Effects of *ANK3* Variation on Gray and White Matter in Bipolar Disorder

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Abstract

The single nucleotide polymorphism rs9804190 in the Ankyrin G (*ANK3*) gene has been reported in genome-wide association studies to be associated with bipolar disorder (BD). However, the neural system effects of rs9804190 in BD are not known. We investigated associations between rs9804190 with gray and white matter structure within a frontotemporal neural system implicated in BD. A total of 187 adolescent and adult European Americans were studied: a group homozygous for the C allele [52 individuals with BD and 56 controls] and a T-carrier group, carrying the high risk T allele (38 BD and 41 controls). Subjects participated in high-resolution structural magnetic resonance imaging and diffusion tensor imaging (DTI) scanning. Frontotemporal region of interest (ROI) and whole brain exploratory analyses were conducted. DTI ROI-based analysis revealed a significant diagnosis by genotype interaction within the uncinate fasciculus ($p < 0.05$), with BD subjects carrying the T (risk) allele showing decreased fractional anisotropy compared to other subgroups, independent of age. Genotype effects were not observed in frontotemporal gray matter volume. These findings support effects of rs9804190 on frontotemporal white matter in adolescents and adults with BD and suggest a mechanism contributing to white matter pathology in BD.

Introduction

Converging evidence implicates abnormalities in a ventral frontotemporal system in the etiology of bipolar disorder (BD). Neuroimaging studies of BD have repeatedly shown structural abnormalities in gray matter (GM), particularly within the amygdala and orbitofrontal cortex (OFC)[1, 2]. Abnormalities within ventral frontotemporal GM structures, and in the connections between them, have also been reported in adolescents with BD[3–8], suggesting these abnormalities may play a role in the neurodevelopment of BD. Recent

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research has increasingly implicated white matter (WM) connecting these regions in the etiology of BD. Diffusion tensor imaging (DTI) studies have shown decreased WM structural integrity within tracts carrying prefrontal cortex (PFC) connections, particularly within the uncinate fasciculus (UF)[9–13], a tract that carries substantial amygdala-ventral PFC connections[14], as well as the anterior limb of the internal capsule (ALIC)[12, 15, 16], corpus callosum and cingulum[17–23]. There have been some conflicting findings in the UF[24, 25]. Genetic variation may be one mechanism leading to phenotypic heterogeneity within BD. Supporting this are studies of families multiply affected with BD[21] and first degree relatives of individuals with BD[13, 26], which implicates genetic liability in UF structural changes in BD.

Variation in the Ankyrin G (*ANK3*) gene, including an intronic single nucleotide polymorphism (SNP), rs9804190, has been reported in genome-wide association studies (GWAS) to be associated with BD[27–32]. The neural system effects associated with *ANK3* variation in the context of BD are not yet understood. *ANK3* may be associated with the development of structural brain abnormalities implicated in BD[33] and may particularly influence WM. *ANK3* is thought to participate in the stabilization and localization of ion channels and cell adhesion molecules to nodes of Ranvier and axonal initial segments[34–36]. *ANK3* may also play a role in the developing cortex[37], onset of myelination[38], and neurogenesis in adults[39]. Additionally, lithium has been shown to alter *ANK3* expression in the mouse brain[40] and decrease hyperactivity observed in mice with altered *ANK3* expression[41], further supporting a role for *ANK3* in BD pathology and as a potential therapeutic target.

Ventral frontotemporal dysfunction associated with rs9804190 in individuals with BD has been reported[42], suggesting rs9804190 can influence frontotemporal systems in BD. In one study including 121 individuals with BD, no associations were detected with amygdala GM volume (GMV)[43], suggesting rs9804190 may have its effects in other pathophysiological aspects of BD development. To our knowledge, there is only one neuroimaging report on effects of rs9804190 on structural integrity of WM. It was in 88 healthy individuals and an effect was not detected[44].

In this study, we used DTI to investigate associations between rs9804190 and structural integrity of frontotemporal WM in BD and healthy control adolescents and adults. We hypothesized that rs9804190*T would be associated with decreased frontotemporal WM fractional anisotropy (FA), with the BD subgroup carrying the risk allele having the lowest UF FA, and with decreases independent of age, suggesting a role in the developmental pathophysiology of BD. Frontotemporal GMV was also assessed with structural magnetic resonance imaging (sMRI). WM FA and GMV in other brain regions were explored in whole brain analyses.

