Journal of Psychiatric Research 45 (2011) 76–82

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

White matter hyperintensities in affected and unaffected late teenage and early adulthood offspring of bipolar parents: A two-center high-risk study

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ARTICLE INFO

Article history:
Received 23 November 2009
Received in revised form
12 April 2010
Accepted 20 April 2010

Keywords:
White matter hyperintensities
MRI
Bipolar disorders
High-risk
Early onset
Endophenotype
Biological risk factor

ABSTRACT

Background: White matter hyperintensities (WMHs) are among the most replicated neuroimaging findings in bipolar disorder (BD). It is not clear whether these lesions are an artifact of comorbid conditions, or whether they are directly associated with the disorder, or even represent biological risk factor for BD.
Methods: To test whether WMHs meet criteria for an endophenotype of BD, we conducted a high-risk design study and recruited 35 affected, 44 unaffected relatives of bipolar probands (age range 15–30 years), matched by age and sex with 49 healthy controls without any personal or family history of psychiatric disorders. The presence of WMHs was determined from Fluid Attenuated Inversion Recovery (FLAIR) scans acquired on a 1.5 Tesla scanner using a validated semi-quantitative scale.
Results: We found mostly low grade WMHs in all groups. The proportion of WMH-positive subjects was comparable between the unaffected high-risk, affected familial and control groups.
Conclusion: White matter hyperintensities did not meet criteria for an endophenotype of BD. Bipolar disorder in young subjects without comorbid conditions was not associated with increased rate of WMHs.

1. Introduction

White matter hyperintensities (WMHs) represent the change of water content in white matter of the brain that appears as foci of high signal intensity on Fluid Attenuated Inversion Recovery (FLAIR) or T_2 weighted magnetic resonance images. These lesions have diverse ethiopathogeneses. White matter hyperintensities are associated with cardiovascular and metabolic (Awad et al., 1986; Sullivan et al., 1990), as well as demyelinating disorders, such as multiple sclerosis (Barkovich, 2000). They are also present in neurodevelopmental conditions, including attention deficit/hyperactivity disorder (Lyoo et al., 2002), and are associated with known genetic underpinnings, such as the *NOTCH3* gene mutation (Opherk et al., 2006), apolipoprotein E (Kuller et al., 1998) or angiotensin-converting enzyme (Markus et al., 1995) gene polymorphisms.

White matter hyperintensities are among the most replicated structural neuroimaging findings in bipolar disorder (BD) (Beyer et al., 2009). Many of the above-mentioned conditions associated with WMHs, however, are also frequent comorbidities of BD, which makes the interpretation of increased rate of WMHs in BD challenging. It is not clear whether WMHs in BD represent inherited biological risk factor/endophenotype for BD or are secondary to the burden of illness, co-morbid conditions or medication exposure (Hajek et al., 2005).

One of the best ways to eliminate confounding effects of comorbid conditions, illness burden, and to test whether WMHs meet criteria for an endophenotype is by studying both affected and unaffected offspring of bipolar parents around the typical age of onset of BD (high-risk design). To our knowledge, no true high-risk design studies of WMHs in BD exist. One previous investigation found high proportion of subjects with WMHs among both affected and unaffected relatives in a single family multiply affected with BD. However no control group was included to assess differences from the general population (Ahearn et al., 1998). A second study found a higher proportion of subjects with WMHs in affected siblings compared to unaffected siblings and healthy controls

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doi:10.1016/j.jpsychires.2010.04.019

(Gulseren et al., 2006). The majority of affected subjects, however, had no family history of mood disorders. From the available data it is thus not possible to assess whether WMHs represent an endophenotype of BD. Carefully controlled high-risk design studies are therefore needed to elucidate the role of WMHs in BD and to provide insight into the pathophysiology of mood disorders.

In order to test whether WMHs represent an endophenotype of BD, we studied late teenage and early adulthood offspring of bipolar parents both affected and unaffected with mood disorders. We controlled for known confounders (higher age, comorbid conditions, medication, and burden of illness) in the design of the study. If WMHs are an endophenotype of BD, than significantly more affected offspring of bipolar parents would show WMHs relative to controls, with intermediate proportion of WMH-positive subjects in unaffected offspring of bipolar parents.

