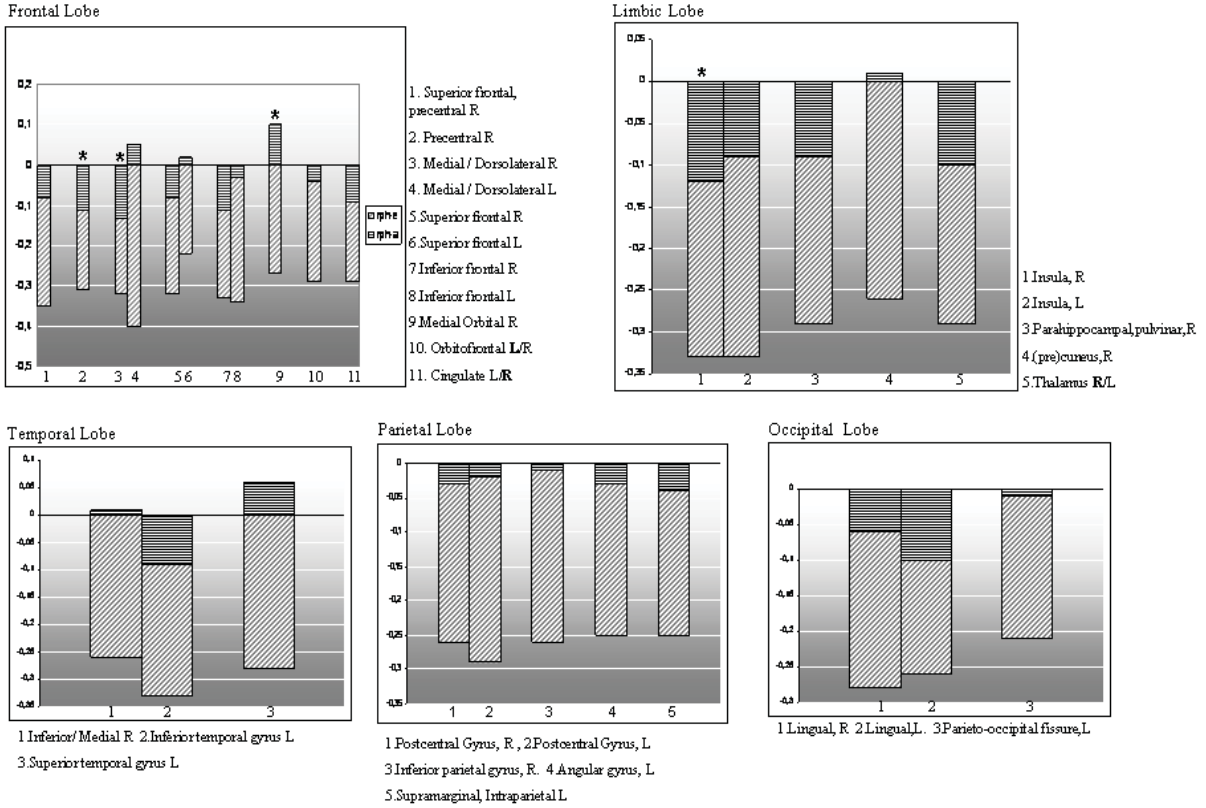
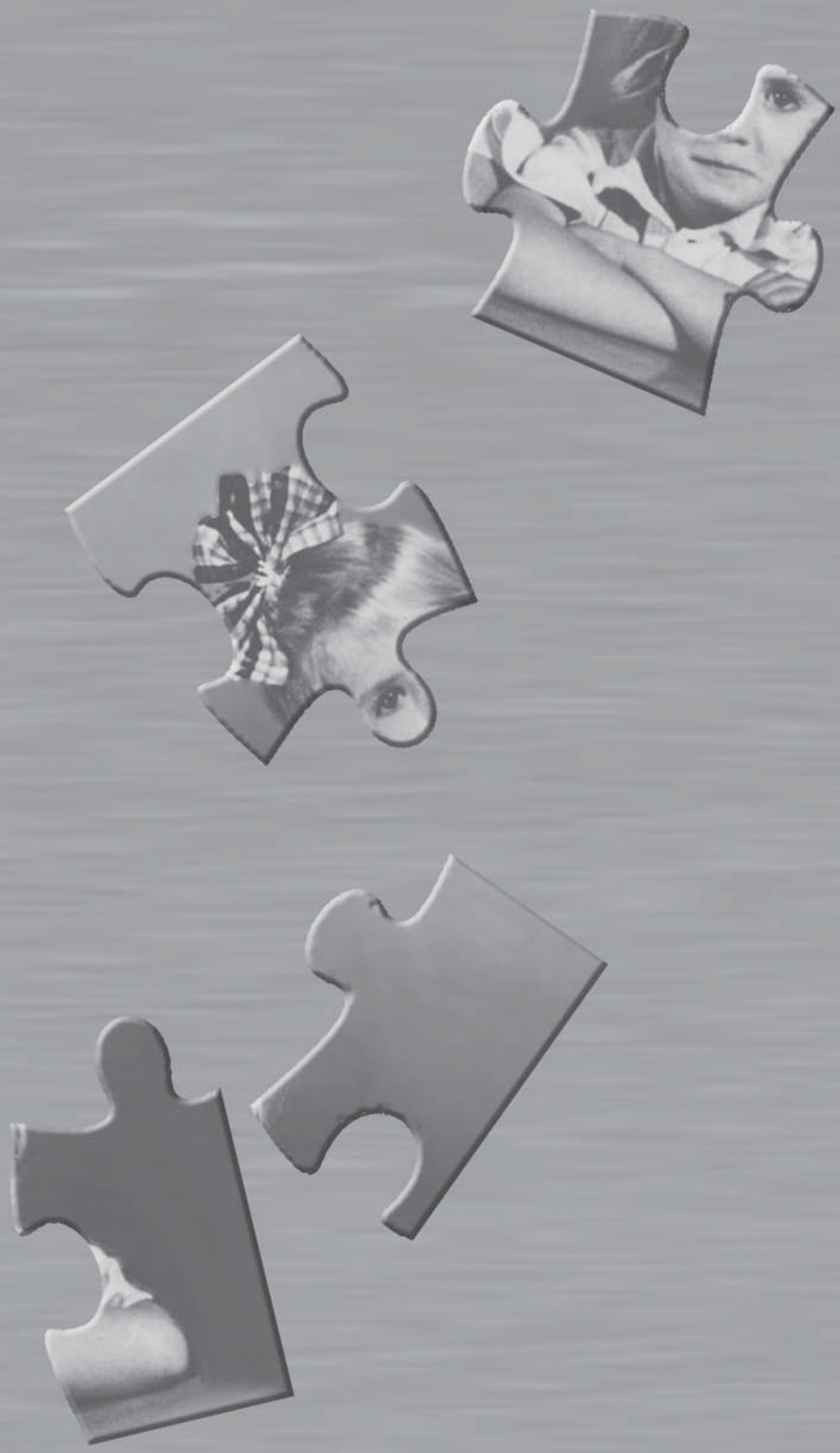
eFigure 1:
Phenotypic correlations between bipolar disorder and gray matter density in focal regions within all lobes, rph-a and rph-e.



eFigure 1. Combining the information from the rg and re with the h2 and e2 the phenotypic correlation (rph) can be decomposed into a genetic contribution (rph-a) and an environmental contribution (rph-e). * significant bivariate heritability indicating common genetic influence to both bipolar disorder and decreased or increased ***gray*** matter density. All regions also showed a significant bivariate environmentability (**Table 2**)



Chapter

4

Dermatoglyphics in relation to brain volumes in twins concordant and discordant for bipolar disorder

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Abstract

Background: Palmar and finger dermatoglyphics are formed between the 10th and the 17th weeks of gestation and their morphology can be influenced by genetic or environmental factors, interfering with normal intrauterine development. As both the skin and the brain develop from the same embryonal ectoderm, dermatoglyphic alterations may be informative for early abnormal neurodevelopmental processes in the brain.

Objective: We investigated whether dermatoglyphic alterations are related to structural brain abnormalities in bipolar disorder and to what extent they are of a genetic and of an environmental origin.

Methods: Dermatoglyphics and volumetric data from structural MRI were obtained in 53 twin pairs concordant or discordant for bipolar disorder and 51 healthy matched control twin pairs. Structural equation modeling was used.

Results: Bipolar disorder was significantly positively associated with palmar a–b ridge count (ABRC), indicating higher ABRC in bipolar patients (rph=.17 (CI .04–.30)). Common genes appear to be involved because the genetic correlation with ABRC was significant (rph-A=.21 (CI .05-.36). Irrespective of disease, ABRC showed a genetically mediated association with brain volume, indicated by a significant genetic correlation rph-A of respectively -.36 (CI -.52 to -.22) for total brain, -.34 (CI -.51 to -.16) total cortical volume, -.27 (CI -.43 to -.08) cortical gray matter and -.23 (CI -.41 to -.04) cortical white matter.

Conclusion: In conclusion, a genetically determined abnormal development of the foetal ectoderm between the 10th and 15th week of gestation appears related to smaller brain volumes in (subjects at risk for) bipolar disorder.

Introduction

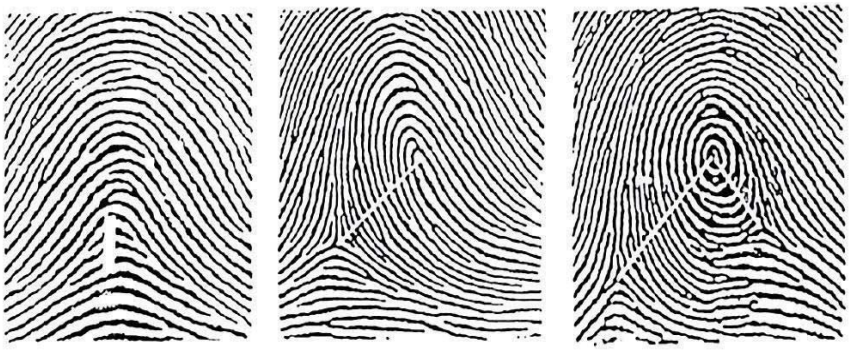
Bipolar disorder is a complex illness in which the core feature is a pathological disturbance in mood ranging from episodes with extreme elation or irritability to severe depressive episodes. In many patients mood episodes can also be accompanied by abnormalities in thinking and behavior, which may include psychotic symptoms. Typically, it is an episodic illness, with (almost) complete recovery of mood and psychotic symptoms in-between episodes (Craddock and Jones, 1999).

Family and twin studies in bipolar disorder have established the importance of genetic factors in its etiology, with heritability estimates in the range of 60–85% (Craddock and Jones, 2001; Smoller and Finn, 2003; McGuffin et al., 2003). Recently, Lichtenstein et al. (2009) estimated heritability to be 59% and influences of common environment 3.4% in a sample of over 40,000 first-degree relatives of patients with bipolar disorder. However, the exact size of the independent and combined effects of relevant risk factors is not known (Craddock and Jones, 2001).

Bipolar disorder is characterized by changes in brain structure (Elkis et al., 1995; McDonald et al., 2004; van der Schot et al., 2009), such as decreases in cortical volume, cerebral white matter, cortical and prefrontal gray matter – particularly in the subgenual and dorsolateral prefrontal cortex – and increased ventricular volumes. These brain changes are present once the illness is established (Elkis et al., 1995; McDonald et al., 2004; van der Schot et al., 2009; Vita et al., 2009) but it is unknown when these brain changes develop since prospective studies prior to illness onset (in high risk subjects) are lacking. However, we (van der Schot et al., 2009) and others (Kieseppa et al., 2003) have shown that some of these brain abnormalities, such as loss of white matter, are related to the genetic risk to develop the illness, whereas gray matter loss appears due to environmental, possibly illness-related, effects (van der Schot et al., 2009). But, again, it is unclear when these brain changes occur, although some suggest that the abnormal brain growth may occur very early in the course of the subject's development, possibly even in utero (Vita et al., 2009). Indeed, it has been suggested that intra-uterine development may be affected in bipolar disorder. This has been mainly been argued on the basis of abnormalities in the development of palmar and finger dermatoglyphics (Jelovac et al., 1999; Chakraborty et al., 2001). Palmar and finger dermatoglyphics are formed early during intrauterine life between the 10th and the 17th weeks of gestation (Babler, 1991; van Oel et al., 2001; Fatjó-Vilas et al., 2008). Once their

formation is complete, dermatoglyphics remain unchanged over the lifetime, except for an increase in size related to general growth (Fatjó-Vilas et al., 2008). Their morphology can be influenced by genetic or environmental factors interfering with normal intrauterine development, such as maternal exposure to rubella, cytomegalovirus, or alcohol (Schaumann and Alter, 1976; van Os et al., 1997). Both skin and brain develop from the same embryonal ectoderm. Interestingly, massive neural cell migration takes place in the brain at the same time as dermatoglyphics are established. Thus, dermatoglyphics are considered fossilized evidence of a specific period of prenatal neurodevelopment (Fatjó-Vilas et al., 2008) and are informative for early abnormal developmental processes to later psychiatric illness such as schizophrenia or bipolar disorder (Van Os et al., 1997).

The most commonly reported dermatoglyphic markers investigated in psychiatric disorders are pattern frequency, ridge simplification (a decreased number of total finger ridge count (TFRC) or palmar a–b ridge count (ABRC)), directional asymmetry (differences between right and left, consistently in the same direction) and fluctuating asymmetry (random differences between right and left (FA)) (Figures 1 and 2) (Gutiérrez et al., 1998, van Oel et al., 2001).



Arch (No Triradius)
Finger Ridge Count 0

Loop (One Triradius)
Finger Ridge Count 13

Whorl (Two Triradii)
Finger Ridge Count 17 (on left)
8 (on right)

Figure 1: Types of finger patterns

Where tree ridge systems meet, they form a triradius. The straight lines crossing the ridges in the loop and in the whorl are the lines used to determine the finger ridge counts (modified from Holt 1968).

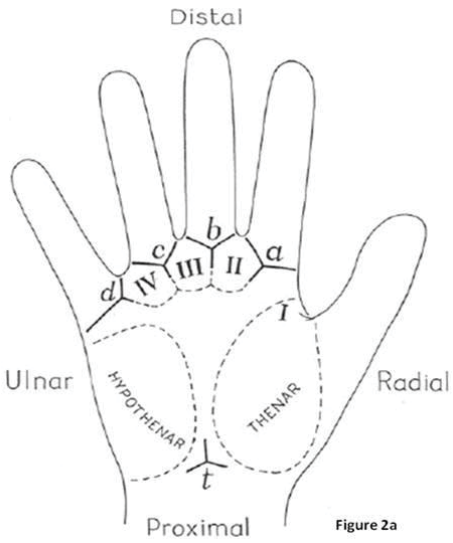


Figure 2a

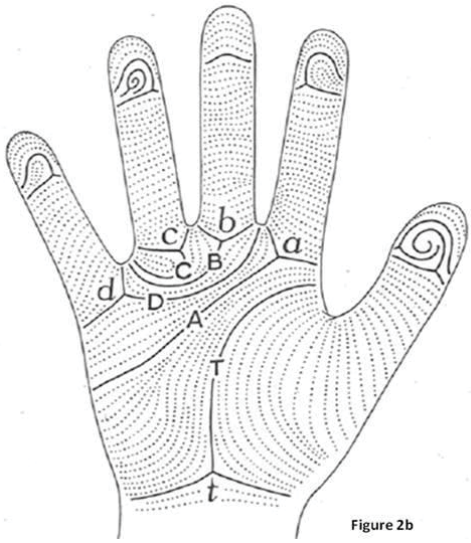


Figure 2b

Figure 2: Example of configuration of the digital and palmar dermatoglyphics

In Figure 2a the dermatoglyphic palmar areas can be seen. In the second palmar area (II), the straight line (Figure 2b) crossing the ridges between triradius *a* and *b* is the line used for determining the *a*-*b* ridge count.

In Figure 2b the finger patterns are represented; the thumb presents a whorl, the index a radial loop, the middle finger an arch, the ring finger a whorl and the little finger an ulnar loop (modified from Holt 1968).

Studies in patients with bipolar disorder have revealed inconsistent results (summarized in Table 1). Some reported different frequencies in finger patterns (Srinivasa Murthy et al., 1974, Balgir, 1982, Chakraborty et al. 2001), lower TFRC (Balgir, 1982, Jelovac et al., 1999) and ABRC (Balgir, 1982, Jelovac et al., 1999) or more fluctuating asymmetry (Yousefi-Nooraie and Mortaz-Hedjri, 2008) in patients with bipolar disorder compared with controls, while others failed to find differences (Markow and Wandler, 1986; Gutiérrez et al., 1998; Saha et al., 2003). The lack of consistency may be due to the differences in sample characteristics (i.e. different diagnostic DSM criteria), methodology (i.e. small groups with lack of power) or analytical techniques (i.e. differentmethods of FA measure) (Saha et al., 2003).

The current study was designed to examine whether dermatoglyphic alterations in bipolar disorder are related to the brain changes that we have shown earlier in this sample (van der Schot et al., 2009). Moreover, we were interested whether these changes reflect a time-linked (i.e. between the 10th and the 17th weeks of gestation) genetic or environmental marker for an abnormal neurodevelopmental origin of bipolar disorder.

Table 1. Dermatoglyphic studies in bipolar disorder

Study	Classification	Sample size (P/C)	Population	Findings
[a] Srinivasa Murthy et al., 1974	Slater and Roth (1969)	35 / 50	India	MDP significantly less arches than controls .
[b] Balgir et al., 1980	DSM-II	120 / 240	India	MDP significantly less arches and loops than schizophrenia MDP slightly higher TFRC than schizophrenia
[c] Balgir et al., 1982	DSM-II	175 / 200	India	MDP (unipolar and bipolar) significantly less arches and more loops than controls MDP (unipolar and bipolar) significantly less TFRC and ABRC than controls
[d] Markow et al, 1986	DSM-III	49 / 69	USA	No differences in ABRC, fluctuating asymmetry of ABRC and fluctuating asymmetry of fingertip pattern between AP and controls
[e] Gutierrez et al., 1998	DSM-III-R	118 / 216	Spain	No differences in TFRC and ABRC. Bipolar disorder significant more ridge dissociation (RD) and abnormal features (AF) than controls
[f] Jelovac et al., 1999	DSM-III-R	92 / 200	Croatia	Bipolar Disorder significant less TFRC and ABRC than controls
[g] Chakraborty et al., 2001	DSM-IV	75 / 102	Malaysia	Bipolar Disorder significant more radial loops than controls
[h] Saha et al., 2003	DSM-III-R	59 / 228	Australia	No differences in TFRC, ABRC and fluctuating asymmetry between AP and controls
[i] Yousefi-Nooraie et al., 2007	DSM-IV	32 / 34	Iran	No differences in TFRC and ABRC. Bipolar disorder significant more fluctuating asymmetry than controls

MDP, Manic Depressive Psychosis (DSM II, 1968) included both unipolar and bipolar patients according criteria Perris (1996)
AP, Affective Psychosis (DSM-III, 1980) included all forms of affective psychoses, but a majority of bipolar disorder
AP, Affective Psychosis (DSM-III-R, 1987) included all forms of affective psychoses (bipolar disorder and mania with psychosis, depression with psychotic features and schizo-affective psychosis)
TFRC, total finger ridge count; ABRC, a-b ridge count

[b] Patients with MDP were compared with patients with schizophrenia .
[f], [i] All patients and controls were males

Experimental procedures

2.1 Subjects

Subjects were twin-pairs, aged 18–60 years, with at least one twin suffering from bipolar I or II disorder according to DSM-IV criteria. Clinical diagnosis for axis I psychiatric disorders was confirmed with the structured clinical interview for DSM-IV (SCID) (First et al., 1996), for axis II personality disorders using the structured inter- view for DSM-IV personality (SIDP) (Pfohl et al., 1997) and for both also using available medical records. The twins had no history of drug or alcohol dependency for the last half year and no severe medical illness, verified by a medical history inventory. Patients were also interviewed on their medication history and their use of medication on the day of the MRI scan.

The 53 twin pairs concordant and discordant for bipolar disorder were recruited via the Dutch Patient's Association for Manic Depressives and Relatives (*n*= 16 twin pairs), via the "Lithium-Plus Working Group", a collaborating group of psychiatrists in The Netherlands with a special interest in bipolar disorder (*n* = 11 twin pairs), via referral by psychiatrists working in several Dutch psychiatric institutes (*n* = 10 twin pairs) and via articles or advertisements in national and regional newspapers (*n* =16 twin pairs).

The bipolar twins were compared to healthy control twins matched on zygosity, gender, age and parental education. The healthy controltwins had no history of axis I psychiatric disorder or axis II personality disorder according to DSM-IV criteria, confirmed with a SCID and SIDP interview respectively and no history of a severe medical illness. Furthermore, they had no first degree relative with a history of a major axis I psychiatric disorder (DSM-IV) such as schizophrenia, psychotic disorder, mood disorder, anxiety disorder or substance related disorder. Family history of both affected and control twins was obtained using the family interview genetic studies (FIGS) (Nurnberger et al., 1994), by interviewing separately and independently the index twin and the cotwin.

Table 2. Lifetime Psychiatric Diagnosis of the Affected Twin pairs (n = 53)

	Index Twin (n = 53)	Cotwin (n = 53)	
		Concordant (n = 13)	Discordant (n = 40)
Diagnosis			
Bipolar I disorder	38	8	
Bipolar II disorder	15	3	
Bipolar disorder NOS		1	
Bipolar disorder NOS and schizophrenia, paranoid type		1	
Major depressive disorder			4
Depressive disorder NOS			4
Schizophrenia, paranoid type			3
Dissociative disorder NOS			1
Comorbid diagnosis			
Depressive disorder NOS			3
Mood disorder due to hyperthyroidism			1
Psychotic disorder NOS	1		
Psychotic disorder due to cannabis	1		
Agoraphobia without history of panic disorder	2		
Panic disorder without agoraphobia			1
Post traumatic stress disorder	1		
Obsessive-compulsive disorder in full remission			1
Alcohol use disorder in full remission	2		1
Cannabis use disorder in full remission	1		
Sedative use disorder	1		
Sedative use disorder in full remission		1	
Anorexia nervosa	1		
Borderline personality disorder	5		1
Obsessive-compulsive personality disorder	1		
Dependant personality disorder	1		
Personality disorder NOS		1	1
No diagnosis			28

Abbreviation: NOS, not otherwise specified

The 51 control twin pairs were recruited from the ongoing twin study on schizophrenia of the UMC Utrecht (*n* = 20 twin pairs), via the Netherlands Twin Register (NTR) in Amsterdam (*n* = 15 twin pairs), via articles or advertisements in national and regional newspapers *n* = 8 twin pairs) and via friends and acquaintances of the researchers (*n* = 8 twin pairs).

A total of 53 affected twin pairs (9 MZ concordant, 15 MZ discordant, 4 DZ concordant and 25 DZ discordant pairs) took part in the study as well as 51 (32 MZ and 19 DZ) healthy control twin pairs. Except for one control twin pair (which was separated at age 12, when both parents died), all twins were reared together.

The study was approved by the Medical Ethical Review Board of the UMC Utrecht and all participants gave written informed consent after full explanation of the study aims and procedures

2.2 Dermatoglyphic assessment

Rolled, inked finger and palm prints obtained by the investigators were scored by 2 experienced dactyloscopists. First pattern type was classified, then the ridges were counted. The raters were blind with respect to the diagnosis and family membership.

2.2.1 Finger ridge pattern

Finger ridge patterns were either classified as an arch, tended arch, ulnar loop, radial loop, whorl or combined figure, using the classification of Henry (1937). Total number of arch and whorl patterns were computed for each subject. In addition, pattern asymmetry was assessed by counting pattern matches on their five pairs of homologous fingers.

2.2.2 Finger ridges

Whorls and combined figures do have two triradii each and thus two ridge counts. For the total finger ridge count (TFRC) only the highest ridge count was used and adds up over the ten fingers. The absolute finger ridge count (AFRC) was computed by adding up the number of ridges between all the digital triradii and the center of the finger ridge pattern. The highest ridge count of each finger was used to test for directional asymmetry. Fluctuating

asymmetry was assessed by totalling the absolute differences between the total ridge counts on the five pairs of homologous fingers (DigAsy).

2.2.3 AB ridges

The epidermal ridges between the index and the middle finger were counted on both side and add up for the AB ridge count (ABRC). Left and right AB ridge count were compared for directional asymmetry. Fluctuating asymmetry was assessed by computing the absolute difference between the left and right AB ridge count (AsyAB).

2.3 MRI acquisition and image analysis

Image acquisition and data processing have been described in previous studies from our group (Hulshoff Pol et al., 2004; van Schot et al., 2009). With magnetic resonance imaging (1.5 T) brain scans quantitative assessments of the intracranium, cerebrum (total brain excluding cerebellum and stem), gray and white matter of the cerebrum, lateral and third ventricular volume, and cerebellum were performed based on histogram analyses and series of mathematical morphology operators to connect all voxels-of-interest (Schnack et al., 2001a, 2001b). All images were checked after measurement and corrected manually if necessary. To evaluate regional contributions, these gray- and white-matter segments from the individual images were used to identify gray and white matter for each individual lobe (frontal, parietal, temporal and occipital). A fully automated warping technique was used. This technique uses non- linear transformations to register every brain scan in the study to a model brain. The model brain was selected earlier from 200 brain images of subjects aged between 16 and 70 years (Mandl et al., 1999). Frontal, parietal, temporal, and occipital lobes were manually demarcated on this image. The borders have been described in detail previously (Palmen et al., 2004). In short, the cingulate gyrus and the insula were excluded from all cortical segments. The prefrontal segment excluded the precentral gyrus, although a frontal segment including the precentral gyrus was also defined. The parietal segment was separated from the frontal lobe by the central sulcus. The parietooccipital fissure defined the boundary with the occipital lobe.

The boundary between the temporal and occipital segments was defined using the temporooccipital notch. Cortical volume is defined as the sum of the gray and white matter of

the separate lobes, cortical gray matter as gray matter of all lobes, and lobar white matter as total white matter of all lobes. Brain images were registered to the model brain using the ANIMAL algorithm (Collins et al., 1994) to remove global differences in the sizes and shapes of the individual brains. The inverse of the transformation process registered the manual segmentations of the model brain to all subjects' brain images (van der Schot et al., 2009).

