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White matter microstructural impairments and genetic liability to familial bipolar I disorder

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Background
Subtle abnormalities in frontal white matter have been reported in bipolar disorder.

Aims
To assess whether impaired integrity of white matter tracts is associated with bipolar disorder and genetic liability for the disorder.

Method
A total of 19 patients with psychotic bipolar I disorder from multiply affected families, 21 unaffected first-degree relatives and 18 comparison individuals (controls) underwent diffusion tensor imaging. Whole brain voxel-based analyses compared fractional anisotropy between patients and relatives with controls, and its relationship with a quantitative measure of genetic liability.

Results
Patients had decreased fractional anisotropy compared with controls in the genu of the corpus callosum, right inferior longitudinal fasciculus and left superior longitudinal fasciculus. Increased genetic liability for bipolar disorder was associated with reduced fractional anisotropy across distributed regions of white matter in patients and their unaffected relatives.

Conclusions
Disturbed structural integrity within key intra- and interhemispheric tracts characterises both bipolar disorder and genetic liability for this illness.

Declaration of interest
None.

Bipolar disorder is a highly heritable illness and white matter abnormalities represent potential endophenotypes that may help to clarify the likely complex pathways from susceptibility genes to the clinical syndrome.[1] White matter hyperintensities and volume deficits identified from structural magnetic resonance imaging (MRI) are reported in bipolar disorder[2,3] and in unaffected relatives of patients at presumed high genetic liability for the illness.[4,5] Diffusion tensor imaging (DTI)[6] uses the microscopic diffusion of water *in vivo*[7] to probe the coherence and integrity of white matter fibres. Previous DTI studies in patients with bipolar disorder have suggested impairments to white matter coherence in frontal regions (online Table DS1). We employed a voxel-based approach to assess changes in fractional anisotropy over the whole brain, in families multiply affected with psychotic bipolar I disorder. Fractional anisotropy changes were identified that were associated with (a) a diagnosis of bipolar disorder and (b) genetic liability for this illness.

Method

Participants

We successfully obtained diffusion-weighted imaging data on 19 participants with bipolar I disorder in remission, 21 of their unaffected first-degree relatives (4 parents, 10 siblings, 7 children) and 18 healthy volunteers (controls). All patients had experienced psychotic symptoms during episodes of illness exacerbation and came from families where there was at least one additional first- and/or second-degree relative with a psychotic disorder. Most participants (16 patients, 18 relatives, 8 controls) had participated in our previous structural imaging studies.[3,5] Patients and their family members were recruited via voluntary support groups, the study website or by direct referral from their mental health services as described elsewhere.[8] Controls were recruited by newspaper advertisements and at a group level were demographically similar in age, gender and parental social class to the combined patient and relative group.

All participants were assessed using the same clinical scales. Structured diagnostic interviews were performed using the Schedule for Affective Disorders and Schizophrenia – Lifetime Version[9] enabling DSM–IV diagnoses. Information regarding family history of psychiatric illness was obtained from the most reliable informants using the Family Interview for Genetic Studies[10] and from medical notes when available. The Beck Depression Inventory (BDI)[11] and the Altman Self-Rated Mania Scale (ASRM)[12] were completed to quantify current psychopathology. Full-scale IQ was estimated using the Wechsler Abbreviated Scale of Intelligence.[13]

Exclusion criteria included organic brain disease, previous head trauma resulting in loss of consciousness for more than 5 min, and fulfilment of DSM–IV criteria[14] for substance or alcohol dependence in the 12 months prior to assessment. No unaffected relatives or controls had ever experienced a psychotic illness. No patients were in-patients at the time of assessment. The study was approved by the local ethics committee and written informed consent was obtained from all participants.

Genetic liability scale

As previously described in detail,[5] we modelled the likely variation in the level of genetic risk among family members with bipolar disorder using a continuous quantitative measure of genetic liability based on each individual's affection status and the number, affection status and genetic relatedness of all adult members of each family. Briefly: a polygenic multifactorial liability threshold model of illness was used in which liability was assumed to be continuous in the population, with a Gaussian distribution. Patients with bipolar disorder were initially imputed a mean liability above a threshold based on the population prevalence rate of the illness, estimated at 0.5%,[15] as were their family members with psychotic disorders, who were assumed to express the same

phenotype as the index patient. Relatives who were without a psychotic disorder were considered unaffected and were initially imputed a mean liability below the threshold. These scores were then adjusted for each individual to account for family size and affection distribution for all individuals older than 16 years and as far as second degree from the index patient.