2. Methods

2.1. Study design

This is a study of subjects with genetic liability for BD using a high-risk design, i.e. a studying affected and unaffected relatives of bipolar patients around the typical age of onset of BD. To be considered an endophenotype, a specific abnormality should be associated with increased genetic liability for the illness, irrespective of affected status, i.e. should be detectable in unaffected relatives as well (Hajek et al., 2005). However, the average liability among unaffected relatives decreases with age, as those with higher liability gradually become affected. Therefore, it is important to include relatively young subjects, ideally at an age lower than the typical age of onset. The inclusion of affected subjects in the sample is also important as it helps to establish association with the illness as well as co-segregation between the illness and abnormality within families (Gottesman and Gould, 2003). Again the affected subjects should be young, as the age close to the onset of their illness minimizes the impact of the illness- and treatment-related factors.

Participants for this study were recruited from two sites: Halifax, Nova Scotia, Canada and Prague, Czech Republic.

2.2. Halifax dataset

2.2.1. Probands

The subjects were recruited through adult bipolar probands according to methods described elsewhere (Duffy et al., 2009, 2010, 2002). Briefly, families were identified through adult probands with bipolar type I (BD I) and type II disorder (BD II), who participated in previous genetic studies and were recruited from outpatient clinics at the Queen Elizabeth II Health Center in Halifax. Each proband completed the Schedule for Affective Disorders and Schizophrenia – lifetime version interview (SADS-L) (Endicott and Spitzer, 1978) conducted by research psychiatrists blind to the identity of the subjects. The final DSM-IV diagnoses were decided using all available clinical materials in a blind consensus fashion by an independent panel of senior clinical researchers. Similar to previous studies, we included probands with BD II (Duffy et al., 2002; Todd et al., 1996), who were similar in their clinical characteristics to patients with BD I. They experienced a low prevalence of comorbid conditions and an episodic course of illness. Bipolar II probands differed from bipolar I subjects only in severity of mania. Family studies using similarly narrow diagnoses generally found BD II to be a part of the same genetic spectrum as BD I (Gershon et al., 1982).

2.2.2. Affected familial subjects

We recruited 20 affected relatives of the above-described probands. These participants came from multiplex families (more than one member affected with BD), and had one parent affected with a primary mood disorder, whereas the other parent was unaffected. Depending on their age, the subjects were interviewed by a child/adolescent or adult psychiatrist using KSADS-PL (Kaufman et al., 1997) or SADS-L format. Diagnoses were made based on DSM-IV, as well as Research Diagnostic Criteria in a blind consensus review that included at least two additional research psychiatrists using all available clinical material. As part of the high-risk study, offspring are re-assessed annually or at any time symptoms develop. The familial affected subjects met criteria for a lifetime Axis I diagnosis of mood disorders (i.e. personal history of at least one episode of depression, hypomania, or mania meeting full DSM IV criteria), or in one case, psychosis not otherwise specified. This definition of affected subject stems from clinical high-risk studies by us and others, which clearly showed, that unipolar depression is most typically the first manifestation of BD in high-risk offspring (Duffy et al., 2009, 2010, 2002) (Hillegers et al., 2005) and about 70% of depressed first-degree relatives of bipolar probands are in fact bipolar (Blacker et al., 1993). Since the single subject with psychosis not otherwise specified had a family history of BD I and no family history of schizophrenia, his presentation is also likely an antecedent of BD. At the time of scanning, all affected subjects were allowed to continue taking their psychotropic medications and all were in remission, as determined by clinical interviews and functioning at school or work, and absence of depressive or manic syndrome (Clinical Global Impression—Severity of Illness score of 0 or 1).

2.2.3. High-risk unaffected group

We recruited 24 high-risk unaffected relatives of the above-described probands. These participants were considered to be at increased genetic risk for development of BD, because they came from multiplex families (more than one member affected with BD), and had one parent affected with a primary mood disorder, whereas the other parent was unaffected. Depending on their age, the subjects were interviewed by a child/adolescent or adult psychiatrist using KSADS-PL (Kaufman et al., 1997) or SADS-L and the interview was discussed in a blind consensus review that included at least two additional research psychiatrists. As part of the high-risk study, offspring are re-assessed annually or at any time symptoms develop. The high-risk unaffected subjects had no lifetime history of any psychiatric disorder.

Exclusion criteria for all groups included: 1. history of closed head injury resulting in loss of consciousness; 2. untreated active medical illness (e.g. diabetes); 3. identified learning disability or diagnosis of Attention Deficit/Hyperactivity Disorder (ADHD); 4. substance-related disorder within the past 6 months; 5. lifetime history of substance dependence; 6. history of any neurological disease; 7. presence of any MRI exclusion criteria.