2.4 Statistical analyses

For comparison of the level of education and the family history for mood disorders between various groups an independent samples *T* test or a Chi-square test was used (SPSS 15.0). (Table 3)

Familial influences on dermatoglyphics may be the result of genetic as well as common environmental effects. In order to estimate the influence of genetic and environmental factors on phenotypic variation in dermatoglyphics, data from groups of individuals who are genetically related is needed. One of the most powerful designs to detect genetic and shared environmental effects is the classical twin design (Martin et al., 1997; Boomsma et al., 2002). A first step towards finding genetic effects on dermatoglyphics is to calculate heritabilityof the trait. This was done by decomposing the variance in dermatoglyphic parameters into genetic (A, additive genetic), common environmental (C) and unique environmental (E) variance, based on the fact that identical, monozygotic twins (MZ) share 100% of their segregating genes and fraternal, dizygotic twins (DZ), share 50% of their segregating genes on average. Heritability was defined as the proportion of genetic over total variance of dermatoglyphic parameters and its calculation – in twin studies – is based on the correlation of the dermatoglyphic parameter in twin 1 with that of twin 2 (Falconer and Mackay, 1996).

As a second step we conducted a bivariate genetic model-fitting analysis to separate the expected correlation/covariance between dermatoglyphic parameters and bipolar disorder liability into genetic and environmental components. The extent to which A, C, and E explained the variance in dermatoglyphics or covariance between dermatoglyphics and bipolar disorder, was expressed as the percentage of the total covariance and variance, resulting in estimates of, respectively, univariate and bivariate h^2 (heritability), c^2 (common or shared environmentability) and e^2 (unique environmentability).

Parameters can be removed from the full ACE model. The aim is to find the most restrictive model that most accurately describes the observed data. This can be tested via likelihood ratio tests (LRT). The LRT statistic follows a chi-square contribution. A Chi- square larger than 3.84 (1 df) indicates a significant difference at alpha=.05, and indicates that the discarded effect cannot be left out of the model without seriously deteriorating the goodness of fit. The first likelihood ratio test for dermatoglyphics was whether the influence of C on dermatoglyphics could be discarded from the model (see results). For the best fitting AE model bivariate heritabilities (the percentage of covariance between bipolar disorder and dermatoglyphics that is accounted for by a common genetic factor $h^2_{DE/BD} = cov_A / cov_A + cov_E$, where DE is dermatoglyphics, BD is bipolar disorder, and cov is covariance) and genetic and environmental correlations between bipolar disorder and dermatoglyphics were obtained. From the estimates of the best fitting model, the "phenotypic correlation due to genetic factors" was calculated: the correlation between dermatoglyphic para- meters and bipolar disorder liability which is exclusively accounted for by genetic factors.

We adjusted the bivariate twin models on selected samples used before in the same set of twins (Van der Schot et al., 2009; Padmos et al., 2009). Bipolar disease was regarded as a dichotomous index for an underlying bipolar liability. Because our sample was selected on bipolar disease, it was not possible to estimate prevalence and heritability, and therefore these were constrained to be the same as reported in the literature (h^2 = 59%, c^2 = 3%, e^2 = 38%, prevalence= 1%) (Lichtenstein et al., 2009; Padmos et al., 2009).

As third step we conducted with the same methodology of a bivariate analysis genetic model-fitting to separate the expected correlation between dermatoglyphic parameters and brain volumes into genetic and environmental components. In this way we tested multiple correlations between dermatoglyphic parameters and brain volumes. As this study was considered as an explorative study, we did no multiple testing corrections.

Results

The diagnostic characteristics of the bipolar twin pairs are presented in Table 2. Forty-six bipolar patients (index twins and concordant cotwins) met DSM-IV criteria for bipolar I, and 18 for bipolar II disorder. Bipolar disorder Not Otherwise Specified (NOS) was diagnosed in 2 bipolar cotwins, one of them also suffering from schizophrenia, paranoid type. Thirty-six bipolar patients (55%)

also had psychotic symptoms. Fifty-four bipolar patients had no lifetime comorbid diagnosis and in 12 bipolar patients one or more comorbid diagnoses was present. Of the (discordant) non-bipolar cotwins (*n*=40), 8 were diagnosed with another mood disorder (4 major depressive disorder and 4 depressive disorder NOS), 3 with schizophrenia, paranoid type(all with a comorbid depressive disorder NOS) and 1 with a dissociative disorder NOS and a borderline personality disorder. Twenty-eight cotwins were healthy with no lifetime psychiatric diagnosis. The demographic and clinical characteristics of all twin pairs are presented in Table 3. Lithium use on the day of the MRI scan could be ascertained most reliably and was quantified as on/off on that day and implemented as such (Table 3).

Table 3. Demographic Data of the Affected Twin Pairs and Control Twin Pairs

	Affected Twin Pairs (n=53)		Control Twin Pairs (n=51)	
	MZ [a] (n=24)	DZ [b] (n=29)	MZ (n=32)	DZ (n=19)
Female, N (%)	34 (71)	38 (66)	40 (63)	25 (66)
Mean age, yrs (SD)	37.8 (10.6)	44.3 (8.5)	40.3 (11.5)	42.0 (7.4)
Mean education father, yrs, (SD)	10.6 (3.8)	10.6 (4.4)	10.6 (3.8)	9.7 (4.2)
Mean education mother, yrs (SD)	9.7 (3.2)	9.0 (2.5)	8.8 (2.7)	9.9 (3.0)
Mean education, yrs (SD)	12.4 (2.0)	13.3 (2.6)	13.6 (2.7)	13.0 (2.4)
First-degree relative, N (%)				
bipolar disorder	8 (33)	5 (17)		
depression	4 (17)	11 (38)		
mood disorder	11 (46)	15 (52)		

	Bipolar patients (n=66)	Non-bipolar cotwins (n=40)	Control twins (n=102)
Female, N (%)	45 (68)	27 (68)	65 (64)
Mean age, yrs (SD)	40.8 (10.1)	42.4 (9.9)	40.9 (10.1)
Mean education, yrs (SD)	13.1 (2.2)	12.5 (2.6)	13.4 (2.6)
Mean age of onset, yrs (SD)	28.3 (9.7) [14-59]		
Psychotic symptoms, N (%)	35 (53%)		

Abbreviations: DZ, dizygotic; MZ, monozygotic [a] Concordant, 9; discordant, 15 [b] Concordant, 4; discordant, 25

3.1 Heritability of dermatoglyphics

Irrespective of bipolar disorder, significant moderate to high heritability's were found for some dermatoglyphics, ranging from 76% for ABRC to 96% for TFRC (Table 4). Unique environmental factors were more important for the variance of fluctuating asymmetry of TFRC (Digasy) and ABRC (AsyAB), respectively 82% and 86%. The influence of common environment was not significant for dermatoglyphics. We therefore report on the AE model for all bivariate analyses.

Table 4. Estimated influences of additive genetic (h^2), common environmental (c^2) and unique environmental (e^2) factors on dermatoglyphics, irrespective of disease

Dermatoglyphics	h^2_{DE} % (95% CI)	c^2_{DE} % (95% CI)	e^2_{DE} % (95% CI)
Whorls	82 (52 to 90)	0 (0 to 27)	18 (10 to 33)
Arches	92 (57 to 97)	0 (0 to 33)	8 (3 to 21)
TFRC	96 (82 to 99)	0 (0 to 14)	4 (1 to 9)
AFRC	91 (74 to 96)	0 (0 to 16)	9 (4 to 20)
ABRC	76 (27 to 91)	8 (0 to 52)	17 (9 to 31)
DigAsy	18 (0 to 48)	0 (0 to 27)	82 (52 to 100)
AsyAB	6 (0 to 43)	8 (0 to 34)	86 (57 to 100)

Abbreviations: DE, dermatoglyphics; CI, confidence interval; TFRC, total finger ridge count; AFRC, absolute finger ridge count; ABRC, a-b ridge count ; DigAsy, fluctuating asymmetry of finger ridge count; AsyAB, fluctuating asymmetry of a-b ridge count

Table 5. Dermatoglyphics of bipolar patients, non-bipolar co-twins and healthy control twins

	Bipolar patients (n=66)	Non-bipolar cotwins (n=40)	Control twins (n=102)
Whorls [a]	2.55 (2.63) (n=65)	2.38 (2.67) (n=40)	2.05 (2.27) (n=102)
Arches [a]	0.58 (1.35) (n=65)	0.55 (1.11) (n=40)	1.07 (1.70) (n=102)
TFRC [a]	138.98 (47.83) (n=63)	132.00 (43.15) (n=36)	126.21 (44.19) (n=98)
AFRC [a]	171.84 (80.28) (n=63)	159.50 (73.63) (n=36)	150.66 (65.14) (n=98)
ABRC [a]	83.17 (10.02) (n=63)	82.71 (11.17) (n=38)	77.95 (8.85) (n=100)
DigAsy [a]	15.95 (8.01) (n=63)	19.28 (8.67) (n=36)	15.39 (7.34) (n=98)
AsyAB [a]	3.59 (3.06) (n=63)	3.08 (3.25) (n=38)	3.24 (2.42) (n=100)

[a] Values are mean (SD)

Abbreviations: TFRC, total finger ridge count; AFRC, absolute finger ridge count; ABRC, a-b ridge count ; DigAsy, fluctuating asymmetry of finger ridge count; AsyAB, fluctuating asymmetry of a-b ridge count

3.2 Association of bipolar disorder and dermatoglyphics

In Table 5 the dermatoglyphics are presented for the bipolar patients, their non-bipolar cotwins and the healthy controls. Bipolar disorder was significantly and positively associated with ABRC, indicating a higher ABRC in bipolar patients (r_{ph} =.17 (.04–.30)) (Table 6). Bipolar disorder did not show significant associations with the other dermatoglyphics. Bipolar disorder showed a genetically mediated association with ABRC (Table 6). Common genes appear to be involved because the genetic correlation with ABRC was significant (r_{ph}-A =.21 (.05–.36). We did not find any significant environmental correlation betweendermatoglyphics and bipolar disorder (Table 6).

Table 6. Correlations and sources of Covariance between Bipolar Disorder and Dermatoglyphics

	Correlation		
Dermatoglyphics	**r_{ph} (95%CI)**	**r_{ph}-A (95%CI)**	**r_{ph}-E (95%CI)**
Whorls	.02 (-.11 to .16)	.01 (-.15 to .18)	.01 (-.12 to .13)
Arches	-.14 (-.28 to .01)	-.09 (-.27 to .09)	-.05 (-.18 to .09)
TFRC	.11 (-.04 to .24)	.09 (-.06 to .24)	.01 (-.05 to .08)
AFRC	.06 (-.07 to .20)	.01 (-.15 to .17)	.05 (-.05 to .15)
ABRC	**.17 (.04 to .30)**	**.21 (.05 to .36)**	-.04 (-.08 to .08)
DigAsy	-.01 (-.14 to .12)	.06 (-.12 to .23)	-.07 (-.26 to .12)
AsyAB	.02 (-.11 to .15)	-.10 (-.26 to .07)	.12 (-.07 to .29)

Abbreviations: CI, confidence intervals; r_{ph}, phenotypic correlation; r_{ph}-A, genetic correlation; r_{ph}-E, environmental correlation; TFRC, total finger ridge count; AFRC, absolute finger ridge count; ABRC, a-b ridge count ; DigAsy, fluctuating asymmetry of finger ridge count; AsyAB, fluctuating asymmetry of a-b ridge count

3.2 Association of ABRC and brain volumes

In Table 7 the raw mean volumes and differences in brain volumes (in percentages) are presented for the bipolar patients, their non-bipolar cotwins and the healthy controls, that we have shown earlier in this sample (van der Schot et al., 2009). Furthermore, the differences between the volumes of the bipolar patients who were taking lithium(L^+; n = 46) and the bipolar patients who did not take lithium (L^-; n=17) are shown in Table 7 (van der Schot et al., 2009).

Table 7. Brain volumes of bipolar patients, their co-twins and healthy comparison subjects (van der Schot et al. 2009).

	Mean (SD) Volume, mL[a]			Increase, %[b]		
	BP HC (n=63)[c]	Co-twins[d] (n=37)	HC (n=134)	BP vs HC	Co-twins[d] vs HC	Difference Between L^- and L^+, %[e]
Intracranium	1420 (174)	1451 (179)	1428 (129)	+1.18	+ 2.62	+ 2.8
Cerebrum	1063 (128)	1089 (129)	1086 (111)	- 1.37	-0.64	**- 3.30** [f]
Gray matter	607 (69)	615 (71)	619 (70)	-1.13	-0.80	- 3.64
White matter	456 (77)	474 (79)	467 (66)	-1.97	-0.78	- 2.77
Lateral ventricle	17.1 (9.7)	16.6 (8.6)	15.2 (7.7)	+ 8.70	+ 4.48	**+ 12.49** [f]
Third ventricle	.89 (.53)	.84 (.41)	.79 (.41)	+ 7.7	- 2.56	**+ 17.18** [f]
Cerebellum	138 (15)	138 (13)	141 (13)	-0.72	-2.17	- 2.32
Cortical Volume[g]	745 (92)	766 (92)	764 (80)	- .47	- 0.54	**- 3.26** [f]
Cortical gray matter[h]	447 (50)	456 (53)	458 (51)	-1.45	- 0.47	- 3.4
Lobar white matter[i]	297 (52)	310 (55)	306 (39)	- 2.44	- 1.25	- 3.05
Gray Matter						
Prefrontal lobe	153 (18)	155 (18)	156 (18)	- 0.89	-0.58	- 2.80
Temporal lobe	133 (14)	136 (16)	136 (14)	- 1.35	-0.08	- 2.92
Parietal lobe	109 (12)	110 (13)	111 (13)	- 1.51	-1.14	- 3.73
Occipital lobe	53 (8)	55 (8)	55 (8)	- 3.07	-0.14	- 5.60
White Matter						
Prefrontal lobe	108 (18)	112 (19)	110 (14)	- 1.16	-0.23	- 3.08
Temporal lobe	66 (13)	69 (14)	68 (10)	- 2.0	-0.97	- 2.52
Parietal lobe	78 (14)	81 (14)	81 (10)	- 3.25	- 2.56	- 4.89
Occipital lobe	45 (9)	48 (10)	46 (7)	- 2.63	-0.04	- 0.54

Abbreviations: BP, patients with bipolar disorder; HC, healthy control subjects (twin and co-twin); L^+, bipolar patients who are taking lithium (n=46); L^-, bipolar patients who did not take lithium (n=17).
[a] Uncorrected for age, sex, or intracranial volume.
[b] Corrected for age, sex, and intracranial volume; based on a mean age of 39.04 years, mean intracranial volume of 1430 mL, and female sex.
[c] Including 26 patients from concordant pairs (9 monozygotic; 4 dizygotic); for one patient, no separation of gray and white matter volume was possible.
[d] Co-twin without bipolar disorder
[e] Difference obtained by subtracting L^+ from L^-.
[f] Values in bold face are significant at α=.05.
[g] Sum of the volumes of the prefrontal, parietal, temporal, and occipital lobes.
[h] Sum of the volumes of the gray matter of separate lobes.
[i] Sum of the volumes of the white matter of separate lobes.

Irrespective of disease, ABRC was negatively associated with total brain (phenotypic correlation rpha = - .29 [CI - .45 to -.13]), total cortical volume (rpha = - .28 [CI - .43 to - .11]), cortical gray matter (rpha = - .24 [CI - .39 to - .07]) and white matter, (rpha = - .24 [CI - .37 to - .03]) (Table 8), indicating a smaller total brain, total cortical volume, cortical gray matter and white matter in twins with higher ABRC.

ABRC showed a genetically mediated association with these brain volumes, indicated by a significant genetic correlation rph-A of respectively -.36 (CI -.52 to -.22) for total brain, -.34 (CI -.51 to -.16) for total cortical volume, -.27 (CI -.43 to -.08) for cortical gray matter and -.23 (CI -.41 to -.04) for white matter (Table 8). We did not find any significant environmental correlation between ABRC and brain volumes (Table 8). We did not find any significant correlation between the other dermatoglyphic parameters and brain volumes.

Table 8. Correlations and sources of Covariance between Brain Volumes and ABRC

			Correlation		
Brain Volume	**h^2_{ABRC} % (95%CI)**	**h^2_{BV} % (95%CI)**	**r_{ph} (95%CI)**	**r_{ph}-A (95%CI)**	**r_{ph}-E (95%CI)**
Total brain	82 (69 to 90)	63 (45 to 77)	-.29* (-.45 to -.13)	-.36* (-.52 to -.22)	.07 (-.03 to .17)
Total cortical volume	81 (68 to 89)	66 (48 to 79)	-.28* (-.43 to -.11)	-.34* (-.51 to -.16)	.06 (-.03 to .17)
Cortical grey matter	82 (69 to 90)	79 (59 to 84)	-.24* (-.39 to -.07)	-.27* (-.43 to -.08)	.03 (-.06 to .13)
Cortical white matter	82 (69 to 90)	62 (41 to 77)	-.20* (-.37 to -.03)	-.23* (-.41 to -.04)	.03 (-.07 to .13)

Abbreviations: ABRC, a-b ridge count ; CI, confidence intervals; BV, brain volume; $h^2_{ABRC,}$ heritability ABRC; r_{ph}, phenotypic correlation; r_{ph}-A, genetic correlation; r_{ph}-E, environmental correlation;

Discussion

This study explored whether dermatoglyphic patterns are related to brain structure in 53 twin pairs with bipolar disorder and 51 healthy control twin pairs and if so whether these are differentially related to (the risk to develop) bipolar disorder.

The first and general finding is a genetically-mediated negative association between palmar a–b ridge count (ABRC) and the volumes of the total brain, cortical gray and white matter irrespective of disease status, indicating that smaller brain volumes and higher ABRC are influenced by common genes. Second, we find that higher ABRC is related to the genetic risk of developing bipolar disorder: at least 70% of the covariance between ABRC and bipolar disorder could be explained by genetic factors influencing both ABRC and (the risk for developing) bipolar disorder. This suggests that genes involved in the etiology of bipolar disorder may contribute to the higher ABRC found in bipolar patients and their cotwins.

Our finding of a genetically mediated (negative) association between ABRC and brain volumes irrespective of disease status supports the view that dermatoglyphics and brain development are (at least in part) regulated by the same sets of genes and that increased ARBC is related to stunted brain development. This has not been reported before. When this result is combined with our finding that higher ABRC is related to the genetic risk of

developing bipolar disorder, our study suggests that cerebral structural abnormalities in bipolar disorder are the result of an early (genetically mediated) pathological process (also) affecting the development of foetal ectodermal structures. Since dermatoglyphics develop between the 10th and 15th week of gestation, we can even be more specific: some of the brain abnormalities related to the genetic risk to develop bipolar disorder (Van der Schot et al., 2009, Kieseppa et al. 2003) are the result of developmental abnormalities originating around the 10th and 15th week of gestation.

The association of higher ABRC with bipolar disorder in this study is not consistent with previous dermatoglyphic studies in bipolar disorder showing lower ABRC in patients with bipolar disorder compared with controls (Balgir, 1982; Jelovac 1999) or reporting no difference in ABRC (Markov and Wandler, 1986; Saha et al., 2003; Gutiérrez et al., 1998; Yousefi-Nooraie and Mortaz-Hedjri, 2008). As far as we know, this is the first study in bipolar disorder that reveals a significantly higher ABRC than controls. It is not clear what the finding of higher ABRC exactly means in the light of the development of the foetal ectoderm. In general, an abnormal development of dermatoglyphics is, on the opposite, defined by "ridge simplification", a decreased number of total finger ridge count (TFRC) or palmar a–b ridge count (ABRC) (Gutiérrez et al., 1998; Fatjó-Vilas et al., 2008). We are not aware of earlier studies with the same finding of an increased number of ridge counts in psychiatric disorders compared to controls.

In addition, this study shows the heritability of the different dermatoglyphic parameters. We found a moderate to high heritability for some dermatoglyphics, ranging from 76% (CI 27–91) for ABRC to 96% (CI 82–99) for TFRC and a low heritability for fluctuating asymmetry of TFRC (Digasy) and ABRC (AsyAB), being 18% (CI 0–48) and 6% (CI 0–43), respectively (Table 4). This study did not find any significant association between bipolar disorder and those dermatoglyphics, which were mediated by (unique) environmental factors (Table 5). This suggests that environmental factors operating during early pregnancy (between 10–17e weeks of gestation) are not related to a higher risk of developing bipolar disorder (Scott et al., 2006).

Comparing the current results with those of our previous twin study in schizophrenia (Van Oel et al., 2001), we found a different neurodevelopmental process between bipolar disorder and schizophrenia. The schizophrenia twin study revealed that non-genetic circumstances early in pregnancy (10–13 weeks of gestation) were associated

with a susceptibility to schizophrenia since both twins with schizophrenia and the unaffected cotwins showed more fluctuating asymmetry of the finger ridges than control twin pairs.

Our findings need to be seen in the context of the strength of this study and several (methodological) limitations. The strength of this study is the fairly large twin sample with the assessment of 53 affected twin pairs (9 monozygotic concordant, 15 monozygotic discordant, 4 dizygotic concordant and 25 dizygotic discordant). However, a first limitation is the low sample number per group. At the start of the study we intended to collect similar numbers of monozygotic and dizygotic concordant and discordant twin pairs per group. Unfortunately we were not able to reach this aim. Second, this twin sample is not a population-based twin sample, but a selected subgroup of bipolar twins and healthy (control) twins in the Netherlands. For instance, more female bipolar twins (68%) and female control twins (71%) than male twins participated in this study. This probably is a result of selection, as in the general population the prevalence of bipolar disorder is the same in men and women. On the other hand, the concordance rates in our sample of 53 affected twins of 55% for MZ twins and 24% for DZ twins is comparable with other concordance rates for bipolar disorder (McGuffin et al., 2003). Also the healthy control twins form a selection, as that they were not only selected for lifetime absence of any psychiatric disorder but also for absence of a family history of any major psychiatric disorder. Third, we tested with a relatively small twin sample multiple correlations between dermatoglyphic parameters and brain volumes. As we did no multiple testing correction, we should consider the results with some caution. Finally, lithium showed considerable effects on the brain changes found in this study, attenuating the decrease in both gray and white matter. As a consequence, findings became more pronounced, but did not change fundamentally, when analyses were corrected for lithium. Because nearly two-thirds of our bipolar patients used lithium, it cannot be ruled out that this is a good compliance sample, having structural brain correlates of their own (van der Schot et al., 2009).

In conclusion, we found that higher ABRC is related to the genetic risk of developing bipolar disorder and is negatively associated with brain volumes total brain, total cortical volume, cortical gray matter and lobar white matter irrespective of disease (or risk) status. Thus, a genetically determined abnormal development of the foetal ectoderm between the 10th and 15th week of gestation appears related to smaller brain volumes in (subjects at risk for) bipolar disorder. This in

turn suggests that some of the brain abnormalities in (subjects at risk for) bipolar disorder may be attributable to a genetically driven early (foetal) disruption in neurodevelopment.

Conflict of interest

There is no conflict of interest related to this work (financial or otherwise).

Contributors

Authors R.Vonk and A.C. van der Schot designed the study and wrote the protocol. Authors G.C.M. van Baal undertook the statistical analysis, and author R.Vonk wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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Funding for this study was provided by the Stanley Medical Research Institute; the Stanley Medical Research Institute had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication

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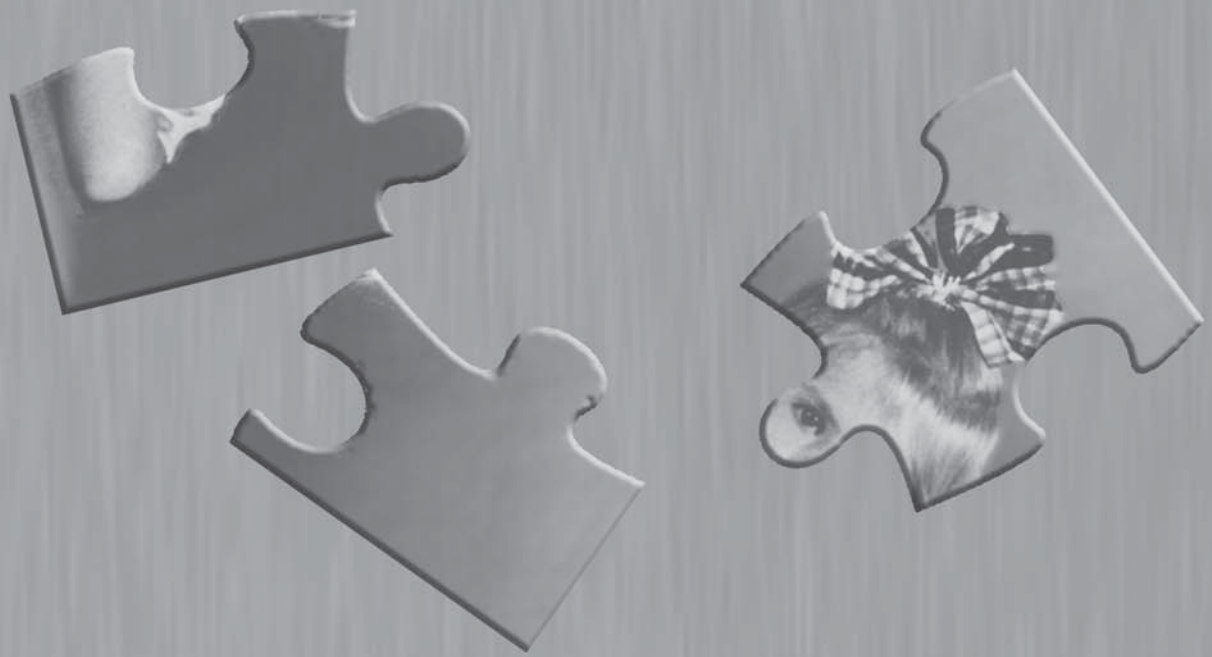
Mr. Vonk takes responsibility for the integrity of the data and the accuracy of the data analysis; all authors had full access to all data in the study.



Chapter

5

Is autoimmune thyroiditis part of the genetic vulnerability (or an endophenotype) for bipolar disorder?

