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Title: Diffusion tensor imaging correlates of early markers of depression in youth at high familial risk for bipolar disorder

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Abstract: Background: mood disorders are familial psychiatric diseases, in which patients show reduced white matter (WM) integrity. We sought to determine whether WM integrity was affected in young offspring at high familial risk of mood disorder before they go on to develop major depressive disorder (MDD).
Methods: the Bipolar Family Study is a prospective longitudinal study examining individuals at familial risk of mood disorder on three occasions two years apart. This study used baseline imaging data, categorizing groups according to clinical outcome at follow-up. Diffusion tensor MRI data were acquired for 61 controls and 106 high-risk individuals, the latter divided into 78 high-risk subjects who remained well throughout the study ("high-risk well"), and 28 individuals who subsequently developed MDD ("high-risk MDD"). Voxel-wise between-group comparison of fractional anisotropy (FA) based on diagnostic status was performed using Tract-based Spatial Statistics (TBSS).
Results: compared to controls, both high-risk groups showed widespread decreases of FA ($P_{corr}<0.05$) at baseline. Although FA in the high-risk MDD group negatively correlated with sub-threshold depressive symptoms at the time of scanning ($P_{corr}<0.05$), there were no statistically significant differences at p-corrected levels between the two high-risk groups.
Conclusions: these results suggest that decreased FA is related to presence of familial risk for mood disorder along with sub-diagnostic symptoms at the time of scanning, rather than predictive of subsequent diagnosis. Due to the difficulties performing such longitudinal prospective studies we note, however, that this latter analysis may be underpowered due to sample size within the high-risk MDD group. Further clinical follow-up may clarify these findings.

Diffusion tensor imaging correlates of early markers of depression in youth at high familial risk for bipolar disorder

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Short title: WM integrity in high-risk subjects for mood disorders

Abstract

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Methods:

The Bipolar Family Study is a prospective longitudinal study examining individuals at familial risk of mood disorder on three occasions two years apart. This study used baseline imaging data, categorizing groups according to clinical outcome at follow-up. Diffusion tensor MRI data were acquired for 61 controls and 106 high-risk individuals, the latter divided into 78 high-risk subjects who remained well throughout the study ("high-risk well"), and 28 individuals who subsequently developed MDD ("high-risk MDD"). Voxel-wise between-group comparison of fractional anisotropy (FA**)** based on diagnostic status was performed using Tract-based Spatial Statistics (TBSS).

Results:

Compared to controls, both high-risk groups showed widespread decreases of FA ($P_{corr}<0.05$) at baseline. Although FA in the high-risk MDD group negatively correlated with sub-threshold depressive symptoms at the time of scanning ($P_{corr}<0.05$), there were no statistically significant differences at p-corrected levels between the two high-risk groups.

Conclusions:

These results suggest that decreased FA is related to presence of familial risk for mood disorder along with sub-diagnostic symptoms at the time of scanning, rather than predictive of subsequent diagnosis. Due to the difficulties performing such longitudinal prospective studies we note, however, that this latter analysis may be underpowered due to sample size within the high-risk MDD group. Further clinical follow-up may clarify these findings.

Introduction

Bipolar disorder (BD) is a mood disorder characterised by manic or hypomanic episodes during which the mood becomes euphoric and labile (1). Depressive episodes are also common in BD during which pleasure-seeking is reduced and experiences are rated as less rewarding. Many studies have sought for the structural and functional brain mechanisms of BD; in which altered connectivity has been proposed to play a key role (2, 3).

Fractional anisotropy (FA) is a sensitive diffusion tensor MRI (DTI) metric of white matter (WM) integrity, which estimates the degree of restriction of water molecule diffusion due to tissue organization. A recent meta-analysis of DTI studies in BD found three large clusters showing decreased FA compared with controls (4), with the main tracts being the inferior longitudinal and fronto-occipital fasciculi located in the temporal lobe, the cingulum, and the anterior thalamic radiations. Some studies have, however, found increases in FA in BD patients compared with healthy individuals (5, 6), while others report no detectable difference (7, 8).

Previous reviews and meta-analyses of DTI studies on patients with major depressive disorder (MDD) have found reduced FA in the left superior longitudinal fasciculus (9), the genu of the corpus callosum (10), bilateral frontal lobe, right fusiform and right occipital lobe (11), and frontal and temporal lobes (2). The majority of studies of brain abnormalities in mood disorder have mostly been conducted on adult patients with longstanding illness *vs.* controls. It is unclear therefore whether brain structure may be altered before the development of the disorder, and whether abnormalities are confounded by secondary effects of long-term illness or treatment, or predictive of subsequent illness.

White matter integrity as indexed by FA is heritable (12, 13), and DTI studies have revealed suggestive evidence of WM integrity reductions in unaffected relatives of BD patients (14, 15). Versace et al. (16) demonstrated age-by-group interactions in the unaffected teenage offspings of bipolar probands. Moreover, the largest studies to date are consistent with the notion of subtle but widespread effects in relatives (17, 18).

The Scottish Bipolar Family study (BFS) is a prospective cohort imaging study precisely examining young individuals (16-25 years) at high familial risk of mood disorder, along with a group of healthy controls with no family history, which aimed to investigate whether brain alterations precede and predict the later development of mood disorders (19).

