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# Genetic and environmental influences on focal brain density in bipolar disorder

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**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.**

**Keywords:** twins; bipolar disorder; brain density; structural equation modelling
**Abbreviation:** FWHM = full width at half maximum

## 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

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~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.*, 2007*a,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.*, 2007*a,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 demographic 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 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 co-twin. 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). $T_1$-weighted 3D fast field echo scans with 160–180 contiguous coronal slices (echo time = 4.6 ms, repetition time = 30 ms, flip angle = 30°, $1 \times 1 \times 1.2\ mm^3$ voxels) and $T_2$-weighted dual-echo turbo-spin-echo scans with 120 contiguous coronal slices (echo $time_1$ = 14 ms, echo $time_2$ = 80 ms, repetition time = 6350 ms, flip angle = 90°, $1 \times 1 \times 1.6\ mm^3$ 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.*, 2006*a,b*). Voxel-based morphometry included the following steps: first all individual brain images were registered to a model brain (Hulshof Poll *et al.*, 2006*a*). The cerebral grey and white matter volumes from

**Table 1 Demographic data**

| | Bipolar twin pairs ($n=49$) | | | | Control twin pairs ($n=67$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Monozygotic[a] ($n=23$) | | Dizygotic[b] ($n=26$) | | Monozygotic ($n=39$) | | Dizygotic ($n=28$) | |
| Female, *n* | 34 | | 34 | | 46 | | 31 | |
| Mean (SD) age, years | 36.9 (10.5) | | 43.8 (8.5) | | 39.0 (9.9) | | 39.0 (7.5) | |
| Mean(SD) parental education, years | 10.9 (3.5) | | 11.2 (3.8) | | 11.3 (3.3) | | 11.5 (3.5) | |
| First-degree relative, *n* (%) | 7 (30) | | 4 (15) | | | | | |
| bipolar disorder | 4 (17) | | 9 (35) | | | | | |
| depression | 10 (44) | | 13(50) | | | | | |
| mood disorder | | | | | | | | |
| | BD patient | Co-twin | BD patient | Co-twin | Twin 1 | Twin 2 | Twin 1 | Twin 2 |
| Mean (SD) education, years | 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, *n* (%) | 15 (47) | | 12 (40) | | | | | |
| Handedness (left/right/both), *n* | 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, years[c] | 26.3 (8.9) | | 31.3 (9.9) | | | | | |
| Lithium/no lithium on day MRI, *n*[d] | 26/16 | | 20/10 | | | | | |
| Psychotic symptoms, *n* | 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) | 0.38 (0.71) | 0.48 (0.97) | 0.14 (0.65) | 0.21 (0.57) | 0.13 (0.34) | 0.29 (0.82) | 0.31 (0.75) |

BD = bipolar disorder; IDS = inventory of depressive symptoms (both groups score below for depressive state); YMRS = young mania rating scale.
a Concordant, 9; discordant, 14.
b Concordant, 4; discordant, 22.
c Age of onset: significant difference between monozygotic and dizygotic [$F(1,60)=4.42$, $P=0.040$].
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 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 monozygotic and dizygotic [$F(1,60)=7.9$, $P=0.01$], but both groups below score for depressive state.

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.*, 2006*a*).

# 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).

Influences of genetic (A) and/or unique environmental (E) factors were tested using structural equation modelling (Neale *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 $h^2_{density/BD}$ and bivariate $e^2_{density/BD}$, 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 $\chi^2$ distribution. A $\chi^2$ 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 $\chi^2$ values are $\chi^2 > 25.3$ (df = 1) and $\chi^2 > 27.7$ (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 current 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.

## 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 $\chi^2$ 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 ($r_{ph}$ 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).

Taking into account the univariate heritabilities of both traits (density and bipolar disorder; bivariate $h^2_{grey\ matter\ density/BD}$), 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).

#### 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 ($r_g = -0.19$) and around the postcentral gyrus, part I of the superior longitudinal fasciculus ($r_g = -0.18$). Bivariate heritabilities showed that 40% (left CI 16–65%) and 45% (right CI 3–71%) of the covariances between decreased white matter density in these regions and bipolar disorder 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.