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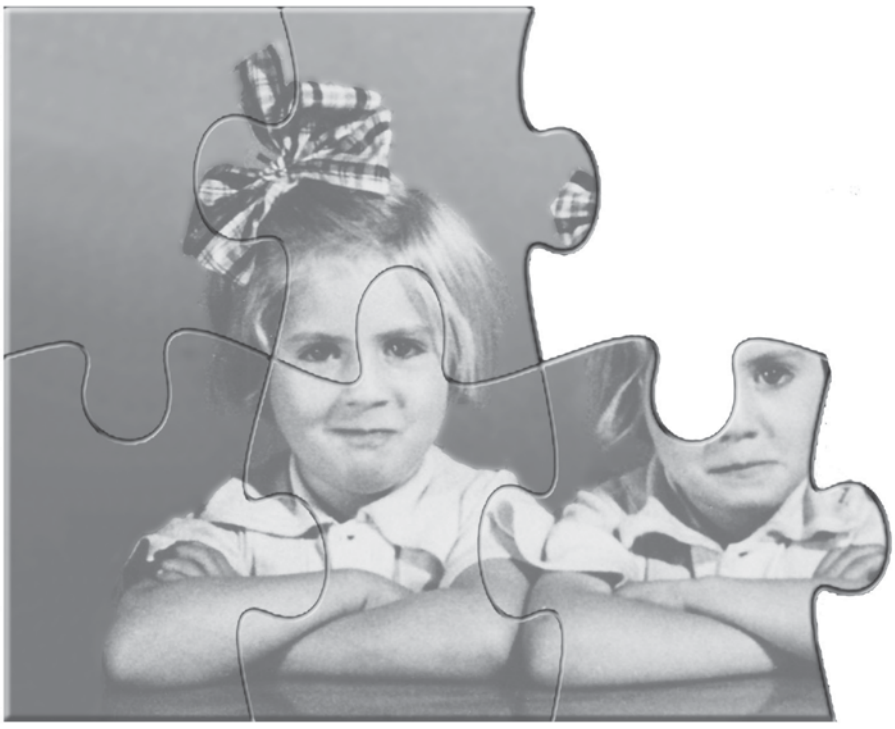

Abstract

Background: Both genetic and environmental factors are involved in the etiology of bipolar disorder; however, biological markers for the transmission of the bipolar genotype ("endophenotypes") have not been found. Autoimmune thyroiditis with raised levels of thyroperoxidase antibodies (TPO-Abs) is related to bipolar disorder and may be such an endophenotype. This study was intended to examine whether autoimmune thyroiditis is related to the disease itself, to the (genetic) vulnerability to develop bipolar disorder, or both.

Method: Blood was collected from 22 monozygotic (MZ) and 29 dizygotic (DZ) bipolar twins and 35 healthy matched control twins to determine TPO-Abs.

Results: The TPO-Abs were positive in 27% of the bipolar index twins, 29% of the monozygotic bipolar cotwins, 27% of the monozygotic nonbipolar cotwins, 25% of the dizygotic bipolar cotwins, 17% of the dizygotic nonbipolar cotwins, and in 16% of the control twins. Repeated measures analysis of covariance on log-transformed absolute TPO-Abs values revealed significantly increased mean TPO-Abs levels in discordant twin pairs as compared with healthy twin pairs, whereas no difference was found between bipolar patients and their (discordant) nonbipolar cotwins.

Conclusions: This study shows that autoimmune thyroiditis is related not only to bipolar disorder itself but also to the genetic vulnerability to develop the disorder. Autoimmune thyroiditis, with TPO-Abs as marker, is a possible endophenotype for bipolar disorder.

Introduction

Bipolar disorder is a complex illness in which the core feature is a pathologic disturbance in mood ranging from extreme elation to severe depression. The illness is usually accompanied by abnormalities in thinking and behavior, which may include psychotic symptoms. Typically, it is an episodic illness, usually with (almost) complete recovery between episodes (Craddock and Jones 1999).

Family and twin studies in bipolar disorder have established the importance of genetic factors in its etiology. Environmental factors are likely to be involved as well, given that the concordance rate for monozygotic (MZ) twins is not 100% but only 40%–70% (Craddock and Jones 2001). The precise interaction between the genetic vulnerability to develop bipolar disorder and environmental factors is not clear, however, nor is the exact size of the independent and combined effects of relevant risk factors known (Craddock and Jones 2001). Thus far, biological markers for the transmission of the bipolar genotype ("endophenotypes") have not been found (Hasler et al. 2006).

Neurophysiologic, biochemical, endocrinologic, neuroanatomic, and cognitive abnormalities often accompany bipolar disorder and may thus be candidates to serve as endophenotypes (Lenox et al. 2002). Five criteria have been described that need to be met for a marker to serve as an endophenotype. The marker 1) is associated with the illness in the population, 2) is heritable, 3) is state-independent (i.e., is manifested in an individual whether or not the illness is active), 4) cosegregates with the illness within families, and 5) is found in nonaffected family members at a higher rate than in the general population (Gershon and Goldin 1986; Gottesman and Gould 2003; Hasler et al. 2006; Leboyer et al. 1998; Lenox et al. 2002).

Such a marker for bipolar disorder could be autoimmune thyroiditis with raised levels of thyroperoxidase antibodies (TPO- Abs). The association of autoimmune thyroiditis, using TPO-Abs as a marker, with bipolar disorder in the population, was demonstrated in a previous study of our group among a largesample (n = 226) of outpatients with bipolar disorder. We found an elevated prevalence of TPO-Abs (28%) compared with a control group from the general population (n = 252) and

compared with psychiatric in patients with any diagnosis (*n* 3190; 3%–18%; Kupka *et al.* 2002).

Interestingly, TPO-Abs is a heritable marker, making it a candidate to serve as an endophenotype. Indeed, family studies have repeatedly shown aggregation of TPO-Abs in healthy first-degree relatives of patients with autoimmune thyroiditis (Brix *et al.* 2004). Moreover, a twin study of discordant twin pairs with autoimmune thyroiditis showed a higher prevalence of thyroid autoantibodies in the healthy monozygotic cotwins than in the healthy dizygotic cotwins (Brix *et al.* 2004). These findings suggest that familial aggregation of thyroid autoantibodies is mainly genetically determined (Brix *et al.* 2004). The prevalence of TPO-Abs in male relatives is less than that of female relatives, and this pattern has been attributed to a dominant inheritance with reduced penetrance in males (Phillips *et al.* 1990).

Moreover, there were no differences in TPO-Abs between bipolar patients in various mood states, including euthymia. The presence of TPO-Abs is more likely a trait marker of bipolar disorder than a state marker of an episode of the disorder (Kupka *et al.* 2002).

Whether autoimmune thyroiditis and bipolar disorder cosegregate within families and whether autoimmune thyroiditis is found in nonbipolar relatives at a higher rate than in the general population is unknown. If so, TPO-Abs would serve as an appropriate endophenotype. To explore these questions we examined the prevalence of autoimmune thyroiditis in an ongoing twin study in bipolar disorder. The primary aim of this study was to investigate relevant risk factors for bipolar disorder in relation to genotype and environment. Autoimmune thyroiditis was considered as a presumed risk factor in this study. The study was performed to elucidate whether autoimmune thyroiditis with raised levels of TPO-Abs was related to the disorder itself, the (genetic) vulnerability to develop bipolar disorder, or both, in which case autoimmune thyroiditis could be a possible endophenotype for bipolar disorder.

Methods and Materials

Subjects

The subjects were twin pairs, aged 18 to 60 years, with at least one twin suffering from bipolar I or bipolar II disorder according to DSM-IV criteria. Clinical diagnosis for Axis I psychiatric disorders was confirmed via the Structured Clinical Interview for DSM-IV (SCID), for Axis II personality disorders via the Structured Interview For DSM-IV Personality (SIDP) and for both also via available medical records. The twins had no history of drug or alcohol dependency for the last half year and no severe medical illness, verified with a medical history inventory. Current mood state was assessed via the Young Mania Rating Scale (YMRS) and the Inventory for Depressive Symptomatology (IDS). Euthymia was defined as a YMRS score of < 4 and IDS score < 12. In addition, current and previous use of medication was assessed. The bipolar twins were recruited via the Dutch Patient's Association for Manic Depressives and Relatives (*n* 16 twin pairs), via the Lithium-Plus Working Group, a collaborating group of psychiatrists in The Netherlands with a special interest in bipolar disorder (*n* 10 twin pairs), via referral by psychiatrists working in several Dutch psychiatric institutes (*n* 9 twin pairs), and via articles or advertisements in national and regional newspapers (*n* = 16 twin pairs).

The bipolar twins were compared with healthy control twins matched on zygosity, gender, and age. The healthy control twins had no history of Axis I psychiatric disorder or Axis II personality disorder according to DSM-IV criteria, confirmed with an SCID and SIDP interview respectively and no history of a severe medical illness. Furthermore, they had no first-degree relative with a history of a major Axis I psychiatric disorder (DSM-IV) such as schizophrenia, psychotic disorder, mood disorder, anxiety disorder, or substance-related disorder. Family history of both affected and control twins was obtained via the Family Interview Genetic Studies interview, performed with both the index twin and cotwin.

The control twins were recruited from the ongoing twin study on schizophrenia of the University Medical Centre (UMC) Utrecht (*n* 5 twin pairs), via The Netherlands Twin Register in Amsterdam (*n* 15 twin pairs), via articles or advertisements in

national and regional newspapers (*n* 8 twin pairs), and via friends and acquaintances of the researchers (*n* 7 twin pairs). The study included 51 affected twin pairs (7 MZ concordant, 15 MZ discordant, 4 DZ concordant, and 25 DZ discordant pairs) and 35 (19 MZ and 16 DZ) healthy control twin pairs. Except for one control twin pair (which was separated at age 12 when both parents died), all twins were reared together.

The study was approved by the Medical Ethical Review Board of the UMC Utrecht, and all participants gave written informed consent after full explanation of the study aims and procedures.

Biological Measurements

Zygosity was determined by DNA fingerprinting, using 9-11 high polymorphic microsatellite markers in the laboratory of the Division Biomedical Genetics, UMC Utrecht. Serum samples were frozen, stored, and later assessed at one laboratory (Department of Immunology, Erasmus University, Rotterdam, The Netherlands) for TPO-Abs, which were measured with an en- zyme-linked immunosorbent assay (ELISA; Immulite, DPC, Breda, The Netherlands). We considered autoimmune thyroiditis present when TPO-Abs were positive at a level of 25 U/mL or higher, using the cutoff level for positivity as indicated by the manufacturer. The threshold for TPO-Abs of this assay was .05 U/mL. All assays were carried out blind to the subject's medical status.

Statistical Analysis

Statistical analyses were performed with use of a standard statistical software package (SPSS 11.0). For comparison of the demographic characteristics between various groups an Independent Samples *t* test or a chi-square test was determined. The prevalence of autoimmune thyroiditis (TPO-Abs positive > 25 U/mL) between various groups was analyzed as a dichotomized variable (positive/negative) with a chi-square test. The absolute TPO-Abs values had a nonnormal distribution, and this variable was successfully normalized using a logarithmic transformation. The TPO-Abs values and variables (age and gender)

were pairwise analysed using repeated measures analysis of covariance (ANCOVA) with Twin (bipolar patient vs. cotwin, control twin 1 vs. control twin 2) as the within-subjects variable, Group (discordant, healthy) and Zyg (monozygotic, dizygotic) as the between subjects variables. The following main and interaction effects were tested: Zyg, Group, Twin, Group X Zyg, Twin X Group, Twin X Zyg, and Zyg X Twin X Group (Hulshoff-Pol *et al.* 2004).
The TPO-Abs values were corrected for age and gender because autoimmune thyroiditis occurs more in women than in men and increases with age (Hornig *et al.* 1999; Rapoport and McLachlan 1996).

Results

The diagnostic characteristics of the bipolar twin pairs are presented in Table 1. Of the bipolar patients (index twins and concordant cotwins), 46 met DSM-IV criteria for bipolar I, and 14 for bipolar II disorder. Bipolar disorder, not otherwise specified, (NOS) was diagnosed in two bipolar cotwins, one ofwhom also suffered from schizophrenia, paranoid type. There was no lifetime comorbid diagnosis in 50 of the bipolar patients, and 12 bipolar patients had one or more comorbid diagnoses.
Of the (discordant) nonbipolar cotwins (n = 40), 8 were diagnosed with another mood disorder (4 major depressive disorder and 4 depressive disorder NOS); 3 with schizophrenia, paranoid type (all with a comorbid depressive disorder NOS); and 1 female cotwin with a dissociative disorder NOS and a borderline personality disorder. Twenty-eight cotwins were healthy with no lifetime psychiatric diagnosis.
At the time of the study, all patients were euthymic (YMRS < 4, IDS < 12), except for four patients, who met criteria for a depressive episode (IDS scores 15, 20, 29, and 38, respectively).
Demographic characteristics of all twins are summarized in Table 2. Groups did not differ significantly from each other on age (p .92), educational level of the father (p .37) and the mother (p .87), nor on years of education (p .08).

Table 1 Lifetime psychiatric diagnosis of the affected twin pairs (n = 51)

	Index Twin (n = 51)	Cotwin (n = 51)	
		Concordant (n = 11)	Discordant (n = 40)
Diagnosis			
Bipolar I disorder	38	8	
Bipolar II disorder	13	1	
Bipolar disorder NOS		1	
Bipolar disorder NOS and schizophrenia, paranoid type		1	
Major depressive disorder			4
Depressive disorder NOS			4
Schizophrenia, paranoid type			3
Dissociative disorder NOS			1
Comorbid diagnosis			
Depressive disorder NOS			3
Mood disorder due to hyperthyroidism			1
Psychotic disorder NOS	1		
Psychotic disorder due to cannabis	1		
Agoraphobia without history of panic disorder	2		
Panic disorder without agoraphobia			1
Post traumatic stress disorder	1		
Obsessive-compulsive disorder in full remission			1
Alcohol use disorder in full remission	2		1
Cannabis use disorder in full remission	1		
Sedative use disorder	1		
Sedative use disorder in full remission		1	
Anorexia nervosa	1		
Borderline personality disorder	5		1
Obsessive-compulsive personality disorder	1		
Dependant personality disorder	1		
Personality disorder NOS		1	1
No diagnosis			28

The prevalence of autoimmune thyroiditis (i.e., TPO-Abs positive > 25 U/mL) in bipolar patients was 27% compared with 16% in the healthy control twins (Figure 1). The prevalence of autoimmune thyroiditis in the monozygotic (discordant) nonbipolar cotwins (27%) was higher than in the dizygotic (discordant) nonbipolar cotwins (17%) and the control twins (16%). Using a chi-square analysis on dichotomized TPO-Abs values (positive–negative), these differences were not significant. However, using a repeated-measures ANCOVA on log-transformed absolute TPO-Abs values of the MZ and DZ discordant twin pairs and the MZ and DZ healthy control twin pairs, a significant main effect for Group was found

Table 2 Demographic characteristics

	Bipolar Twin Pairs			Control Twin Pairs		
	Total (n=51)	MZ (n=22)	DZ (n=29)	Total (n=35)	MZ (n=19)	DZ (n=16)
Female gender (in %)	68 (67)	30 (68)	38 (66)	55 (79)	32 (84)	23 (72)
Age (years) [a]	41.3 (10.0)	37.3 (10.6)	44.3 (8.5)	41.2 (9.3)	39.1 (10.5)	43.6 (7.0)
Education (years) [a]	12.8 (2.4)	12.2 (2.0)	13.3 (2.6)	13.5 (2.5)	14.0 (2.5)	12.9 (2.4)
Education father (years) [a]	10.6 (4.1)	10.6 (3.7)	10.6 (4.4)	10.0 (4.1)	10.1 (3.9)	9.9 (4.4)
Education mother (years) [a]	9.2 (2.8)	9.4 (3.2)	9.0 (2.5)	9.2 (2.9)	8.4 (2.4)	10.2 (3.2)
Fdr [b] bipolar disorder	12 (24%)	7 (32%)	5 (18%)			
Fdr [b] depression	14 (28%)	3 (14%)[#]	11 (37.9%)[#]			
Fdr [b] mood disorder	25 (49%)	10 (46%)	15 (51.7%)			

[a] Values are mean (SD) [b]Fdr = First degree relative

	Bipolar patients				
	Total (n=62)	MZ (n=29)	DZ (n=33)	Index twin (n=51)	Cotwin (n=11)
Female gender (in %)	41 (66)	19 (66)	22 (67)	35 (69)	6 (55)
Age (years)[a]	40.6 (10.1)	36.5 (10.4)	44.2 (8.4)	41.3 (10.1)	37.5 (10.1)
Age of onset (years) [a]	28.6 (9.6)	26.5 (9.0)	30.4 (10.0)	28.9 (10.0)	27.1 (8.2)
Lithium use current	46 (74.2%)	23 (79.3%)	23 (69.7%)	37 (72.5%)	9 (81.8%)
- Mean dosis of lithium (mg/day)[a]	953 (339)	915 (410)	991(254)	937 (326)	1022 (406)
Lithium use ever	52 (83.8%)	26 (89.7%)	26 (78.8%)	43 (84%)	9 (81.8%)
- Duration of lithium use (years) [a]	5.8 (5.6)	5.3 (5.8)	6.4 (5.6)	5.6 (5.3)	7.2 (7.1)
Anticonvulsant use current	17 (27.4%)	9 (31.0%)	8 (24.2%)	15 (29.4%)	2 (18.2%)
Anticonvulsant use ever	24 (38.7%)	10 (34.5%)	14 (42.4%)	22 (43.1%)	2 (18.2%)
- Duration of anticonvulsant use (years) [a]	3.5 (3.4)	4.4 (3.1)	2.9 (3.6)	3.1 (3.3)	8.0 (.0)

[a] Values are mean (SD)

because the discordant bipolar twin pairs having higher mean TPO-Abs levels than the control twin pairs (F 4.08, $p < 0.05$; Figure 2). This was particularly prominent for monozygotic twin pairs. No significant main effect was found for Twin, indicating that bipolar patients and their cotwins did not differ from each other. There was no main effect for Zyg or interaction effects for Twin and Zyg. The absolute TPO-Abs values (using a logarithmic transformation) of the bipolar patients, the (discordant) nonbipolar cotwins, and the control twins are presented in Figure 3.

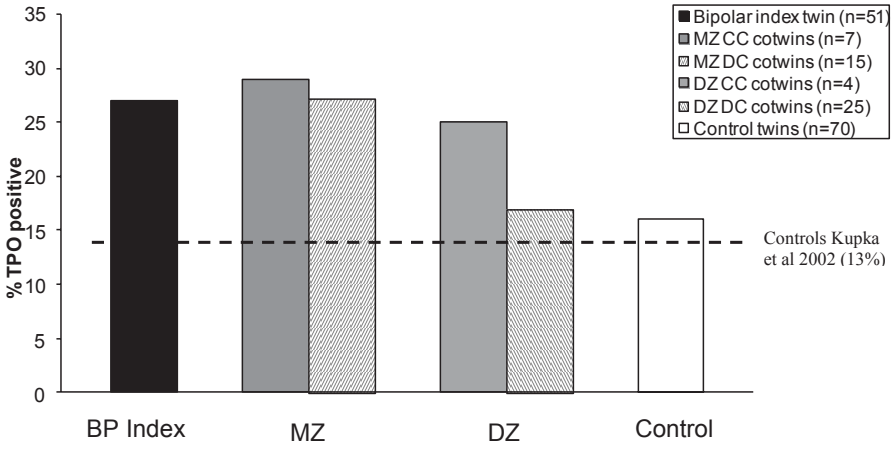

Figure 1 : Prevalence of autoimmune thyroiditis (TPO antibodies positive ≥ 25 U/ml) in bipolar index twins, (bipolar) cotwins and control twins.

BP index= bipolar index twin, MZ= monozygotic, DZ=dizygotic, CC=concordant, DC=discordant, Control=control twin

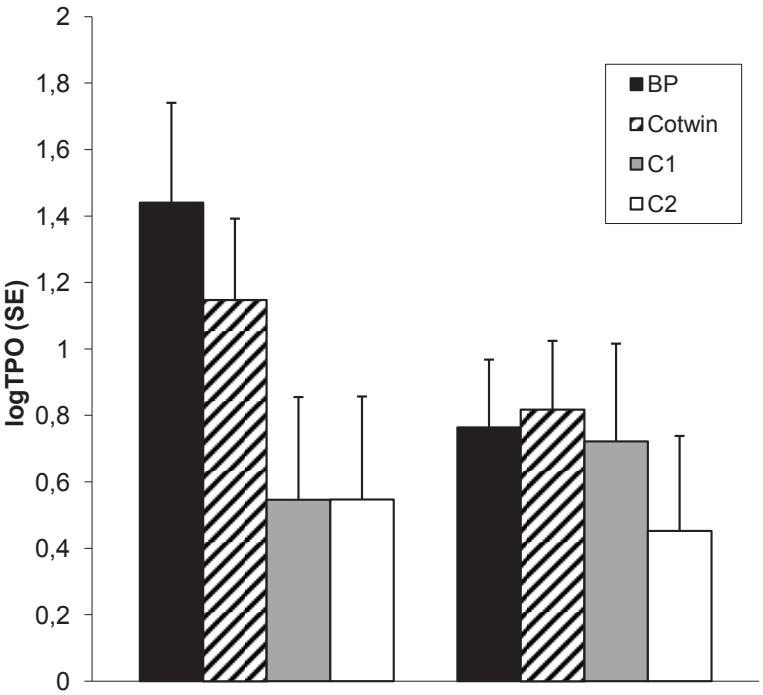

Figure 2 : Log TPO-abs (SE=standard error) for monozygotic (MZ) and dizygotic (DZ) discordant twin pairs and MZ and DZ healthy control twin pairs.

BP=bipolar index twin (n=15 MZ, 25 DZ); Cotwin = non-bipolar cotwin (n=15 MZ, 25 DZ); C1=healthy control index twin (n=19 MZ, 16 DZ);C2=healthy control cowin (n=19 MZ, 16 DZ).

Bipolar patients (*n* = 62) had significantly higher TPO-Abs levels compared with the control twins (*n* = 70) (*df* 130, *p* .01) and increased (although not significant) TPO-Abs levels compared with the nonbipolar cotwins (*n* = 40) (*df* 100, *p* .64). The nonbipolar cotwins had higher TPO-Abs levels but did not differ significantly from the control twins (*df* 108, *p* .12). In our total sample of 86 twin pairs (51 bipolar and 35 control), the pairwise concordance rate for positive TPO-Abs in monozygotic twins was 50% compared with 20% for dizygotic twin pairs.

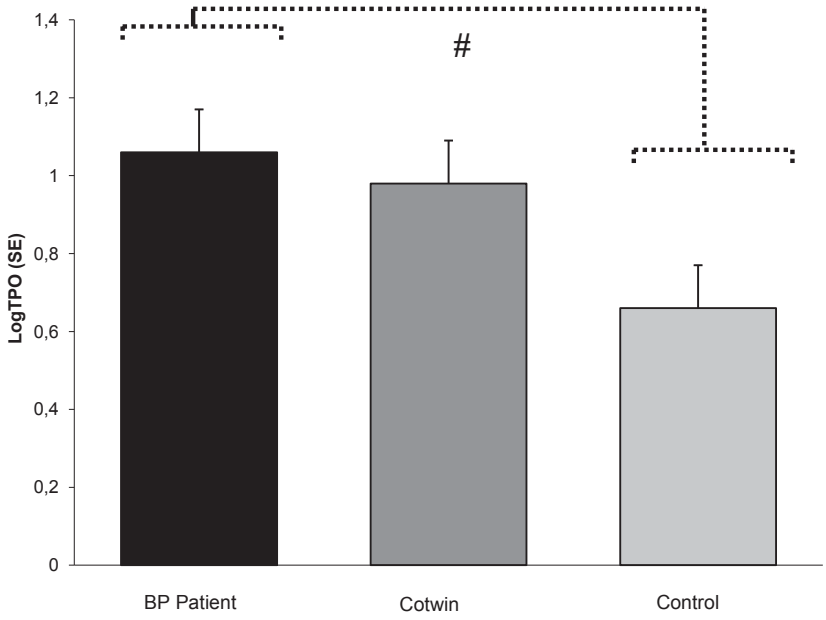

Figure 3 : LogTPO-Abs (SE = standard error) for bipolar patients, non-bipolar cotwins and control twins
BP Patient = Bipolar Patient (n=62) ; Cotwin = non-bipolar cotwin (n=40) ;
Control = control twin (n=70)

Bipolar patients differ significantly from control twins (T-test, df=130, p=0,02)
Bipolar patients did not differ significantly from non-bipolar cotwins (T-test, df=100, p=0,64)
Non-bipolar cotwins did not differ significantly from control twins (T-test, df=108, p=0,12)

Discussion

This study compared TPO-Abs levels and the prevalence of autoimmune thyroiditis (i.e., TPO-Abs > 25 U/mL) in 51 bipolar twin pairs and 35 matched healthy control twin pairs. The study was performed to elucidate whether

autoimmune thyroiditis was related to the disease itself, the (genetic) vulnerability to develop bipolar disorder, or both.

The main finding was that the discordant twin pairs showed significantly higher mean levels of TPO-Abs than the control twin pairs, with no difference in TPO-Abs levels between bipolar patients and their (discordant) nonbipolar cotwins. We also found a higher prevalence of autoimmune thyroiditis in monozygotic (discordant) nonbipolar cotwins (27%) compared with dizygotic (discordant) nonbipolar cotwins (17%) and with matched healthy control twins (16%). Finally, this study confirmed significantly higher TPO-Abs levels in bipolar patients versus control twins, with TPO-Abs levels in the (discordant) nonbipolar cotwins between those of the bipolar patients and the control twins.

Because TPO-Abs levels were significantly increased in the discordant twin pairs compared with the healthy twin pairs, whereas patients did not differ from their (discordant) nonbipolar cotwins, increased TPO-Abs levels may be related more to the genetic vulnerability to develop the disease than to the disease process itself. This is supported by the finding that the prevalence of autoimmune thyroiditis in the monozygotic (discordant) nonbipolar cotwins (27%), who have the same genetic vulnerability for bipolar disorder as the monozygotic bipolar index twins, was higher than the prevalence in the dizygotic (discordant) nonbipolar cotwins (17%), who share half of their genes with the bipolar index twins.

We found a higher prevalence of autoimmune thyroiditis in bipolar patients (27%) than in control twins (16%) and confirmed a significantly higher TPO-Abs level in bipolar patients compared with control twins. The prevalence of autoimmune thyroiditis in bipolar patients was 27%, similar to that in a large sample (n= 226) of bipolar patients (28%; Kupka *et al.* 2002). Contrary to an equal prevalence in female and male bipolar patients in that study, however, the female bipolar patients showed a significantly higher prevalence than male patients in our study [37% vs. 10%; x^2 5.11, df(1), p .02], corresponding with the results of earlier studies (Hornig *et al.* 1999; Rapoport and McLachlan 1996). Autoimmune thyroiditis was present in 16% of the control twins, with 6% in the

male control twins and 18% in the female control twins, which is not different from the previously found prevalences of 13% in the general population and of 7% and 18%in male and female subjects, respectively (Kupka *et al.* 2002). In summary, there is thus a clear gender effect in our results because a significantly higher prevalence of autoimmune thyroiditis was found in female bipolar patients than female controlsubjects [37% vs. 18%; x^2 4.13, *df*(1), *p* .02] but not to such extent in male bipolar patients versus male control subjects (10%vs. 6%).