In a hypothetical situation where absolute information on all affected individuals over several generations was available, we would expect the distribution to be normally distributed. In the absence of such detail, the distribution was found to be bimodal (relatives, 0.13–0.77; patients, 1.62–2.20: Kolmogorov–Smirnov test, $P=0.025$). In order to remove the possibility of correlations between the genetic liability scale (GLS) and fractional anisotropy being driven by group differences in the GLS, rather than variation within the patient and relative groups, the GLS values from each group were separately standardised to their respective subgroup means. This standardisation gave z-score values for relatives ($-1.46<z<2.14$) and patients ($-1.44<z<1.90$), resulting in a normally distributed continuous variable for genetic liability (Kolmogorov–Smirnov test, $P=0.587$).

Data acquisition and preprocessing

Diffusion-weighted imaging data were acquired using a GE Signa 1.5 T LX MRI system (General Electric, Milwaukee, Wisconsin, USA), using an echo planar imaging acquisition, peripherally gated to the cardiac cycle and optimised for the acquisition of diffusion tensor magnetic resonance of white matter. At each of the 60 slice locations, 7 non-diffusion-weighted images were acquired ($b=0$), along with 64 images with diffusion gradients ($b=1300$ s/mm^2) applied in 64 optimised directions uniformly distributed in space.[16] Whole head acquisition gave isotropic (2.5 mm^3) voxels, reconstructed to a 1.875×1.875 mm in-plane pixel size. Following correction of the diffusion-weighted images for image distortions introduced by the diffusion-weighting gradients, in-house software was used to (a) remove non-brain tissue and (b) determine the diffusion tensor in each voxel (based on the calculations of Basser *et al*[6]). Images of (a) mean T_2-weighted intensity (i.e. with no diffusion gradients applied) and (b) fractional anisotropy[16] were computed for each participant.

To facilitate a voxel-based approach, images were preprocessed using SPM2 (Wellcome Department of Imaging Neuroscience, London; www.fil.ion.ucl.ac.uk/spm). A two-stage registration process was performed. First, a custom fractional anisotropy template was created by normalising each participant's average T_2-weighted ($b=0$) image to the standard T_2-weighted echo planar imaging template supplied within SPM2, applying the resulting transformation parameters to the corresponding (inherently co-registered) fractional anisotropy images, smoothing these with an 8 mm full width half maximum (FWHM) isotropic Gaussian kernel and then averaging over all participants at each voxel to create a mean intensity image. The original fractional anisotropy images were subsequently re-normalised to this custom template and smoothed by a 4 mm FWHM isotropic Gaussian kernel to increase the signal:noise ratio and compensate for any residual anatomical variation not removed by normalisation. The smoothing kernel size was chosen *a priori* to match the approximate diameter of the major white matter tracts of interest. During the second normalisation phase, each participant's image was segmented (using SPM2's default *a priori* tissue probability information) and thresholded (at a level of 10%) to provide a liberal binary mask of white matter; along with the requirement that all participants contribute data at a particular location (see below), this limits statistical testing to core white matter only.

Voxel-based statistical analyses

Group analyses

To assess the statistical significance of between-group differences in fractional anisotropy, data were analysed using a non-parametric permutation-based method[18,19] (XBAM version 3.4, Brain Imaging Analysis Unit, Institute of Psychiatry, London). A non-parametric approach for data of this type is more suitable than parametric approaches, because of potential non-normality of the residuals of fit to the general linear model. At each voxel in standard space for which data were present in all individuals, an analysis of variance (ANOVA) model was fitted with fractional anisotropy as the dependent variable and group classification as the key predictor variable. Analyses were performed separately for patients and relatives in comparison with the control group. First, a voxel-wise test statistic was computed by regressing the model onto the observed data, and only those voxels that exceeded a relatively lenient probability threshold ($P<0.05$) were retained for future analyses. These suprathreshold voxels were clustered and the 'mass' (sum of suprathreshold voxel values) of each 3-dimensional voxel cluster was then tested by permutation (using a one-tailed randomisation test) against the null hypothesis of no fractional anisotropy differences between the groups. Analyses are reported at an adaptive cluster-level threshold where the expected number of false-positive clusters is less than one per analysis. Significant clusters were ascribed the coordinates of the centroid voxel, with their location determined by comparison with known anatomical pathways.[20]

GLS correlation to fractional anisotropy

We explored the relationship between the quantitative GLS and fractional anisotropy in families with bipolar disorder using multiple regression models at each voxel with fractional anisotropy as the dependent variable and the GLS as the key predictor variable, employing the same permutation-based method described above.

Multivariate analysis of significant clusters

The average fractional anisotropy value from each significant cluster for each individual was recorded. To explore the relationship between patients with bipolar I disorder, their unaffected relatives and controls, the spatial locations of the clusters identified in the patient–control contrast were used as binary masks to extract fractional anisotropy values from the relatives group and these values were entered into a non-parametric test for a linear trend (groups ordered: bipolar disorder < relatives < controls), based on a Wilcoxon rank sum test.[21] Where multiple clusters were present, principal components analysis without rotation was performed to explore the extent of correlation between regions and to reduce the data to a single value for each individual.