2.2.4. Controls (offspring of well parents)

Thirty-one healthy offspring from families without any history of psychiatric disorder were recruited by word of mouth. Healthy controls had similar characteristics to the experimental groups with regards to age, sex, and sociodemographic background. Similar to the protocol described above, they were interviewed by a child/adolescent or adult psychiatrist, and determined to be well upon blind consensus review. Exclusion criteria for this group were the same as for the experimental groups with the addition of exclusion of personal or family history of psychiatric disorders.

All subjects were screened for personal and treatment history of comorbid somatic conditions including diabetes, hypertension, and hypothyroidism. We did not screen for personal history of migraines. Prior to conducting the assessments, all interviewers underwent extensive training consisting of participation in interviews, interviews under supervision, and blind consensus diagnostic reviews.

After complete description of the study, written informed consent was obtained from every subject. The study was approved by the Research Ethics Boards of IWK Health Center and Capital District Health Authority, Halifax, Nova Scotia.

2.3. Prague data set

2.3.1. Probands

Subjects were identified through adult probands with BD I or BD II who participated in the Czech Bipolar Disorder Case Registry, a database of patients with BD confirmed by SADS-L interviews (Endicott and Spitzer, 1978) when entering. Subsequent to registration, all subjects were prospectively followed up. Thirty-two families, in which one of the parents was affected with BD (27 BD I, 5 BD II), participated in this study. In nine cases, the parents were not available for direct interviews (e.g. death, health complications). Diagnosis of these parents was established based on available hospital charts and information from treating psychiatrist and family members.

2.3.2. Affected familial subjects

We recruited 15 affected offspring of the above-described bipolar probands. Subjects were interviewed using the SADS-L interview conducted by experienced research psychiatrists (T.N, M.K.) and where available, hospital records were also reviewed. The familial affected subjects met criteria for a lifetime Axis I diagnosis of mood disorders (i.e. personal history of at least one episode of depression, hypomania, or mania meeting full DSM IV criteria). This definition of affected subject stems from clinical high-risk studies by us and others, which clearly showed, that unipolar depression, is most typically the first manifestation of BD in high-risk offspring (Duffy et al., 2009, 2010, 2002) (Hillegers et al., 2005) and about 70% of depressed first-degree relatives of bipolar probands are in fact bipolar (Blacker et al., 1993). At the time of scanning, all affected familial subjects were allowed to continue taking their psychotropic medications, and all were in remission, which was determined according to current symptoms description in SADS-L interview (Endicott and Spitzer, 1978) conducted ± 1 week from the MRI scanning.

2.3.3. Unaffected high-risk subjects

We recruited 20 unaffected high-risk offspring of the above-described bipolar probands. These participants were considered to be at increased genetic risk for development of BD, because they had a bipolar parent. Subjects were interviewed using the SADS-L interview conducted by experienced research psychiatrists (T.N, M.K.). The unaffected high-risk subjects had no lifetime history of any psychiatric disorders.

Inclusion criterion for all groups was the age range of 15 to 30 years. Exclusion criteria were 1. any serious medical illness (e.g. Cushing's disease, conditions treated with corticosteroids); 2. neurological disorder (e.g., epilepsy, head trauma with loss of consciousness, demyelinating disorders); 3. substance abuse or dependence during last 6 months (except nicotine dependence); 4. presence of any MRI exclusion criteria.

2.3.4. Controls (offspring of well parents)

Through advertisement, we recruited 18 healthy offspring from families without any history of psychiatric disorders. Healthy controls had similar characteristics to the experimental groups with regards to age, sex, and sociodemographic background. Similar to the protocol described above, they were interviewed by an adult psychiatrist in accordance with SADS-L format, and determined to be well. Negative psychiatric family history was evaluated by acquiring family history from the subjects, and if possible, also from one of the parents. Exclusion criteria for this group were the same as for the other experimental groups with the addition of exclusion of a personal or family history of psychiatric disorders.

All subjects were screened for personal and treatment history of comorbid somatic conditions including diabetes, hypertension, hypothyroidism, and migraine.

Following complete description of the study to the subjects, written informed consent was obtained, prior to inclusion in the study. The study was reviewed and approved by The Prague Psychiatric Centre Institutional Review Board and was performed in accordance with 1964 Declaration of Helsinki.