METHODS AND MATERIALS

Participants

Participants included 187 non-Hispanic European Americans: 90 with BD (76 BDI, 14 BDII), age$_{mean}$ ± standard deviation=27.1 ± 11.7 years, range=13–54 years, 68% female; 97

controls without personal history of an Axis I disorder or first-degree family member with a major mood or psychotic disorder, $age_{mean}=25.9 \pm 11.0$ years, range=12–55 years, 53% female. Psychiatric diagnoses and mood state at scanning were confirmed with the Structured Clinical Interview for DSM-IV Diagnosis[45] for participants 18 years and the Kiddie-Schedule for Affective Disorders and Schizophrenia[46] for participants <18 years. No subject had a history of a neurological illness, including head trauma with loss of consciousness for 5min, or major medical illness except treated hypothyroidism in BD subjects (N=7, 7.8%). No HC subject had substance (including alcohol) abuse/dependence history and no BD subject had a history within 6 months prior to scanning. On the scan day, urine toxicology screens were negative for substances of abuse for all subjects. After complete description of the study, written informed consent was obtained from subjects 18 years, and assent and parental permission from subjects <18 and their parent/guardian, in accordance with human investigation committees of the Yale School of Medicine and Department of Veterans Affairs.

DNA was extracted from whole blood. Genotypes at rs9804190 were determined by standard Taqman methods (Applied Biosystems; http://www.appliedbiosystems.com/). BD and control groups were each further subdivided into two groups: rs9804190 CC homozygotes (n=52 BD, 56 controls; age_{mean} of whole CC group=25.6 ± 10.8 years; 58% female), and rs9804190 T-carriers (CT and TT genotypes) (n=38 BD, 41 controls; age_{mean} of whole T-carrier group=27.6 ± 12.0 years, 62% female). Minor allele frequencies were 25% in the BD group and 26% in the control group. Genotype frequencies were consistent with Hardy-Weinberg equilibrium expectations. Table 1 provides demographic and clinical characteristics of the subgroups.

MRI Acquisition

High-resolution sMRI and DTI data were acquired in the same scanning session for each subject with a 3-Tesla Siemens Trio MR scanner (Siemens, Erlangen, Germany). The sMRI sagittal images were acquired with a 3D magnetization prepared rapid acquisition gradient echo (MPRAGE) T_1-weighted sequence with parameters: repetition time (TR)=1500ms, echo time (TE)=2.83ms, matrix=256x256, field of view (FOV)=256mm x 256mm ^{2}, and 160 one-mm slices without gap and two averages. DTI data were acquired in alignment with the anterior commissure-posterior commissure plane with diffusion sensitizing gradients applied along 32 non-collinear directions with b-value=$1000s/mm^2$, together with an acquisition without diffusion weighting (b-value=0) (TR=7400ms; TE=115ms; matrix=128×128; FOV=256mm × $256mm^2$ and 40 three-mm slices without gap).

SMRI Processing

Images were processed with the Statistical and Parametric Mapping 5 (SPM5) (http://www.fil.ion.ucl.ac.uk/spm). The SPM segmentation function was implemented for bias correction, spatial normalization and segmentation of the original structural images in the same model. SPM tissue probability maps (voxel size $2x2x2mm^3$) guided normalization and segmentation. To ensure the overall amount of tissue in a class was not altered, a "modulation" step was used during spatial normalization. After segmentation, normalization, and modulation steps, GM images were smoothed with an 8mm full-width-at-half-maximum

(FWHM) isotropic kernel. Right and left *a priori* amygdala and OFC region of interests (ROIs) were defined with the WFU PickAtlas Tool (http://www.fmri.wfubmc.edu/download.htm). For OFC ROIs, the inferior, middle and superior orbital ROIs were combined with the rectus.