Linear regression analyses were carried out with the regress command and combined 'robust' and 'cluster' options in STATA (version 9.2 for Windows). This uses the Huber–White sandwich[22,23] estimate of variance, which maintains correct type 1 error rates when data are observed in clusters (in this case, families). Within the linear regression analyses the principal components scores were specified as the dependent variable, and affection status or GLS as key predictors, with effects of gender and a group × gender interaction also tested. Results are reported using a two-tailed statistical significance threshold of $P<0.05$. The effect of mood ratings in patients at the time of scanning and illness severity on mean fractional anisotropy values identified

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in the case–control analysis were explored by entering total score on the BDI and ASRM and the number of hospitalisations into the regression analyses previously described.

Results

Participants

Participants' sociodemographic and other details are given in Table 1. There were no significant differences between the three groups in age, gender, handedness, full-scale IQ, years of education or parental social class. Patients and their relatives were recruited from 21 families in which the index patient had had at least one additional first- or second-degree relative with a psychotic disorder (family history of bipolar disorder: $n=13$ families; schizophrenia or schizoaffective disorder: $n=6$; psychosis not otherwise specified: $n=2$).

All patients had a lifetime DSM–IV diagnosis of bipolar I disorder and had experienced delusions and/or hallucinations during at least one episode of illness exacerbation. Patients had experienced on average 4 hospitalisations (range 0–13) with a mean duration of illness, as measured from the time of diagnosis, of 15.6 years. Lifetime comorbidity was detected in two patients, one with anxiety disorder and one with alcohol dependence syndrome (both recovered). Overall, 15 patients at the time of scanning were taking at least one psychotropic medication (lithium $n=9$; other mood stabilisers, including sodium valproate, $n=8$; antidepressants $n=5$; antipsychotics $n=3$). Four patients were not receiving medication. Four of the unaffected relatives fulfilled criteria for a non-psychotic Axis 1 disorder during their lifetime – three with major depressive disorder and one with substance-induced mood disorder. No relatives were taking psychotropic medication at the time of scanning. In the control group, one participant fulfilled lifetime DSM–IV criteria for major depressive disorder, and one participant for alcohol misuse (both recovered), with no controls ever receiving psychotropic medication.

Symptom scales

All patients were clinically in illness remission. However, many patients with bipolar disorder continue to experience subsyndromal symptoms in between episodes of illness[24] and this was reflected in higher mean BDI and ASRM scores in patients compared with controls.

Fractional anisotropy changes in bipolar I disorder

Significant reductions in fractional anisotropy in patients compared with controls were detected in three spatially extensive 3-dimensional clusters (Fig. 1 and Table 2). These were: a bilateral frontal cluster extending from deep frontal white matter to include the genu of the corpus callosum and a left lateralised portion of the internal capsule; a right temporal cluster which extended superiorly towards the parietal lobe; and a superior frontal cluster. No clusters of increased fractional anisotropy were detected in patients.

Table 1 Sociodemographic and clinical characteristics of each group

	Group				
	Patients ($n=19$)	Relatives ($n=21$)	Controls ($n=18$)	Statistic	P
Age, years Mean (s.d.) Range	43.3 (10.2) 30–62	42.5 (13.6) 21–64	41.7 (12.2) 26–63	$F(2,57)=0.07$	0.929
Gender, male : female	9 : 10	12 : 9	10 : 8	$\chi^2(2)=0.42$	0.810
Left-handed, *n* (%)	1 (5.3)	3 (14.3)	3 (16.7)	$\chi^2(2)=1.52$	0.469
Full-scale IQ, mean (s.d.)	114.6 (15.4)	118.8 (7.5)	114.9 (13.9)	$F(2,53)=1.02$	0.366
Education, years: mean (s.d.)	14.4 (3.3)	15.5 (3.6)	16.7 (3.8)	$F(2,57)=1.91$	0.157
Parental SES,[a] *n* (%)	9 (47.3)	13 (61.9)	11 (61.1%)	$\chi^2(2)=1.03$	0.597
Beck Depression Inventory	7. 9 (7.0)*	5.0 (3.5)	3.4 (3.7)	$F(2,51)=3.49$	0.038†
ASRM	3.5 (2.6)*	1.8 (2.5)	1.0 (1.8)	$F(2,51)=4.95$	0.011†
Age at diagnosis, years: mean (s.d.)	27.7 (10.3)	NA	NA	NA	NA
Number of hospitalisations, mean (s.d.)	4.1 (3.8)	NA	NA	NA	NA

ASRM, Altman Self-Rating Mania Scale; NA, not applicable; SES, socioeconomic status.
a. Class I or II (professional, managerial and technical occupations). Based on details of parental occupation at the time of the individual's birth.
*Mean difference between patients and controls is significant at $P<0.05$ in *post hoc* (Bonferroni) analyses.
†Group comparisons significant at $P<0.05$; two-tailed continuous data were assessed with a one-way ANOVA, and categorical data were assessed using a chi-squared test.