3. MR procedures

3.1. Scanning protocol

3.1.1. Halifax data

All MR scans were preformed on a General Electric 1.5 Tesla Signa System equipped with standard single- channel head coil. Following the localizer scan, a FLAIR sequence (flip angle $= 90^\circ$, TI $= 2200$, TE $= 140$ ms, TR $= 9000$ ms, FOV $= 22$ cm $\times$ 22 cm, matrix $= 256 \times 256$ pixels, NEX $= 3$, no inter-slice gap, 28 images with 5 mm slice thickness) was acquired.

3.1.2. Prague data

All MR scans were performed on a General Electric 1.5 Tesla Signa System equipped with standard single- channel head coil. Following the localizer scan, a FLAIR sequence (flip angle $= 90^\circ$, TI $= 2000$, TE $= 127$ ms, TR $= 8000$ ms, FOV $= 24$ cm $\times$ 24 cm, matrix $= 256 \times 256$ pixels, NEX $= 2$, no inter-slice gap, 54 images with 3 mm slice thickness) was acquired.

3.1.3. Hyperintensity measurement

FLAIR images were reconstructed using NeuroLens software (Hoge and Lissot, 2004). Scans were rated for the presence of WMHs by an experienced neuroradiologist (M. S.) blind to identity and group (Fig. 1) assignment of the subjects. Scans were evaluated two times (92.7% agreement between the two assessments) by the same radiologist (M.S.). Criteria for WMH grading followed the Fazekas scale (Fazekas et al., 1987) with the modification of separating WMHs close to cortex from lesions occupying deep white matter tracts:

1. Periventricular hyperintensity (PVWMHs): absent, (1) 'caps' or pencil-thin lining, (2) smooth 'halo', (3) irregular periventricular hyperintensity extending into the deep white matter.
2. Deep white matter hyperintensity (DWMHs): absent, (1) punctuate foci, (2) beginning confluence of foci, (3) large confluent areas.
3. Subcortical white matter hyperintensity (SCWMHs): absent, (1) punctuate foci, (2) beginning confluence of foci, (3) large confluent areas.

3.2. Statistical analyses

Analyses were completed using BMDP statistical software. Due to small numbers of subjects with WMHs, and in order to decrease the number of comparisons, we grouped the DWMHs, SCWMHs, and PVWMHs into one category: 'presence of WMHs: yes vs. no'.

To compare categorical demographic variables, proportions of subjects with WMHs between groups (affected familial, unaffected high-risk, control subjects), as well as in medicated versus non-medicated subjects, in subjects with family history of BD I versus BD II, and in subjects with versus without family history of

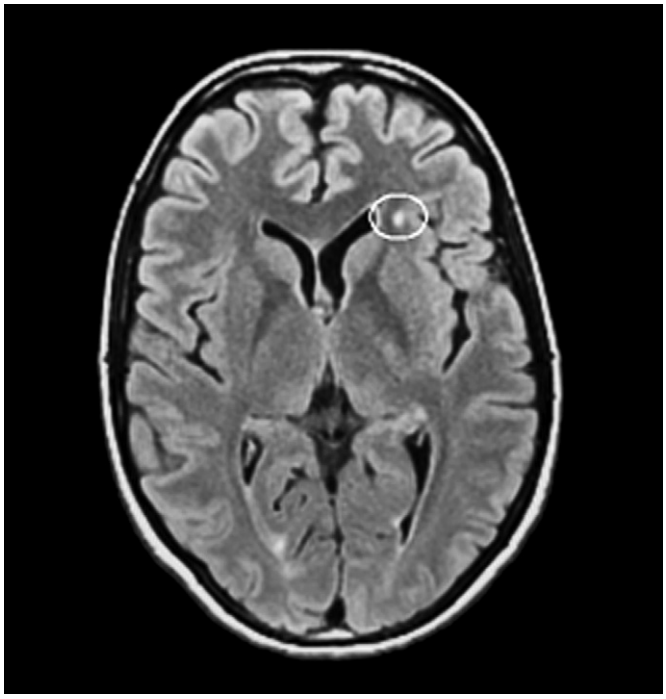

Fig. 1. Typical white matter hyperintensity from one of the studied subjects.

psychosis, we used Pearson Chi-Square test (χ^2). To compare age between groups (unaffected high-risk vs. affected familial vs. controls), we performed one-way ANOVA. The statistical significance level was set at $p < .05$, two tailed. For the a priori hypothesis (i.e. comparing proportion of subjects with WMHs in affected familial, unaffected high-risk, and control groups), we performed separate analyses for each site (i.e. Halifax and Prague) and for the combined dataset, in order to evaluate consistency of findings between the sites, as well as to maximize statistical power. In order to maximize power and decrease the number of tests, exploratory analyses regarding effects of sex, medication, family history of psychotic mood disorders, or bipolar disorders subtype on presences of WMHs were completed only in the combined dataset.