In a previous DTI study on this sample at baseline, we reported widespread FA reductions in unaffected young relatives of BD compared to subjects without family history of psychiatric disorders. Although the

effects of genetic risk were diffuse, the associations with cyclothymia were more localized to fronto-temporal and prefrontal-thalamic connections (20). These findings therefore suggest that WM integrity is a marker of genetic risk for mood disorder with additional behavioral association linked to the etiology of the condition.

The Young Mania Rating Scale (21) (YMRS) and the Hamilton Rating Scale for Depression (22) (HAM-D) are the most frequently utilized rating scales to assess manic and depressive symptoms that are the core features of BD. In the previous study (20), high-risk healthy subjects for mood disorders showed significantly higher scores in both these clinical scales compared to controls, suggesting that manic and depressive subclinical symptoms could also be a potential endophenotype for BD.

Individuals in the study, by virtue of the shared genetic architect of MDD/BD, are at risk for both mood disorders. Over the course of the BFS, a number of individuals have developed one or the other, with MDD being the predominant outcome (defined as meeting formal diagnostic criteria, Diagnostic and Statistical Manual of Mental Disorder, 4th edition, DSM-IV) and in sufficient numbers as to make a prospective comparison of well *vs*. affected individuals.

The current study presents findings for the same individuals, as acquired in the BFS, categorised into those at high familial risk who remained asymptomatic over the course of the study (“high-risk well”), compared to those who developed a mood disorder at either of the follow-up assessments (“high-risk MDD”). We hypothesized, based on our previous study and the wider literature, that abnormalities in WM integrity assessed using DTI, in particular fronto-limbic and fronto-thalamic connections would precede subsequent illness in initially unaffected familial high-risk individuals.

Methods and Materials

Participants

Participants were recruited as part of the BFS (BFS; (Principal investigator A. McIntosh, U. of Edinburgh) (19, 20). Participants, including controls, have been followed up on three occasions two years apart (23). Only the first two occasions contained imaging assessments, the third assessment being primarily a follow-up clinical evaluation. At the first assessment, patients with a clinical diagnosis of BD I were identified from the case loads of psychiatrists across Scotland. Each patient was asked to identify members of their close family between 16 and 25 years of age and to consent to either a review of their case notes or to a structured clinical interview. The diagnosis of all affected subjects was then confirmed with the Structured Clinical Interview for DSM-IV (SCID) or the OPCRIT symptom checklist (24). After informed consent, unaffected relatives of the proband with at least one first-degree or two-second degree relatives with BD I were then invited to participate in the study.

Wherever possible, to optimize matching on key confounds, control subjects were recruited from the social networks of the high-risk subjects themselves.

All participants, controls and high-risk subjects, were interviewed by one of two experienced psychiatrists (A.M.M., J.E.S.) using the SCID (25) to confirm the lifetime absence of any Axis I disorders at the baseline (T1), and at the fist follow-up (T2) to determine the presence of any mood disorder meeting diagnostic criteria over the intervening period. At the second follow-up (T3) diagnostic status was determined either by face-to-face assessment, or through accessing clinical records at the National Health Service (NHS) as to whether a clinical diagnosis had been made or not (23).

For a number of individuals (n = 43), it was not possible to determine the clinical status at T3; either the general practitioner (GP) did not provide details or the GP address was unknown. In the absence of further clinical information indicating that they had become unwell, and since they had remained well over the previous two assessments, these individuals were presumed to have remained well.

Manic and depressive symptoms were rated using the Young Mania Rating Scale and the Hamilton Rating Scale for Depression. Estimates of trait-liability to mood disorder (cyclothymia, neuroticism and extraversion) were measured using the TEMPS-A and NEO-FFI (26, 27).

The final sample consisted of 167 individuals categorized into three groups: (i) healthy controls who remained well (n = 61; note that four control individuals developed MDD or BD at the follow-up; however, due to small group size these individuals were not included in the current analysis); (ii) familial high-risk participants who remained well throughout the study (*high-risk well*, n = 78); and (iii) familial high-risk

participants who developed a mood disorder (*high-risk MDD*) at any time throughout the period of study (20 by T2, a further eight by T3; high-risk MDD, n = 28; also of note is that three high-risk individuals had developed BD over the course of the study, similar to above these individuals were not included in the current analyses).

Written informed consent was provided by all participants, and the study was approved by committee of the Multicentre Regional Ethics Committee for Scotland.

Scan Acquisition and Preprocessing

The current study reports findings from imaging data collected at entry in the study ('baseline'). The MRI data were collected on a 1.5-T GE Signa Horizon HDX (General Electric, Milwaukee, Wisconsin) clinical scanner equipped with a self-shielding gradient set (22 mT/m maximum gradient strength) and manufacturer-supplied "birdcage" quadrature head coil. Whole brain DTI data were acquired for each subject with a single-shot pulsed gradient spin-echo echo-planar imaging sequence with diffusion gradients (b = 1000 sec/mm2) applied in 64 non-collinear directions and seven non-diffusion weighted (b=0) echo-planar baseline volumes. Fifty-three 2.5-mm contiguous axial slices were acquired with a field-of-view of 240 x 240 mm, acquisition matrix of 96 x 96 (zero-filled to 128 x 128), giving an isotropic acquisition voxel dimension of 2.5 mm. In addition, a T1-weighted volume was acquired with time of inversion = 500 msec, echo time = 4 msec, flip angle = 8°, and voxel-size = 1.25 x 1.25 x 1.20mm (192 x 192voxels, 180 slices).