It is interesting to note that the pairwise concordance rate for positive TPO-Abs in all (bipolar and healthy) monozygotic twins was 50% compared with 20% for all dizygotic twin pairs, indicating that the production of TPO-Abs are (in part) genetically determined.

Furthermore, this study showed that autoimmune thyroiditis and bipolar disorder cosegregate within the twin pairs and that the prevalence of autoimmune thyroiditis was found in nonaffected cotwins at a higher rate than in control subjects. The prevalence of autoimmune thyroiditis in the monozygotic (discordant) nonbipolar cotwins (27%) was higher than in the nonbipolar dizygotic cotwins (17%) and control twins (16%). In addition, the TPO-Abs level of the nonaffected cotwins was higher than the control twins.

Finally, the state independence of the presence of TPO-Abs was already demonstrated in our group's previous study because there were no differences in TPO-Abs between bipolar patientsin various mood states (Kupka *et al.* 2002).

Our results suggest that autoimmune thyroiditis, with raised TPO-Abs levels as a marker, fulfill the criteria that need to be met for this marker to serve as an endophenotype (Gershon andGoldin 1986; Gottesman and Gould 2003; Hasler *et al.* 2006; Leboyer *et al.* 1998; Lenox *et al.* 2002).

Our findings must be viewed in light of several methodologic limitations. First, although our bipolar disorder twin sample is, as far as we know, the largest twin sample studied to date (51 bipolar twin pairs), the sample is still not large. Second, this twin sample is not a population-based twin sample but a selected subgroup of bipolar twins and healthy (control) twins in The Netherlands. Nevertheless, the whole sample of affected twins can be considered representative with probandwise concordance rates for bipolar disorder of 48% for

MZ twins and 24% for DZ twins (Craddock and Jones 2001). Third, more female bipolar twins (67%) and female control twins (79%) than male twins participated in this study. This probably is a result of selection because in the general population, the prevalence of bipolar disorder is the same in men and women. Therefore, the results of this study should be carefully generalized to the total populationof bipolar patients because the design might be prone to specific selection biases. Fourth, differences in TPO-Abs assessment methodology are a reason for minor discrepancies in prevalencefigures of autoimmune thyroiditis between various studies. In this study, we compared the prevalence of autoimmune thyroiditis with the prevalence found in the study of Kupka *et al.* (2002). In both studies, different ELISA assays with different cutoff levels for TPO-Abs positivity were used (this study: Immulite, 25 U/mL, Kupka et al: Millenia, 10 U/mL), yet the prevalence figures of autoimmune thyroiditis in bipolar patients (27% vs. 28%) and healthy control subjects (16% vs. 13%) are comparable. In fact, tested on a same series of sera, we found excellent correlations between the Millenia and Immulite assay (p 43, r .92, data not shown). Prevalence figures of TPO-Abs positivity in general populations are approximately 10%–13% (Pedersen *et al.* 2003; Vanderpump *et al.* 1995). Also, previous data from our group are in accord with this figure; using yet another TPO-Ab ELISA (Orgentec GMBH, Mainz, Germany, cutoff level of 50 U/mL), a prevalence figure of 9.4% of TPO-Ab positivity was found in Dutch women aged 20 to 40 years (Kuijpens *et al.* 1998), whereas using a radioimmunoassay (Lumitest, Henning, Berlin, Germany, cutoff level of 100 U/mL), a prevalence of 13.7% was found in a healthy control group consisting of inhabitants aged over 55 years selected at random from the Rotterdam suburb Ommoord (Oomen *et al.* 1996). All these data clearly support the view that gender and age (older women have higher prevalences of TPO-Abs) are more important determinants of the prevalence ofTPO-Abs than the assay used, but the data also point out the importance of the inclusion of a healthy control group matchedfor age and gender and the usage of the same TPO-Ab assay ineach study.

A higher prevalence of autoimmune thyroiditis compared with healthy control subjects is not unique for bipolar disorder. Unipolar depression studies are inconsistent, whether or not TPO-Abs are elevated. Some studies reported an

association with autoimmune thyroiditis (Nemeroff *et al.* 1985; Pop *et al.* 1998), others found no increase in TPO-Abs positivity compared with healthy control subjects (Brouwer *et al.* 2005; Haggerty 1997). A high prevalence of TPO-Abs positivity is well known in other organ-specific autoimmune diseases such as type I diabetes mellitus and autoimmune atrophic gastritis, forming together the so called autoimmune polyendocrine syndrome (APS) type 3 (Lam-Tse *et al.* 2003). In addition, a high prevalence of TPO-Ab positivity has been found in somatic illnesses such as fibromyalgia (24%) and rheumatoid arthritis (29%; Pamuk and Cakir 2005). In summary, the discordant bipolar twin pairs had higher mean TPO-Abs levels than control twin pairs with no difference in TPO-Abs levels between bipolar patients and their (discordant) nonbipolar cotwins. In addition, the prevalence of autoimmune thyroiditis was higher and the TPO-Abs levels were increased in discordant nonbipolar (especially monozygotic) cotwins compared with control twins. Therefore, autoimmune thyroiditis with increased TPO-Abs levels seems to be related to the genetic vulnerability to develop bipolar disorder rather than to the disease process itself and may serve as a potential endophenotype.

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Chapter

6

Premorbid school performance in twins concordant and discordant for bipolar disorder

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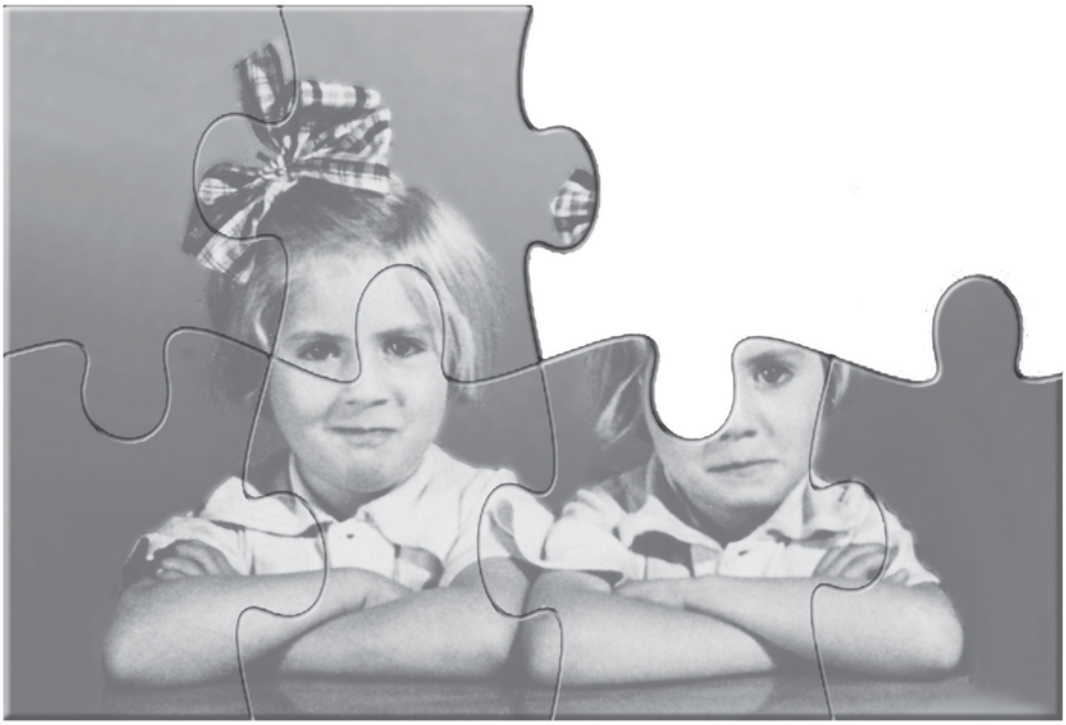

Abstract

Background: Although the genetic risk to develop bipolar disorder is present from conception, the first frank symptoms of the illness generally become evident in late adolescence or early adulthood. However, except for pediatric bipolar disorder (PBD), it is still unclear when the first signs of the illness in adults become apparent and whether these are related to the genetic risk to develop bipolar disorder. This study examined whether underperformance at school precedes the onset of the illness and is a genetically related risk marker for developing bipolar disorder.

Methods: Information on school performance was obtained using objective archival data from 53 bipolar twin pairs (24 monozygotic (MZ), 29 dizygotic (DZ)) and 42 healthy matched control twin pairs (23 MZ, 19 DZ).

Results: Affected twin pairs completed significantly fewer years of education than did control twin pairs with no difference between bipolar patients and their non-bipolar cotwins. The underperformance at school in the affected twin pairs occurred in early adolescence at a significantly younger age than the control twin pairs and preceded the onset of the first frank episode of bipolar disorder by thirteen years. Median age at onset of underperformance was not different in the patients and their non-bipolar cotwins. The association between liability of bipolar disease and age of first underperformance was significant and could be explained by genetic factors.

Limitations: The sample is not a population based twin sample.

Conclusion: Underperformance at school during early adolescence may be a genetic marker for the vulnerability to develop bipolar disorder.

Introduction

Bipolar disorder is a complex illness in which the core feature is a pathological disturbance in mood ranging from extreme elation or irritability to severe depression. The illness is usually accompanied by abnormalities in thinking and behavior, which may include psychotic symptoms. Typically, it is an episodic illness, with almost complete recovery of mood and psychotic symptoms in-between episodes (Craddock and Jones, 1999).

Family and twin studies in bipolar disorder have established the importance of genetic factors in its etiology, with heritability estimates in the range of 60–85% (Craddock and Jones, 2001; McGuffin et al., 2003; Smoller and Finn, 2003). Recently, Lichtenstein et al. (2009) estimated heritability to be 59% and influences of common environment 3.4% in a sample of over 40,000 first-degree relatives of patients with bipolar disorder. The precise interaction between the genetic vulnerability to develop bipolar disorder and environmental factors is not clear, nor is the exact size of the independent and combined effects of relevant risk factors known (Craddock and Jones, 2001).

Although the genetic risk to develop bipolar disorder is obviously present from conception, the first frank symptoms of the illness generally become evident in late adolescence or early adulthood (Goodwin and Jamison, 2007; Hillegers, 2007; Kennedy et al., 2005). However, when the first signs of (the risk for) the illness in adults become apparent is still unclear. On the other hand, there is growing literature describing the validity and nature of pediatric bipolar disorder (PBD), which could be an early-onset subtype of bipolar disorder with atypical symptoms different from the symptoms in adults. There is an ongoing controversy about the prevalence of PBD, resulting in the view of a very rare illness or an illness even affecting an estimated 1% of children and adolescents (Biederman et al., 2003; Chang and Ketter, 2001; Hillegers et al., 2005), At present, when it arises in childhood or early adolescence the long-term course or outcome of bipolar disorder is not clear (Strober et al., 2006).

Some argue that bipolar disorder is a neurodevelopmental illness (Blumberg et al., 2004; Van Os et al., 1997). Were this to be the case one would expect the first

signs of the illness to manifest themselves early in development and before the onset of the first (hypo)manic or depressive episode. To capture these first signs in subjects who will later go on to develop bipolar disorder, large prospective studies in subjects at risk are generally needed since retrospective studies are usually hampered by informant recall-bias. However, studies in twins of whom at least one has developed bipolar disorder may also clarify the possible neurodevelopmental nature of the disorder, particularly when information is used that does not rely on recall but uses objective archival data, since the cotwin of the future patient is an ideal genetic and environmental control (Van Oel et al., 2002).

Earlier, we examined school performance in elementary and secondary school as an objective measure for general functioning in twins concordant and discordant for schizophrenia (Van Oel et al., 2002). School performance, we argued, especially in the highly regulated Dutch schooling system, may be a rather objective measure of the development of general cognitive functioning. In 90% of the twin pairs discordant for schizophrenia, the twin who underperformed at school was the one who later developed schizophrenia. These findings confirmed the decline in premorbid IQ in schizophrenia compared to controls reported in other studies (Murray et al., 2004) and suggest that the first signs of schizophrenia may manifest as cognitive dysfunction many years prior to the onset of the first psychosis.

In pediatric bipolar disorder (PBD) academic functioning also is compromised, resulting in increased use of special education services (Faedda et al., 2004; Findling et al., 2001; Pavuluri et al., 2006; Wozniak et al., 1995). PBD children have lower full scale IQ (verbal and performance IQ) compared with healthy controls (Pavuluri et al., 2006). Patients with PBD showed at a 3 year follow-up a developmental delay in neurocognitive functioning with reduced functional ability compared to healthy controls (Pavuluri et al., 2009).

The current study was designed to examine whether a decline in functioning, specifically school performance, precedes the onset of the illness in adults and is a (genetic) risk marker to develop bipolar disorder.

Methods

2.1 *Subjects*

Subjects were twin-pairs, aged 18 to 60 years, with at least one twin suffering from bipolar I or bipolar II disorder according to DSM-IV criteria. Clinical diagnosis for axis I psychiatric disorders was confirmed with the Structured Clinical Interview for DSM-IV (First et al., 1996), for axis II personality disorders using the Structured Interview For DSM-IV Personality (Pfohl et al., 1997) and for both also using available medical records. The twins had no history of drug or alcohol dependency for the last half year and no severe medical illness, verified by a medical history inventory.

The 53 twin pairs concordant and discordant for bipolar disorder were recruited via the Dutch Patient's Association for Manic Depressives and Relatives (n = 16 twin pairs), via the "Lithium-Plus Working Group," a collaborating group of psychiatrists in The Netherlands with a special interest in bi- polar disorder (n = 11 twin pairs), via referral by psychiatrists working in several Dutch psychiatric institutes (n= 10 twin pairs) and via articles or advertisements in national and regional newspapers (n= 16 twin pairs).

The bipolar twins were compared to healthy control twins matched on zygosity, gender, age and parental education. The healthy control twins had no history of axis I psychiatric disorder or axis II personality disorder according to DSM-IV criteria, confirmed with a SCID and SIDP interview respectively and no history of a severe medical illness. Furthermore, they had no first degree relative with a history of a major axis I psychiatric disorder (DSM-IV) such as schizophrenia, psychotic disorder, mood disorder, anxiety disorder or substance related disorder. Family history of both affected and control twin was obtained using the Family Interview Genetic Studies (Nurnberger et al., 1994), by interviewing separately and independently the index twin and the cotwin.

The 42 control twin pairs were recruited from the ongoing twin study on schizophrenia of the UMC Utrecht (N= 11 twin pairs), via the Netherlands Twin Register (NTR) in Amsterdam (N= 15 twin pairs), via articles or advertisements in

national and regional newspapers (N= 8 twin pairs) and via friends and acquaintances of the researchers (N= 8 twin pairs).

A total of 53 affected twin pairs (9 MZ concordant, 15 MZ discordant, 4 DZ concordant and 25 DZ discordant pairs) took part in the study as well as 42 (23 MZ and 19 DZ) healthy control twin pairs. Except for one control twin pair (which was separated at age 12, when both parents died), all twins were reared together. The study was approved by the Medical Ethical Review Board of the UMC Utrecht and all participants gave written informed consent after full explanation of the study aims and procedures.

2.2 *School performance*

In the Netherlands, school performance may be a correct indicator of general cognitive functioning. Primary, secondary, and nearly all tertiary education is provided by state schools (Fig. 1). From 6 until 12 years of age, everyone receives the same primary education. After 12 years the results of the last grade of primary education and the results of a national state- sponsored exam administered at most schools will determine the child's admission to one of the four existing levels of secondary education. These four levels each demand increasing intellectual and scholastic abilities. Following secondary school, there are three levels of tertiary education possible if one passes the exams at secondary level (around age 16–18). In secondary education there is only one level that prepares the students for university. Unlike the school system in many other countries, it is quite common for children to repeat a grade if they do not meet the final standards for that year. This can happen in primary, but mostly in secondary education, where children also can move up to a higher schooling level or move down to a lower level. There is continuous assessment throughout the school year, and the final decision to repeat a year is based on this assessment. Special education is offered at both primary and secondary levels of education. The children who attend these schools do so because of attention deficits, learning disabilities, and behavioural or pediatric problems.

To determine school performance of our subjects, we used a questionnaire to collect information on the school history (educational level, grade repetition,

diplomas, academic performance, years spent at school, attendance and the kind of special education). In addition, we obtained copies of diplomas and school records of nearly all the twins. Information on deviant behavioural development was obtained from the psychiatric interviews and medical records.

We defined underperformance at school on the basis of the following criteria:

a) a twin was taken into special education
b) a twin moved down to a lower level of education
c) a twin repeated a grade

Every criterion applied to all ages. We rated the age when underperformance occurred for the first time when at least one of the criteria was met. In 60 (29 MZ, 31 DZ) (co)twins underperformance had not occurred at the time they left education. These twins were censored in further analysis.

Since we wanted to examine a possible underperformance at school before the onset of the illness, we classified the affected twin pairs into two subgroups.

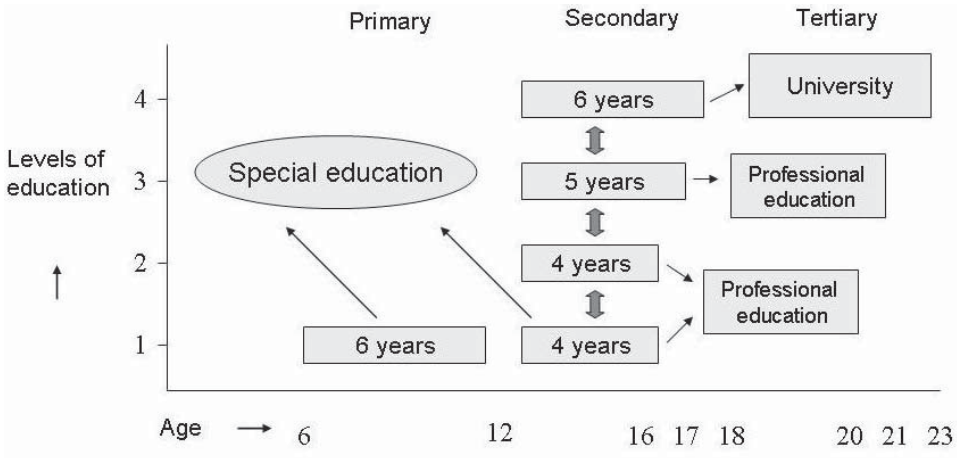

Figure 1 School system in the Netherlands

When underperformance took place before the onset of a mental illness in one of the twins (bipolar patients and non-bipolar cotwins), we named this subgroup "underperformance at school." However, if the age of onset of the mental illness in one of the twins preceded the underperformance at school, we classified the subgroup "mental illness associated underperformance". This latter subgroup included 6 bipolar patients (5 MZ, 1 DZ) and 2 non-bipolar cotwins (2 MZ, 0 DZ) and was excluded from further analysis on the age of first underperformance. We also examined the level of education of all twin pairs at the beginning of secondary education (at age 13) and de- fined three standards; low (level 1), middle (level 2) and high (level 3 and 4) (Fig. 1).

2.3 ***Statistical analyses***

For comparison of the level of education, completed years of education and frequency of underperformance between various groups an Independent Samples *T* Test or a Chi- square test was used (SPSS 15.0). Data on event occurrence were analyzed using discrete-time survival analysis (Singer and Willett, 2003). This type of analysis is suitable to study influences of independent factors (gender, zygosity (MZ/ DZ), educational level at age 13 (low/middle/high), patient status (disease yes/no) and group (patients and cotwins vs. healthy controls)) on event occurrence (in this case: under- performance) which is measured on a discrete instead of a continuous time scale (in this case: one measurement per year). Survival analysis needs to be used when data can be censored, that is, when an event does not occur in the time range of which data are available but may occur before or after that. For example, in the current study, a subject may have to repeat a grade in the second year of tertiary education, which is then recorded as an event. But another subject may graduate from secondary education and not continue with tertiary education, which would not give him the "opportunity" to repeat a grade in tertiary education. No event is recorded, but it might have occurred if given the chance (although precise time will still be unknown; right censoring). This specific type of missing data is accounted for in survival analysis.

For each time period the hazard was calculated, which is the proportion of subjects that showed underperformance over all subjects that followed education

during that year. This was used to calculate the proportion of subjects that were still present at the end of the period, which resulted in the survivor function. The year when the survivor function crosses the proportion .50 was defined as the median lifetime. The 40th percentile (40% survived up to that age) was also reported. These calculations can be done on the whole sample as well as on subgroups (e.g., males and females, patients and controls).

2.4 ***Genetic analyses***

Familial influences on onset of underperformance at school may be the result of genetic as well as common environmental effects. In order to estimate the influence of genetic and environmental factors on phenotypic variation in age of first underperformance, data from groups of individuals who are genetically related is needed. One of the most powerful designs to detect genetic and shared environmental effects is the classical twin design (Boomsma et al., 2002; Martin et al., 1997). A first step towards finding genetic effects on underperformance is to calculate heritability of the trait. This was done by decomposing the variance in the age at onset of underperformance into genetic (A, additive genetic), common environmental (C) and unique environmental (E) variance, based on the fact that identical, monozygotic twins (MZ) share 100% of their segregating genes and fraternal, dizygotic twins (DZ), share 50% of their segregating genes on average. Heritability was defined as the proportion of genetic over total variance of underperformance age and its calculation - in twin studies - is based on the correlation of underperformance age in twin 1 with that of twin 2 (Falconer and Mackay, 1996). We used the method described in Falcaro and Pickles (2007), which is an extension of Structural Equation Modeling (SEM) of ordinal data (Neale and Cardon,1992) that takes censoring into account. Briefly, we categorized the data into six 3-year bins. Censored data automatically fell into the last bin, which was defined with a left border of the age of censoring. Using a probit link function these categories were then transformed to a (multivariate) standard normal distribution (i.e., mean and SD are 0 and 1) and these were decomposed into three different sources of variance and then tested for significance by comparing full models with nested ones (e.g., ACE with CE, testing the significance of the A-effect). Twice the

difference of the Log-Likelihoods of these models is chi square distributed. A p-value of 0.05 (corresponding to a Chi-square of 3.84) was adopted as the threshold of significance. 95% likelihood confidence intervals were also calculated (Neale and Miller, 1997).

As a last step, we conducted a bivariate ordinal-survival twin analysis to study the association between age at onset of underperformance and bipolar disorder liability. To this end we adjusted the bivariate ordinal twin models on selected samples used before in the same set of twins (Padmos et al., 2009; Van der Schot et al., 2009). Bipolar disease was regarded as a dichotomous index for an underlying bipolar liability. The co- variance of this liability with onset of underperformance was decomposed into A, C and E. From the estimates of the best fit- ting model, the "phenotypic correlation due to genetic factors" was calculated: the correlation between age at onset of under- performance and bipolar disorder liability which is exclusively accounted for by genetic factors.

Because our sample was selected on bipolar disease, it was not possible to estimate prevalence and heritability, and therefore these were constrained to be the same as reported in the literature (h2 = 59%, c2 = 3%, e2 = 38%, prevalence= 1%) (Lichtenstein et al., 2009; Padmos et al., 2009).

Results

The diagnostic characteristics of the bipolar twin pairs are presented in Table 1. Forty-six bipolar patients (index twins and concordant cotwins) met DSM-IV criteria for bipolar I, and 18 for bipolar II disorder. Bipolar disorder Not Otherwise Specified (NOS) was diagnosed in 2 bipolar cotwins, one of them also suffering from schizophrenia, paranoid type. Thirty-six bipolar patients (55%) also had psychotic symptoms. Fifty-four bipolar patients had no lifetime comorbid diagnosis and in 12 bipolar patients one or more comorbid diagnoses was present. Of the (discordant) non-bipolar cotwins (n = 40), 8 were diagnosed with another mood disorder (4 major depressive disorder and 4 depressive disorder NOS), 3 with schizophrenia, paranoid type (all with a comorbid depressive disorder NOS) and 1 with a dissociative disorder NOS and a borderline

Table 1. Lifetime Psychiatric Diagnosis of the Affected Twin pairs (n = 53)

	Index Twin (n = 53)	Cotwin (n = 53)	
		Concordant (n = 13)	Discordant (n = 40)
Diagnosis			
Bipolar I disorder	38	8	
Bipolar II disorder	15	3	
Bipolar disorder NOS		1	
Bipolar disorder NOS and schizophrenia, paranoid type		1	
Major depressive disorder			4
Depressive disorder NOS			4
Schizophrenia, paranoid type			3
Dissociative disorder NOS			1
Comorbid diagnosis			
Depressive disorder NOS			3
Mood disorder due to hyperthyroidism			1
Psychotic disorder NOS	1		
Psychotic disorder due to cannabis	1		
Agoraphobia without history of panic disorder	2		
Panic disorder without agoraphobia			1
Post traumatic stress disorder	1		
Obsessive-compulsive disorder in full remission			1
Alcohol use disorder in full remission	2		1
Cannabis use disorder in full remission	1		
Sedative use disorder	1		
Sedative use disorder in full remission		1	
Anorexia nervosa	1		
Borderline personality disorder	5		1
Obsessive-compulsive personality disorder	1		
Dependant personality disorder	1		
Personality disorder NOS		1	1
No diagnosis			28

Abbreviation: NOS, not otherwise specified

personality disorder. Twenty-eight cotwins were healthy with no lifetime psychiatric diagnosis. There were no differences in symptom severity between recruitment sources.

The demographic characteristics of the affected twin pairs and control twin pairs are presented in Table 2, SES data were not available. The mean age of onset of bipolar disorder in this sample was 28.3 (9.7) [14–59] years (Table 2). In four patients the age of onset was before 18 years (14, 15, 16 and 17 years, respectively).

Table 2. Demographic characteristics of affected twin pairs and control twin pairs

	Affected Twin Pairs (n=53)	Control Twin Pairs (n=42)
Female sex (in %)	72 (68)	60 (71)
Age (years) [a]	41.4 (10.0)	40.4 (10.1)
Education father (years) [a]	10.6 (4.1)	10.3 (4.0)
Education mother (years) [a]	9.3 (2.9)	9.4 (2.8)
Level of education at age 13		
- low (%)	32 (30,2%)	18 (21,4%)
- medium (%)	43 (40,6%)	41 (48,8%)
- high (%)	31 (29,2%)	25 (29,8%)
Education (completed years) [a]	12.9 (2.4) [#]	13.6 (2.4) [#]

[a] Values are mean (SD)

[#] Affected twin pairs differ significantly from the control twin pairs (T(188)=2.06, p<0.05)

	Bipolar patients (n=66)	Non-bipolar cotwins (n=40)	Control twins (n=84)
Female gender (in %)	45 (68)	27 (68)	60 (71)
Age (years)[a]	40.8 (10.1)	42.4 (9.9)	40.4 (10.1)
Level of education at age 13			
- low (%)	19 (28,8%)	13 (32,5%)	18 (21,4%)
- medium (%)	28 (42,4%)	15 (37,5%)	41 (48,8%)
- high (%)	19 (28,8%)	12 (30%)	25 (29,8%)
Education (completed years) [a]	13.1 (2.2)	12.5 (2.6) [**]	13.6 (2.4) [**]
Age of onset (years) [a]	28.3 (9.7) [14-59]		
Psychotic symptoms	35 (53%)		

[a] Values are mean (SD)

[**] Non-bipolar cotwins differ significantly from the control twins (T(122)=2.32, p=0.02).