Table 2 Anatomical location, representative Montreal Neurological Institute (MNI) coordinates, cluster size and loading scores on first principal component for significant clusters of reduced fractional anisotropy in patients with bipolar I disorder compared with healthy volunteers

		MNI coordinates				
Cluster location	Side	x	y	z	Cluster size (voxels)	First principal component loading
Bilateral deep frontal white matter and genu of the corpus callosum: corresponding to anterior portions of the fronto-occipital fasciculus and superior longitudinal fasciculus	Right/Left	−14	37	28	1576	0.935
Superior frontal white matter: corresponding to the superior longitudinal fasciculus and corona radiata	Left	−25	−9	36	538	0.932
Parietotemporal junction: incorporating temporal white matter and extending to parieto/occipital regions: corresponding to the inferior longitudinal fasciculus and posterior portions of the inferior fronto-occipital fasciculus	Right	31	−53	4	624	0.948

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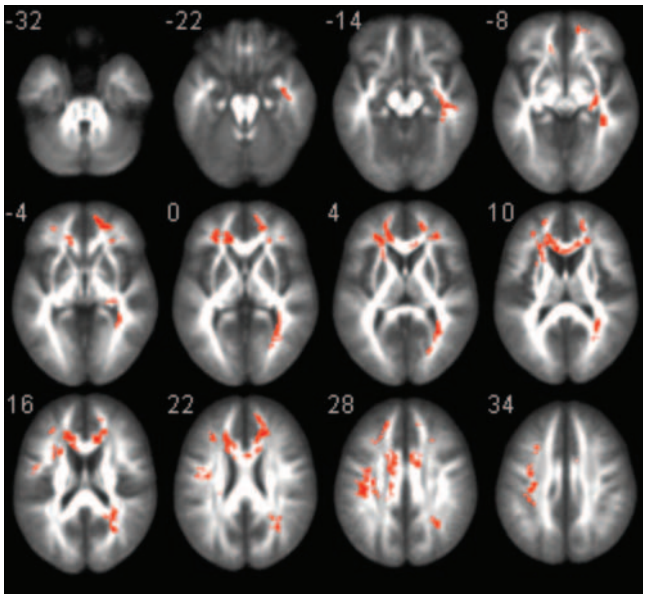

Fig. 1 Regions of decreased fractional anisotropy in patients with bipolar I disorder compared with healthy volunteers.

Regions of decreased (red voxels) fractional anisotropy in bipolar I disorder when compared with controls. Cluster-wise probability was thresholded at <1 false positives. The *z*-coordinate for each axial slice, in the space of the International Consortium for Brain Mapping non-linear average brain template (ICBM152), is given in millimetres and the images are displayed in neurological format (right side of each panel represents the right side of the brain).

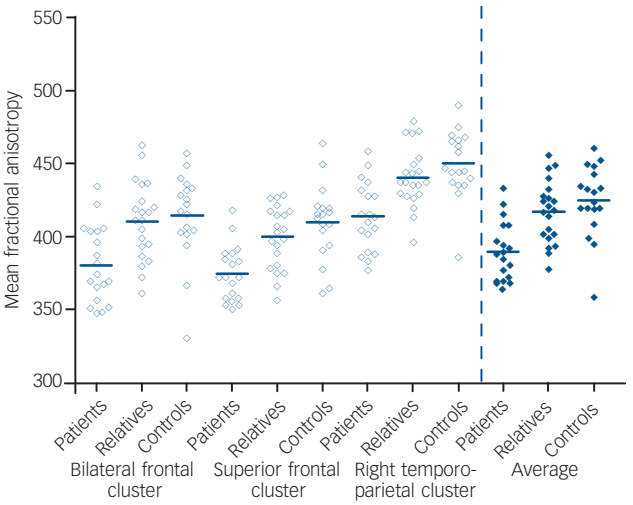

Fig. 2 Mean fractional anisotropy values for each participant, extracted from clusters of decreased fractional anisotropy in bipolar I disorder.

Extracted mean fractional anisotropy values (x 1000) plotted for each participant, extracted from the three spatially invariant clusters (◇) that showed reduced fractional anisotropy in patients with bipolar I disorder when compared with controls (see Fig. 1), and average of clusters (◆) Values were also extracted from unaffected relatives in the same areas, and whose mean values were seen to be intermediary between patients and controls as tested by a linear trend analysis ($P=0.001$).