We carried out a priori sample size calculation to estimate the number of subjects needed to achieve sufficient power (80%) to detect the effect size reported in single previous study similar to ours in design and demographic characteristics of the sample (Gulseren et al., 2006), as statistically significant (alpha = 0.05). As a measure of effect size we used $w = \sqrt{\sum_{i-1}^{m} \frac{(p0_i - p1_i)^2}{p0_i}}$ where m denotes the number of cells, p0(i) and p1(i) denote the cell probabilities for the *i*th cell according to H_0 and H_1, respectively (Cohen, 1988). We also performed a sensitivity analysis to calculate the smallest effect size (w) detectable with our sample of 128 subjects in three groups, as statistically significant (alpha = 0.05). We used G-Power software for these analyses (Erdfelder et al., 1996).

4. Results

4.1. Demographics

Affected familial, unaffected high-risk subjects and controls did not differ significantly in age, sex, or in family history in the Halifax, Prague or in the combined dataset. The two datasets were comparable in the proportion of females and age. None of the subjects had any personal or treatment history of cardiovascular disorders or diabetes. One control in the Prague dataset had a personal history of migraines. We did not screen for migraines in the Halifax dataset.

For detailed description of the subjects, see Table 1.

4.2. Description of WMHs

In the combined data, fifteen subjects had one lesion, four had two lesions, one had three lesions, two had four lesions, one subject had five lesions, and one healthy control showed multiple lesions. One control had a grade 3 lesion, four subjects had grade 2 lesions (three unaffected high-risk and one control), and twenty-one subjects had grade 1 lesions (six unaffected high-risk, six affected familial, and nine controls).

With respect to location, nine subjects had PVWMHs (four unaffected high-risk, one affected familial, and four controls), five had DWMHs (one unaffected high-risk and four controls), and sixteen subjects had SCWMHs (four unaffected high-risk, six affected familial and six controls). Seventeen subjects had WMHs in the left hemisphere (six unaffected high-risk, four affected familial, and seven controls), while sixteen subjects had WMHs in the right hemisphere (five unaffected high-risk, four affected familial, and seven controls). Fourteen subjects had lesions in the frontal lobes (four unaffected high-risk, five affected familial, and five controls), one control had a lesion in the parietal lobe, one affected offspring and one control had lesions in the occipital lobe, and five subjects (three unaffected high-risk and two controls) had lesions in multiple lobes.

4.3. Frequency of WMHs

Since the numbers of WMHs per group were small, we grouped the PVWMHs, DWMHs, and SCWMHs into one category WMHs 'yes vs. no'. There were no differences in proportions of subjects with WMHs among unaffected high-risk, affected familial, and control subjects in the Halifax, Prague, or combined datasets ($\chi^2 = 0.80$, DF = 2, $p = 0.67$; $\chi^2 = 0.36$, DF = 2, $p = 0.83$; $\chi^2 = 0.44$, DF = 2, $p = 0.80$, respectively). The relative risk (RR) for having WMHs in unaffected high-risk relative to control subjects was RR = 0.81, 95% Confidence Interval (CI) = 0.35–1.83 and RR = 0.76, 95% CI = 0.31–1.87 for affected familial versus control subjects. The same pattern of numerically, but not statistically significantly fewer subjects with WMHs in affected familial and unaffected high-risk subjects relative to controls was seen in each dataset, without significant differences in rates of WMHs between sites.

In the combined dataset, among subjects with WMHs, there were more females (84%) compared to males (16%) ($\chi^2 = 5.00$, DF = 1, $p = 0.03$). The increased frequency of females with WMHs was seen in each group (affected familial, unaffected high-risk, control subjects), and each site without differences between affected familial, unaffected high-risk and control subjects or between sites in the proportion of WMH-positive females.