3.1 Level of education at onset of secondary school (age 13)

At the age of 13, there was no difference in level of education between affected twin pairs (n = 53 pairs) and control twin pairs (n= 46 pairs) (Table 2). Within the affected twin pairs there was no difference in the level of education at age 13 between bipolar patients and non-bipolar cotwins(Table

2). There was no effect of zygosity on the level of education at the age of 13 (Table 2).

3.2 Completed years of education

Affected twin pairs completed significantly fewer years of education (12.9 years) than did control twin pairs (13.6 years) ($T(188) = 2.06$, $p < 0.05$) (Table 2). The non-bipolar cotwins ($n = 40$) completed significantly fewer years of education (12.5 years) than control twins ($n = 84$)(13.6 years) ($T(122) = 2.32$, $p < 0.05$). Within the affected twin pairs there was no difference between bipolar patients and non-bipolar cotwins (Table 2).

3.3 Frequency of underperformance

Affected twin pairs (78.3%) significantly more often displayed underperformance at school than did control twin pairs (56%) (Chi-square 10.8, $df = 1$, $p < 0.01$) (Table 3). Within the affected twin pairs there was no difference in frequency of underperformance between bipolar patients and non-bipolar cotwins (Table 3). Of all twins, who displayed underperformance, 5 twins (3,8%) were taken into special education (3 bi- polar twins, 1 non-bipolar cotwin and 1 healthy control twin), all mainly because of learning disabilities (Table 3). There were no differences in frequency of underperformance between recruitment sources.

Table 3. Incidence of underperformance at school of affected twins (bipolar patients and non-bipolar cotwins) and control twins

	Affected twins (n=106)	Bipolar patients (n=66)	Non-Bipolar cotwins (n=40)	Control twins (n=84)
Underperformance	83 (78,3%)[#]	51 (77,3%)	32 (80%)	47 (56%) [#]
- Move to special education (n, %) [1]	4 (4,8%)	3 (5,8%)	1 (3,1%)	1 (2,1%)
- Repeat a grade (n, %) [1]	71 (85,6%)	44 (86,3%)	27 (84,4%)	40 (85,1%)
- Move down to a lower level (n, %) [1]	8 (9,6%)	4 (7,9%)	4 (12,5%)	6 (12,8%)

[#] Affected twin pairs had significant more frequent underperformance at school than control twin pairs (Chi-square 10.8, df=1, p < 0,01)

[1] The percentage reported here are the number of individuals, who moved to special education/repeated a grade/ moved down to a lower level, divided through the number of individuals who showed underperformance X 100%

3.4 Age of first underperformance

The effects of several predictors (gender, patient status and group) on age of first underperformance are displayed in Table 4. Gender effects (Wald= 1.998, p = .158) and patient status effect were not significant (Wald= 1.223, p = .269), but the effect of group was (Wald= 11.358, p = .001), with an odds ratio= 2.356 (CI 1.431 to 3.878). The effects of zygosity and educational level (at age 13) were included in the original analyses, but did not reach significance and are not reported here.

Fig. 2 shows the survival functions of patients, cotwins and healthy controls. After the age of 15 patients and their cotwins show increased risk of event occurrence (i.e. underperformance at school) compared to healthy controls. Median life time risk was reached at similar ages for patients (15.44) and their cotwins (15.07) but occurred later in healthy controls (17.42). These differences increased with age: the 40% life time risk (40% survived), which we chose post-hoc, were 16.79, 15.69 and 21 years respectively. This is plotted in the survivor functions of these groups.

Table 4. Predictors of occurrence of underperformance per timer period by logistic regression

Predictors #	B (s.e.) ##	Odds ratio (95% CI)	Wald statistic	p value
Gender	-0.293 (0.208)	0.746 (0.496 to 1.120)	1.998	0.158
Patient status	-0.281 (0.254)	0.755 (0.459 to 1.242)	1.223	0.269
Group	0.857 (0.254)	2.356 (1.431 to 3.878)	11.358	0.001

Variables entered in the analysis were D6 to D21, which indicate the baseline event occurrence at that age (not reported in the table) and gender, patient status and group
Gender: 1 = female, 0 = male
Patient status: 1 = patient, 0 = cotwin or healthy control
Group: 1 = patient or cotwin, 0 = healthy control

s.e. = standard error, CI = confidence interval

Positive scores indicate more events for each time period, and thus faster declining survivor curves, which is the worst scenario

3.5 Heritability of underperformance at school

In the whole group the MZ twin correlation of underperformance was .85 (CI .71 to .92) with a DZ twin correlation of .42 (CI .07 to .67). Structural equation

modeling revealed that a model containing genetic (A) and unique environmental (E) influences was sufficient to describe the data: heritability was estimated to be 85% (CI 33 to 92), whereas influence of common environment was not significant and estimated at 0% (CI 0 to 48). Influences of unique environment were estimated at 15% (CI 8 to 29). Based on these results and on the fact that only minor evidence for common environmental effects on liability for bipolar disease has been reported, C was discarded from the bivariate genetic analyses. The association between liability of bipolar disease and age of first underperformance as significant and could be explained by genetic factors (χ^2 (df= 1) = 5.093, p= 0.024): the phenotypic correlation due to genetic factors was − 0.19 (CI − 0.35 to − 0.03), which translates to a moderate effect size of −0.4 (Cohen's D). This indicates that subjects with a higher liability for bipolar disease (patients and to a lesser extent cotwins) have an elevated probability to underperform at school at an earlier age than unaffected subjects, which can be attributed to their genetic background.

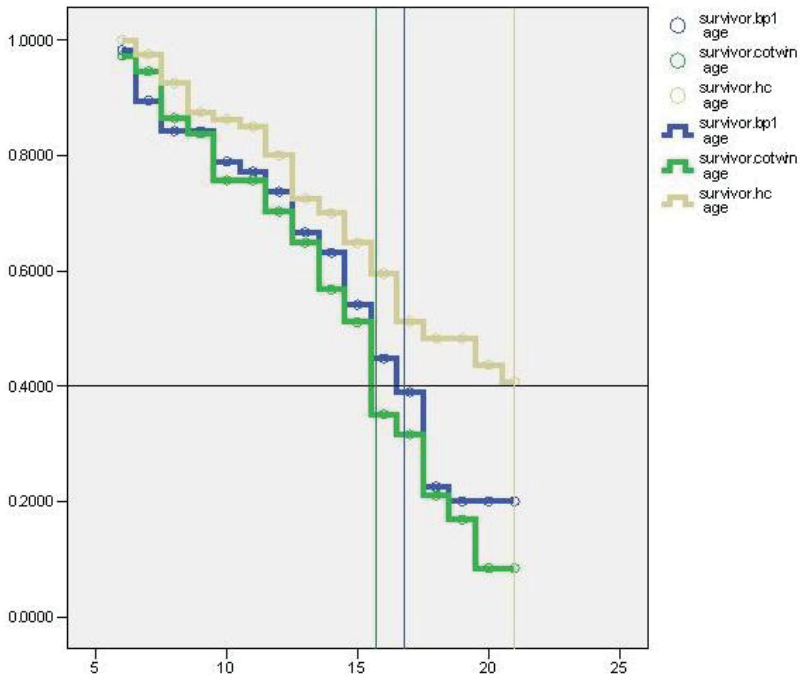

Figure 2 Survivor functions of patients (n=57), cotwins (n=37) and healthy controls (n=78). Vertical lines represent 40% life time risks (60% has had the event in the past).

Discussion

This study examined school performance in 53 bipolar and 42 matched healthy control twin pairs in order to elucidate whether underperformance at school precedes the onset of the illness and is a genetically related risk factor for developing bipolar disorder.

We found that while there was no difference in level of education between affected and control twin pairs at the start of secondary education (i.e. age of 13), ultimately the affected twin pairs completed significantly fewer years of education than did control twin pairs. This was due to the fact that affected twin pairs underperformed at school both at an earlier age and more frequently than the control twin pairs. Interestingly, within the affected twin pairs, school performance was similar in the twins who would go on to develop bipolar illness and their non-bipolar cotwins. Remarkable, even among the control twin pairs the percentage who displayed underperformance at school was rather high (56%). This demonstrates the narrow criteria in the well organized Dutch schooling system for children to meet the final standards for that year.

This study also showed that age at onset of underperformance at school, irrespective of affected status, was highly heritable, with a heritability estimate of 85% (CI 33 to 92). Structural equation modeling revealed that a model containing genetic and unique environmental influences was sufficient to describe the data of the whole sample whereas influence of common environment was not significant and estimated at 0% (CI 0 to 48). Our data indicate that subjects at increased genetic risk to develop bipolar disease may show a genetically mediated elevated liability to underperform at school during early adolescence. It should be kept in mind that this effect is based on a twin model with a number of assumptions, e.g. equal treatment of MZ and DZ twins (MZ twins may be treated more alike by teachers than DZ twins and MZ twins might be kept together in the same grade longer than warranted), which may have caused a possible overestimation of the genetic influence on the age of divergence in school performance (Van Oel et al., 2002).

The underperformance at school in the bipolar twins occurred in early adolescence and preceded the onset of the first frank episode of bipolar disorder

in the affected twin by a mean of thirteen years. Interestingly, and supporting the notion that underperformance at school is not a precursor of the illness per se but instead is related to the genetic risk to develop the disorder, the underperformance occurred just as frequently in the bipolar twins as it did in the non-bipolar cotwins. Indeed, the underperformance at school in early adolescence does not lead to a pronounced and permanent decline in school performance, since the affected twins eventually lost less than one year of education as compared to the healthy twins. Clearly, (increased genetic risk for) bipolar disorder is not associated with a permanent or pronounced decline in cognitive functioning preceding the onset of the illness.

We are not aware of other studies examining a relationship between school performance, increased genetic risk and the development of bipolar disorder. However, several studies suggest that bipolar disorder is not preceded by a decline in school performance and indicate that even the opposite may be true. Maccabe et al. (2006, 2010) reported in a retrospective cohort study using national Swedish population registers that excellent school performance (based on school records) at sixteen years preceded the development of bipolar disorder. A population-based study of Finnish conscripts found an association between increased risk of bipolar disorder and premorbid high arithmetic intellectual performance (Tiihonen et al., 2005). Since we consider school performance to reflect (at least in part) general cognitive functioning, our data are consistent with the studies examining childhood development preceding frank bipolar disorder or mania (Cannon et al., 1997, 2002). In a study based on maternal recall, Cannon et al. (1997) found that 28 patients with bipolar disorder had displayed significant impairment in premorbid social adjustment as compared with controls, but were not different from controls on premorbid IQ or school performance. In a birth cohort study, 20 children who later fulfilled diagnostic criteria for mania exhibited difficulties in social, behavioural and emotional development, but not in motor performance, language or cognition (Cannon et al., 2002). This normal premorbid intellectual function in subjects who later go on to develop bipolar disorder has also been reported in population-based studies of Israeli (Reichenberg et al., 2002) and

Swedish conscripts (Zammit et al., 2004) and in a study of 39 patients with bipolar disorder from multiply affected families (Toulopoulou et al., 2006).

Comparing the current results with those of our previous twin study in schizophrenia (Van Oel et al., 2002), bipolar twins attained almost the same level of education as healthy control twins, while schizophrenia twins had a pronounced lower level of education than control twins. In contrast to what we found in the schizophrenia twin sample, here we found no significant difference in school performance between bipolar twins and their non-bipolar cotwins. This finding is in line with those of Maccabe et al. (2006, 2008) reporting that excellent school performance is a risk factor for bipolar disorder, whereas poor performance predicted schizophrenia.

Obviously, the findings of this study raise the question of the clinical relevance. Can these findings improve the identification and early intervention of youth at risk for developing bipolar disorder? Although 78% of the affected twin pairs displayed temporary underperformance at school, also did more than half of the healthy control twins (56%). With that, temporary underperformance at school seems not suitable to identify youth at risk in the general population. But in a high-risk population of youth with a positive family history of bipolar disorder, temporary underperformance could be an early sign of the genetic risk in the children or an early sign for developing bipolar disorder. These children should be carefully monitored during their youth, especially if they showed some prodromal emotional, social or behavioural problems besides the underperformance. Especially mid- adolescence seems an important window for identification and intervention of at-risk youth, since the affected twin pairs showed increased risk of underperformance compared to healthy controls after the age of fifteen. Possibly, the genetic risk for bipolar disorder is expressed after the age of fifteen because of increased academic stress in the Dutch schooling system. Our findings also suggest that, despite temporary underperformance, ultimately patients developing bipolar disorder show at the end only a slightly lower level of education than healthy controls.

Academic performance may be impaired in individuals with PBD or individuals at risk for adult bipolar disorder (Pavuluri et al., 2006), Underperformance at school

could function as a biomarker for developing bipolar disorder, that may reflect underlying pathophysiologic illness processes (Phillips, 2010). Interestingly, in our twin study we previously found, that a decrease in cortical white matter volume was related to the genetic risk of developing bipolar disorder (van der Schot et al., 2009). In addition, in healthy offspring having a parent with bipolar disorder Versace et al. (2010) found altered developmental patterns of white matter (WM) with age during adolescence. Previous studies in bipolar disorder examining white matter abnormalities in relation to the functional outcomes, specific cognitive functions, showed contradictory results. Some studies found that white matter lesions underlie cognitive deficits in bipolar patients (Dupont et al., 1995; Liu et al., 2010), while others did not find this relation (Krabbendam et al., 2000). It seems conceivable, that cognitive deficits, based on developmental white matter abnormalities, elicit the (temporary) slight underperformance in those at risk for bipolar disorder. Indeed, (temporary) academic underperformance could be considered as a cognitive endophenotype for bipolar disorder.

Our findings need to be seen in the context of several (methodological) limitations. First, this twin sample is not a population-based twin sample, but a selected subgroup of bipolar twins and healthy (control) twins in the Netherlands. For instance, more female bipolar twins (68%) and female control twins (71%) than male twins participated in this study. This probably is a result of selection, as in the general population the prevalence of bipolar disorder is the same in men and women. On the other hand, the concordance rates in our sample of 53 affected twins of 55% for MZ twins and 24% for DZ twins is comparable with other concordance rates for bipolar disorder (McGuffin et al., 2003). Also the healthy control twins form a selection, as that they were not only selected for lifetime absence of any psychiatric disorder but also for absence of a family history of any major psychiatric disorder. Second, this study was not a longitudinal prospective study, as we assessed school performance retrospectively, with the possibility of a recall bias. However, since we confirmed the subject's information on school performance by examining school reports and diplomas of nearly all twins, we believe that the assessment of school performance was not biased. Third, we argued that the Dutch school system provides a unique opportunity to examine

school performance as a rather objective measure of global cognitive functioning of the child. However, underperformance at school seems multiply determined and could be a reflection of the presence of behavioural or attendance problems of the child. Also prodromal emotional difficulties or difficulties in social situations may distract them from the optimal academic performance at school. Prodromal or subsyndromal (BP-NOS) states of bipolar disorder are associated with significant psychosocial and psycho educational impairment (Chang et al., 2003). However, Cannon et al. (1997) found that bipolar patients had significant impairment in premorbid social adjustment as compared with controls, but were not different from controls on premorbid IQ or school performance. Exact information about the possible existence of subtle emotional, social en behavioural symptoms in the twins was not captured in this study. Nevertheless, especially in the highly regulated Dutch schooling system there is much attention for these problems in relation to optimal school performance. Furthermore, in this sample only a few children with more severe learning difficulties were taken into special education. For this reason we believe that in our sample underperformance reflects more the academic difficulties. Fourth, in this sample the mean age of onset of bipolar disorder is fairly high, 28.3 (9.7) [14–59] years. Only in four patients the age of onset was before 18 years (14, 15, 16 and 17 years, respectively). This could be the result of selection bias, but the Netherlands Mental Health Survey and Incidence Study (NEMESIS) also showed a rather high average age of onset of bipolar disorder of 26.2 years (Ten Have et al., 2002). Consequently, this sample is particular relevant for examining premorbid school performance in adult bipolar disorder.

In conclusion, results of this study suggest that school performance in early adolescence is affected in subjects at increased genetic risk to develop bipolar disorder. However, this underperformance at school is temporary and not related to a permanent impairment in intellectual functioning since those at increased risk, including the subjects who will later develop the illness, eventually do scholastically (almost) as well as their healthy peers. In this respect bipolar disorder clearly contrasts with schizophrenia where deterioration in school performance, although also preceding the onset of the first (psychotic) episode by many years, is permanent. Taken together, temporary underperformance at school

in early adolescence may be a genetic marker for the vulnerability to develop bipolar disorder.

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Conflict of interest

This study was financially supported by the Stanley Medical Research Institute.

There is no conflict of interest related to this work (financial or otherwise).

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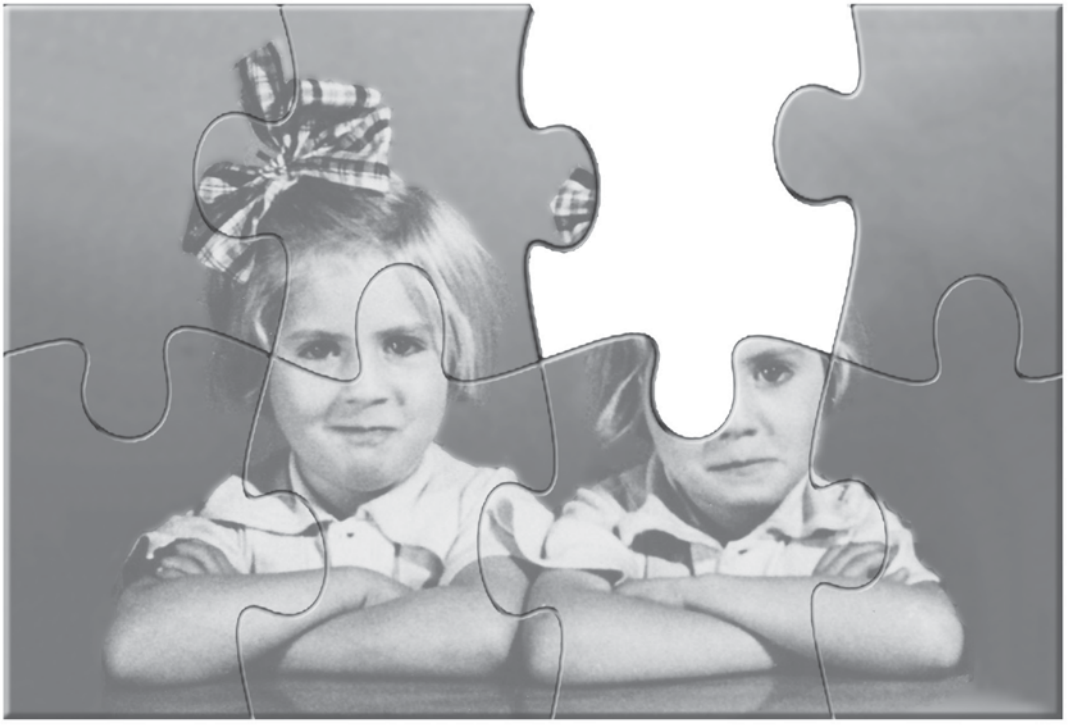
Chapter 7

Summary and discussion



Summary and Discussion

First, I will recapitulate the original research questions of this dissertation and describe to what extent they have been answered in the preceding chapters. Then, I will focus on the relevance of the study results and their consequences for clinical practice. Finally future perspectives for research will be discussed.

Answers to the research questions:

Do genes and environment have an Influence on the global and on focal brain structures in bipolar disorder?

In our study we found that white matter volume decrease is related to the genetic risk of developing bipolar disorder, while environmental factors, including the effects of illness, lead to decreased cortical gray matter volume. Interestingly, lithium showed considerable effects on the brain changes found in this study, attenuating the decrease in both gray and white matter (Chapter 2).

Bipolar disorder is associated with widespread decreases in grey matter density, most prominent in frontal and limbic regions, while white matter abnormalities were mostly limited to decreases in density in the superior longitudinal fasciculi. Density loss in the latter was also related to the increased genetic risk to develop the illness, as were grey matter density decreases in small areas of the frontal lobe. White matter pathology in the frontal lobe may be central to the genetic risk to develop bipolar disorder, while most of the

widespread grey matter abnormalities may be related to environmental effects and the illness itself (Chapter 3).

Are dermatoglyphic alterations related to structural brain abnormalities in bipolar disorder and have they a genetic or environmental origin?

If so, are dermatoglyphic alterations a time-linked (i.e. between the 10th and the 17th weeks of gestation) genetic or environmental marker for an abnormal neurodevelopmental origin of bipolar disorder?

We found that a higher palmar a-b ridge count (ABRC) is related to the genetic risk of developing bipolar disorder and is negatively associated with brain volumes total brain, total cortical volume, cortical grey matter and lobar white matter irrespective of disease (or risk) status. Thus, a genetically determined abnormal development of the foetal ectoderm between the 10^{th} and 15^{th} week of gestation appears related to smaller brain volumes in (subjects at risk for) bipolar disorder. This in turn suggests that some of the brain abnormalities in (subjects at risk for) bipolar disorder may be attributable to a genetically driven early (foetal) disruption in neurodevelopment (Chapter 6).

Is autoimmune thyroiditis related to the disease itself or to the (genetic) vulnerability to develop bipolar disorder?

If so, is autoimmune thyroiditis a possible endophenotype for bipolar disorder?

We found that autoimmune thyroiditis is related not only to bipolar disorder itself but also to the genetic vulnerability to develop the disorder. Autoimmune thyroiditis, with TPO-Abs as marker, is a possible endophenotype for bipolar disorder (Chapter 4).

Does underperformance at school precede the onset of the bipolar disorder? If so, is underperformance at school a genetically related risk marker for developing bipolar?

We found that school performance in early adolescence is affected in subjects at increased genetic risk to develop bipolar disorder. However, this underperformance at school is temporary and not related to a permanent impairment in intellectual functioning since those at increased risk, including the subjects who will later develop the illness, eventually do scholastically (almost) as well as their healthy peers.

Taken together, temporary underperformance at school in early adolescence may be a genetic marker for the vulnerability to develop bipolar disorder (Chapter 5).

General Discussion

Generalisability of the results

Initially, whether there is a different rate of bipolar disorder in twins or singletons is a basic question for the generalisability of the results of this twin study in bipolar disorder. In other words, is being a part of a twin pair a risk factor for developing (a specific form of) bipolar disorder?

Several studies addressed the question of whether twins differ from singletons in their risk for bipolar disorder and found no difference between the rate of bipolar disorder in twins and in singletons and between monozygotic and dizygotic twins (Luxenburger 1928, Essen-Moller 1941, Bertelsen et al. 1977, Kendler et al. 1992, 1996).

However, the statistical power in these studies has been limited because of small sample sizes; furthermore, there have been difficulties in estimating the rate of illness or the rate of

twinning in the background population (Kläning et al. 2004). A recent, methodological more accurate, nationwide register-based Danish study showed an equal rate of bipolar disorder in (dizygotic and monozygotic) twins and in singletons, as well as for parents and siblings of dizygotic twins, monozygotic twins and singletons (Kläning et al. 2004).

In contrast, for schizophrenia an increased rate has been described in DZ twins compared to the rate in singletons, whereas the rate in MZ twins with schizophrenia was equal to the rate in singletons (Kläning 1999). A differential relationship between bipolar disorder and DZ twinning on one hand and schizophrenia and DZ twinning on the other hand may suggest differences in the genetic basis of the two diseases, assuming that DZ twinning is under some genetic influence (Kläning et al. 2002, 2004).

In summary, being a part of a MZ or DZ twin pair is not a risk factor for developing bipolar disorder.

Unfortunately, our twin sample is not a population-based twin sample, but a selected subgroup of bipolar twins and healthy (control) twins in the Netherlands. For instance, more female bipolar twins (68%) and female control twins (71%) than male twins participated in our study. This probably is a result of selection, as in the general population the prevalence of bipolar disorder is the same in men and women. On the other hand, the whole sample of affected twins can be considered representative as the concordance rates in our sample of 53 affected twins of 55% for MZ twins and 24% for DZ twins is comparable with other reported concordance rates for bipolar disorder (McGuffin et al. 2003). Also the healthy control twins form a selection, as they were selected for lifetime absence of any psychiatric disorder as well as for absence of a family history of any major psychiatric disorder. Therefore, the results of our study should only carefully be generalized to the total

population of bipolar patients because the design might be prone to specific selection biases.

Interestingly, during the recruitment several twin pairs signed up for participation in the study, where both twins themselves had no psychiatric disorder but a parent with bipolar disorder. In this way, in any case to their disappointment, they did not meet the criteria for the bipolar twin group, neither for the control twin group. Of course, we considered the examination of this special group of twins with a high genetic risk for developing bipolar disorder, but the research capacity was not sufficient to amend the original protocol.

Bipolar phenotype a methodological problem

Precise definition of the bipolar phenotype is an issue of critical importance for the success of (genetic) studies of bipolar disorders (Duffy and Grof 2001). Since more than a century, the bipolar disorder phenotype is defined solely according to reported or observed clinical features as assessed in a diagnostic interview, while diagnostic tests (e.g. lab tests, brain scans) for bipolar disorder do not (yet) exist (Craddock and Sklar 2013).

With the methodology used in our study, it is crucial to classify a twin pair as concordant or discordant. Therefore, a methodological dilemma in our sample is the comorbidity within a twin pair.