The DTI data were converted to 4D NIfTI volumes and preprocessed with standard tools available from the FMRIB Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl). This included the following processes: correction for eddy current induced distortions and bulk subject motion in the scanner by registering the diffusion weighed volumes to the first T2-weighted volume within each subject; brain extraction; and calculation of diffusion tensor characteristics, including principal eigenvectors and FA values with DTIFIT.

Tract-Based Spatial Statistics

Tract-based Spatial Statistics (TBSS) was carried out according to standard FSL procedures (28) (http://www.fmrib.ox.ac.uk/fsl). First, FA volumes of all subjects were nonlinearly registered to a standard template. Secondly, a mean of all registered FA volumes was calculated, and a white matter "skeleton" created. This was achieved by searching for the maximum FA values in directions perpendicular to the local tract direction in the mean FA map. A threshold of FA > 0.25 was applied to the FA skeleton to exclude predominantly non-white matter voxels. Thirdly, the maximum voxel FA value perpendicular to the local direction was projected onto the skeleton at each point in all subjects. This resulted in one FA

skeleton map per subject, assumed to contain the anatomically corresponding centroids of the WM structure, which we used to test for between-group differences and correlations with clinical scores.

With the obtained TBSS skeletons we performed two statistical tests. Firstly, to test for between-group differences in FA, nonparametric voxel-wise T tests were applied to the skeletons using the FSL "randomize" function, with three regressors in the design matrix, one for each group. Secondly, we examined the relationship between FA and measures of depressive symptoms at the time of the scan (from HAM-D scores), and measures of trait-liability (cyclothymia, neuroticism and extraversion). This was performed in 'FSL' testing separately for association in each group and interactions between groups by adding three regressors to the design matrix, each containing the scores of one group.

Threshold-free cluster enhancement (TFCE) was applied to obtain cluster-wise statistics corrected for multiple comparisons. This method transforms local T-statistics into TFCE statistics that reflect both the size of the local effect (or "height") and the cluster extent (29). The major advantages are that no predefined T-threshold is required, and that TFCE is sensitive to detect both large clusters of modest effects and single voxels of large effects, at the same time.

With the obtained TFCE maps, "randomize" then calculates a *p*-value (*p*-corrected) for each voxel, corrected for whole-brain family wise error (FWE) rate via permutation testing (5,000 permutations). These TFCE corrected p-maps were thresholded at $pFWE < 0.05$. We report the sizes of contiguous clusters of suprathreshold voxels. Significant results were localized to white matter tracts/structures with the Johns Hopkins University DTI-based white matter atlas and the Johns Hopkins University white matter tractography atlas (30) digitally available in FSL.

Statistical analysis of demographic and clinical data was conducted using SPSS software, version 23.0 (http://www-01.ibm.com/software/analytics/spss/). Differences between groups were tested using one-way ANOVA, Kruskal-Wallis or chi-squared tests as appropriate.

Results

Demographic and clinical features

There were no significant differences between groups with respect to gender, age, handedness, IQ, or substance use. Scores for the YMRS, HAM-D, cyclothmic, depressive, irritability, and anxious traits as measured by the TEMPS-A scale, as well as for neuroticism and agreeableness factors as measured using the NEO-FFI differed significantly between groups (Table 1).Post-hoc analysis revealed that high-risk MDD individuals had greater scores for the YMRS and HAM-D scales versus the controls; and for cyclothymic, depressive, and irritability traits, as well as neuroticism, they differed from both the controls and high-risk well groups. Moreover, the two high-risk groups were significantly different on anxious traits and agreeableness (Table 2).

TBSS comparisons groups (controls, high-risk well, and high-risk MDD)

WM integrity differences between groups surviving the permutation testing are shown in Figure 1, and Table 3 ($p_{corr} < 0.05$). Compared to controls, at baseline neither high-risk group showed significantly *higher* FA values

Both HR groups however demonstrated significant decreased FA in comparison with the control group. The largest WM *reduction* in high-risk well *vs* controls was in a cluster that included the posterior thalamic radiation, extending to the posterior corona radiata and the superior longitudinal fasciculus in the right hemisphere (K=7,340, $p_{corr} = 0.02$). Other significant differences at p-corrected levels included smaller clusters in the right superior longitudinal fasciculus (K=53, $p_{corr} = 0.05$), and right anterior corona radiata (K=15, $p_{corr} = 0.05$), the left posterior thalamic radiation, and left posterior cingulum until the body of the corpus callosum (K= 839, $p_{corr} = 0.03$; and K=234, $p_{corr} = 0.05$, respectively).

High-risk MDD subjects compared with controls showed reduction of WM integrity mainly located in four large clusters in the right hemisphere. The largest cluster included the uncinate fasciculus and extended to the anterior corona radiata and inferior fronto-occipital fasciculus (K=4,092, $p_{corr} = 0.03$). Other clusters involved the inferior longitudinal fasciculus (K=2,849, $p_{corr} = 0.04$), posterior limb of internal capsule, extending to the anterior limb of internal capsule, anterior corona radiata and thalamic radiation (K=2,190, $p_{corr} = 0.04$), and the last one comprised posterior cingulum (k=2,014, p = 0.04). Smaller clusters were also found in the splenium and the body of corpus callosum, and bilaterally in the superior cortical spinal tract, in the brainstem corticospinal tract extending to the cerebellar peduncle, in the inferior and superior longitudinal fasciculi, and in fronto-occipital fasciculi.