The principal components analysis of voxel clusters identified by the patient–control comparison demonstrated that fractional anisotropy was strongly correlated between brain regions, with the first principal component accounting for 88% of the total variance. Values of this first component were strongly related to affection status (group) ($F(1,34)=11.77$, $P=0.002$), but there was no effect of gender ($F(1,34)=0.00$, $P=0.978$) or gender × group interaction ($F(1,34)=0.00$, $P=0.999$). There was no difference in fractional anisotropy scores between those patients who were currently medicated ($n=15$) and those who were not medicated ($n=4$) ($F(1,17)=0.02$, $P=0.899$). Within the patient group, there was no relationship between values on the first component and total score on the BDI ($F(1,16)=0.26$, $P=0.616$), ASRM ($F(1,16)=0.31$, $P=0.582$) or in correlation to the number of hospitalisations ($F(1,16)=1.34$, $P=0.200$).

Fractional anisotropy changes in unaffected relatives of bipolar I patients

Using a whole brain voxel-based analysis, no significant clusters of either increased or decreased fractional anisotropy were detected between the unaffected relatives of patients and controls. Figure 2 displays a plot of mean fractional anisotropy values of the three groups, extracted from the spatial locations identified in the patient–control comparison. The principal components analysis of voxel clusters again showed high correlation between the clusters in the three groups (first principal component accounting for 88% of the variance).

There were no significant differences between controls and relatives for the principal component summarising all three clusters ($F(1,38)=1.21$, $P=0.278$); however, a linear trend was confirmed within all clusters (Cuzick's trend test: bilateral frontal, $z=3.45$, $P=0.001$; superior frontal, $z=4.04$, $P<0.001$; right temporo-parietal, $z=4.35$, $P<0.001$), indicating that the mean fractional anisotropy value of the relatives are intermediary to the patients and controls.

Fractional anisotropy changes with genetic liability scale

Increasing genetic liability for bipolar disorder was significantly associated with lower fractional anisotropy in 70 distributed clusters incorporating several of the major white matter tracts of the brain (Fig. 3 and online Table DS2). These regions included: the cerebellum and brainstem; bilateral temporal lobe corresponding to the inferior and superior longitudinal fasciculi and uncinate; bilateral deep frontal white matter, extending to the genu of the corpus callosum corresponding to the anterior regions of the fronto-occipital fasciculus, superior longitudinal fasciculus, and superior fronto-occipital fasciculus; posterior brain regions corresponding to bilateral portions of the inferior fronto-occipital and inferior longitudinal fasciculi; splenium of the corpus callosum and corona radiata.

The principal components analysis demonstrated that the extracted clusters were substantially correlated, with the first component accounting for 39% of the total variance (second component accounted for 6% of total variance). Values of the first principal component were strongly associated with the GLS ($F(1,20)=20.02$, $P<0.001$). There was no evidence of an interaction between group (patient/relative) and GLS ($F(1,20)=0.91$, $P=0.351$), indicating that this pattern of fractional anisotropy reductions was not determined solely by abnormalities in the patients.

Figure 4 demonstrates a highly significant negative correlation between genetic liability and mean fractional anisotropy values, for both patients ($r=-0.814$, $P<0.001$, 95% CI -0.926 to -0.570) and unaffected relatives ($r=-0.717$, $P<0.001$, 95% CI -0.877 to -0.412). The relationship remained highly significant within the relatives group, after excluding the four volunteers with a lifetime DSM–IV diagnosis ($r=-0.730$, $P<0.001$, 95% CI -0.896 to -0.384).

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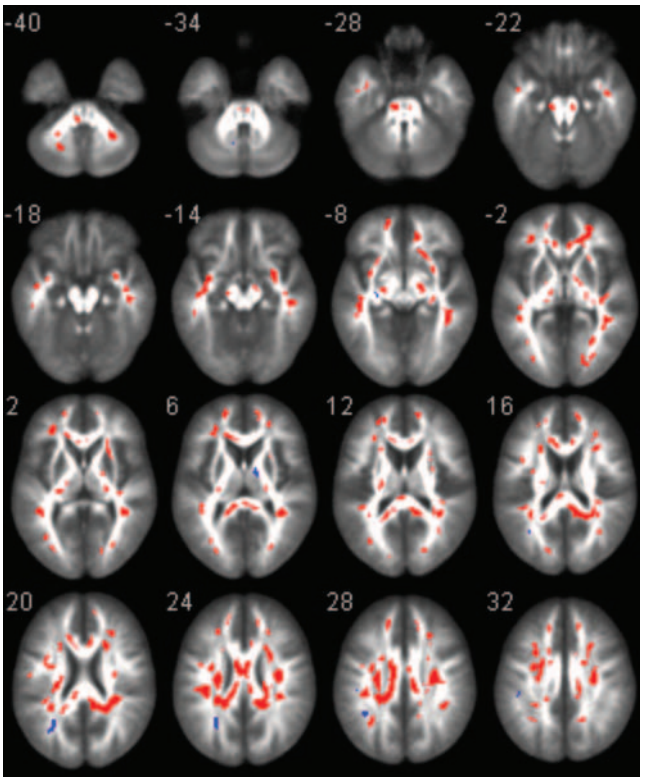

Fig. 3 Fractional anisotropy correlations with genetic risk for bipolar disorder.