There were no differences in proportions of WMH-positive subjects among offspring with vs. without family history of psychotic mood disorders ($\chi^2 = 0.20$, DF = 1, $p = 0.66$), or among subjects with family history of BD I versus subjects with family history of BD II ($\chi^2 = 2.34$, DF = 1, $p = 0.12$). Excluding the medicated subjects did not change the results and there were no differences between medicated and unmedicated subjects in the rates of WMHs ($\chi^2 = 0.23$, DF = 1, $p = 0.63$).

4.4. Power analyses

We would need a total sample size of 55 subjects to achieve 80% power to show an effect size of w = 0.42 reported in the only

Table 1
Characteristics of the unaffected high-risk, affected familial, and healthy controls without personal or family history of mood disorders.

		Unaffected high-risk subjects	Affected familial subjects	Controls	*p*
Halifax	*N*	24	20	31	N/A
	Female sex *N* (%)	17 (70.8)	15 (75.0)	20 (64.5)	NS
	Age (years), mean (SD)	19.6 (3.1)	21.0 (3.7)	20.6 (3.3)	NS
	Age range – years	15.0–25.6	15.1–30.4	15.8–30.2	N/A
	Age of onset (years), mean (SD)	N/A	16.9 (3.1)	N/A	N/A
	Age of onset range – years	N/A	12.0–24.0	N/A	N/A
	Diagnoses	N/A	11 MD, 3 BD I, 3 BD II, 1 BD(NOS), 1 Dysthymia, 1 Psych(NOS)	N/A	N/A
	Family history (bipolar 2nd degree relatives, bipolar I parent, bipolar II parent.)	5, 15, 6	2, 14, 4	0, 0, 0	N/A
	Treatment at the time of scanning	N/A	2 Li, 2 AD, 1 AP, 1 AC, 14 no treatment	N/A	N/A
	Illness duration – years, mean (SD)	N/A	4.43 (2.85)	N/A	N/A
	% Right handed	72	89	90	NS
	WMHs N (%)	4 (16.7)	2 (10)	6 (19.4)	NS
Prague	*N*	20	15	18	N/A
	Female sex *N* (%)	11 (55)	11 (73.3)	11 (61.1)	NS
	Age (years), mean (SD)	20.2 (4.2)	22.1 (4.8)	23.0 (3.5)	NS
	Age range – years	15.0–30.0	15.0–30.0	16.0–29.0	N/A
	Age of onset (years), mean (SD)	N/A	18.5 (3.3)	N/A	N/A
	Age of onset range – years	N/A	14.0–25.0	N/A	N/A
	Diagnoses	N/A	6 MD, 5 BD I, 4 BD II	N/A	N/A
	Family history (bipolar 2nd degree relatives, bipolar I parent, bipolar II parent.)	0, 16, 4	0, 13, 2	0, 0, 0	N/A
	Treatment at the time of scanning	N/A	1 Li, 5 AD, 3 AC, 6 AP, 4 no treatment[a]	N/A	N/A
	Illness duration – years, mean (SD)	N/A	3.16 (3.46)	N/A	N/A
	% Right handed	93.8	100	100	NS
	WMHs N (%)	4 (20)	4 (26.7)	5 (27.8)	NS
Halifax + Prague	*N*	44	35	49	N/A
	Age (years), mean (SD)	19.8 (3.6)	21.5 (4.1)	21.5 (3.5)	NS
	WMHs *N* (%)	8 (18.2)	6 (17.1)	11 (22.4)	NS

Abbreviations: AC –anticonvulsants, AD – antidepressants, AP – antipsychotics, BD I – bipolar type I disorder, BD II – bipolar type II disorder, BD(NOS) – bipolar disorder not otherwise specified, Li – lithium, MD – major depression, N/A – not applicable, NS – not significant, Psych(NOS) – psychosis not otherwise specified, SD – standard deviation.
[a] Overall 11 patients were medicated and 4 of these were on combination treatment.

previous study comparable in design to ours (Gulseren et al., 2006) as statistically significant (alpha = 5%). With $n = 128$ as total sample size for the combined data, we can detect a lower effect size of $w = 0.27$ as statistically significant.