Figure 2 demonstrates these two contrasts (high-risk well versus controls and high-risk MDD versus

controls) overlaid onto a single image; however no differences were observed that survived permutation testing comparing directly the two high-risk groups.

TBSS correlation with clinical measures

A widespread negative association of HAM-D scores and FA values within the TBSS skeleton was found in the MDD high-risk group (Figure 3, Table 4). The three larger significant clusters were located in (i) the body of the corpus callosum comprising the genu and the splenium (K=6,860 voxels, $p_{corr} = 0.01$), (ii) in the right posterior thalamic radiation extending over the external capsule and the uncinate fasciculus (K=6441, $p_{corr} = 0.02$), and (iii) in the left inferior longitudinal and fronto-occipital fasciculi (K=2958, $p_{corr} = 0.03$).

Smaller significant clusters contained the superior longitudinal fasciculus bilaterally, and the forceps major, the inferior fronto-occipital fasciculus, and the anterior thalamic radiation in the right side, and the left cingulum, and left posterior thalamic radiation were also detected (Table 4).We did not find any association between FA and cyclothymia, neuroticism or extraversion.

Discussion

This study investigates WM integrity in a relatively large sample of young subjects at high genetic risk of mood disorder with and without a subsequent diagnosis of MDD. The main aim of the project was to determine whether there was any distinguishing white matter microstructure features associated with the onset of subsequent mood disorder.

High-risk versus control differences

The comparison between high-risk subjects who remained well and controls, and high-risk individuals who subsequently develop MDD and controls revealed FA reductions in both high-risk groups. These results confirmed our previous baseline findings where we examined the high-risk subjects as an entire group, rather than sub-dividing based on outcome of clinical follow-up. In that study we reported significantly reduced integrity in the major WM association pathways, extending from frontal to occipital lobes in unaffected youth with familial risk for BD when compared to healthy controls without a family history of psychiatric disorders (20).

In particular in the present study, smaller FA values in posterior WM tracts such as the posterior portion of the thalamic radiation, superior longitudinal fasciculus, cingulum, and in fibers belonging the body of the corpus callosum were detected in both high-risk groups compared with controls. High-risk individuals who developed MDD compared with controls however showed further reduced integrity in WM tracts located in the brainstem and anterior brain regions, such as the uncinate fasciculus, the anterior limb of the internal capsule, the anterior corona radiata and thalamic radiation, and sections of the inferior fronto-occipital and superior longitudinal fasciculi located in the middle and orbital frontal lobe.

To date, few other studies have examined WM integrity in unaffected youth individuals with familial risk of BD (14, 16). Frazier et al. (14) compared seven unaffected relatives with eight control subjects and found reduced FA in two clusters in the superior longitudinal fasciculi. Versace et al. observed that asymptomatic youth with familial risk for BD had a linear decrease between age and FA in the left corpus callosum, whereas healthy controls showed a linear increase in the same regions (16). Another study detected increased FA in youth having both a parent with BD and mood dysregulation compared to controls (31). Unaffected siblings, of patients with BD, once they are adults and mostly past the typical age onset of BD (mean age of 30 years old), also showed subtle FA reduction when compared to controls, which are most apparent in the corpus callosum (17, 18). FA reductions were therefore observed using TBSS in siblings, mainly restricted to the corpus callosum, posterior thalamic radiations, and left superior longitudinal fasciculus (17). Using tractography analysis, reduced FA were not detected in the siblings

compared to the controls, except for a trend in the corpus callosum (18).

Our findings support the suggestion that alterations in WM integrity may be an endophenotype for BD and encourage future studies assessing its relationship with the onset of mood disorders.

Despite we did not find formally statistically significant differences in FA comparing directly the two high-risk groups (high-risk well *vs* high-risk MDD), when we compared the high-risk MDD group to controls we found reduced FA values in the anterior fronto-thalamic connections, especially in the right side. We did not find WM disintegration in these tracts in the high-risk well compared to controls.

In vivo structural and functional imaging studies, as well as postmortem investigations of adults suggest that cortical–subcortical neural circuits play an important role in the pathogenesis of depression, especially limbic–thalamic–frontal networks (43, 44). Structural and functional imaging data in pediatric populations, although limited, also support these models (45, 46). WM abnormalities constitute one element of these dysfunctional networks (2, 47, 48).

A more widespread disconnection involving selectively fronto-thalamic tracts would be consistent with a temporally close MDD onset and mild sub threshold depressive symptoms; however, in our samples these emerging differences in high-risk MDD vs controls comparison are too subtle to be detected when we compare the two high-risk groups directly. It is important to note, nevertheless, that this analysis may be underpowered due to the relatively small sample size within the high-risk MDD group. The difficulties in conducting prospective longitudinal high-risk studies on young individuals with a family history of mental illness particularly relating to cumulative attrition over the course of the study should not be underestimated. Clinical longitudinal follow-up of these high-risk cohort and larger samples could clarify this point and relationships between WM integrity and the onset of mood symptoms.

Associations with symptoms

Although the 28 subjects who later developed MDD did not meet diagnostic criteria at baseline, these individuals had significantly increased levels of symptoms as measured by the clinical scales (HAM-D and YMRS) as well as greater scores for the cyclothymic, depressive traits, and neuroticism. These subclinical symptoms may indicate predictors of subsequent illness, or they could reflect the prodromal phase of a mood disorder.