DTI Processing

Images were processed with SPM8. Diffusion-weighted data were interpolated to 1.5-mm isotropic voxels and denoised by a three-dimensional isotropic Gaussian kernel with Sigma 2mm FWHM Gaussian kernel. Diffusion eigenvectors and corresponding eigenvalues ($\lambda 1$, $\lambda 2$, $\lambda 3$) were acquired after diagonalization of the DTI data. FA was then calculated according to previously published methodology[9, 47]. Whole brain FA maps were normalized to the standard Montreal Neurological Institute (MNI) space using a tissue probability map of WM as a template. FA maps were resampled to $2x2x2mm^3$ during normalization. Each FA map was spatially smoothed with a 10mm FWHM isotropic Gaussian kernel. The *a priori* UF ROI (right and left calculated separately) was obtained from the Johns Hopkins University DTI-based WM atlas (http://cmrm.med.jhmi.edu/)[48].

ROI-Based and Voxel-Based sMRI and DTI Analyses

For ROI-based sMRI analysis, a linear mixed model was used, with diagnostic group (controls, BD) and genotype (CC, T-carriers) as between-subjects factors, hemisphere (left, right) as a within-subjects factor, and amygdala and OFC GMV as dependent variables. Main effects and the interaction between diagnostic group and genotype were modeled. Age and sex were considered as covariates with both main effects and interactions with genotype modeled. An equivalent model was used for ROI-based DTI analysis to assess UF FA.

Voxel-based exploratory analyses were completed with SPM8. Diagnostic group by genotype factorial models in SPM, covarying age and sex, with GMV or FA values as dependent variables, were run to assess for non-hypothesized regions showing a significant diagnostic group by genotype interaction. To further explore localization of gene effects within BD, two-sample (CC vs. T-carriers) *t* tests in SPM were conducted, covarying age and sex, with GMV or FA values as dependent variables. Findings were considered significant at p<0.001, ≥50voxels. Parallel *t* tests were conducted in control subjects.

Analyses of Demographic and Clinical Factors

A 2x2 (diagnostic group, genotype) analysis of variance (ANOVA) was used to examine differences in age. Chi-square tests were used to examine sex between diagnostic groups, between genotypes, and between genotypes within each diagnostic group. Within the BD group, Chi-square or Fisher's exact tests were used to examine if clinical factors differed by genotype. Additionally, 2x2 (clinical factor, genotype) ANOVAs were performed for ROIs showing a significant gene effect. Clinical factors were assessed only if both CC and T-carrier groups had N>5 subjects with history of the factor. Mean GMV or FA from clusters showing significant gene effects in exploratory analyses were calculated and associations with clinical factors also explored.

RESULTS

Participant Characteristics

There were no significant differences in age between diagnostic or genotype groups. Overall, the BD group had more females (61%) (χ^2=4.5, df=1, p<0.05). However, no sex differences were observed between the CC group and T-carriers within BD (χ^2=0.01, df=1, p=0.91) or control (χ^2=0.35, df=1, p=0.55) groups. There were no significant differences in other clinical factors by genotype (Table 1).

ROI-Based Analyses

SMRI—For amygdala and OFC GMV, no main effect of genotype or significant diagnosis by genotype interactions were detected (p 0.1). A main effect of diagnosis was observed for OFC GMV ($F_{(1,180)}$=4.44, p<0.05); OFC GMV was lower in the BD than the control group. No main or interactive effects of diagnostic group or genotype were observed for amygdala GMV. No significant genotype interactions with age, sex, or hemisphere were observed.

DTI—UF FA levels were lower in the T-carriers than CC homozygotes ($F_{(1,181)}$=14.6, p<0.0005) and BD than control group ($F_{(1,181)}$=14.7, p<0.0005). A significant diagnosis by genotype interaction was observed ($F_{(1,181)}$=3.88, p=0.05); FA levels were lowest in BD T-carriers compared to BD CC homozygotes and both control genotype groups (p<0.0005, Figure 1). No other groups differed (p 0.15). No genotype interactions with age, sex, or hemisphere were observed.

Effects of Demographic and Clinical Factors—Clinical factors including whether subjects were medicated at the time of scan (yes/no, overall and medication subclasses) and history (yes/no) of rapid-cycling, lifetime psychosis, suicide attempt, mood states, alcohol abuse/dependence, or cannabis abuse/dependence, did not show significant effects on UF FA measures within BD. These clinical variables did not show significant genotype interactions.