Regions of decreased (red voxels) and increased (blue voxels) fractional anisotropy associated with increasing genetic liability for bipolar disorder. Cluster-wise probability was thresholded at <1 false positives. The *z*-coordinate for each axial slice, in the space of the International Consortium for Brain Mapping non-linear average brain template (ICBM152), is given in millimetres, and the images are displayed in neurological format (right side of each panel represents the right side of the brain).

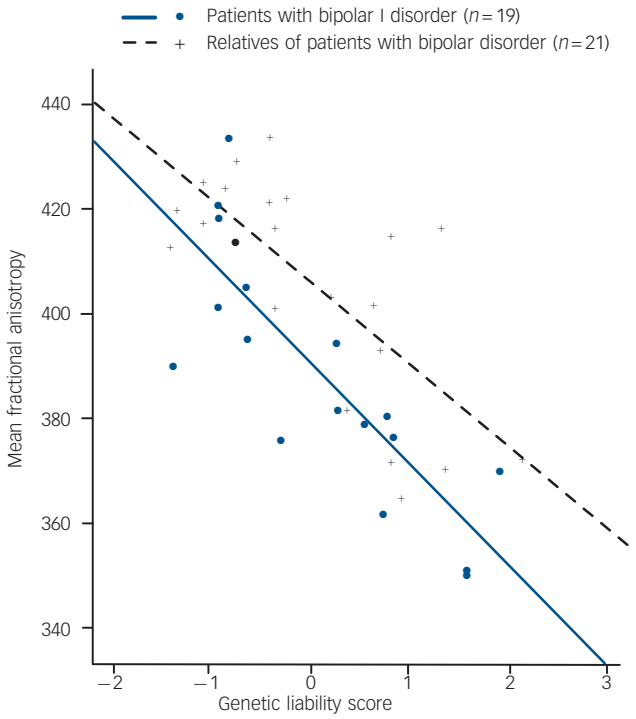

Fig. 4 Plot of mean fractional anisotropy of clusters that were negatively correlated with genetic liability to bipolar disorder.

Linear association between mean fractional anisotropy values (×1000) and genetic liability score demonstrating similar negative correlation in both patients with bipolar I disorder and their unaffected relatives. Mean fractional anisotropy score represents correlated fractional anisotropy deficits identified by voxel-based analysis of genetic liability scale and fractional anisotropy in patients and relatives. The genetic liability score was standardised to the subgroup means for patients and relatives.

A positive correlation between fractional anisotropy and the GLS was identified in 11 clusters (Fig. 3), with only one cluster having a spatial threshold of greater than 50 voxels. This was located in left parietal white matter, posterior to the splenium of the corpus callosum (online Table DS2).

Discussion

In our study, we used a whole brain voxel-based analysis to assess changes in white matter microstructure as indicated by fractional anisotropy values, in patients with bipolar I disorder and their unaffected relatives, in order to assess the likely genetic contributions to white matter abnormalities in the illness. Reductions in fractional anisotropy were identified in patients when compared with controls in white matter incorporating several major inter- and intrahemispheric tracts. The affected white matter regions were consistent with anterior portions of the fronto-occipital and superior longitudinal fasciculi; the genu of the corpus callosum, which connects anterior portions of the brain interhemispherically; and the left anterior limb of the internal capsule, which contains corticothalamic projections. Fractional anisotropy reductions were also noted in the right temporal portion of the inferior longitudinal fasciculus which extended to the temporal–parietal border, and in superior frontal regions, in locations consistent with the superior longitudinal fasciculus and corona radiata. Our findings also confirm our previous report that utilised voxel-based analyses of T_1-weighted magnetic resonance images, in an overlapping sample of patients at a different time point.[3] A reduction in fractional anisotropy in these regions could signify changes in the organisation or orientation of white matter tracts, a reduction in density of axons or impairments in myelination and consequent variation in membrane permeability to water.[7]

Previous DTI studies in bipolar I disorder have used manual placement of regions of interest, which has constrained the number of areas of white matter sampled. A review of previous DTI studies of bipolar disorder can be found in online Table DS1 and largely support our findings. A reduction in fractional anisotropy in superior frontal regions has previously been found in a chronic[25] and a first-episode study[26] and this may be specific to the left hemisphere, as indicated in our study and by Adler *et al*.[26] The locations of these reductions are consistent with impairments in the left superior longitudinal fasciculus which connect frontal to predominantly temporo-parietal regions. Two studies have specifically assessed fractional anisotropy in the corpus callosum, with one study supporting our finding of reductions in genu rather than splenium[27] and another reporting increases in the genu.[28] In addition, an increase in local anisotropy relative to controls has also been identified in one study in anterior superior white matter.[29] Increased fractional anisotropy can be caused by an actual change in the packing and myelination of the white matter fibres or by secondary mechanisms such as unmasking of regions due to die back of crossing fibres, differing methodologies and acquisition parameters. Heterogeneity within clinical populations can also cause variation in neuroimaging findings, and fractional anisotropy reductions may be more expected within our study, owing to the ascertainment of a