In the current study, we detected in the high-risk MDD subjects a significant negative association between severity of depressive symptoms measured by the HAM-D scale and WM integrity, mainly located in the corpus callosum and in the cortical and thalamic pathways.. This finding could suggest that FA levels are sensitive to behavioural changes associated with clinical measures. The largest cluster of negative

association in our group was found in the corpus callosum comprising the genu, the body, and the splenium.

The relationship between severity of depressive symptoms and WM integrity has previously been reported in the corpus callosum of patients with recurrent MDD (32, 33). Interestingly, Lamar et al. (33) studied a sample consisting solely of euthymic patients with major depressive disorder, indicating that the relationship persists into remission.

In our sample, high-risk MDD subjects showed reduced FA levels in the body and the splenium of corpus callosum when compared to controls. As stated previously, Versace et al. found that asymptomatic youth with familial risk for BD had a linear decrease between age and FA in the corpus callosum (16), and other studies using TBSS have also reported WM integrity reduction in the corpus callosum in siblings of BD patients (17, 18), adolescents with a parental history of depression (34), and in adolescent patients with MDD (35). Reduced volume and deformations in the corpus callosum have been observed also in MDD (36), suicidal behaviour (37), bipolar depression (38), and dysthymia (39).Together, these findings suggest that impairments in corpus callosum tracts may be involved early in the aetiology of depressive symptoms.

Other findings of increased severity of depressive symptoms with decreased WM integrity in the same regions that we found in the current study has been previously reported, such as the posterior thalamic radiation (32), inferior frontal regions (40), cingulum (41), superior longitudinal fasciculus, and uncinate (18, 42), The varied pattern of WM tracts implicated in the literature indicates that further research is necessary to clarify the specificity of the relationship between depressive symptoms severity and brain changes and their association to functional impairments in people with depressive symptoms.

Limitations

Firstly, the early diagnosis of MDD in a young group of individuals at risk of BD may herald the onset of BD (49), where often first episode is depressive (49, 50). Moreover, the high-risk subjects who subsequently became ill had higher clinical scores than controls at baseline. It is difficult therefore to dissociate neural markers underlying genetic risk for the disease trait from those co-occurring with the development of MDD symptoms, and indeed the two may be inherently related. Continued clinical longitudinal follow-up of the sample will ultimately contribute to a better understanding of prodromal phases of illness and associated disease pathways.

Finally, although DTI is currently our best tool for estimating WM integrity, DTI results should be interpreted with caution because of the limitations of this in vivo technique: crossing fibers may occur

within the region measured, and the resolution does not allow for a physiological interpretation at the cellular level. Thus a lower FA measurement is not necessarily always synonymous for “lower connectivity”.

In conclusion, using DTI we report altered microstructure within specific WM tracts in youth with high-risk for mood disorders that will or will not develop MDD. Although we did not find a different pattern of FA reduction in high-risk individuals who subsequently met MDD criteria compared to those who remained well, these findings provide evidence for an association between sub-threshold depressive symptoms and WM integrity. These FA reductions therefore may reflect subtle differences in current mood state (at baseline) rather than predictors of future state.

Further follow-up studies will allow us to identify other individuals in the high-risk group who develop mood disorders and who remain healthy in order to clarify the early phases of mood disorder pathways.

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Other authors RG, TN, ES, MEB, SG, AM, and JS have no competing interests to declare.

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Table 1. Demographic and Historical Characteristic

Variable	Controls (n=61)	High-risk well (n=78)	High-risk MDD (n=28)	P value
age[a]	20.8 (2.3)	21.4 (2.7)	21.1 (2.9)	0.50
Gender[b]				
Male	27 (44.35)	41 (52.6%)	12 (42.9%)	0.52
Female	34 (55.7%)	37 (47.4%)	16 (57,1%)	
Handedness[b]				
Left	4 (6.6%)	7 (9%)	1 (3.6%)	0.50
Right	57 (93.4%)	69 (88.5%)	27 (96.4%)	
Mixed	0	2 (2.5%)	0	
NART IQ[c]	110 (8)	110 (10)	107 (12)	0.58
Clinical measures				
YMRS[c]	0 (0)	0 (0)	0 (1)	0.02
HAM-D[c]	0 (1)	0 (2)	1.5 (6)	0.01
Temperament and personality measures				
Cyclothymia[c]	1 (4)	2 (3)	2 (7)	< 0.001
Depressive[c]	0 (2)	0 (1)	2 (3)	< 0.001
Irritability[c]	1 (2)	1 (2)	2 (2)	< 0.001
Hyperthymia[c]	2.5 (3)	2 (2)	2 (2)	0.29
Anxious[c]	1 (2)	0 (1)	1 (3)	0.04
NEO - Five Factor Inventory				
Neuroticism[a]	20.4 (9.3)	21.04 (9.2)	30.5 (10.5)	< 0.001
Extraversion[c]	31 (7)	30 (7)	27.5 (12)	0.21
Agreeableness[c]	33 (6)	34 (8)	30.5 (7)	0.03
Coscientiousness[c]	28 (9)	29.5 (12)	23 (12)	0.19
Openness[c]	29 (10)	28 (7)	29.5 (3)	0.07
Substance Use				
Alcohol[b]	55 (91.7%)	69 (88.2%)	21 (75%)	0.08
if yes: units/week[c]	15 (13)	12 (16.5)	15 (15.5)	0.68
Cigarettes[b]	20 (33.3%)	21 (26.9%)	11 (39.3%)	0.51
If yes:cig/day[c]	10 (6)	10 (11)	10 (18)	0.80
Cannabis[b]				
past	40 (66.7%)	53 (67.9%)	17 (60.7%)	0.78
current	5 (8.3%)	13 (16.7%)	6 (21.4%)	0.20
Stimulants[b]				
past	17 (28.3%)	23 (29.5%)	10 (35.7%)	0.77
current	6 (10%)	5 (6.4%)	1 (3.6%)	0.71
Hallucinogenic[b]				
past	10 (16%)	12 (15.4%)	6 (21.4%)	0.76
current	0	2 (2.6%)	2 (7.1%)	0.13
Opiates[b]				
past	1 (1,7%)	4 (5.1%)	1 (3.6%)	0.56
current	1 (1,7%)	0	1 (3.6%)	0.31
Sedatives[b]				
past	1 (1,7%)	5 (6.4%)	2 (7.1%)	0.91
current	0	1 (1.3%)	1 (3.6%)	0.36