Voxel-Based Analyses

SMRI—No significant diagnostic group by genotype interaction was observed. Compared to BD CC homozygotes, BD T-carriers showed significantly smaller GMV bilaterally in the thalamus (MNI coordinates: x=4mm, y=−10mm, z=−2mm, cluster=286voxels). Compared to control CC homozygotes, control T-carriers showed significantly smaller GMV in the right thalamus (x=16mm, y=−14mm, z=10mm, cluster=82voxels). No regions showing greater GMV in BD or control T-carrier groups were observed.

DTI—Clusters in non-hypothesized regions that showed significant diagnostic group by genotype interactions, with lower FA in BD T-carriers than all other subgroups, were in areas of right temporoparietal WM (z=32mm, y=−32mm, z=20mm, cluster=130voxels), left posterior dorsal cingulum (z=−10mm, y=−28mm, z=30mm, cluster=54voxels) and posterior corpus callosum/fornix (x=−6mm, y=−34mm, z=12mm, cluster=90voxels). Compared to BD CC homozygotes, BD T-carriers had lower FA in the left UF cluster extending into ventral frontal WM, including anterior cingulum and corpus callosum, and dorsal frontal

WM, including forceps minor (x=−16mm, y=−2mm, z=−12mm, cluster=53voxels; x= −16mm, y=48mm, z=0mm, cluster=453voxels), and in the right UF cluster into ventral frontal WM, including anterior cingulum and corpus callosum, and ALIC (x=20mm, y=28mm, z=−4mm, cluster=747voxels). There was also a decrease within right superior temporal WM (x=44mm, y=−8mm, z=−12mm, cluster=103voxels).

Lower FA in BD T-carriers, compared to BD CC homozygotes, was observed in dorsomedial frontal WM, including areas of right dorsal anterior cingulum (x=20mm, y=4mm, z=42mm, cluster=522voxels) and left dorsal anterior cingulum extending into the corona radiata and external capsule (x=−16mm, y=12mm, z=34mm, cluster=862voxels), of left temporoparietal WM (x=−34mm, y=−42mm, z=24mm, cluster=247voxels), and of posterior dorsomedial WM, including areas of left dorsal cingulum (x=−12mm, y=−30mm, z=32mm, cluster=65voxels), left parietal/occipital WM (x=−18mm, y=−60mm, z=32mm, cluster=777voxels), and right parietal WM (x=24mm, y=−56mm, z=34mm, cluster=1117voxels) extending into temporoparietal WM. There were no areas showing greater FA in BD T-carriers and no areas of significant differences by genotype in controls.

Effects of Demographic and Clinical Factors—Individuals with lifetime history of rapid-cycling, compared to those without, showed higher FA in the right dorsal anterior cingulum ($F_{(1,84)}$=3.97, $p<0.05$) and left parietal/occipital WM ($F_{(1,84)}$=5.90, $p<0.05$). Individuals with a history of suicide attempt, compared to those without, showed lower FA in the left UF ($F_{(1,80)}$=4.44, $p<0.05$) and higher FA in left parietal/occipital WM ($F_{(1,80)}$=5.98, $p<0.05$). Individuals with a history of alcohol abuse/dependence showed higher FA in left temporoparietal WM ($F_{(1,84)}$=5.55, $p<0.05$). No other clinical factors showed significant effects on clusters showing gene effects in DTI exploratory analysis. Clinical factors did not show significant effects on thalamic GMV. There were no significant clinical variable by genotype interactions.

DISCUSSION

Decreases in UF FA were observed among BD subjects carrying the *ANK3* rs9804190 risk allele (T), compared to CC and T-carrier control subjects and BD CC homozygotes. Additionally, exploratory analyses showed decreases in BD T-carriers extending further into ventral and dorsal WM regions, including the ALIC, anterior and posterior cingulum, and corpus callosum. Findings were independent of age.

UF FA decreases in the BD T-carrier group suggest the risk allele is associated with decreased frontotemporal WM integrity in BD. Negative DTI findings in healthy individuals is consistent with a previous report[44], suggesting rs9804190 may not be associated with WM deficits in typical development. Decreased UF FA in BD was observed in this and previous BD studies[9–13]. The UF has been suggested to play a role in emotional regulation, reward processing, and impulsive decision-making[49], processes disrupted in BD. More research is needed to identify mechanisms by which WM in individuals with BD may be more vulnerable to effects of rs9804190. Effects of rs9804190 specifically among BD subjects may be due to an effect of rs9804190 on BD progression or an effect of higher genetic liability for BD among the BD subjects compared to control subjects. Changes in FA are