**Table 2 Phenotypic correlations between bipolar disorder and grey matter density, genetic and environmental contributions ($r_g$, $r_e$, $h^2_{\text{grey matterD/BD}}$, $e^2_{\text{grey matterD/BD}}$)**

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

The fixed genetic model for bipolar disorder used 85% for an estimate of the heritability of bipolar disorder. Fifteen percent of the variance in the underlying liability can be explained by unique environmental factors. BA = Brodmann area; BD = bipolar disorder; $e^2_{\text{grey matterD/BD}}$ = bivariate environmentability (unique environmental influence on both bipolar disorder and grey matter density, 1- $h^2_{\text{grey matterD/BD}}$); $h^2$ = heritability (estimated influence of additive genetic influence on grey matter density irrespective of disease). $h^2_{\text{grey matterD/BD}}$ = bivariate heritability [common genetic influence on bipolar disorder and grey matter density (GMD)]; L = left; R = right. $r_e$ = environmental correlation; $r_g$ = genetic correlation; $r_{ph}$ = phenotypic correlations.

a If the 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.

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

| 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/superior longitudinal fasciculus (SLF) I[b] R | 22 (0–45) | 31 | **−0.28** (−0.37 to −0.24) | −0.11 (−1 to 1) | **−0.69** (−0.91 to −0.35) | 16 (0–54) | **84** (46–100) |
| Superior frontal/precentral gyrus/SLF I/II R | **73** (57–84) | 48 | **−0.33** (−0.43 to −0.23) | **−0.19** (−0.32 to −0.05) | **−0.91** (−1 to −0.60) | **45** (16–65) | **55** (35–84) |
| (Pre)central gyrus, SLF II[b] L | 18 (0–44) | 30 | **−0.27** (−0.37 to −0.18) | −0.04 (−1 to 1) | **−0.75** (−0.93 to −0.41) | 5 (0–31) | **95** (69–100) |
| Postcentral gyrus, SLF I[b] L | **49** (28–65) | 32 | **−0.30** (−0.40 to −0.20) | **−0.18** (−0.37 to −0.01) | **−0.66** (−0.89 to −0.29) | **40** (3–71) | **60** (29–97) |
| Postcentralgyrus, corticospinal tract L | **54** (34–69) | 40 | **−0.28** (−0.37 to −0.18) | −0.03 (−0.18 to 0.12) | **−0.97** (−1 to −0.72) | 8 (0–36) | **92** (64–100) |
| Postcentral/supramarginal gyrus, SLF III[b] L | 11 (0–36) | 32 | **−0.30** (−0.39 to −0.20) | −0.21 (−1 to 1) | **−0.65** (−0.91 to −0.26) | 21 (0–36) | **79** (64–100) |
| Inferior frontal gyrus L | **60** (41–73) | 28 | **−0.24** (−0.34 to −0.14) | −0.06 (−0.22 to 0.10) | **−0.81** (−1 to −0.44) | 18 (0–55) | **82** (45–100) |
| Inferior frontal gyrus R | **45** (21–63) | 29 | **0.24** (0.14 to 0.34) | 0.02 (−0.17 to 0.20) | **0.79** (0.46 to 0.96) | 6 (0–44) | **94** (56–100) |
| Superior occipital gyrus/optic radiation R | 24 (0–45) | 28 | **−0.26** (−0.35 to −0.16) | 0.04 (−0.23 to 1) | **−0.81** (−0.97 to −0.47) | 6 (0–29) | **94** (71–100) |

a The white matter peaks were overlaid onto post-mortem histological probability maps (Zilles, 2002).
b 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).

### 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 ($r_e$) and bivariate $e^2_{\text{white matter density/BD}}$ are presented in Table 2 for grey matter and in Table 3 for white matter. Environmental correlations ranged from $r_e = -0.63$ in the left lingual gyrus (CI −0.86 to −0.27) for grey matter density up to $r_e = -0.97$ (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 environmental factors, $r_e = 0.24$ (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 environmental 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