Table shows statistical comparisons of demographic, clinical, behavioral measures and substance use in the three comparison groups at baseline.

[a]Group means and standard deviation for variables normally distributed (ANOVA).

[b]Frequency and percentages for categorical variables (chi-squared).

[c]Medians and interquartiles for variables not normally distributed (Kruskal-Wallis test).

MDD, Major Depressive Disorder; IQ, intelligent Quotient; YMRS, Young mania rating scale; HAM-D, Hamilton Scale for Depression.

Table 2. Post-hoc comparisons of clinical and temperamental measures

Variable	Comparison group	Post-hoc p value
Clinical measures, and childhood trauma measures		
YMRS	Controls *vs* High-risk well	0.228
	Controls *vs* High-risk MDD	0.021*
	High-risk well *vs* High-risk MDD	0.474
HAM-D	Controls *vs* High-risk well	0.458
	Controls *vs* High-risk MDD	0.009*
	High-risk well *vs* High-risk MDD	0.149
Temperament and personality measures		
Cyclothymia	Controls *vs* High-risk well	1.000
	Controls *vs* High-risk MDD	0.002*
	High-risk well *vs* High-risk MDD	0.008*
Depressive	Controls *vs* High-risk well	1.000
	Controls *vs* High-risk MDD	0.003*
	High-risk well *vs* High-risk MDD	0.000*
Irritability	Controls *vs* High-risk well	1.000
	Controls *vs* High-risk MDD	0.021*
	High-risk well *vs* High-risk MDD	0.003*
Anxious	Controls *vs* High-risk well	0.517
	Controls *vs* High-risk MDD	0.451
	High-risk well *vs* High-risk MDD	0.037*
NEO - Five Factor Inventory		
Neuroticism	Controls *vs* High-risk well	1.000
	Controls *vs* High-risk MDD	0.000*
	High-risk well *vs* High-risk MDD	0.000*
Agreableness	Controls *vs* High-risk well	0.357
	Controls *vs* High-risk MDD	0.646
	High-risk well *vs* High-risk MDD	0.031*

Table shows results from post-hoc comparisons on variables found significantly different among the three groups (*cf.* Table 1).

The Bonferroni and the Dunn-Bonferroni corrections were respectively used for variables normally distributed and not normally distributed.

*indicates a significant statistical difference with $p \leq 0.05$,

MDD, Major Depressive Disorder; YMRS, Young mania rating scale; HAM-D, Hamilton Scale for Depression.

Table 3. Regions showing significant reductions in fractional anisotropy (FA) at p-corrected levels in high-risk subjects who remained well (High-risk well) at follow-up and high-risk subjects who met diagnostic criteria for a major depressive disorder at follow-up (High-risk MDD) when compared with controls.

Cluster size (voxels)	WM region	Side	X	Y	Z	P value
Reduced FA in high-risk well vs controls						
7340	Posterior thalamic radiation	R	30	-71	9	0.02
53	Superior longitudinal fasciculus		40	-51	13	0.05
15	Anterior corona radiata		25	31	13	0.05
839	Posterior thalamic radiation	L	-33	-67	-2	0.03
234	Posterior cingulum		-21	-59	30	0.05
Reduced FA in high-risk MDD vs controls						
4092	Uncinate fasciculus	R	13	35	-14	0.03
2849	Inferior longitudinal fasciculus		45	-23	4	0.04
2190	Posterior limb of internal capsule		21	-16	-2	0.04
2014	Posterior cingulum		16	-41	40	0.04
713	Splenium of corpus callosum		22	-47	8	0.04
630	Superior longitudinal fasciculus		36	-62	28	0.04
596	Inferior fronto-occipital fasciculus		27	-67	26	0.04
105	Superior longitudinal fasciculus		47	-39	19	0.05
13	Corticospinal tract		6	-28	-34	0.05
3	Inferior longitudinal fasciculus		40	-53	1	0.05
2	Body of corpus callosum		3	1	25	0.05
767	Corticospinal tract	L	-22	-30	44	0.04
518	Inferior fronto-occipital fasciculus/ Inferior longitudinal fasciculus		-41	-31	-6	0.04
497	Posterior thalamic radiation		-34	-63	-3	0.04
29	Inferior fronto-occipital fasciculus/ Inferior longitudinal fasciculus		-19	-84	15	0.05
18	Superior longitudinal fasciculus		-32	-13	52	0.05
7	Inferior longitudinal fasciculus		-52	-13	-1	0.05
2	Superior longitudinal fasciculus		-35	-10	55	0.05
1	Superior longitudinal fasciculus		-39	-7	51	0.05

WM, White Matter; FA, Fractional Anisotropy; R, Right; L, Left; MDD, Major Depressive Disorder.