suggested to reflect altered myelination, fiber density, axonal damage and/or axonal diameter[50–53]. A role for *ANK3* has been implicated during the onset of myelination[38]. Postmortem studies in BD have reported reduced glia cells, including oligodendrocytes, and downregulation of genes related to myelination[54–57], particularly in frontal brain regions. We suggest a possible multi-hit hypothesis in BD. Increased vulnerability to *ANK3* variation in the neural circuitry implicated in BD in individuals with the disorder could result from interactions with effects of other myelination genes, oligodendrocyte function or other mechanisms contributing to neural circuitry pathology in the disorder. *ANK3* variation may cross diagnostic boundaries[58–60], although results are mixed[28]. Negative findings in HCs reported here suggest some gene specificity; future work including other psychiatric diagnoses is needed to investigate specificity of *ANK3* pathophysiological mechanisms to BD.

DTI exploratory analyses suggest that, in BD, regions of the ALIC, anterior and posterior cingulum, and corpus callosum are also affected by rs9804190. Decreased FA within these regions has been observed in BD[12, 15–23], with studies suggesting genetic variation may contribute to heterogeneity[21, 61, 62]. Within BD, diffusivity within cingulum and corpus callosum has been suggested to relate to differences in attention and executive functions[63], diffusivity in ALIC to working memory[63], altered set shifting and greater risk-taking[13]. Rs9804190 has been shown previously to be associated with externalizing behavior, including substance abuse[64]. Studies also support a role of the ankyrin gene family in nicotine dependence and alcohol use-related phenotypes[65–69]. We did not observe significant differences between BD CC and T-carrier groups with respect to histories of alcohol or cannabis abuse/dependence, and history of alcohol abuse/dependence did not contribute to the FA decreases observed. More research is needed to understand behavioral consequences of gene-associated brain changes in BD.

We did observe an effect of genotype on GMV in exploratory analyses: both control and BD T-carrier groups showed smaller thalamic GMV than control and BD CC homozygotes. Decreased thalamic volume has been observed in BD subjects and their unaffected family members[70]. The thalamus plays a key role in sensory processing and dysfunction can affect cognition and emotional behavior[71]. It is possible that *ANK3* variation may contribute to thalamic effects in individuals with, and those at risk for, BD.

The developmental timing of genetic effects on brain structure cannot be determined from this study. No significant effects of age, and no interactions with age, were observed for UF FA. This suggests gene effects observed are present by adolescence in BD and could be associated with the development of BD pathology. Exploratory analyses controlled for age and therefore gene effects observed in dorsal WM and ALIC appear to also be independent of age. However, care must be taken with making developmental inferences from this study's cross-sectional design and future longitudinal studies are needed.

Diagnostic group differences, independent of genotype, in frontotemporal GMV were observed. Supporting previous research[1, 2], we found individuals with BD had smaller OFC GMV compared to controls. While not shown, adolescents/young adults (<22 years) with BD showed significantly smaller amygdala GMV, compared to adolescent/young adult

controls. Reports of GMV differences in adolescents with BD have been relatively consistent in showing amygdala decreases, whereas reports in adults have varied[1]. The source of this heterogeneity is not clear, but could in part be related to age of onset, course of illness, and medication history[72]. No effects of genotype were observed on amygdala GMV in this study suggesting *ANK3* may not contribute to this heterogeneity.

Other *ANK3* SNPs have been associated with altered GM and WM structure. Rs1938526 has been reported to be associated with cortical thinning in inferior frontal, orbital frontal, and temporal gyrus in individuals with first episode psychosis[73]; however, in that study only one BD subject had the risk allele. Another *ANK3* SNP, rs10994336, also supported by GWAS studies as conferring risk for BD[32], has been reported to be associated with variation in ALIC FA and greater risk-taking in a study of healthy individuals without BD[44]. Studies in BD implicate rs10994336 in altered ventral anterior cingulate cortex responses during a working memory task[42] and ventral PFC activation and connectivity during a facial affect-processing task[74]. These studies suggest other *ANK3* SNPs may contribute to altered deficits within corticostriatal-limbic systems implicated in BD, including frontotemporal deficits. Previous studies suggest high linkage disequilibrium (LD) between rs10994336 and rs1938526[73], but low LD between rs10994336 and rs984190, indicating rs984190 may contribute independently to BD[27, 75]. More work is needed examining differences between, and effects of, multiple SNPs in BD and HCs.