Studies of genetically identical subjects illustrate the phenotypic spectrum, which may be associated with susceptibility genes for bipolar disorder. In addition to bipolar disorder, MZ co-twins or co-triplets have been described who have a diagnosis of unipolar depression, schizoaffective disorder or even schizophrenia (Craddock and Jones 1999). However, reports of such twin pairs with bipolar disorder and schizophrenia are rare, suggesting that an

identical set of genes does not confer liability for both schizophrenia and bipolar disorder (O'Reilly et al. 2013) and supporting the biological distinctness of the two diseases (Lohr and Bracha 1992). Otherwise, when diagnostic hierarchies were relaxed the twin study of Cardno et al. (2002), they clearly showed evidence for a degree in overlap in the genes contributing to schizophrenic, schizoaffective and manic syndromes as defined by Research Diagnostic Criteria (RDC), which are comparable to the DSM criteria. Also the results of family studies indicate that it is not only bipolar disorder that is more common among relatives of bipolar probands, but also other psychiatric conditions. These are usually milder forms of bipolar disorder, not meeting the full criteria and together regarded as bipolar spectrum, which may share a genetic basis (McQueen et al. 2005). Thus, although psychiatric disorders such as schizophrenia, bipolar disorder, major depressive disorder, attention-deficit/hyperactivity disorder and autism spectrum disorder are currently classified into distinct disorder categories, they show clinical overlap and familial co-aggregation, and probably share genetic risk factors (Doherty and Owen 2014, Asherson et al. 2015).

In our twin study we classified a twin pair as concordant when both twins had a diagnosis of Bipolar I, Bipolar II or Bipolar NOS according to DSM-IV. If the cotwin was healthy with no lifetime psychiatric diagnosis or had another axis I or II diagnosis according to DSM-IV, the twin pair was classified as a discordant pair.

The diagnostic characteristics of the bipolar twin pairs are presented in Table 2. Forty-six bipolar patients (index twins and concordant cotwins) met DSM-IV criteria for bipolar I and 18 for bipolar II disorder. Bipolar disorder Not Otherwise Specified (NOS) was diagnosed in 2 concordant bipolar cotwins, one of them also suffering from schizophrenia, paranoid type.

Table 2. Lifetime Psychiatric Diagnosis of the Affected Twin pairs (n = 53)

	Index Twin (n = 53)	Cotwin (n = 53)	
		Concordant (n = 13)	Discordant (n = 40)
Diagnosis			
Bipolar I disorder	38	8	
Bipolar II disorder	15	3	
Bipolar disorder NOS		1	
Bipolar disorder NOS and schizophrenia, paranoid type		1	
Major depressive disorder			4
Depressive disorder NOS			4
Schizophrenia, paranoid type			3
Dissociative disorder NOS			1
Comorbid diagnosis			
Depressive disorder NOS			3
Mood disorder due to hyperthyroidism			1
Psychotic disorder NOS	1		
Psychotic disorder due to cannabis	1		
Agoraphobia without history of panic disorder	2		
Panic disorder without agoraphobia			1
Post traumatic stress disorder	1		
Obsessive-compulsive disorder in full remission			1
Alcohol use disorder in full remission	2		1
Cannabis use disorder in full remission	1		
Sedative use disorder	1		
Sedative use disorder in full remission		1	
Anorexia nervosa	1		
Borderline personality disorder	5		1
Obsessive-compulsive personality disorder	1		
Dependant personality disorder	1		
Personality disorder NOS		1	1
No diagnosis			28

Abbreviation: NOS, not otherwise specified

Of the non-bipolar cotwins (n=40), 28 cotwins were healthy with no lifetime psychiatric diagnosis and obviously classified as part of discordant twin pairs. Furthermore 12 non-bipolar cotwins had at least one another DSM-IV diagnosis with no or more comorbid diagnoses; 8 were diagnosed with another mood disorder (4 major depressive disorder and 4 depressive disorder NOS), 3 with schizophrenia, paranoid type (all with a comorbid depressive disorder NOS) and 1 with a dissociative disorder NOS and a borderline personality disorder. Comorbid diagnoses of these 12 non-bipolar cotwins contain mood disorder due to hyperthyroidism, panic disorder, obsessive-compulsive disorder in full remission, alcohol use

in full remission, borderline personality disorder or personality disorder NOS.
We discussed the possibility to amplify the boundaries of the diagnoses of the concordant twin pairs, including a diagnosis of depressive disorder or psychotic disorder, which likely would have given other results. A broader phenotype of bipolar disorder, for example also including depressive disorder, as inclusion criterion for the study, would have facilitated the recruitment of the index twins. Definitely, this choice would have raised the amount of participating twins, increasing the power of the study. However, studies examining the genetic vulnerability of a disorder require a narrowly defined phenotype, increasing the likelihood of an association with susceptibility genes. Although gathering a relatively homogeneous sample of bipolar patients is a major challenge, they more likely share the same susceptibility genes (Duffy and Grof 2001).
Ultimately, we have chosen a relatively narrow boundary of the bipolar spectrum, namely Bipolar I, Bipolar II or Bipolar NOS to classify a twin pair as a concordant pair.

There is another important question about the meaning of discordance: how much time must pass after the first twin becomes ill, before we can confidently declare a twin pair to be discordant? The time interval between the moment when a twin became ill and when the cotwin displayed significant psychopathology indicates discordance for the (age of) onset of the illness. While it is never possible to be sure that any twin pair will remain discordant, follow-up studies of identical twins discordant for schizophrenia reported that the majority of twins who become concordant do so within five years following the onset of schizophrenia in the first twin (Gottesman and Shields 1972, O'Reilly et al. 2013). However, a rare interval of more than thirty years between the onset of schizophrenia in MZ twins has been reported (Slater and Shields 1953, O'Reilly et al. 2013). Yet, for bipolar disorder these follow-up studies of discordant twins do not exist, so we do not known which time interval

we logically should adhere before a discordant twin pair will almost definitely not change anymore into a concordant pair.

In our twin study the mean time between the age of onset of bipolar disorder in the index twins and the current age of the 40 discordant cotwins was more than 13 years (median 10,3 years, 25 percentiles 6,6 years), whereby in 8 discordant twin pairs (20%) this time was less than 5 years. In the 13 concordant twin pairs the mean time between the ages of onset of both twins was 4.9 years (median 3 years, 75 percentiles 9.2 years), which is remarkably shorter than in the discordant pairs, indicating that probably most or our discordant twins will remain so in the future. This is further supported by the follow-up study of our twin sample (Bootsman et al. 2015) in which 15 MZ (8 concordant, 7 discordant) and 14 DZ bipolar twin pairs (2 concordant, 12 discordant) of the original sample participated. The mean follow-up time was seven years and none of the discordant bipolar twin pairs had changed from discordant to concordant during the interval.

Markers for the genetic risk for developing bipolar disorder (endophenotypes)

Endophenotypes are traits that are associated with the expression of an illness and are believed to represent the genetic liability of the disorder among non-affected subjects (Leboyer 1998). Neurophysiologic, biochemical, endocrinologic, neuroanatomic, and cognitive abnormalities often accompany bipolar disorder and may thus be candidates to serve as endophenotypes (Lenox et al. 2002). Five criteria have been described that need to be met for a marker to serve as an endophenotype. The marker 1) is associated with the illness in the population, 2) is heritable, 3) is state-independent (i.e., is manifested in an individual whether or not the illness is active), 4) cosegregates with the illness within families, and 5) is found in nonaffected family members at a higher rate than in the general

population (Gershon and Goldin 1986, Gottesman and Gould 2003, Hasler et al. 2006, Leboyer et al. 1998, Lenox et al. 2002).

There still are a lot of unresolved issues associated with the underlying pathogenic mechanisms of bipolar disorder (Hranov et al. 2013).The endophenotype concept might help to resolve questions about etiology (Hasler et al. 2005), as endophenotypes are measurable components along the pathophysiological pathway between etiology and psychopathology (Gottesman and Gould 2003, Hranov 2013).

The aim of this twin study was to investigate putative risk factors for bipolar disorder and to explore to what extent the risk factors were genetically or environmental mediated. The design of a twin study is an accurate design to identify bipolar endophenotypes, which in fact are markers of the genetic vulnerability to develop bipolar disorder.

First, this study showed that autoimmune thyroiditis, with TPO-Abs as marker, is related to the genetic vulnerability to develop the disorder and is a possible endophenotype for bipolar disorder (Chapter 4).

Second, also white matter abnormalities (decreases in density in the superior longitudinal fascicule) were related to the increased genetic risk to develop the illness. White matter pathology in the frontal lobe may be central to the genetic risk to develop bipolar disorder (Chapter 2 and 3).

Third, temporary underperformance at school in early adolescence may be a genetic marker for the vulnerability to develop bipolar disorder (Chapter 5).

Fourth, higher palmar a-b ridge count (ABRC) is related to the genetic risk of developing bipolar disorder and is negatively associated with brain volumes total brain, total cortical volume, cortical grey matter and lobar white matter. Higher ABCR seems a time-linked

(between the 10th and the 15th weeks of gestation) genetic marker for a genetically driven early (foetal) disruption in neurodevelopment in bipolar disorder (Chapter 6).
This study shows that the search for discrete endophenotypes in bipolar disorder is a fruitful and promising path leading to understand the underlying neurobiology and more specifically to elucidate the genetic vulnerability for this disease (Hranov et al. 2013). Furthermore, bipolar endophenotypes can be helpful for prediction, to identify those at higher (genetic) risk and to enhance early diagnosis.

Are the neurodevelopmental trajectories of schizophrenia and bipolar disorder overlapping or different?

The last two decades there is an ongoing dispute whether bipolar disorder and schizophrenia are separate entities or different manifestations of a single underlying pathological process (Demjaha et al. 2012).
Some have argued that just like schizophrenia, bipolar disorder is a neurodevelopmental illness (Blumberg et al. 2004, van Os et al. 1997). Were this to be the case, one would expect the first signs of the illness to manifest themselves early in development and before the onset of the first (hypo)manic or depressive episode. To capture these first signs in subjects who will later develop bipolar disorder, large prospective studies in subjects at risk are generally needed since retrospective studies are usually hampered by informant recall-bias. However, studies in twins of whom at least one has developed bipolar disorder may also clarify the possible neurodevelopmental nature of the disorder, since the co-twin of the future patient is an ideal genetic and environmental control (Van Oel et al. 2002).

For our twin study in bipolar disorder we were in the unique opportunity to compare the results with the results of the similar twin study in schizophrenia also

performed at the University Medical Center Utrecht (Baaré et al. 2001, van Oel et al. 2001, 2002, Hulshoff Pol et al. 2004). More specifically, we were able to compare the neurodevelopment of both disorders at various point of the development of the brain. First, early neurodevelopment during the first trimester of gestation by investigation dermatoglyphics in relation to brain structures and the further neurodevelopment of the brain during puberty and adolescence by examining the school performance in both bipolar twins and twins with schizophrenia. Second, we could compare the structural brain abnormalities in relation to genetic and environmental factors in both disorders, as the result of (impaired) neurodevelopment during life.

The early neurodevelopment of the brain during gestation was investigated by the investigation of dermatoglyphics in relation to brain structures (Chapter 6) and we found that higher palmar a-b ridge count (ABRC) is related to the genetic risk of developing bipolar disorder and is negatively associated with brain volumes total brain, total cortical volume, cortical grey matter and lobar white matter. Higher ABCR seems a time-linked (between the 10th and the 15th weeks of gestation) genetic marker for a genetically driven early (foetal) disruption in neurodevelopment in bipolar disorder.

Comparing these results of the dermatoglyphics in the bipolar twin study with those of the previous twin study in schizophrenia (Van Oel et al. 2001), we found a different neurodevelopmental process in bipolar disorder versus schizophrenia. The schizophrenic twin study revealed that non-genetic circumstances early in pregnancy (10-13 weeks of gestation) were associated with a susceptibility to schizophrenia since both twins with schizophrenia and the unaffected cotwins showed more fluctuating asymmetry of the finger ridges than control twin pairs. However, we did not find any significant environmental correlation between dermatoglyphics and bipolar disorder. Indeed, obstetric complications,

including indicators of fetal growth, have been associated with an increase in schizophrenia but a systematic review did not show robust evidence that exposure to obstetric complications increases the risk of developing bipolar disorder (Scott et al. 2006). Our results confirm the missing role of environmental factors during early pregnancy in the development of bipolar disorder, but demonstrate, also in bipolar disorder, a genetically mediated impaired neurodevelopment of the foetal ectoderm (Chapter 6). This is in contrast with the model of Demjaha et al. (2012) wherein, on a background of a shared genetic liability for both disorders, neurodevelopment is impaired only in patients with schizophrenia, and influenced by additional genetic and/or environmental factors.
Thus, comparing the results of the dermatoglyphics in the bipolar twins and the schizophrenic twins we found for both disorders an early (foetal) disruption in neurodevelopment, but in schizophrenia caused by environmental factors early in gestation, and in bipolar disorder genetically medicated (Chapter 6). This suggests a different neurodevelopmental trajectory for bipolar disorder and schizophrenia early in gestation (between the 10th and the 15th weeks of gestation).

The neurodevelopment of the brain during puberty and adolescence was further studied by examining the school performance in the twins (Chapter 5). We found that school performance in early adolescence was affected in subjects at increased genetic risk to develop bipolar disorder. However, this underperformance at school is temporary and not related to a permanent impairment in intellectual functioning since those at increased risk, including the subjects who will later develop the illness, eventually do scholastically (almost) as well as their healthy peers.
In the previous twin study in schizophrenia we found that in 90% of the discordant twin pairs, the twin who underperformed at school was the one who later developed

schizophrenia (Van Oel et al. 2002). These findings confirmed the decline in premorbid IQ in schizophrenia compared to controls reported in other studies (Murray et al. 2004) and suggest that the first signs of schizophrenia may manifest as cognitive dysfunction many years prior to the onset of the first psychosis.

Comparing the results of our bipolar twin study with those of the previous twin study in schizophrenia (Van Oel et al. 2002), bipolar twins attained almost the same level of education as healthy control twins, while schizophrenia twins had a pronounced lower level of education than control twins. In contrast to what we found in the schizophrenia twin sample, we found no significant difference in school performance between bipolar twins and their non-bipolar cotwins. In this respect, bipolar disorder clearly contrasts with schizophrenia where deterioration in school performance, although also preceding the onset of the first (psychotic) episode by many years, is permanent. (Chapter 5). This finding is in line with those of Maccabe et al. (2006, 2008) reporting that excellent school performance is a risk factor for bipolar disorder, whereas poor performance predicted schizophrenia.

We also investigated the contribution of genetic and environmental factors on brain volume in bipolar disorder (Chapter 2 and 3) and found that white matter volume decrease is related to the genetic risk of developing bipolar disorder, while environmental factors, including the effects of illness, lead to decreased cortical gray matter volume.

Interestingly, white matter pathology had also been suggested to be central to the genetic risk of developing schizophrenia (Davis et al. 2003)(Chapter 3). Indeed, in an earlier MRI study in twins discordant for schizophrenia, white matter decrease was associated with the increased genetic risk of developing schizophrenia (Hulshoff Pol et al. 2004). Similarly, a voxel-based morphometry study that included patients with schizophrenia, bipolar patients, and their unaffected relatives found the genetic risk for both disorders to be associated with

a white matter decrease in the left frontal and temporoparietal regions (McDonald et al. 2004). White matter pathology seems to constitute a common genetic risk factor for bipolar disorder and schizophrenia. Indeed, findings from genetic association studies suggested considerable overlap in risk genes for bipolar disorder and schizophrenia, particularly regarding oligodendrocyte- and myelin-related genes (Tkachev et al. 2003, Sokolov 2007, Carter 2007)(Chapter 3).

So, our findings in bipolar twins mirror those reported in studies of schizophrenia and support the notion that the disorders share pathophysiological processes as well as vulnerability genes that are related to deficient myelination or abnormal white matter integrity. Interestingly, lithium greatly attenuated the brain volume changes, suggesting that its use may, in fact, obscure even greater similarities in brain pathology between bipolar disorder and schizophrenia (Chapter 3).

Whether patients with schizophrenia and patients with bipolar disorder display overlapping abnormalities in brain volumes and cortical thickness and whether these are caused by shared genetic or environmental influences was further investigated in a combined study of the bipolar and schizophrenic twin cohort of the University Medical Center Utrecht (Hulshoff-Pol et al. 2012). Decreased white matter volume, thinner orbitofrontal and medial temporal cortices, and a thicker temporoparietal cortex seemed to be markers for genetic risk factors that are shared between schizophrenia and bipolar disorder. Right parietal cortical thickness best differentiated disease liabilities for schizophrenia and bipolar disorder: a thicker cortex was associated with increased genetic liability for schizophrenia. Thus, while there was some degree of genetic specificity, the overlapping smaller white matter and common areas of thinner cortex suggested that both disorders share genetic (neurodevelopmental) roots (Hulshoff-Pol et al. 2012) (chapter 8 Addendum).

Taken all together, comparing the results of our twin study in bipolar disorder with the results of the similar twin study in schizophrenia at the University Medical Center Utrecht, we found indications for time-linked overlapping as well as different neurodevelopmental trajectories for bipolar disorder and schizophrenia.

Implications for clinical practice

The aim of this twin study was to investigate putative (genetic and environmental) risk factors for bipolar disorder. A risk factor is a variable associated with an increased risk for developing a disease. Although the word "risk factor" implies causality, the term risk factor includes both causal and predictive factors. Most risk factors are correlational and not necessarily causal, because correlation does not prove causation. Causal risk factors directly reflect the underlying biology of the disease, while other risk factors are used solely for prediction, to identify those at higher risk. Typically in infectious diseases, a single causative agent or primary cause is identified, for example the tubercle bacillus as the cause of tuberculosis. On the other hand, having yellow fingers might predict risk of myocardial infarction. However, yellow fingers don't directly reflect the underlying biology of this disease, but identifies heavy cigarette smokers, a well known risk factor for cardiovascular diseases. So, there is clear distinction between diseases with a single cause and multifactorial diseases as cardiovascular diseases and bipolar disorder, where the risk factor concept is more complex (Stampfer 2004).

Another useful conceptual distinction is between risk factors and markers of early disease. These markers can be predictors of the future occurrence of clinical outcomes, yet they typically are not considered risk factors, but rather are seen as reflections of the disease process. At this time, such markers could potentially be useful in prediction to identify high-

risk individuals for aggressive intervention. In addition, they may be informative for the biological progression of disease (Stampfer 2004).

Identification of a risk factor does not have, necessarily, any immediate clinical implication. If a risk factor is associated with occurrence of disease but is not itself causal, then changing that factor may have no impact on the disease (Stampfer 2004).

Obviously, the findings of our study raise the question of the implications for clinical practice. Our study aimed to identify risk factors for developing bipolar disorder and to determine whether they are genetically or environmental caused, may help clarifying the complex pathogenesis of bipolar disorder and possibly also improving the early identification people at risk for developing bipolar disorder.

In the early identification of psychosis specific instruments and risk criteria have been generated and validated. As the first signs of bipolar disorder often start before adulthood, such efforts are especially important in the vulnerable pediatric and adolescent population (Hauser et al. 2013). Compared to schizophrenia, bipolar disorder has received limited attention in this regard (Conus et al. 2013) and is still in its early stages (Hauser et al. 2013). Family history of BD I and presence of syndromal depression, ADHD, disruptive behaviour disorders, and cyclothymic hypersensitive temperamental traits are risk factors for future bipolar development, but most subjects exhibiting these rather aspecific traits or states do not develop bipolar disorder. Therefore, the identification of endofenotypes (neuroimaging, neurochemistry, and neurocognition) is necessary to further enhance early identification of bipolar disorder in subsyndromal phases in the future (Hauser et al. 2013). In our study we showed that white matter volume decrease is related to the genetic risk of developing bipolar disorder, while environmental factors, including the effects of illness, is related to decreased cortical gray matter volume.

Interestingly for clinical practice, lithium showed considerable effects on the brain changes found in our study, attenuating the decrease in both gray and white matter (Chapter 2), even suggesting that its use may, in fact, obscure similarities in brain pathology between bipolar disorder and schizophrenia. Several reports have suggested a neurotrophic and neuroprotective effect of lithium; its use in bipolar patients has been associated with increases in cortical gray matter (Sassi et al. 2002, Moore et al. 2000) and hippocampal volume (Yucel et al. 2007). In fact, this neuroprotective effect of lithium as also suggested by our study is an important finding to inform the bipolar patients, who have to use lithium medication daily and frequently have concerns whether its use would harm their brain. In fact, available data suggest the opposite: a beneficial effect of lithium (Van der Schot et al. 2009).

Another main finding of our twin study is that autoimmune thyroiditis is related not only to bipolar disorder itself but also to the genetic vulnerability to develop the disorder. Autoimmune thyroiditis, with TPO-Abs as marker, is a possible endophenotype for bipolar disorder (Chapter 4). This finding reveals the etiology of the frequent clinical comorbidity between autoimmune thyroiditis and bipolar disorder. Therefore, this finding helps to understand why autoimmune thyroiditis and lithium exposure are two independent but cumulative risk factors for hypothyroidism in patients with bipolar disorder (Kupka et al. 2002).

These findings are relevant for bipolar patients and their relatives. Relatives of patients with bipolar disorder and autoimmune-thyroiditis have a risk of developing themselves bipolar disorder as well as autoimmune-thyroiditis. As long as they remain asymptomatic, i.e. without mood symptoms or symptoms of thyroid pathology, it is not necessary to take a blood sample for controlling thyroid function and TPO antibodies.

However, when they develop mood symptoms or symptoms of thyroid pathology, they should be aware of this family history of associated bipolar disorder and autoimmune-thyroiditis and a blood sample for controlling thyroid function and TPO antibodies should be considered.

Our finding that temporary underperformance at school in early adolescence may be a genetic marker for the vulnerability to develop bipolar disorder (Chapter 5) does also lead to the question whether this might improve the identification and early intervention of youth at risk for developing bipolar disorder. While 78% of the affected twin pairs displayed temporary underperformance at school, this was also the case in more than half of the healthy control twins (56%)(Chapter 5). Therefore, temporary underperformance at school does not seem suitable to identify youth at risk in the general population. But in a high-risk population of youth with a positive family history of bipolar disorder, temporary underperformance could be an early sign of the genetic risk in the children or an early sign for developing bipolar disorder. These children should be carefully monitored during their youth, especially if they showed some prodromal emotional, social or behavioural problems besides the underperformance. Especially midadolescence seems an important window for identification and intervention of at-risk youth, since the affected twin pairs showed increased risk of underperformance compared to healthy controls after the age of fifteen.

Our findings also suggest that, despite temporary underperformance, ultimately patients developing bipolar disorder show at the end only a slightly lower level of education than healthy controls**.** Thus, although temporary underperformance at school could be an early sign for developing bipolar disorder for those children at higher risk, they may get the positive motivational message of the possibility to reach their maximum level of education, irrespective the possible development of the illness.

Implications for future research

Because of the relatively long period to finish this thesis, several ideas and perspectives for future research after completion this twin study, already have been developed and in the mean time even carried out.

In this way, to study whether the brain abnormalities in bipolar disorder are progressive and whether these changes over time are under genetic or environmental influence, a longitudinal follow-up study of this twin cohort had to be revealed. Bootsman et al. (2015) achieved the first longitudinal twin study, examining genetic and environmental contributions to the association between liability to bipolar disorder (BD) and changes over time in brain structures. The results of this follow-up study are described in chapter 8 Addendum. Similarly, there was a unique opportunity to combine twin cohorts (bipolar disorder, schizophrenia, healthy) of our group, to test the effect of genotype on brain volume (change) and to investigate associations that are either specific for bipolar disorder or schizophrenia or shared between these two illnesses. Hulshoff-Pol et al (2012) performed a study with twins concordant and discordant for schizophrenia or BD, to examine whether the genetic risk of these disorders was reflected in brain volumes and cortical thickness (chapter 8 Addendum).

One of the presumed relevant risk factors for developing bipolar disorder, originally investigated in this twin study was life events with social rhythm disruption. Numerous studies have demonstrated that life events play a role in the onset and course of both unipolar depression and bipolar disorder (Brown and Harris 1989, Hillegers et al. 2004, Malkoff-Schwartz et al. 1998, Kemner et al. 2015). The twins in our study were interviewed with the investigator-based Bedford College Life Events and Difficulties Schedule (LEDS) (Brown and Harris 1978, 1989), a semi structured interview for assessing life events and long

term difficulties in adults. In this way, Kemner et al. (2015) obtained detailed life event information throughout the life span of our twin pairs and tested the influence of life events on first and recurrent admissions in bipolar disorder (chapter 8 Addendum).

The finding of our study that autoimmune thyroiditis is related to the genetic vulnerability of bipolar disorder has thrown new light on the association between mood disturbances and an activated inflammatory response system (IRS). The “Macrophage-T cell theory of depression “ considers an activated inflammatory response system to be a driving force behind mood disorders, because proinflammatory cytokines are capable of destabilizing brain function (Smith 1991, Padmos et al. 2008). Searching for a causal explanation of this association between an activated inflammatory response system and mood disorders, the shared genetic vulnerability for auto-immune thyroiditis and bipolar disorder founded in our study, specifically has given interest to the investigation of genes and gene expression involved in bipolar disorder as well as in the immune system. Padmos et al. (2008) performed such a study of inflammatory gene expression levels in monocytes of bipolar patients (partly recruited from this twin study) and described an inflammatory monocyte gene expression signature in patients with bipolar disorder reflecting their activated inflammatory response system. This mRNA signature represents a set of proinflammatory genes that discriminate bipolar patients from healthy controls. This signature is also present in bipolar offspring, including those who later develop a mood disorder, suggesting that the pro-inflammatory state of monocytes precedes the actual mood symptoms. These findings support the theory that an activated inflammatory response system is a causal factor for mood symptoms in bipolar disorder. Consequently, the altered immune signature could be helpful as a biomarker for disease prediction in individuals genetically at risk for developing bipolar disorder and for detection of those

bipolar patients that could possibly benefit from anti-inflammatory treatment (chapter 8 Addendum). Subsequently, in the bipolar and healthy control twins of our study, Padmos et al. (2009) investigated the contribution of genetic and environmental influences on the association between this monocyte pro-inflammatory state and bipolar disorder. Common environmental factors are the main contributing factors to the association of the pro-inflammatory monocytes with bipolar disorder. When acting on a susceptible genetic background, the environmentally induced pro-inflammatory monocyte activation can be seen as a factor precipitating disease (chapter 8 Addendum). Since the concept of an activated IRS also extends to schizophrenia (Smith and Maes 1995), Drexhage et al. (2010) tested the similar abnormal inflammatory gene fingerprint, founded in bipolar disorder, in the circulating monocytes of naturalistically treated patients with schizophrenia. Their approach shows that monocytes of patients with schizophrenia or bipolar disorder overlap in a high inflammatory set point, but also differ in inflammatory gene expression.

All together, these studies (Vonk et al. 2007, Padmos et al. 2008, 2009, Drexhage et al. 2010) show accumulating evidence that activation of the immune system plays an important role in the pathogenesis of bipolar disorder, contributing to the interesting view of bipolar disorder as a multi-organ inflammatory disease (Leboyer et al. 2012). Ultimately, future research in this field may lead to innovative diagnostic biomarkers for disease prediction, to new methods of prevention and to personalized (anti-inflammatory) treatments.