Table 4. Regions showing significant negative correlation at p-corrected levels between fractional anisotropy (FA) and HAM-D scores in high-risk subjects who met diagnostic criteria for a major depressive disorder at follow-up (High-risk MDD)

Cluster size (voxels)	WM region	Side	X	Y	Z	P max-value
6860	body of corpus callosum	R	7	-21	25	0.01
6441	Posterior thalamic radiation		35	-59	8	0.02
546	Forceps major		10	-88	19	0.05
201	Inferior fronto-occipital fasciculus		35	24	19	0.05
68	Inferior fronto-occipital fasciculus		33	33	11	0.05
33	Superior longitudinal fasciculus		50	-6	22	0.05
10	Anterior thalamic radiation		11	-3	-6	0.05
2958	Inferior longitudinal fasciculus/inferior fronto-occipital fasciculus	L	-43	-24	14	0.03
700	Superior longitudinal fasciculus		-38	-24	-14	0.04
698	Posterior thalamic radiation		-32	-64	9	0.04
218	Cingulum		-6	-8	35	0.05
112	Superior longitudinal fasciculus		-46	-53	1	0.05
105	Superior longitudinal fasciculus		-40	-52	11	0.05
83	Cingulum		-14	-54	22	0.05
51	Posterior thalamic radiation		-36	-49	5	0.05

WM, White Matter; R, Right; L, Left.

Figure 1. Reduced FA at baseline in unaffected BD patients' relatives who did not develop psychiatric symptoms at follow-up ('High-risk well') compared to control subjects (Top row; High-risk Well < Controls); and in BD patients' relatives who met the criteria for MDD ('High-risk MDD') at follow-up compared with controls (Bottom row; High-risk MDD < controls). Corrected P value < 0.05. For better visibility the results are thickened with the "tbss-fill" command. The images are display in radiological convention.

Figure 2. Sagittal view of reduced FA at baseline in unaffected BD patients' relatives who did not develop psychiatric symptoms at follow-up compared to control subjects (in blue); reduced FA in BD patients' relatives who met the criteria for MDD at follow-up compared to controls (in red); and the overlap between the previous comparisons (in purple). Note that WM integrity is reduced in high-risk subjects, and more so for the deep connections and frontal regions in high risk subjects who became depressed.

Corrected P value < 0.05. For better visibility the results are thickened with the "tbss-fill" command.

The images are in radiological convention.

R, Right side; L, Left side; MDD, Major Depressive Disorder.

Figure 3. Axial (A) and sagittal (B) view of negative correlation between FA values and scores obtained by the Hamilton scale of depression in high-risk MDD subjects

Corrected P value < 0.05. For better visibility the results are thickened with the “tbss-fill” command. The images are in radiological convention.

R, Right side; L, Left side.

Figure

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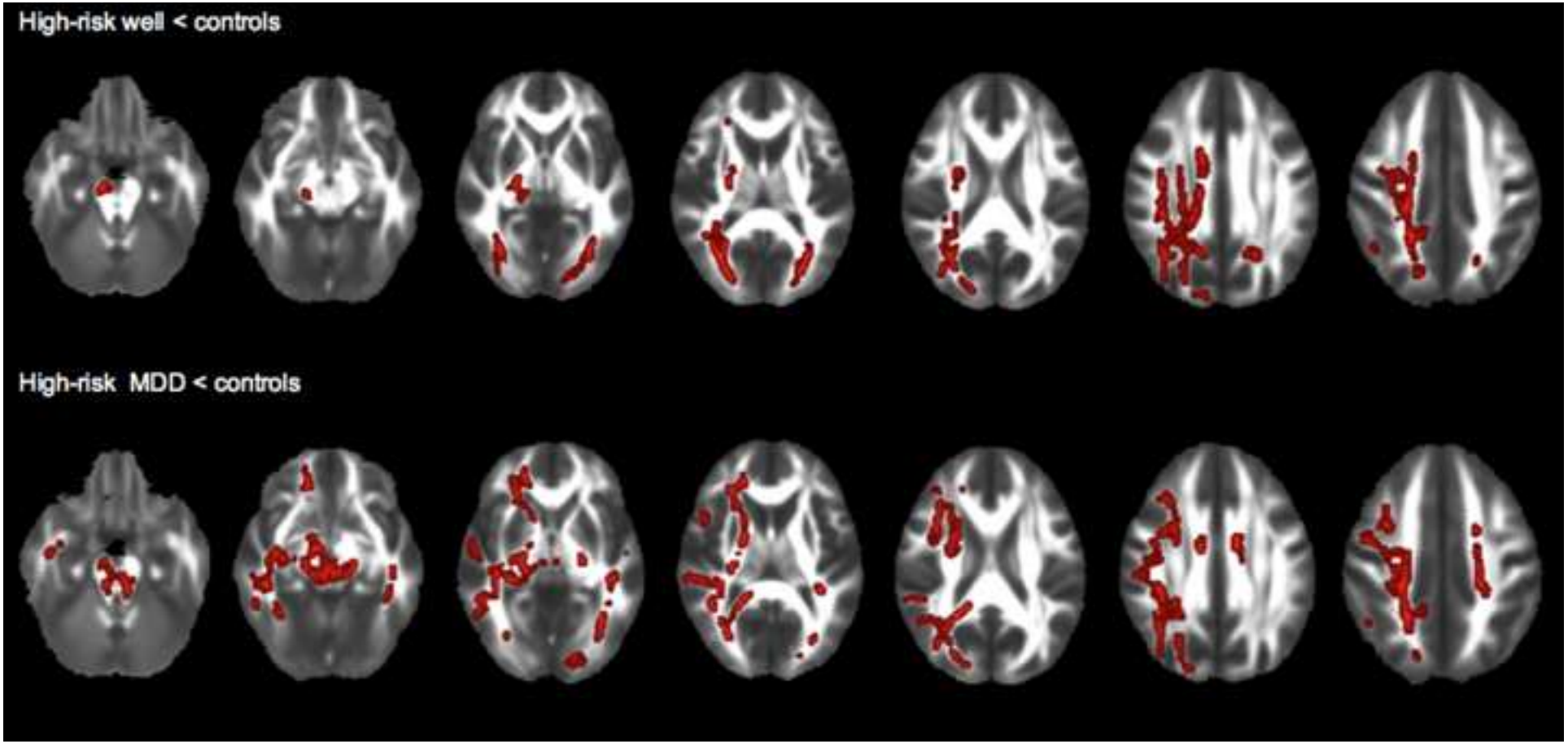