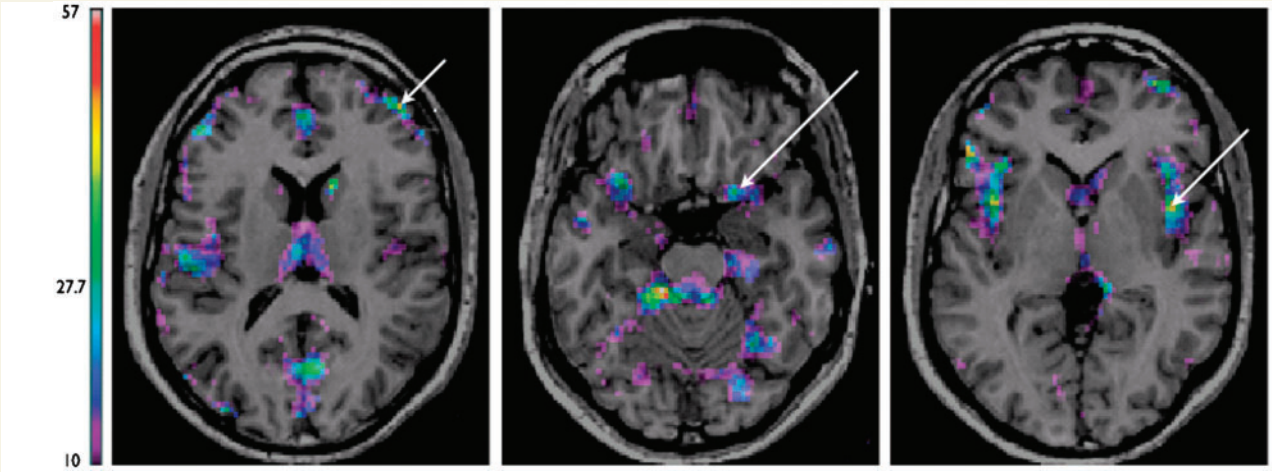


**Figure 1** Negative phenotypic associations between liability to bipolar disorder and grey matter density ($r_{ph}$). Phenotypic correlations between liability to bipolar disorder and grey matter density. Areas with a significant genetic contribution are indicated by an arrow. For visualization purposes, $\chi^2$ values in this picture range from 15 to 57 (significant $\chi^2 > 27.7$). *Left*: right medial/dorsolateral prefrontal gyrus. Brodmann areas 9, 46. Peak value $\chi^2 = 44$, $r_{ph} = -0.33$, $r_g = -0.18$, $r_e = -0.81$. *Middle*: right medial orbital gyrus. Brodmann area 11. Peak value $\chi^2 = 32$, $r_{ph} = -0.17$,$r_g = 0.22$, $r_e = -0.95$. *Right*: right insula, Brodmann area 13. Peak value $\chi^2 = 44$, $r_{ph} = -0.33$, $r_g = -0.17$, $r_e = -0.83$.

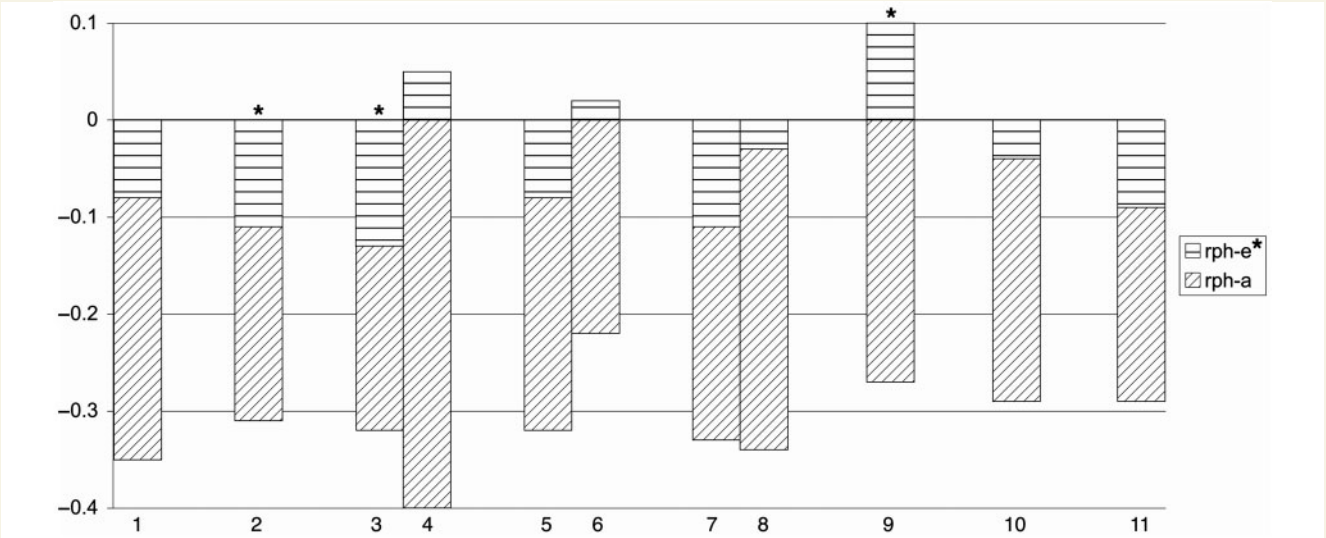


**Figure 2** Phenotypic correlations between bipolar disorder and grey matter density in the frontal lobe. Asterisk indicates significant bivariate heritability indicating common genetic influence to both bipolar disorder and decreased or increased grey matter density in frontal lobe regions. All regions also showed a significant bivariate environmentability (Table 2). 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**. L = Left; R = Right.