Chapter 8

Samenvatting

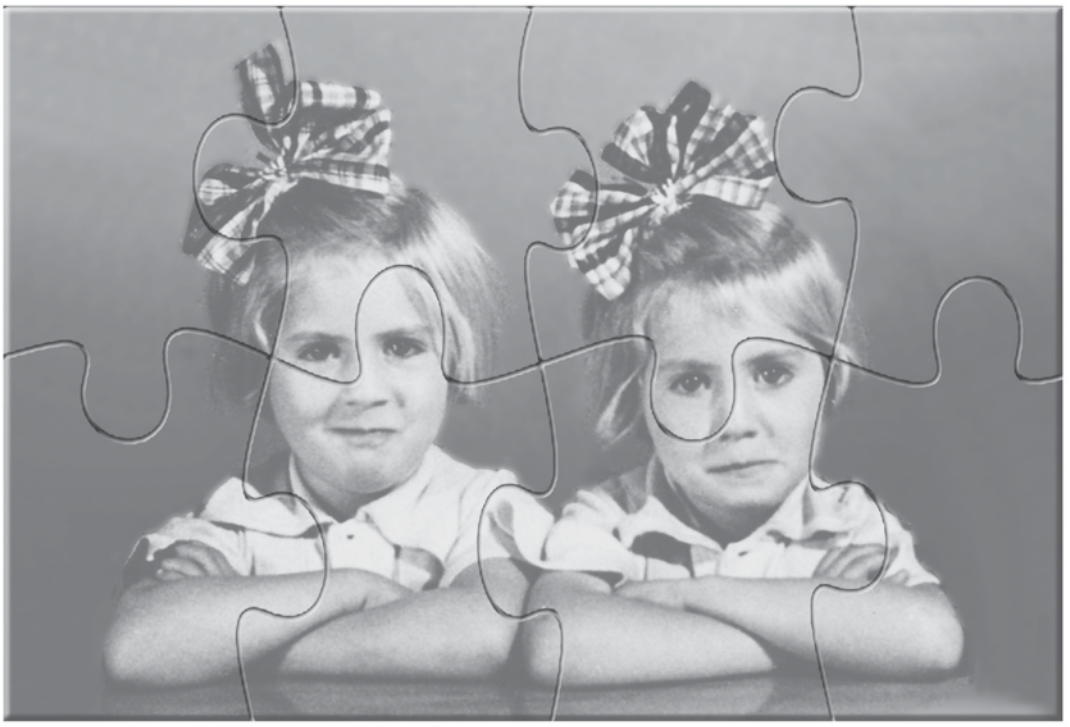
References

Addendum

List of Publications

Dankwoord

Curriculum Vitae



Nederlandse Samenvatting

Inleiding

De bipolaire stoornis (ofwel manisch-depressieve stoornis) is een ernstige en complexe stoornis van de stemming, waarin episodes van (hypo)manie (o.a. verhoogde stemming en overactiviteit) en depressie (o.a. verlaagde stemming en verminderde activiteit) afgewisseld worden met episodes van normale stemming en normaal functioneren. In zowel een depressieve als een manische episode kunnen psychotische verschijnselen zoals wanen en hallucinaties voorkomen.

Er bestaat een grote individuele variatie in de frequentie en ernst van de episodes, maar de aandoening heeft in de meeste gevallen een aanzienlijke invloed op de kwaliteit van leven en het functioneren van zowel de patiënt als de naast betrokkenen (Kupka 2008). De bipolaire stoornis komt bij ongeveer 1-2% van de algemene bevolking voor en begint meestal tussen het 15^{e} en 25^{e} jaar.

Familie- en tweelingstudies hebben aangetoond dat zowel erfelijke factoren als omgevingsfactoren een rol spelen bij het ontstaan van de bipolaire stoornis. De exacte omvang van de afzonderlijke en gecombineerde risicofactoren is echter niet bekend. Endofenotypen kunnen een rol spelen in het ontrafelen van de vragen naar het ontstaan van de bipolaire stoornis. Endofenotypen zijn kenmerken die geassocieerd zijn met de ziekte en die gekoppeld zijn aan het genetische risico voor de ziekte (genotype). Endofenotypen zijn minder complexe en meer meetbare kenmerken in vergelijking met de gedragskenmerken (fenotype) van de bipolaire stoornis, zoals beschreven in de DSM-IV en DSM-5. Neurofysiologische, neuroanatomische, neuropsychologische, endocrinologische of biochemische veranderingen komen vaak samen voor met de bipolaire stoornis en kunnen

dienen als kandidaat endofenotypen voor de bipolaire stoornis.
De bipolaire stoornis dient te worden onderscheiden van andere stoornissen, zoals de (unipolaire) depressie. Bij de patiënten die tijdens hun episodes ook psychotische verschijnselen vertonen, is ook het onderscheid t.o.v. psychotische stoornissen zoals schizofrenie van belang. Daarbij staat ter discussie in hoeverre de bipolaire stoornis en schizofrenie unieke afzonderlijke ziektebeelden zijn, danwel verschillende manifestaties van een gezamenlijk onderliggend proces, c.q. een rijpingsstoornis van de hersenen, met een (deels) gemeenschappelijke genetische achtergrond.

Het doel van de in dit proefschrift beschreven tweelingstudie is om factoren te onderzoeken, die gerelateerd lijken te zijn aan het ontwikkelen van (de ziekte) bipolaire stoornis of aan de kwetsbaarheid (het risico) voor het ontwikkelen van de bipolaire stoornis. Vooral wilden we de relatieve invloed van genetische en omgevingsfactoren op deze (risico)factoren bepalen. We hebben verschillende risicofactoren onderzocht; obstetrische complicaties, structurele hersenafwijkingen, veranderingen in vinger- en handafdrukken, autoimmuun thyroiditis met verhoogde thyroperoxidase antilichamen (TPO-Abs), (onder)presteren op school (als maat van ontwikkeling van cognitief functioneren) en levensgebeurtenissen. Door in het onderzoek gebruik te maken van een tweelingonderzoeksopzet is het mogelijk om de relatieve invloed van genetische en omgevingsfactoren op deze risicofactoren te bepalen. Op deze manier is het eveneens mogelijk kenmerken (markers) vast te stellen voor het genetische risico op het ontwikkelen van een bipolaire stoornis (endofenotypen).
Aanvullend doel van deze tweelingstudie is een vergelijking te maken tussen de bipolaire stoornis en schizofrenie ten aanzien van de onderzochte risicofactoren, door de resultaten van deze studie te vergelijken met de uitkomsten van een vergelijkbare tweelingstudie bij

schizofrenie van het UMC Utrecht (Baare et al., 2001, van Oel et al., 2001, 2002, Hulshoff-Pol et al., 2004).

In een 'klassiek' tweelingonderzoek wordt de overeenkomst van een ziekte of eigenschap (fenotype) binnen monozygote (eeneiige, MZ) tweelingparen vergeleken met de overeenkomst binnen dizygote (twee-eiige, DZ) tweelingparen. MZ tweelingen zijn ontstaan uit een eicel en delen nagenoeg 100% van hun genen, terwijl DZ tweelingen uit twee aparte eicellen ontstaan zijn en gemiddeld de helft van hun genen delen zoals 'gewone' broers en zussen (Martin et al. 1997). Beide tweelingen groeien vaak samen op, waardoor ze ook hun (gezins)omgeving delen, zoals o.a. de ouderlijke zorg en opvoeding, voedingspatroon en sociaaleconomische status. Natuurlijk staan MZ en DZ tweelingen ook bloot aan omgevingsinvloeden die voor ieder individu uniek (kunnen) zijn, zoals ziektes of bepaalde levensgebeurtenissen. Indien in een "klassiek" tweelingonderzoek de mate van overeenkomst (concordantie) van een ziekte of eigenschap bij MZ tweelingen duidelijk groter is dan bij DZ tweelingen, is dit een sterke aanwijzing voor een genetische achtergrond van deze ziekte of eigenschap. In de vorige eeuw toonden diverse tweelingstudies naar de bipolaire stoornis een duidelijke hogere concordantie aan bij MZ tweelingen (40-70%) dan bij DZ tweelingen (0-19%), een belangrijke aanwijzing in die tijd voor een deels genetische achtergrond van de ziekte.

Moderne geavanceerde statistische analyses maken het binnen een tweelingdesign mogelijk de relatieve invloed van genetische en omgevingsfactoren op een eigenschap exacter te bepalen. Indien een eigenschap geassocieerd is met de bipolaire stoornis, kan op dezelfde manier de relatieve invloed van genetische en omgevingsfactoren op de gevonden associatie worden vastgesteld. Zo kunnen gemeenschappelijke genetische of omgevingsfactoren bijdragen aan de ontwikkeling van zowel de eigenschap als de bipolaire stoornis.

In onze tweelingstudie hebben we uiteindelijk 53 bipolaire tweelingparen en 67 gezonde controle tweelingparen onderzocht. MZ en DZ tweelingparen, waarbij bij minimaal 1 tweelinghelft de diagnose bipolaire stoornis gesteld was, konden in de studie worden geincludeerd. Werving van de bipolaire tweelingparen vond tussen 2000-2006 plaats in heel Nederland middels de vereniging voor manisch-depressieve en betrokkenen (VMDB), de 'Lithium-plus' werkgroep als voorloper van het Kenniscentrum Bipolaire Stoornissen (KenBis), collega psychiaters in de diverse instellingen in het land en via advertenties en artikelen in psychiatrische vakbladen en regionale en landelijke kranten.
Op leeftijd en geslacht gematchte gezonde controle tweelingen werden in dezelfde periode geworven vanuit de groep controletweelingen van de schizofrenie tweelingstudie van het UMC Utrecht, vanuit het Nederlands Tweeling Register (NTR) van de VU Amsterdam, via advertenties in regionale en landelijke kranten en via mond op mond reclame in de familie en kennissenkring van de onderzoekers.

In de **hoofdstukken 2 en 3** wordt de studie beschreven naar de vraag in hoeverre genetische en omgevingsfactoren van invloed zijn op de globale en focale hersenafwijkingen bij de bipolaire stoornis. Hiervoor werd bij alle tweelingen een MRI ('Magnetic Resonance Imaging') scan van de hersenen gemaakt. Middels verschillende analysemethoden van de MRI scans hebben we zowel op globaal (volume) en focaal (dichtheid) niveau gekeken naar verschillen tussen patiënten met een bipolaire stoornis en gezonde personen.
Verschillende studies hebben subtiele hersenafwijkingen aangetoond bij de bipolaire stoornis (o.a. McDonald et al., 2004) De meeste structuren maken deel uit van netwerken in de hersenen die betrokken zijn bij o.a. de regulatie van stemming, zoals gebieden in het limbisch systeem en de frontale cortex. Binnen de hersenen kan een onderscheid gemaakt worden in grijze en witte stof. Grijze stof bestaat uit de cellichamen van de neuronen,

waarin de verwerking van informatie plaats vindt. De vezels (axonen) die de neuronen over lange afstand met elkaar verbinden vormen de witte stof, welke informatieoverdracht tussen de neuronen als functie heeft.

Het is al bekend dat hersenstructuren voor een groot deel door genetische factoren worden bepaald. Ondanks de grote genetische invloeden op zowel de hersenen als de bipolaire stoornis is nog nauwelijks bekend of het genetisch risico op het ontwikkelen van de bipolaire stoornis samenhangt met de hersenafwijkingen die gevonden worden bij de bipolaire stoornis. Door gebruik te maken van een tweelingdesign kan gekeken worden of en welk deel van deze associatie verklaard kan worden door genetische en/of omgevingsfactoren.

In **hoofdstuk 2** worden de resultaten van de studie gepresenteerd, waarin bij 50 bipolaire tweelingen en 67 gezonde controle tweelingparen gekeken is naar globale breinvolumes. Allereerst is er een associatie gevonden tussen een verminderd totaal corticaal volume en de bipolaire stoornis. Dalingen in corticale witte stof volume waren gerelateerd aan het genetische risico op het ontwikkelen van de bipolaire stoornis. Omgevingsfactoren hadden vooral invloed op verminderde grijze stof volumes (corticale grijze stof).

Uit andere studies was al gebleken dat het gebruik van het medicijn lithium een grote invloed heeft op de hersenen, vooral op de grijze stof (Moore et al., 2000, 2009). Van onze patiënten gebruikte 2/3 deel het medicament lithium. Na een correctie voor het gebruik van lithium bleken alle gevonden effecten nog sterker te zijn. Deze resultaten laten zien dat het medicament lithium een neuroprotectief effect heeft op de hersenen door het afzwakken van de vermindering van grijze en witte stof bij de bipolaire stoornis.

In **hoofdstuk 3** is er bij 49 bipolaire tweelingen en 67 gezonde controle tweelingparen op focaal niveau gekeken naar de grijze en witte stof dichtheid. De dichtheid van grijze stof was verminderd door het hele brein heen, maar het meest prominent in de frontale en limbische

gebieden. De verminderde witte stof dichtheid werd meer focaal gevonden, vooral in delen van de superieure longitudinale fasciculus (SLF), dit zijn witte stof banen die de frontale gebieden verbindt met occipitale, temporale en parietale delen van het brein. Delen van de SLF waren ook gerelateerd aan het genetische risico op het ontwikkelen van de bipolaire stoornis, zoals ook verminderde grijze stof dichtheid in kleine subgebiedjes van de frontaalkwab. De resultaten suggereren dat vooral (witte stof) pathologie in de frontaal kwab gerelateerd is aan het genetische risico terwijl de meer diffuse vermindering van grijze stof dichtheid aan omgevingsfactoren en de ziekte zelf gerelateerd is.
Het is echter onduidelijk wanneer deze veranderingen in de hersenstructuren optreden, waarbij gesuggereerd wordt dat deze abnormale ontwikkeling van het brein al heel vroeg in de ontwikkeling van het individu kan plaatsvinden, mogelijk zelfs al gedurende de zwangerschap in utero (Vita 2009).

In **hoofdstuk 4** wordt de studie beschreven, waarin gekeken is of veranderingen in vinger– en handafdrukken (dermatoglyfen) zijn geassocieerd met de in onze studie gevonden structurele hersenafwijkingen bij de bipolaire stoornis (beschreven in hoofdstuk 2). Dermatoglyfen worden gevormd tussen de 10^{e} en 17^{e} week van de zwangerschap en eenmaal gevormd blijven ze onveranderd aanwezig gedurende de rest van het leven. De voor ieder mens unieke vormen van de dermatoglyfen worden bepaald door genetische factoren en omgevingsfactoren gedurende de vroege fase van de zwangerschap, zoals o.a. virusinfecties en gebruik van alcohol. Zowel de huid als de hersenen ontwikkelen zich uit het embryonale ectoderm en gedurende de fase van de vorming van de dermatoglyfen vindt er in de hersenen een massale cel migratie plaats van neuronen. Hierdoor kunnen dermatoglyfen worden beschouwd als fossiele bewijzen voor een mogelijk tijdgebonden abnormale ontwikkeling van de hersenen.

Voor onze studie waren we geïnteresseerd of verandering in dermatoglyfen een tijdgebonden (tussen de 10e en 17^{e} week van de zwangerschap) genetische of omgevingsbepaalde marker zou kunnen zijn voor een verstoorde ontwikkeling van de hersenen bij de bipolaire stoornis, leidend tot structurele hersenafwijkingen. Bij 53 bipolaire tweelingen en 51 gezonde controle tweelingparen werden vinger- en handafdrukken afgenomen, welke handmatig werden geanalyseerd door 2 ervaren dactyloscopisten. Allereerst kon bij de verschillende dermatoglyfen nauwkeurig de relatieve invloed van genetische en (unieke of gedeelde) omgevingsinvloeden worden vastgesteld. In onze studie vonden we geen enkele associatie tussen de bipolaire stoornis en die dermatoglyfen, welke door omgevingsinvloeden werden beïnvloed. Dit suggereert dat omgevingsfactoren opererend gedurende de vroege zwangerschap niet bijdragen aan een verhoogd risico op het ontwikkelen van de bipolaire stoornis, conform eerdere studies (Scott et al., 2006). Verder vonden we onafhankelijk van de aan- of afwezigheid van ziekte, een genetisch gemedieerde, negatieve associatie tussen het aantal AB lijntjes in de handpalm en de volumes van de totale hersenen en de corticale grijze en witte stof. Dit duidt erop dat een verhoogd aantal AB lijntjes in de handpalm en kleinere hersenvolumes worden veroorzaakt door gemeenschappelijke genen. Daarnaast bleek een gemeenschappelijke genetische achtergrond voor de associatie tussen bipolaire stoornis en een verhoogd aantal AB lijntjes in de handpalm. Hieruit kan worden afgeleid dat genen betrokken bij de ontwikkeling van de bipolaire stoornis bijdragen aan het verhoogde aantal AB lijntjes, welke werden gevonden bij de bipolaire tweelingen en hun niet-bipolaire tweelingbroers of zussen.

Samengevat blijkt een genetisch gemedieerde, abnormale ontwikkeling van het foetale ectoderm tussen de 10^{e} en 15^{e} week van de zwangerschap gerelateerd aan de kleinere

hersenvolumes bij personen met (een verhoogd risico op de ontwikkeling van) een bipolaire stoornis.

In **hoofdstuk 5** beschrijven we de studie bij 51 bipolaire tweelingparen en 35 gezonde controle tweelingparen naar de aanwezigheid van autoimmuun thyroiditis, een autoimmuun stoornis van de schildklier met een verhoogd voorkomen van antischildklier antilichamen, zogenaamde thyroperoxidase antilichamen (TPO-Abs). Eerder onderzoek had al een verhoogde prevalentie van autoimmuun thyroiditis aangetoond bij patiënten met een bipolaire stoornis in vergelijk met gezonde controles (Kupka et al., 2002). De aanwezigheid van autoimmuun thyroiditis bleek onafhankelijk te zijn van de stemmingsepisode van de patiënt en onafhankelijk van het gebruik van lithium medicatie (Kupka et al., 2002), waardoor verhoogde TPO-Abs kon worden beschouwd als een marker voor de bipolaire stoornis.

Voor ons onderzoek stelden we de vraag of autoimmuun thyroiditis met verhoogde TPO-Abs alleen gekoppeld was aan de ziekte bipolaire stoornis of ook aan de (genetische) kwetsbaarheid voor het ontwikkelen van de bipolaire stoornis. Zo ja, zou dan autoimmuun thyroiditis met verhoogde TPO-Abs een endofenotype kunnen zijn voor de bipolaire stoornis? Er zijn vijf criteria beschreven, waaraan een marker moet voldoen om als endofenotype te worden aangemerkt (Lenox et al., 2002). Een marker moet; 1) geassocieerd zijn met de ziekte in de populatie, 2) erfelijk bepaald zijn, 3) onafhankelijk zijn van de ziekte toestand, 4) in families samen overerven met de ziekte en 5) bij niet aangedane familieleden in een verhoogde prevalentie voorkomen in vergelijk met de algemene bevolking.

Middels bloedonderzoek werd bij de bipolaire en gezonde tweelingparen TPO-Abs bepaald. Bij de tweelingen met een bipolaire stoornis bleek een verhoogde prevalentie van autoimmuun thyroiditis (TPO-Abs >25 U/mL, ook wel TPO positief genoemd) in vergelijking

met gezonde controle tweelingen, conform het eerdere onderzoek (Kupka et al., 2002). Echter ook bij de niet-bipolaire tweelinghelften bleek een hogere prevalentie van autoimmuun thyroiditis dan bij de gezonde tweelingen. Vooral gold dit voor de MZ niet-bipolaire tweelinghelften, welke een vergelijkbare prevalentie hadden als de bipolaire tweelingen. Bij de discordante tweelingparen (de ene tweelinghelft wel een bipolaire stoornis, de andere tweelinghelft niet) vonden we een significant hogere waarde van de TPO antilichamen titer in vergelijk met de gezonde controle tweelingen, waarbij er geen verschil was tussen de bipolaire tweelingen en hun niet-bipolaire tweelinghelften.
Hiermee werd duidelijk dat autoimmuun thyroiditis met verhoogde TPO-Abs niet alleen gekoppeld was aan de ziekte bipolaire stoornis, maar ook aan de (genetische) kwetsbaarheid voor het ontwikkelen van de bipolaire stoornis. Autoimmuun thyroiditis met verhoogde TPO-Abs als marker bleek te voldoen aan de criteria om als endofenotype voor de bipolaire stoornis te mogen worden beschouwd.

De studie beschreven in **hoofdstuk 6** is opgezet om te onderzoeken of een vermindering in cognitief functioneren, in dit geval 'onderpresteren op school', gekoppeld is aan het genetische risico op het ontwikkelen van een bipolaire stoornis en voorafgaat aan het begin van de ziekte. Het genetisch risico om een bipolaire stoornis te ontwikkelen is immers aanwezig vanaf de conceptie, echter de eerste echte ziekte verschijnselen openbaren zich meestal in de late adolescentie of vroege volwassenheid. Het is echter niet bekend op welke leeftijd dit precies is en of er premorbide al symptomen aanwezig zijn.
Middels een specifieke vragenlijst naar schoolopleiding en het zelf onderzoeken van oude rapporten en diploma’s werd bij 53 bipolaire tweelingbanen en 42 gezonde controle tweelingparen gekeken naar het behaalde schoolniveau, het totaal aantal jaren afgemaakte schoolopleiding, de frequentie van onderpresteren en specifiek naar de leeftijd waarop het

onderpresteren plaatsvond. Onderpresteren op school werd op 3 manieren gedefinieerd, namelijk wanneer sprake was van: 1) een verwijzing naar het speciaal onderwijs, 2) een verwijzing naar een lager onderwijsniveau of 3) een doublure.

De resultaten tonen dat er op 13 jarige leeftijd (het begin van het voortgezet onderwijs) geen verschil was in het schoolniveau tussen de bipolaire tweelingparen en de gezonde controle tweeling paren. De bipolaire tweelingparen rondden uiteindelijk wel een (weliswaar gering maar toch significant) kleiner aantal jaren onderwijs af dan de gezonde tweelingparen, respectievelijk gemiddeld 12.9 jaren versus 13.6 jaren. Dit verschil was echter vooral toe te schrijven aan de niet bipolaire tweelinghelften die minder jaren onderwijs afrondden dan hun tweelingbroer of zus, die later een bipolaire stoornis ontwikkelde, namelijk respectievelijk gemiddeld 12.5 jaren versus 13.1 jaren.

De frequentie van onderpresteren was hoger bij de bipolaire tweelingparen dan bij de gezonde tweelingparen en vond plaats op een jongere leeftijd. Binnen de bipolaire tweelingparen was er echter geen verschil in de frequentie van onderpresteren op school tussen de tweelinghelft die later de diagnose bipolaire stoornis zou krijgen en zijn niet-bipolaire tweelingbroer of zus. Onafhankelijk van de ziekte status bleek de leeftijd van onderpresteren in hoge mate genetisch bepaald. We vonden een associatie tussen de bipolaire stoornis en de leeftijd van onderpresteren, die verklaard werd door gemeenschappelijke genetische factoren. Hieruit blijkt dat personen met een verhoogde kwetsbaarheid voor het ontwikkelen van een bipolaire stoornis een verhoogde, door genetische factoren gemedieerde, kwetsbaarheid vertonen om in de vroege adolescentie onder te presteren op school. Het betreft echter kortdurend en niet permanent onderpresteren op school, aangezien de bipolaire tweelingparen uiteindelijk slechts minder dan 1 jaar verliezen op de gezonde tweelingparen. Het onderpresteren op school in de

bipolaire tweelingparen vond plaats ruim 13 jaar voor de eerste duidelijke symptomen van de bipolaire stoornis.
Opmerkelijk in deze studie was de relatief hoge frequentie van onderpresteren bij meer dan de helft van de gezonde controle tweelingen, hetgeen de strenge criteria van het Nederlandse schoolsysteem laat zien om elk schooljaar de vaste standaarden te halen voor overgang naar het volgende niveau. Daarmee is het Nederlandse systeem, in tegenstelling tot bijvoorbeeld het onderwijssysteem in de Verenigde Staten, uitermate geschikt om in deze leeftijdsfase de ontwikkeling van cognitief functioneren middels schoolprestaties jaarlijks te monitoren.
We kunnen concluderen dat (kortdurend) onderpresteren op school in de vroege adolescentie een marker zou kunnen zijn voor de genetische kwetsbaarheid om een bipolaire stoornis te ontwikkelen.

In **hoofdstuk 7** worden de belangrijkste onderzoeksvragen en bevindingen nog eens samengevat. In de discussie wordt achtereenvolgens aandacht besteed aan 1) de generaliseerbaarheid van de resultaten, 2) het methodologische dilemma van het bipolaire fenotype, 3) de verschillende markers voor de genetische kwetsbaarheid voor de bipolaire stoornis (endofenotypen) en 4) de vraag in hoeverre de bipolaire stoornis en schizofrenie overlappende of verschillende trajecten hebben van de rijping van de hersenen.

Voor de generaliseerbaarheid van de resultaten is allereerst de vraag aan de orde in hoeverre het deel uit maken van een tweeling een risicofactor is voor de ontwikkeling van een (specifieke vorm van) bipolaire stoornis. Eerder onderzoek heeft getoond dat deel uit maken van een DZ tweeling wel een risicofactor is voor het ontwikkelen van schizofrenie, maar dat voor de bipolaire stoornis geen verhoogd risico is gevonden bij zowel MZ als DZ

tweelingen (Klaning 1999, 2004).

Helaas zijn de door ons verzamelde tweelingen niet aselect geworven onder de Nederlandse bevolking, maar vormen zij een selectie uit de in Nederland aanwezige bipolaire tweelingparen en gezonde controle tweelingparen. Zo deden er relatief veel vrouwelijke bipolaire tweelingparen (68%) mee, terwijl de prevalentie van bipolaire stoornis niet verschillend is tussen mannen en vrouwen. Anderzijds was de concordantie van bipolaire stoornissen binnen de MZ tweelingen (55%) en DZ tweelingen (24%) wel vergelijkbaar met andere studies (McGuffin et al. (McGuffin et al. 2003), waarmee we onze tweelingen toch als relatief representatief mogen beschouwen. Gezien de selectiebias moet echter toch enige voorzichtigheid worden betracht bij de generaliseerbaarheid van onze resultaten naar de hele groep van patiënten met bipolaire stoornis.