Lower UF FA was associated with both risk allele and history of suicide attempt. Future work is needed to directly investigate this relationship to determine whether *ANK3* variation increases vulnerability to suicide-related behavior. This study included individuals with BDI and BDII. Genetic effects remained the same within BD when covarying for these BD subtypes and there were no significant BD subtype by genotype interactions (data not shown) in ROIs, suggesting genetic effects may cross subtype classifications. No relationships between other clinical factors and gene effects on GMV or WM FA were observed: however, sample size may have limited power to detect effects. We did not observe significant effects of medication, which was further limited by lack of systematic study of medications. Regions identified in whole-brain analyses may include false positives as we did not account for multiple comparisons since analyses were exploratory. Future studies are needed to confirm these findings, including of larger samples, with more homogeneous clinical backgrounds, and systematic study of medication and other clinical features.

In summary, *ANK3* rs9804190 variation was associated with decreased WM structural integrity within the UF, as well as in ventral and dorsal frontal, ALIC, cingulum and corpus callosum regions. Effects of rs9804190 on WM were observed in participants with BD, not controls, with results independent of age, suggesting there may be vulnerability to alterations in WM development in these regions in BD. Effects of the genotype were also detected in thalamus GMV within BD and control groups. More work, including preclinical, is needed to understand mechanisms underlying the emergence of genetic effects and associated behavioral consequences. Dissecting the roles of genetic variation on neural and behavioral pathophysiology of BD could identify new targets for detecting and treating BD that are more specific to an individual based on their genetics.