5. Discussion

Overall, we found relatively few and mostly low grade WMHs across all groups. Furthermore, comparable proportions of WMH-positive subjects were seen in affected familial, unaffected high-risk, and control groups. With 128 subjects, this is one of the largest studies of hyperintensities in BD, and the first study using a true high-risk design. The small effect sizes found in our study, combined with adequate sample size, sufficiently powered to detect a much smaller than previously reported effect, the lack of pattern of changes consistent with our a priori hypothesis (i.e. greatest differences between affected offspring and controls, with intermediate proportion of WMH-positive subjects in the unaffected high-risk group), and lower rate of WMHs in at risk groups relative to healthy controls, make it unlikely that this is a false negative finding. This is further supported by the fact that our findings are congruent with comparable rates of WMHs relative to healthy controls in familial patients (Chang et al., 2005; Sassi et al., 2003), unaffected high-risk subjects (Gulseren et al., 2006), young bipolar patients at the early stages of the disease (Botteron et al., 1995; Chang et al., 2005; Persaud et al., 1997), as well as in bipolar patients free from comorbid conditions, such as alcohol and substance abuse (Aylward et al., 1994; Breeze et al., 2003; Chang et al., 2005; Krabbendam et al., 2000; Persaud et al., 1997; Sassi et al., 2003), cardiovascular disease or diabetes (Aylward et al., 1994; Breeze et al., 2003; Krabbendam et al., 2000; Persaud et al., 1997; Sassi et al., 2003).

Our findings are contrary to studies showing a higher rate of WMHs in bipolar patients compared to controls (Beyer et al., 2009). This discrepancy may stem from clinical, as well as demographic heterogeneity. Many of the positive studies examined patients with a higher mean age, late illness onset, and with a disorder complicated by comorbid conditions. It is important to highlight these factors, as associations between WMHs and higher age (Aylward et al., 1994; de Asis et al., 2006; Deicken et al., 1991; Dupont et al., 1995; Sassi et al., 2003; Takahashi et al., 2008; Woods et al., 1995), longer duration of illness (Sassi et al., 2003), greater severity of illness (Dupont et al., 1995), and later illness onset (de Asis et al., 2006; Dupont et al., 1995; Takahashi et al., 2008; Tamashiro et al., 2008) have been reported previously. Further, white matter lesions are also associated with metabolic and cardiovascular disorders in the general population (Bokura et al., 2008; Claus et al., 1996; Fazekas et al., 1988; Schmidt et al., 1992; Swan et al., 2000), as well as among psychiatric patients (Deicken et al., 1991). Many of the previous studies did not control for these potentially confounding factors. We, on the other hand, investigated young offspring of well-characterized patients at the early stages of the illness with no cardiovascular or metabolic comorbidities.

Contrary to our study, some previous reports indicated higher rate of WMHs in pediatric bipolar patients (Lyoo et al., 2002; Pillai et al., 2002). One of these studies did not introduce a control group, but contrasted patients with BD to patients with other psychiatric conditions making inferences relative to general population impossible (Lyoo et al., 2002). While the second study used a proprietary scale for assessment of WMHs, different from scales applied by us and the majority of other studies (Pillai et al., 2002), which makes cross-study comparisons difficult. Even more importantly, the discrepancy between this and our study may be related to clinical heterogeneity. Pillai et al.

did not provide information about family history, included patients with very early onset of BD, in most cases less than 14 years of age, yielding an average age of onset of 10.7 ± 3.8 years. In our study, all high-risk subjects had family history of BD. In a larger prospective investigation, including the scanned subjects, the mean age of onset among offspring of bipolar parents was 17.2 ± 4.3 years for any mood disorder, 19.0 ± 3.8 for the first hypomanic or manic episode. No patient manifested a major depressive episode prior to age 12 and hypomanic or manic episode prior to age 14 years (Duffy et al., 2009, 2010). The importance of these clinical differences is underscored by the fact that similar to our investigation, studies among familial patients showed comparable rates of WMHs relative to controls (Chang et al., 2005; Sassi et al., 2003).

More females than males showed WMHs. This was unrelated to the presence of personal or family history of mood disorders, as there were no differences in proportion of WMH-positive females between unaffected, affected or control subjects. The increased rate of WMHs in females relative to males is congruent with several epidemiological studies among older subjects (de Leeuw and Birkenhager, 2001; Sachdev et al., 2009; Wen and Sachdev, 2004), and with studies in young psychiatric patients (Breeze et al., 2003). The reason for this replicated sexual dimorphism is not clear, partly because overall more than 80% of the variance in causation of WMHs remains unexplained (Sachdev et al., 2009). It could be related to the presence of a disorder, which is associated with both WMH and female gender, such as migraine or multiple sclerosis. These conditions, however, are unlikely to have confounded our findings. Personal history of MS was an exclusion criterion in both the Prague and Halifax datasets. A personal history of migraine was controlled for in the Prague dataset. We did not specifically screen for migraines in the Halifax dataset, however there were no differences in proportion of females among WMH-positive subjects between the Prague and Halifax samples. Alternatively such dimorphism could be related to as yet unknown X chromosome associated genetic effects.