(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.*, 2007*a*, *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

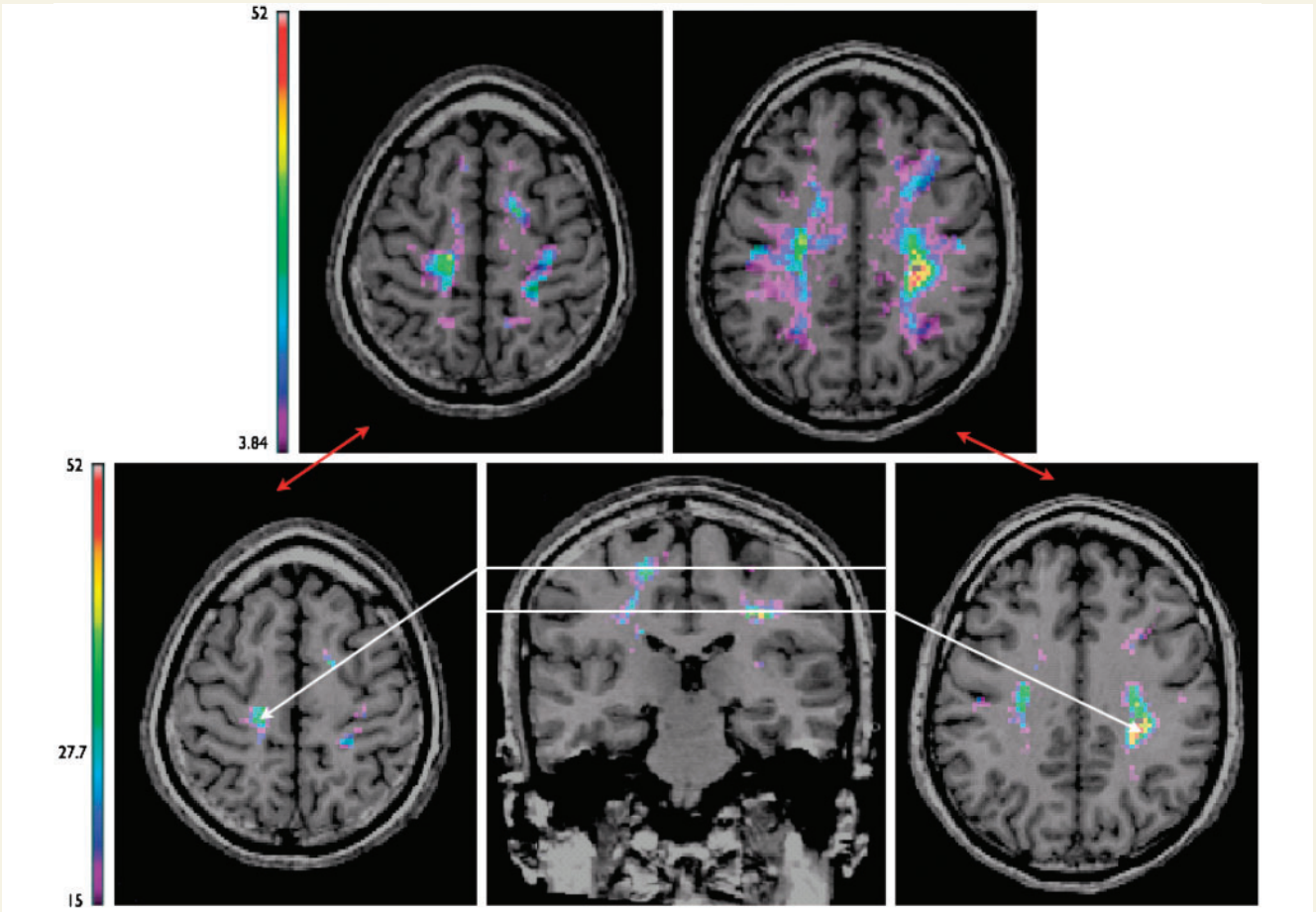


**Figure 3** Negative phenotypic correlations between liability to bipolar disorder and white matter density. The top two pictures show the superior longitudinal fasciculus at uncorrected level ($\chi^2 > 3.84$). The bottom show the two regions within the superior longitudinal fasciculus where a significant contribution of genetic factors was found. *Left*: postcentral gyrus, superior longitudinal fasciculus I. MNI coordinates −21, −32, 56. peak value $\chi^2 = 32$, $r_{ph} = -0.30$, $r_g = -0.18$, $r_e = -0.66$. *Right*: superior frontal, precentral gyrus, superior longitudinal fasciculus I/II. MNI coordinates 27, −36, 36, peak value $\chi^2 = 48$, $r_{ph} = -0.33$, $r_g = -0.19$, $r_e = -0.91$.