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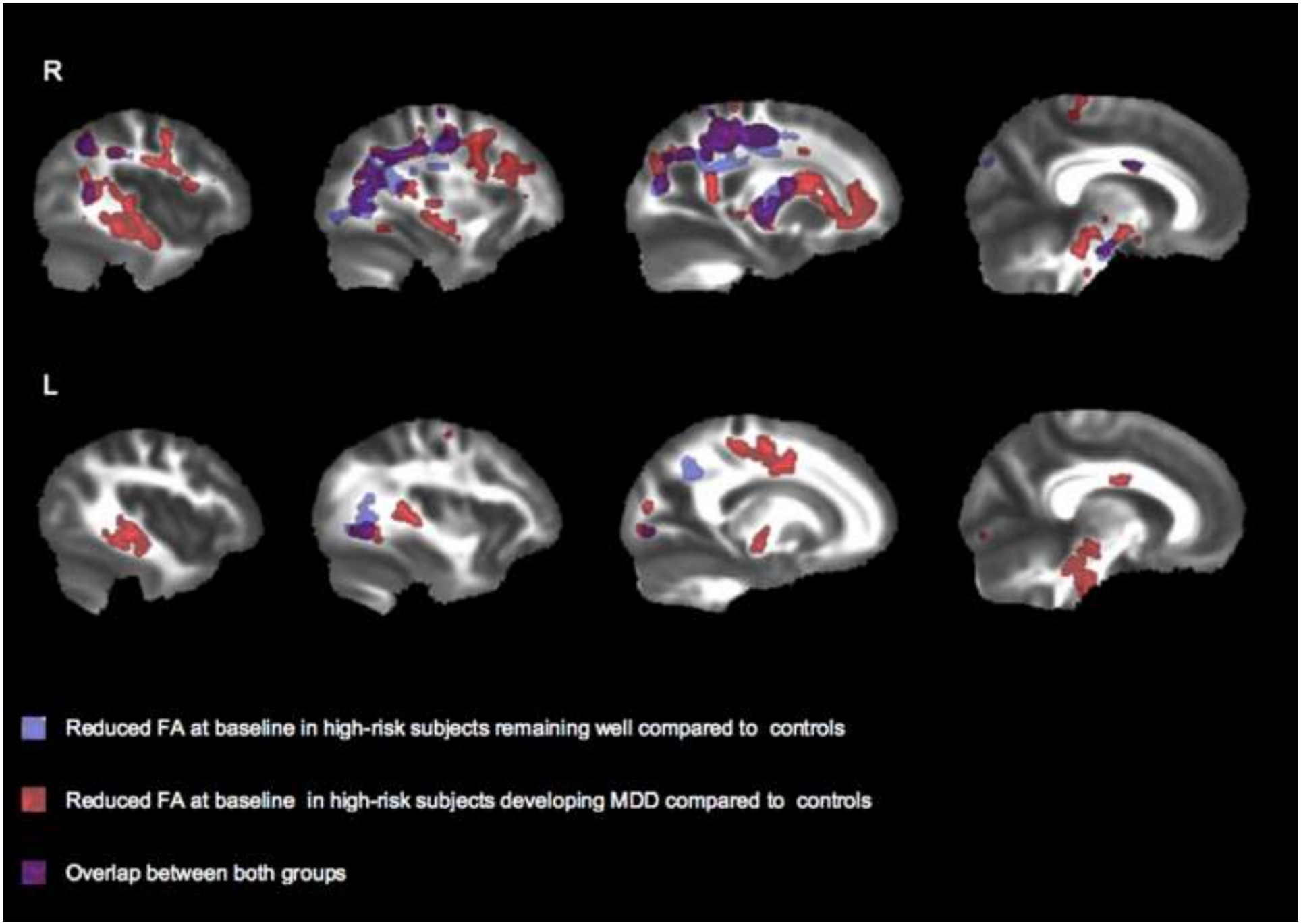

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