Nauwkeurige definitie van het bipolaire fenotype is een belangrijke factor voor het succes van (genetische) studies bij de bipolaire stoornis. Met de in onze tweelingstudie gebruikte methodologie was het van cruciaal belang om vast te stellen met welke criteria een tweelingpaar als concordant of als discordant werd benoemd. We hebben ervoor gekozen een tweelingpaar als concordant te beschouwen indien bij beide tweelinghelften een diagnose was gesteld van Bipolaire I, Bipolaire II of Bipolaire stoornis NAO (volgens DSM-IV). We hebben overwogen een veel ruimer fenotype van de bipolaire stoornis als concordant te beschouwen en ook patiënten met een diagnose depressieve stoornis of psychotische stoornis te includeren. Echter studies naar de genetische kwetsbaarheid van psychiatrische stoornissen vragen om een nauw gedefinieerd fenotype, leidend tot een meer homogene groep van patiënten, welke meer kans hebben dezelfde kwetsbaarheids genen te delen.
Een volgende methologische kwestie is de vraag na hoeveel tijd je zeker weet dat een discordant paar toch niet alsnog wijzigt in een concordant paar, als bij de aanvankelijk niet-

bipolaire tweelinghelft alsnog na jaren een diagnose bipolaire stoornis wordt gesteld. Bij beloopstudies van tweelingen met schizofrenie bleken de meeste tweelingparen concordant te worden binnen 5 jaar na het ontstaan van schizofrenie bij een van beiden. Voor de bipolaire stoornis zijn deze beloopstudies bij discordante paren echter niet gedaan. Bij onze tweelingen bedroeg het gemiddelde verschil in leeftijd van ontstaan van de bipolaire stoornis binnen de concordante paren 4.9 jaar (mediaan 3 jaar, 75e percentiel 9.2 jaar). Bij de discordante paren bedroeg de tijd tussen de leeftijd van ontstaan van de bipolaire stoornis bij de index-twin en de huidige leeftijd van de niet-bipolaire tweelinghelft gemiddeld 13 jaar (mediaan 10,3 jaar, 25e percentiel 6,6 jaar), waarbij bij 20% van de discordante paren dit minder dan 5 jaar was. Dit suggereert dat het merendeel van de discordante paren van onze tweelingen ook in de toekomst discordant zullen blijven. Dit wordt ondersteund door de vervolgstudie van onze tweelingen, waaraan 15 MZ en 14 DZ tweelingen hebben deelgenomen. Na een beloop van gemiddeld 7 jaar is geen enkel paar gewijzigd van discordant naar concordant of omgekeerd.

Doel van deze tweelingstudie is het zoeken naar markers voor de genetische kwetsbaarheid voor het ontwikkelen van de bipolaire stoornis, zogenaamde endofenotypen. Het vaststellen van endofenotypen is waardevol om de onderliggende neurobiologische ontwikkeling van de bipolaire stoornis beter te begrijpen. Tevens om mensen met een verhoogd risico op de ontwikkeling van een bipolaire stoornis eerder te kunnen identificeren, waardoor mogelijk eerder de diagnose kan worden gesteld.

Onze tweelingstudie laat zien dat de genetische kwetsbaarheid voor de ontwikkeling van een bipolaire stoornis al heel vroeg in de ontwikkeling van de foetus zichtbaar wordt, namelijk door de abnormale ontwikkeling tussen de 10^{e} en 15 e week van de zwangerschap van het embryonale ectoderm, leidend tot veranderingen in dermatoglyfen (verhoogd aantal AB

lijntjes in de handpalm) en veranderingen in hersenstructuren op volwassen leeftijd (verkleining van de volumes van de totale hersenen evenals van corticale grijze en witte stof). In de vroege adolescentie zou (kortdurend) onderpresteren op school een marker kunnen zijn voor de genetische kwetsbaarheid om een bipolaire stoornis te ontwikkelen. Op volwassen leeftijd uit het genetische risico zich door witte stof afwijkingen in de vorm van vermindering van het corticale volume en vermindering van de dichtheid in de (frontale) superior logitudinal fascicule (SLF) en tevens in vermindering van grijze stof dichtheid in kleine subgebieden van de frontaal kwab. Genen betrokken bij de ontwikkeling van een bipolaire stoornis kunnen ook leiden tot het ontstaan op volwassen of oudere leeftijd van autoimmuun thyroiditis met verhoogd TPO-Abs als marker.

De laatste 2 decennia speelt sterk de vraag in hoeverre de bipolaire stoornis en schizofrenie unieke afzonderlijke ziekte beelden zijn of verschillende manifestaties van eenzelfde onderliggend proces. Er is beargumenteerd dat bipolaire stoornis net als schizofrenie beschouwd kan worden als een rijpingsstoornis (neurodevelopmental disorder) van de hersenen (Van Os et al., 1997, Blumberg et al., 2004).
Door de resultaten van onze studie bij de bipolaire stoornis te vergelijken met de uitkomsten van de vergelijkbare tweelingstudie bij schizofrenie van het UMC Utrecht, zijn we in de unieke situatie om de ontwikkeling van het brein bij beide stoornissen te vergelijken op verschillende momenten gedurende deze ontwikkeling.
Bij de tweelingstudie naar schizofrenie bleken omgevingsfactoren tijdens de 10-13^{e} week van de zwangerschap geassocieerd met de kwetsbaarheid voor schizofrenie, aangezien zowel de tweelingen met schizofrenie als hun niet-schizofrene tweeling broers of zussen meer asymmetrie vertoonden van de vingerlijntjes (Van Oel et al., 2001). Daarentegen vonden we bij de bipolaire stoornis juist geen omgevingsinvloeden tijdens deze vroege fase

van de ontwikkeling, maar een genetisch gemedieerde, verandering in de 10-15^{e} week van de zwangerschap van de dermatoglyfen in de handpalm. Dit suggereert een verschillend rijpingstraject van de hersenen bij bipolaire stoornis en schizofrenie in deze vroege fase van de foetale ontwikkeling (10^{e}-15^{e} week).

De rijping van de hersenen gedurende de puberteit en vroege adolescentie kunnen we bij beide stoornissen bestuderen door een vergelijking van de schoolprestaties bij de tweelingen. Bij 90% van de discordante tweelingen met schizofrenie bleek de tweelinghelft, die onderpresteerde op school, de tweelinghelft te zijn die later schizofrenie zou ontwikkelen (Van Oel et al., 2002). Bij de discordante tweelingen met bipolaire stoornis trad onderpresteren juist in gelijke mate op bij de tweelinghelft die later een bipolaire stoornis zou ontwikkelen als bij zijn niet-bipolaire tweelingbroer of zus. De bipolaire tweelingen ronden uiteindelijk een vrijwel gelijk aantal jaren opleiding af als de gezonde controle tweelingen, dit in tegenstelling tot de tweelingen met schizofrenie, welke een duidelijk aantal jaren minder schoolopleiding voltooiden (Van Oel et al., 2002). Zowel bij de bipolaire stoornis als bij schizofrenie gaat het onderpresteren op school vele jaren vooraf aan het optreden van de eerste symptomen van de ziekte. Echter het onderpresteren bij de bipolaire stoornis is slechts tijdelijk, terwijl het onderpresteren bij de schizofrenie meer permanent is, leidend tot een duidelijk lager niveau van afgeronde schoolopleiding. Dit stemt deels overeen met de studie van Maccabe et al. (2006), waarbij excellent presteren op school een risicofactor was voor het ontwikkelen van een bipolaire stoornis en slechte schoolprestaties voor schizofrenie.

Op volwassen leeftijd werden witte stof afwijkingen gevonden zowel bij de tweelingen met schizofrenie als met een bipolaire stoornis en bleken deze witte stof afwijkingen gekoppeld aan het genetisch risico op het ontwikkelen van zowel schizofrenie (Hulshoff-Pol et al., 2004)

als een bipolaire stoornis.

Samenvattend laat de vergelijking van de beide tweelingstudies zien dat er aanwijzingen zijn voor zowel tijdgebonden verschillen als overlap in de rijping van de hersenen bij de bipolaire stoornis en schizofrenie.

Het hoofdstuk wordt afgesloten met een beschouwing over de consequenties van dit onderzoek voor de klinische praktijk en implicaties voor toekomstig onderzoek.

Onze studie naar genetische en omgevingsbepaalde risicofactoren voor de bipolaire stoornis en mogelijk markers voor het genetische risico (endofenotypen) kan bijdragen aan een vroege identificering van personen met een verhoogd risico op het ontwikkelen van een bipolaire stoornis, waardoor de diagnose in een zo vroeg mogelijk stadium kan worden gesteld of idealiter de ontwikkeling van de ziekte kan worden voorkomen.

Zo blijkt kortdurend onderpresteren op school in de puberteit een mogelijk kenmerk (marker) voor het genetisch risico op het ontwikkelen van een bipolaire stoornis, welke voorafgaat aan het pas vele jaren later ontwikkelen van de eerste ziektesymptomen. Gezien echter de hoge prevalentie van tijdelijk onderpresteren bij de gezonde controle tweelingen, is kortdurend onderpresteren op school niet geschikt om personen met een verhoogd risico op bipolaire stoornis te identificeren in de algemene bevolking. Het komt in Nederland met ons schoolsysteem simpelweg te vaak voor. Echter in een populatie van hoog risico kinderen, zoals kinderen van ouders met een bipolaire stoornis, kan het wel een vroeg signaal zijn van het genetisch risico op de ontwikkeling van bipolaire stoornis en deze kinderen dienen daarom zorgvuldig gevolgd te worden. Deze kinderen kunnen op basis van de resultaten van onze studie ook gemotiveerd worden om ondanks tijdelijk onderpresteren hun school opleiding wel te vervolgen teneinde uiteindelijk een vergelijkbaar opleidingsniveau te behalen als hun klasgenoten.

Bij onze studie naar de hersenafwijkingen bleek duidelijk de neuroprotectieve werking van lithium bij de bipolaire stoornis door een vermindering van de afname van het volume van zowel de corticale grijze als witte stof, wat aansluit bij de bevindingen uit eerdere studies (Moore et al., 2000, Yucel et al., 2007). In de dagelijkse praktijk kan deze kennis bijdragen aan het optimaal informeren van de patiënten met een bipolaire stoornis, die dagelijks hun lithium medicatie 'moeten' nemen en veelal zorgen hebben of er geen schadelijke effecten optreden in hun hersenen bij langdurig gebruik. Het tegendeel is dus waar.

Een belangrijke bevinding in onze tweelingstudie is de gemeenschappelijk genetische kwetsbaarheid voor zowel bipolaire stoornis als autoimmuunthyroiditis, waardoor we een beter begrip hebben van de reeds bekende comorbiditeit tussen beide stoornissen. Hetzelfde geldt voor het gegeven dat autoimmuunthyroiditis en lithiumgebruik onafhankelijke cumulatieve risicofactoren zijn bij bipolaire stoornis voor het ontwikkelen van een hypothyroidie (Kupka et al., 2002). Ook familieleden van een patiënt met een bipolaire stoornis hebben een verhoogd risico op het ontwikkelen van een autoimuunthyroiditis met uiteindelijk de mogelijkheid van klachten passend bij hypothyroidie. Het lijkt niet passend om een patiënt met bipolaire stoornis al zijn familieleden te laten adviseren een bloedonderzoek te laten plaatsvinden naar de schildklierfunctie en de TPO-Abs. Dit lijkt echter wel zinvol, indien deze familieleden symptomen ontwikkelen passend bij een stemmingsstoornis of schildklierstoornis.

Door de lange duur van dit promotieonderzoek zijn verschillende ideeën en suggesties voor verder onderzoek al opgepakt, in gang gezet of zelfs al afgerond. In het Addendum van **hoofdstuk 8** worden deze studies beschreven.
De relevante vraag in hoeverre de in onze studie gevonden hersenafwijkingen bij bipolaire

stoornis veranderen in de loop van de tijd en in hoeverre deze veranderingen genetisch of omgevingsbepaald zijn, heeft geleid tot de follow-up studie bij onze tweelingsample, waarvan de bevindingen onlangs zijn beschreven (Bootsman et al., 2015).

Door de tweelingsamples van bipolaire stoornis en schizofrenie samen te voegen kon een rechtstreekse vergelijking worden gemaakt van de hersenafwijkingen en kon worden vastgesteld in hoeverre deze gedeeld werden, danwel specifiek waren voor een van beide stoornissen (Hulshoff-Pol et al., 2012).

Een van de relevante (omgevingsbepaalde) risicofactoren voor de bipolaire stoornis, welke we in deze tweelingstudie hebben onderzocht, zijn life-events met een ontregeling van het sociale ritme. Hiervoor werden alle bipolaire en gezonde controle tweelingen onderzocht met het Life Events and Difficulties Schedule (LEDS) interview (Brown and Harris 1978, 1989), een semi-gestructureerd interview naar life events en langdurige levensproblemen. Recent zijn door Kemner et al. (2015) de eerste resultaten beschreven.

De bevinding in onze tweelingstudie van de gemeenschappelijk genetische kwetsbaarheid voor zowel de bipolaire stoornis als autoimmuun thyroiditis, heeft een nieuw licht geworpen op de reeds bekende associatie tussen stemmingsstoornissen en een geactiveerd immuunsysteem. Hierdoor zijn vooral studies interessant naar genen en genetische expressie betrokken bij zowel de bipolaire stoornis als het immuunsysteem.

Met data van onze tweelingstudie werd op deze manier een ‘bipolaire’ pro-inflammatoire gen-expressie signatuur vastgesteld in monocyten van patienten met een bipolaire stoornis (Padmos et al., 2008), welke met name het gevolg bleek te zijn van omgevingsfactoren (Padmos et al., 2009). Een vergelijking met de monocyten van patiënten met schizofrenie liet zien dat de monocyten bij zowel de bipolaire stoornis als schizofrenie een verhoogd inflammatoir setpoint hebben, maar ook verschillen in inflammatoire genexpressie

(Drexhage et al., 2010). Al deze studies samen tonen de intrigerende samenhang tussen een geactiveerd immuunsysteem en de bipolaire stoornis, passend in de visie van bipolaire stoornis als een multi-oraan inflammatoire ziekte (Leboyer et al., 2012). Verder onderzoek in dit veld zou biomarkers voor de ziekte kunnen opleveren met beter mogelijkheden voor identificering van personen met een verhoogd risico op het ontwikkelen van bipolaire stoornis en mogelijk op het individu toegespitste anti-infammatoire behandeling.

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Addendum

Eight studies, build on data of the original twin cohort are described:

Padmos RC, Hillegers MH, Knijff EM, Vonk R, Bouvy A, Staal FJ, de Ridder D, Kupka RW, Nolen WA, Drexhage HA. A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. Arch Gen Psychiatry 2008, 65(4), 395-407.

Mood disturbances are associated with an activated inflammatory response system. The objective of this study was to identify a discriminating and coherent expression pattern of proinflammatory genes in monocytes of patients with bipolar disorder. A quantitative polymerase chain reaction (QPCR) case-control gene expression study was performed on purified monocytes of 42 bipolar patients, 54 offspring of bipolar patients, 25 healthy control participants and 70 healthy children after having selected 22 discriminating inflammatory genes using whole genome analyses. The monocytes of a large proportion of bipolar patients and offspring of bipolar parents showed an inflammatory gene expression signature. This coherent set of genes opens new avenues for biomarker development with possibilities for disease prediction in individuals genetically at risk and for the subclassification of bipolar patients who could possibly benefit from anti-inflammatory treatment.

Padmos RC, Van Baal GC, Vonk R, Wijkhuijs AJ, Kahn RS, Nolen WA, Drexhage HA. Genetic and environmental influences on pro-inflammatory monocytes in bipolar disorder: a twin study. Arch Gen Psychiatry 2009, 66(9), 957-65.

This twin study shows that common environmental factors are most likely responsible for the proinflammatory monocyte activation seen in bipolar disorder. By using a bivariate model, we showed that of the total covariance between bipolar disorder and PDE4B-asociated signature positivity, 94% (95% CI, 45%-100%) was due to shared common environment and not to genetic effects (h2=1%; 95% CI, 0%-50%) or unique environmental factors (e2=5%; 95% CI,0%-24%). When acting on a susceptible genetic background, the environmentally induced pro-inflammatory monocyte activation can be seen as a factor precipitating disease.

S.M. Kemner, N.E.M. van Haren, F. Bootsman, M.J.C. Eijkemans, R. Vonk, A.C. van der Schot, W.A. Nolen, R.S. Kahn and M.H.J. Hillegers. The influence of life events on first and recurrent admissions in bipolar disorder. International Journal of Bipolar Disorders 2015, 3:6, DOI 10.1186/s40345-015-0022-4

Life events play an important role in the onset and course of bipolar disorder. We collected information about life events and admissions across the life span in 51 bipolar patients. We constructed four models to explore the decay of life events effects on admissions. To test this effect we used the Andersen-Gill model. The relationship between life events and admissions was best described with a model in which the effects of life events gradually decayed by 25% per year. Life event load and recurrent admissions significantly increased the risk of hospitalization. No significant interaction between life event load and number of hospitalizations was found. Life event load and number of admissions increase the risk on (re-)admission. We found no evidence for a lower life event load on recurrent admissions in bipolar disorder (i.e,, Kindling hypothesis).

Florian Bootsman, Rachel Brouwer, Hugo G. Schnack, G. Caroline M. van Baal, Astrid C. van der Schot, R. Vonk, Dorret I. Boomsma, Hilleke E. Hulshoff Pol, Willem Nolen, René S. Kahn and Neeltje E.M. van Haren. Genetic and Environmental Influences on Cortical Surface Area and Cortical Thickness in Bipolar Disorder. Psychological Medicine, 2015, 45, 193–204.

The risk to develop bipolar disorder (BD) has been linked to structural brain abnormalities. The degree to which genes and environment influence the association of BD with cortical surface area and cortical thickness remains to be elucidated. In this magnetic resonance imaging twin study, genetic and environmental contributions to the association between liability to develop BD and surface area, thickness and volume of the cortex was examined. Genetic liability to develop BD was associated with larger cortical surface in limbic and parietal regions, and thicker cortex in central and parietal regions. Environmental factors related to BD were associated with larger and smaller (orbito)frontal, and larger parietal cortical surfaces. Thinner frontal, limbic and occipital cortex, and larger frontal and parietal, and smaller orbitofrontal volumes were also associated with environmental factors related to BD. Cortical volume appeared to be primarily dependent on surface and not thickness.

Florian Bootsman, Rachel Brouwer, Hugo G. Schnack, G. Sanne M. Kemner, Astrid C. van der Schot, R. Vonk, Dorret I. Boomsma, Hilleke E. Hulshoff Pol, Willem A. Nolen, G.Sarkisyan and Neeltje E.M. van Haren. Genetic and environmental contributions to structural brain changes over time in bipolar disorder. Journal of Affective Disorders, submitted.

This is the first longitudinal twin study examining genetic and environmental contributions to the association between liability to bipolar disorder (BD) and changes over time in global brain volume, and global and regional measures of cortical surface area, cortical thickness and cortical volume. The liability to BD was not significantly associated with global or regional measures of structural brain change over time. A trend association was found between BD and increase in cerebral white matter. No significant associations between liability to BD and global or regional brain changes were found, indicating structural brain change to follow a similar trajectory in BD patients and healthy controls. Further study with large cohorts is recommended to assess the influence of genes and environment on subtle morphological abnormalities in global and regional brain measures in BD, where the influence of lithium use on brain measures in BD should be accounted for.

Florian Bootsman, Rachel M. Brouwer, Sanne M. Kemner, Hugo G. Schnack, Astrid C. van der Schot, Ronald Vonk, Manon H.J. Hillegers, Dorret I. Boomsma, Hilleke E. Hulshoff Pol, Willem A.Nolen, René S. Kahn, Neeltje E.M. van Haren. Contribution of genes and unique environment to cross-sectional and longitudinal measures of subcortical volumes in bipolar disorder. European Neuropsychopharmacology, in press.

The influence of genes and environment on the association between bipolar disorder (BD) and subcortical brain volume has rarely been studied. Furthermore, as far as we know, longitudinal twin studies of subcortical brain volume change in BD have not been carried out at all. In this study, we focused on the genetic and environmental contributions to cross-sectional and longitudinal measures of subcortical brain volumes in BD. At baseline, BD was phenotypically and genetically associated with smaller volumes of the thalamus, putamen and nucleus accumbens. BD was not phenotypically associated with subcortical brain volume change over time in any of the examined regions. Heritability of subcortical volumes at baseline was high, which was not the case for volume change. Further evaluation of genetic contributions to abnormalities in subcortical brain volumes assumed to

be involved in emotion processing is recommended. Future studies should take into account the influence of lithium on the brain, in order to more reliably assess morphological abnormalities in BD.

Rosa A, Picchioni MM, Kalidindi S, Loat CS, Knight J, Toulopoulou T, Vonk R, van der Schot AC, Nolen W, Kahn RS, McGuffin P, Murray RM, Craig IW. Differential methylation of the X-chromosome is a possible source of discordance for bipolar disorder female monozygotic twins. Am J Med Genet B Neuropsychiatr Genet 2008, Jun 5, 147B(4), 459-62.

Monozygotic (MZ) twins may be subject to epigenetic modifications that could result in different patterns of gene expression. Several lines of evidence suggest that epigenetic factors may underlie mental disorders such as Bipolar Disorder (BD) and Schizophrenia (SZ). One important epigenetic modification, of relevance of female MZ twins, is X-chromosome inactivation. Some MZ female twin pairs are discordant for monogenetic X-linked disorders because of differential X-inactivation. We postulated that similar mechanisms may also occur in disorders with more complex inheritance like BD and SZ. Examination of X-chromosome inactivation patterns in DNA samples from blood and / or buccal swabs in a serie of 63 female MZ twin pairs concordant or discordant for BD or SZ and healthy MZ controls suggest a potential contribution from X-linked loci to discordance within twin pairs for BD, but is inclusive for SZ. Discordant female bipolar twins showed greater differences in the methylation of the maternal and paternal X-alleles than concordant twin pairs and suggest that differential skewing of X-chromosome inactivation may contribute to the discordance observed for bipolar disorder in female MZ twin pairs and the potential involvement of X-linked loci in the disorder.

Hilleke E. Hulshoff Pol, G. Caroline M. van Baal, Hugo G. Schnack, Rachel G. H. Brans, Astrid C. van der Schot, Rachel M. Brouwer, Neeltje E. M. van Haren, Claude Lepage, D. Louis Collins, Alan C. Evans, Dorret I. Boomsma, Willem Nolen, Rene´ S. Kahn. Overlapping and Segregating Structural Brain Abnormalities in Twins With Schizophrenia or Bipolar Disorder. Archives of General Psychiatry 2012, 69(4), 349-359.

The nosologic dichotomy between schizophrenia and bipolar disorder (BD) as formulated by Kraepelin is currently being questioned, stimulated by the finding that schizophrenia and BD partly share a common genetic origin. Although both disorders are characterized by changes in brain structure, family studies suggest more segregating than overlapping neuroanatomical abnormalities in both disorders. The objectives of this study was to investigate whether patients with schizophrenia and patients with BD display overlapping abnormalities in brain volumes and cortical thickness and whether these are caused by shared genetic or environmental influences. Magnetic resonance imaging findings of monozygotic (MZ) and dizygotic (DZ) twin pairs discordant for schizophrenia, twin pairs concordant and discordant for BD, and healthy twin pairs were compared using structural equation modeling. Brain structures reflect overlapping and segregating genetic liabilities for schizophrenia and BD. The overlapping smaller white matter volume and common areas of thinner cortex suggest that both disorders share genetic (neurodevelopmental) roots.

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S.M. Kemner, M.H.J. Hillegers, **R.Vonk**, A.C. van der Schot, F.Bootsman, W.A. Nolen, The influence of genes and environment on life events in health and disease: the Dutch Bipolar Twin Study. Bipolar Disorders 2013, 15, 87-88.

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A.C. van der Schot, **R. Vonk**, H.E.H. Pol, V. Nuboer, W. Nolen, R.S. Kahn. Volumes of brain structures in twins discordant for bipolar disorder. European Neuropsychopharmacology 2005, 15, S69-S70.

A.C. van der Schot, **R. Vonk**, H.E. Hulshoff Pol, N. Haren, R. Brans, V. Nuboer, W.A. Nolen, R.S. Kahn. Volumes of brain structures in twins discordant for bipolar disorder. Schizophrenia Research, 2006, 81, 6-7.

Vonk R, van der Schot AC, van Oel CJ, Nolen WA, Kahn RS. Dissimilarities in premorbid school performance between bipolar disorder and schizophrenia: A twin study. Schizophrenia Bulletin 2007, 33, 548-548.

R.S. Kahn, H.E. Hulshoff Pol, R. Brans, H.Schnack, W.A.Nolen, A.C. van der Schot, **R.Vonk.** Brain changes in schizophrenia: Are they genetic and are they specific? Biological Psychiatry, 2007, 61, 18S-18S.

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Beste **Arthur**, op de kliniek was je mijn maatje van het eerste uur. Twee enthousiaste, naar ons idee talentvolle, jonge hardwerkende psychiaters met goede ideeën hoe de zorg op de kliniek optimaal vorm te geven. Ondanks dat je veel voor me moest waarnemen bij de start van de studie, heb je me altijd gestimuleerd het door te zetten en kon ik de kliniek met een gerust hart bij je achterlaten. Helaas kun je de afronding van het onderzoek niet meer meemaken.
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Curriculum Vitae

Ronald Vonk werd geboren op 2 juli 1961 te Nijmegen. Hij rondde in 1979 zijn middelbare school af aan het Stedelijk Gymnasium te Nijmegen. Hij studeerde geneeskunde aan de Radboud Universiteit te Nijmegen en behaalde in 1987 het artsexamen. Hij volgde van 1990 tot 1995 de opleiding tot psychiater binnen achtereenvolgens het Delta Psychiatrisch Centrum te Poortugaal, de RIAGG 's-Hertogenbosch en het Erasmus Medisch Centrum Rotterdam.
Vanaf 1995 tot heden werkt hij als psychiater binnen de GGZ instelling Reinier van Arkel te 's-Hertogenbosch, deels op de HIC afdeling en deels op het poliklinische centrum voor bipolaire stoornissen. Binnen Reinier van Arkel fungeert hij als coördinator van het zorgprogramma bipolaire stoornissen en participeert hij in het landelijke Kenniscentrum Bipolaire Stoornissen (KenBis).
In 2000 startte hij samen met Astrid van der Schot in het UMC Utrecht het promotieonderzoek naar de invloed van genen en omgeving op de ontwikkeling van een bipolaire stoornis onder supervisie van prof.dr. Willem Nolen en prof. dr. René Kahn.
Hij is getrouwd met Ingrid Swaans en heeft 3 kinderen, Lars, Sanne en Rens. Zijn vrije tijd besteedt hij veelal aan de basketbalvereniging Bourgondië als bestuurslid, trainer-coach van een jeugdteam en supporter van zijn kinderen.