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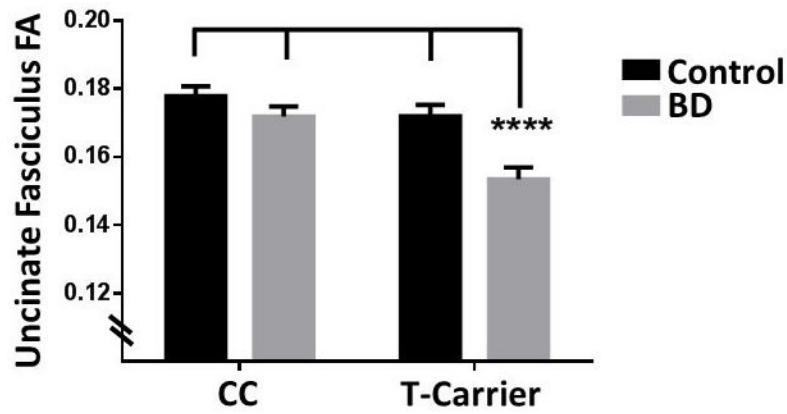
Figure 1. Fractional Anisotropy Genotype by Diagnosis Effects in the Uncinate Fasciculus
A significant diagnostic group [control, bipolar disorder (BD)] by genotype group [homozygous for the rs9804190 C allele (CC), heterozygous and homozygous carriers of the T (risk) allele (T-carriers)] interaction was observed for fractional anisotropy (FA) in the *a priori* uncinate fasciculus (UF) region of interest. T-carriers with BD showed lower UF FA compared to each of the other groups (****p<0.0005, between group comparisons). Control CC homozygotes N=56, BD CC homozygotes N=52, Control T-carriers N=41, BD T-carriers N=38.
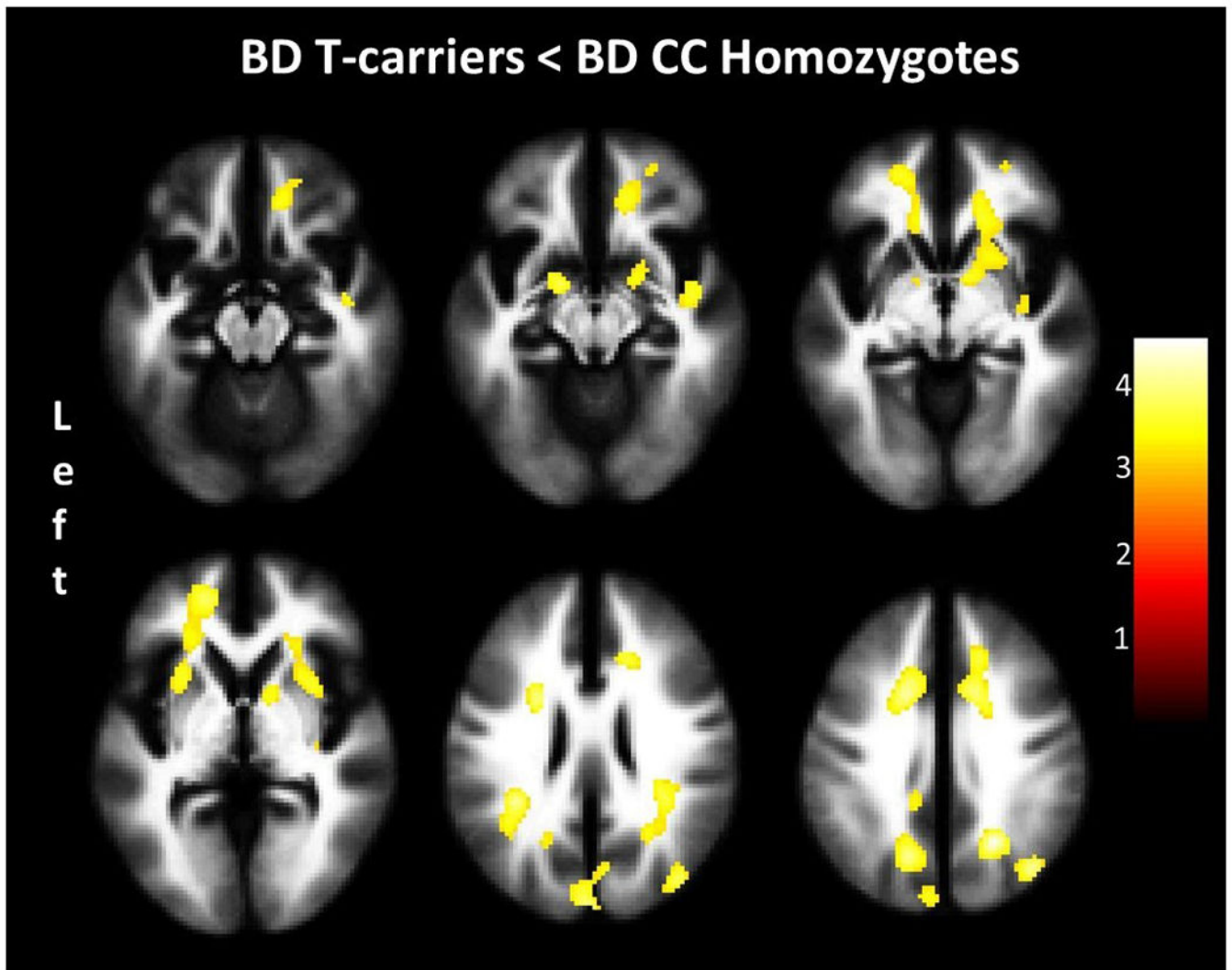
Figure 2. Fractional Anisotropy Decreases in Bipolar Disorder by ANK3 Genotype
The images show the regions of fractional anisotropy decreases in the bipolar disorder (BD)
rs9804190 T-carrier group [heterozygous and homozygous carriers of the T (risk) allele],
compared to bipolar disorder CC homozygotes. No regions of fractional anisotropy increases
were observed in the BD T-carrier group compared to BD CC homozygotes. Significance
threshold is p<0.001, cluster >50 voxels. 'Left' on left of figure denotes left side of brain.
The color bar represents the range of T values. BD CC homozygotes N=52, BD T-carriers
N=38.