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 fronto-limbic 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.*, 2003*b*; 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, 2007*a*) 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-Arán *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

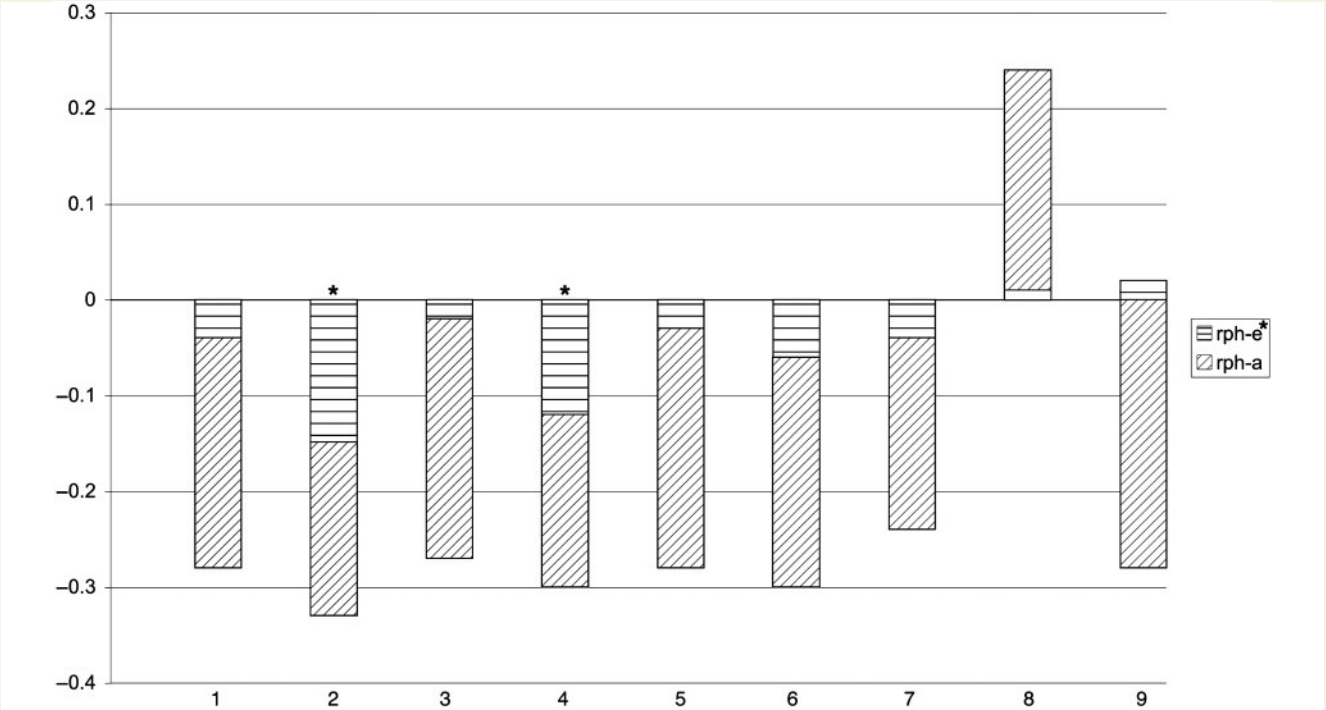


**Figure 4** Phenotypic correlations between bipolar disorder and white matter density, *rph*-a and *rph*-e. Combining the information from $r_g$ and $r_e$ with $h_2$ and $e_2$, the phenotypic correlation (*rph*) can be decomposed into a genetic contribution (*rph*-a) and an environmental contribution (*rph*-e). Asterisk indicates 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 3). 1 = superior frontal gyrus, superior longitudinal fasciculus I, R; 2 = superior frontal gyrus, precentral gyrus, superior longitudinal fasciculus II, R; 3 = (pre)central gyrus, superior longitudinal fasciculus II, L; 4 = postcentral gyrus, superior longitudinal fasciculus I, L; 5 = postcentral gyrus, corticospinal tract, L; 6 = postcentral/supramarginal gyrus, superior longitudinal fasciculus II, L; 7 = inferior frontal gyrus, L; 8 = inferior frontal gyrus, R; 9 = superior occipital gyrus, optic radiation, R. R = Right; L = Left.

prefrontal areas encompassing fronto-striatal 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.*, 2009*a*, *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, 2007*b*; 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.*, 2003*a*,*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.

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# Supplementary material

Supplementary material is available at *Brain* online.

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