There are several limitations of this study. Patients with migraine have approximately four times higher risk for developing WMHs than controls (Swartz and Kern, 2004), and 12–47% of all patients with migraine manifest WMHs (Paemeleire, 2009). Importantly, the prevalence of migraine among bipolar patients is approximately double the prevalence in the general population (McIntyre et al., 2006) and, according to some studies, well over 60% (Fasmer, 2001; Low et al., 2003). We controlled for the presence of migraine only in the Prague dataset, where only a single control suffered from migraine. Considering the population prevalence of migraine and the increased risk of migraines in bipolar patients, it is unlikely that presence of migraine would lead to false negative findings.

We did not use T_2 or proton density weighted imaging techniques. FLAIR images used in this study are less sensitive to lesions in the brainstem and the cerebellum relative to T_2 or proton weighted scans (Gawne-Cain et al., 1998). FLAIR on the other hand, improves sensitivity in periventricular regions, due to elimination of the signal from CSF and reduction of image degradation from partial volume effects (Hajnal et al., 1992). Greater sensitivity to detect WMHs in periventricular regions is critical, as previous studies in BD have reported white matter lesions predominantly in deep white matter and periventricular areas.

The number of slices and slice thickness were different between the two datasets. The 5 mm slice thickness used in Halifax is the typical slice thickness used in the majority of other investigations of WMHs in bipolar subjects (Beyer et al., 2009). The 3 mm slice thickness, as used in the Prague dataset, provides a better coverage and was used in a minority of previous reports. Both of these slice thicknesses are sufficient to evaluate WMHs, especially since there were no gaps between slices. Furthermore, the regional differences in scanning parameters are equivalent for all groups in each site. The fact that the pattern of WMHs across groups was similar in both sites and there were no differences in proportion of WMH-positive subjects between Prague and Halifax datasets indicates that scanning parameters did not affect our findings.

Because the numbers of WMHs per group were small, we were not able to test for differences in lesion location or phenotype of WMHs between the groups. The pattern of lesions seen in high-risk offspring (i.e. mostly grade 1 lesions without any predilected location), however, was not suggestive of lesions associated with demyelination or dysmyelination.

Some of the affected familial patients suffered from unipolar depression. Unipolar depression however, is most typically the first manifestation of the BD in high-risk offspring (Duffy et al., 2009, 2010, 2002) (Hillegers et al., 2005), about 70% of depressed first-degree relatives of bipolar probands are in fact bipolar (Blacker et al., 1993). If we want to study early manifestations of BD, inclusion of these likely pseudo-unipolar patients with family history of BD is thus inevitable. Besides bipolar I patients, we also included probands with conservatively defined bipolar II disorders. As there were no differences in proportion of WMH-positive subjects when comparing the relatives of bipolar I vs. bipolar II patients, the inclusion of bipolar II probands likely did not affect our results.

In nine cases of the Prague dataset, probands were not available for direct SADS-L interviews. In these instances, we used available hospital charts and information from treating psychiatrists and family members.

Finally, a prospective study of high-risk offspring would be better able to capture changes in white matter related to the neurodevelopment or burden of illness. We will address this issue in subsequent studies.

In conclusion, we found a comparable proportion of subjects with WMHs among unaffected, affected relatives of bipolar patients and controls without family or personal history of mood disorders. White matter hyperintensities therefore did not meet criteria for an endophenotype of BD. After eliminating the effects of illness burden, comorbid conditions, and higher age, the proportions of subjects with WMHs were comparable among young bipolar patients and healthy controls, suggesting that WMHs may not be directly related to BD.

Role of funding source

Supported by NARSAD Young Investigator Award to Dr. Hajek, Dalhousie Clinical Research Scholarship to Dr. Hajek and grant NR8786, from the Internal Grant Agency of Ministry of Health, Czech Republic. None of these granting agencies had any further role in the study design; in the collection, the analysis, and in the interpretation of the data; in the writing of the report; or in the decision to submit the paper for publication.

Financial disclosures

None of the authors has any conflict of interest to disclose.

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