Table 1

Participant Characteristics

Age was examined by a 2 [diagnostic group: control, bipolar disorder] x 2 [genotype: homozygous for the rs9804190 C allele (CC), carriers of the T (risk) allele (T-carriers)] ANOVA. All other factors were examined with Chi-square or Fisher's exact tests.

| | Healthy Control | | Bipolar Disorder | | p value |
	CC group (n=56)	T-carrier Group (n=41)	CC Group (N=52)	T-carrier Group (n=38)	
Age (SD)	23.9 (9.0)	28.6 (12.9)	27.5 (12.2)	26.5(11.1)	0.193
Female (%)	28 (50)	23 (53)	35 (67)	26 (68)	**0.034 (HC vs. BD)** 0.611 (CC vs. CT+TT) 0.911 (BD: CC vs. CT −TT) 0.552 (HC: CC vs. CT +TT)
Demographics					
Rapid Cycling (%) [1]	--	--	27 (52)	12 (32)	0.054
Lifetime Psychosis (%)	--	--	18 (35)	13 (34)	0.998
Suicide Attempt (%)	--	--	11 (22)	12 (32)	0.252
Mood State [Euthymic(%) / Depressed(%) / Elevated(%)]	--	--	28 (54)/11 (21)/13 (25)	20 (53)/10 (26)/8 (21)	0.817
Medicated at scan (%)	--	--	42 (81)	30 (79)	0.831
Lithium (%)	--	--	17 (33)	6 (16)	0.069
Anticonvulsant (%)	--	--	22 (42)	13 (34)	0.436
Antipsychotic (%)	--	--	26 (51)	13 (35)	0.140
Medications					
Antidepressant (%)	--	--	15 (29)	14 (37)	0.423
Stimulant (%)	--	--	6 (12)	8 (21)	0.219
Benzodiazepine (%)	--	--	8 (15)	11 (29)	0.119

| | Healthy Control | | Bipolar Disorder | | |
	CC group (n=56)	T-carrier Group (n=41)	CC Group (N=52)	T-carrier Group (n=38)	p value
Dopamine Agonist (%)	--	--	2 (4)	0 (0)	0.507[F]
Opiate (%)	--	--	1 (2)	1 (3)	1.000[F]
Anticholinergic (%)	--	--	1 (2)	1 (3)	1.000[F]
Ketamine (%)	--	--	1 (2)	0 (0)	1.000[F]
Non-Benzodiazepine Hypnotic (%)			1 (2)	1 (3)	1.000[F]
Adrenergic Agonist (%)			0 (0)	1 (3)	0.422[F]
Levothyroxine (%)[2]	--	--	5 (10)	2 (5)	1.000[F]
Alcohol Abuse or Dependence (%)	--	--	9 (17)	9 (24)	0.455
Cannabis Abuse or Dependence (%)	--	--	6 (12)	8 (21)	0.219
Polysubstance Abuse or Dependence (%)	--	--	1 (2)	1 (3)	1.000[F]
Stimulant Abuse or Dependence (%)	--	--	2 (4)	2 (5)	1.000[F]
Cocaine Abuse or Dependence (%)	--	--	3 (6)	2 (5)	1.000[F]
Sedative/Hypnotic Dependence (%)	--	--	1 (2)	1 (3)	1.000[F]
Opioid Dependence (%)	--	--	1 (2)	0 (0)	1.000[F]
Post Traumatic Stress (%)	--	--	6 (12)	2 (5)	0.459[F]
Generalized Anxiety (%)	--	--	4 (8)	1 (3)	0.392[F]
Substance Induced Anxiety (%)	--	--	1 (2)	0 (0)	1.000[F]
Specific Phobia (%)	--	--	4 (8)	1 (3)	0.397[F]

Lifetime Substance Use Disorders

Lifetime Other Psychiatric Disorders

	Healthy Control		Bipolar Disorder		
	CC group (n=56)	T-carrier Group (n=41)	CC Group (N=52)	T-carrier Group (n=38)	p value
Social Phobia (%)	--	--	0 (0)	3 (8)	0.072[F]
Panic Disorder (%)	--	--	2 (4)	3 (8)	0.647[F]
Panic Disorder with Agoraphobia (%)	--	--	0 (0)	3 (8)	0.072[F]
Obsessive Compulsive (%)	--	--	3 (6)	0 (0)	0.260[F]
Anorexia Nervosa (%)	--	--	1 (2)	2 (5)	0.571[F]
Bullmia (%)	--	--	2 (4)	1 (3)	1.000[F]
Binge Eating (%)	--	--	1 (2)	1 (3)	1.000[F]

[F] represents p-value calculated with Fisher's exact test. Clinical factors assessed in both adolescents and adults are reported.

[1] Rapid-cycling reported is lifetime history of rapid cycling

[2] Individuals on levothyroxine had hypothyroidism.