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subgroup of patients who had experienced a psychotic episode. Corticothalamic projections have shown a reduction in anisotropy in the posterior internal capsule[29] and in our study in the anterior limb of the internal capsule. The anterior limb of the internal capsule carries frontothalamic projections that form a portion of the cortico–thalamo–striato–cortical circuits which have an important role in cognition and psychiatric illnesses.[30] Other outcome measures from DTI data support the findings that bipolar disorder is associated with greater diffusivity in frontal regions of interest but not parietal, occipital or temporal regions.[31,32] Another DTI study to have assessed white matter throughout the whole brain using a voxel-based approach confirmed our finding of reduced fractional anisotropy in right temporal white matter, consistent with an impairment within the right inferior longitudinal fasciculus[33] with additional evidence for impaired frontotemporal connectivity from a finding of reduced fractional anisotropy in the anterior cingulum.[34] Taken together, our findings coupled with the previous literature using DTI to investigate white matter integrity in bipolar disorder indicate strong evidence for impairments in white matter in frontal regions, including the left superior longitudinal fasciculus, genu of the corpus callosum and right temporal portions of the inferior longitudinal fasciculus.

Fractional anisotropy as a marker of genetic liability to bipolar disorder

This study provides strong evidence that reduced fractional anisotropy is associated with genetic liability for bipolar disorder. Although we failed to identify significant differences in fractional anisotropy of the unaffected relatives in a straightforward group-level comparison with controls, unaffected relatives had intermediate fractional anisotropy values (between patients and controls) over the clusters that showed reduced fractional anisotropy in bipolar I disorder. Furthermore, by modelling the likely variable genetic liability that a patient or relative carries (rather than treating all relatives or patients as homogeneous for genetic risk), we were able to identify significant negative correlations between the quantitative GLS and fractional anisotropy over many of the major white matter tracts. This widespread association of reduced fractional anisotropy with increasing genetic liability for bipolar disorder is consistent with the findings from our previous study, which linked an identical GLS to white matter volume reductions ascertained from T_1-weighted magnetic resonance images,[5] which were identified in temporoparietal regions, medial frontal regions, including the genu, and in thalamo–cortical connections. The association between fractional anisotropy and GLS was more extensive in this current study, possibly because of increased sensitivity of DTI to white matter microstructure. Interestingly, we have previously identified, using an electrophysiology measure (auditory P300 wave), an increase in latency indicative of slower neuronal transmission speed in both patients with bipolar disorder and their unaffected relatives, which is consistent with impaired white matter.[8] We therefore present consistent findings, which directly or indirectly indicate that impaired structural and functional connectivity is associated with susceptibility genes for bipolar disorder. In addition, our finding of reduced fractional anisotropy as a marker of risk for bipolar disorder is supported by the only previous DTI study to assess unaffected siblings of patients with bipolar disorder (childhood onset), which identified fractional anisotropy reductions in both patients and their siblings in a region of interest placed in bilateral superior longitudinal fasciculus.[35]

Functional significance of white matter abnormalities in bipolar disorder

The phenotype of bipolar I disorder typically encompasses emotional dysregulation and psychosis. Both of these symptom clusters are likely to be underpinned by large-scale neurocognitive networks. Disruption within specialised modules, or alternatively changes in the organisation of white matter tracts between these modules, may be involved in eliciting these symptom dimensions. Evidence from functional MRI and positron emission tomography have generally identified overactivations in bipolar disorder within a ventral 'affective system' (amygdala, thalamus, ventrolateral and orbitofrontal prefrontal cortex) and reductions within the regulatory 'cognitive system' (dorsal prefrontal cortex, anterior cingulate and hippocampus).[36] Our study found reduced fractional anisotropy within tracts linking frontal regions to thalamic regions (e.g. anterior limb of the internal capsule) and, in addition, clusters in regions consistent with the uncinate were identified that showed a significant negative correlation between fractional anisotropy and the GLS. The uncinate, which connects the amygdala, uncus and temporal pole to the orbitofrontal gyrus, is a key tract within the emotion regulation network. Houenou *et al* were unable to identify fractional anisotropy changes within the uncinate in a sample of patients with bipolar disorder, using a tractographic technique that reconstructed fibres *in vivo*.[37] Psychosis has been postulated to be underpinned by abnormal connections between the language areas in the temporal lobe and frontal lobe structures.[38] All of the patients in our study had experienced psychosis in illness exacerbation, and we identified fractional anisotropy reductions in patients in right temporal white matter and in patients and their relatives in bilateral temporal white matter in association with increasing liability for bipolar disorder, in particular in the superior longitudinal fasciculus, which links Broca's and Wernicke's language areas, and in the inferior longitudinal fasciculus. Interestingly, there have been replicated findings of reduced fractional anisotropy in white matter in schizophrenia in similar frontotemporal tracts,[39] which could reflect the impact of susceptibility genes that are shared between schizophrenia and bipolar disorder.[40] Within our study, there were no significant correlations between fractional anisotropy and the symptom rating scale, indicating that the severity of subsyndromal residual symptoms does not correlate with fractional anisotropy differences in bipolar disorder. However, fractional anisotropy reductions may be conferring susceptibility to these types of symptoms which become apparent at times of illness exacerbation. Additionally, a measure of illness severity, the number of hospital admissions, did not show a significant effect in modulating fractional anisotropy, which is supported by the strong negative correlation between fractional anisotropy and the genetic liability scale, indicating that reductions are not a consequence of illness exposure, medication or chronicity of the disorder.

It is not possible to clarify the exact cause of a reduction in fractional anisotropy, as this can be influenced by a change in the organisation or orientation of white matter tracts, a reduction in density of white matter fibres or a reduction in myelination. In bipolar disorder, there is some evidence pointing to a role of disrupted myelination, with increased apoptosis and necrosis of oligodendrocytes[41] and a downregulation of myelination and oligodendrocyte-related genes[42] previously identified.

Limitations

There are some limitations to the current study. First, our sample sizes for all three groups were modest, and for whole brain voxel-based analyses this may explain why we were unable to identify

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absolute differences in fractional anisotropy when comparing relatives of the patients with controls. An absence of group-level reductions of fractional anisotropy in the relatives group may therefore represent a type II error, which was overcome by the use of the potentially more sensitive quantitative scale of genetic liability; however, replication of fractional anisotropy reductions in an independent sample is warranted. Second, it is possible that some of the fractional anisotropy changes in the patients were related to medication effects. We were unable to detect a difference in fractional anisotropy values between the four patients who were unmedicated and those that were taking predominantly mood stabilisers; however, studies of larger samples of unmedicated patients are required to confirm this finding. Changes in fractional anisotropy have not been studied after lithium treatment; however, white matter density has been shown to increase post-treatment,[43] which is in support of lithium's neuroprotective properties. Further evidence against medication driving the low fractional anisotropy values is provided by our finding of predominantly reduced fractional anisotropy in those unaffected and unmedicated relatives with high genetic liability as indicated by high GLS values. Third, although there were no significant differences in the mean ages of the groups, these spanned a large age range. White matter's maturational pattern has recently been characterised, and within the age range of this study fractional anisotropy is seen to decrease predominantly in association and callosal, but not projection, fibres.[44] However, within the clusters that showed reduced fractional anisotropy in patients with bipolar I disorder, there was no evidence for an interaction between group and age, with similar reductions with age identified in each group (data not shown).

We utilised a voxel-based analysis of DTI data to assess changes in fractional anisotropy across whole brain white matter. Similar methods have been criticised as being susceptible to 'edge effects' caused by misregistration of tracts,[45] which is observable when an increase in fractional anisotropy lies adjacent to an area of decreased fractional anisotropy. However, the role of edge effects appears minimal in our study, as we found very few clusters of increased fractional anisotropy. Tractography is one method of removing the possibility of misregistration of white matter tracts, and also confers the benefits of testing anatomically defined hypotheses and potentially increasing sensitivity to detect fractional anisotropy differences.[45] Such approaches may prove beneficial in future studies.

Owing to converging evidence of white matter abnormality in bipolar disorder, we suggest that future studies should attend to those genes involved in the regulation of white matter structure. Proof that such susceptibility genes for bipolar disorder act by altering the structure of white matter would confirm that the observed changes in cortical connectivity are primary factors leading to the downstream affects of symptomatology and cortical misactivation patterns as observed using functional MRI. As it has not been possible to identify a specific cortical area within the brain that is abnormal in structure or function, it is likely that a hodological approach, studying dysfunction within neural networks, will prove more successful. Future studies may also benefit by aiming to uncover whether similar white matter changes are identifiable in schizophrenia, and therefore generic to psychosis, or whether specific networks are affected in bipolar disorder and schizophrenia.

In conclusion, we have demonstrated that bipolar I disorder is associated with significant reductions of fractional anisotropy, indicative of impaired white matter integrity, in key inter- and intrahemispheric tracts. We present evidence that similar distributed abnormalities are present in relatives as indicated by the strong association noted between increasing genetic liability for bipolar disorder and reduced fractional anisotropy. White matter abnormalities that are genetically driven are therefore proposed as a pathophysiological process underlying affective psychosis, and as potential endophenotypic markers of bipolar disorder.

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