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A Schizophrenia Risk Gene, *ZNF804A*, is Associated with Brain White Matter Microstructure

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Abstract

Genome-wide association studies have provided strong evidence for association of the SNP rs1344706 in the *ZNF804A* gene with schizophrenia and bipolar disorder. Neuroimaging studies have suggested that variation at rs1344706 may be associated with neural endophenotypes such as white matter volumes and densities. However, analyses of white matter microstructure using diffusion tensor imaging (DTI) have produced conflicting results. We examined the association between rs1344706 and white matter microstructure in 107 healthy individuals using Tract-Based Spatial Statistics (TBSS). TBSS analysis showed significant association between the risk allele and lower fractional anisotropy in the corpus callosum, left forceps minor, and right parietal white matter ($p < .05$; FWE corrected). Post-hoc analyses indicated that this association was largely driven by alterations in radial diffusivity, consistent with an effect of genotype on myelination. In light of the strong DTI evidence for white matter microstructural abnormalities in schizophrenia,

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Contributors: Dr. Ikuta designed the study, conducted the literature search and imaging analyses, and wrote the first draft of the manuscript. Drs. Szeszko, Lencz and Malhotra wrote the study protocol and edited the manuscript. Drs. Peters and Karlsgodt recruited participants and edited the manuscript. Drs. Guha and John conducted the genetic and statistical analyses. All authors contributed to and have approved the final manuscript.

the current results implicate a potential mechanism for schizophrenia risk formation by *ZNF804A* rs1344706 genotype.

Keywords

schizophrenia; genetics; endophenotype; *ZNF804A*; diffusion tensor imaging; white matter

Introduction

The *ZNF804A* single nucleotide polymorphism (SNP) rs1344706 has been associated with schizophrenia, and to a lesser degree, bipolar disorder, across multiple studies (O'Donovan et al., 2008; Riley et al., 2010; Schwab et al., 2013; Williams et al., 2010). However, the biological functions of *ZNF804A* and the mechanisms through which it conveys the illness risk remain to be elucidated.

White matter abnormalities have also been strongly linked with schizophrenia (Davis et al., 2003). For example, an early post-mortem study found smaller corpus callosum fiber density in women with schizophrenia (Highley et al., 1999). Within the last decade, diffusion tensor imaging (DTI) studies have consistently reported lower white matter integrity as measured by fractional anisotropy (FA) in patients with schizophrenia compared to healthy cohorts (Buchsbaum et al., 2006; Kubicki et al., 2007; Lee et al., 2013). FA deficits have been reported in the first episode of schizophrenia, prior to treatment with antipsychotic medication, as well as in unaffected first-degree relatives of patients with schizophrenia, consistent with a genetic underpinning for this abnormality (Karlsgodt et al., 2012; Peters et al., 2010). Moreover, FA deficits have been correlated with symptom severity, underscoring the potential clinical relevance of understanding this mechanism (Cheung et al., 2011; Whitford et al., 2010).

Prior studies have shown that rs1344706 is associated with brain white matter volume (Lencz et al., 2010; Wassink et al., 2012) and density (Wei et al., 2012). Findings from DTI studies of white matter microstructure, however, are inconsistent. One study, which utilized a 3T MRI scanner, showed association between rs1344706 genotype and white matter microstructure (Kuswanto et al., 2012), while three studies using 1.5T MRI have reported negative results (Sprooten et al., 2012; Voineskos et al., 2011; Wei et al., 2013). This inconsistency in DTI findings may be related to differences in methodology between studies (Table 1), including magnet strength and resultant image resolution and signal-to-noise. Moreover, analytic approaches have varied as two studies employed Tract-Based Spatial Statistics (TBSS) (Sprooten et al., 2012; Wei et al., 2013), with one study using tractography (Voineskos et al., 2011), and another using an approach based on large regions of interest (ROI) (Kuswanto et al., 2012). A number of tracts has been examined in Voineskos et al. (2011) to examine association between risk allele and white matter FA using deterministic tractography, as well as cortical gray matter thickness measures from T1 structural brain images. The risk variant showed negative association with thickness in the superior temporal and cingulate gyri, while it failed to show association with white matter FA. In Kuswanto et al. (2012), FA in frontal, temporal and parietal lobules and cingulate gyrus were examined

between risk allele homozygotes vs. others in patients of schizophrenia and healthy controls. Risk homozygotes showed higher FA than others in right temporal lobe among healthy controls, and showed lower FA in left and right parietal lobe and left cingulate gyrus among schizophrenia patients. Sample sizes were moderate in these studies (healthy controls n=50-69), and appeared not related to the variability in finding s between these studies. In summary, previous 1.5T studies showed no association between rs1344706 and DTI measurements (Sprooten et al., 2012; Voineskos et al., 2011; Wei et al., 2013), and one 3T study showed association between rs1344706 and lobular averages of FA (Kuswanto et al., 2012). The current study is, to our knowledge, the first 3T TBSS study to test the association between rs1344706 and voxelwise FA, in the largest sample to date (n = 107). Whereas tractography and ROI approaches can have greater sensitivity to differences across an entire tract or region, TBSS is a voxel-wise approach that is therefore more sensitive to differences in smaller sub-regions, as well as areas that are not demarcated *a priori*.

The original GWAS studies reporting the association of *ZNF804A* with schizophrenia utilized an additive model (O'Donovan et al., 2008). However, each of the previous DTI studies have used dominant models, in which heterozygous and homozygous risk allele carriers were grouped together and compared to non-risk allele homozygotes, potentially reducing sensitivity. Moreover, it has been shown that age and white matter microstructure show a non-linear relationship across the lifespan in most of the major white matter tracts (Kochunov et al., 2012; Lebel et al., 2012; Peters et al., 2013; Peters et al., 2012; Salat et al., 2005). In samples with wide age ranges, genetic effects on DTI white matter microstructure may be masked by considerable variance derived from age effects.

In the present study, we employed 3T MRI and TBSS to examine associations between rs1344706 genotype and brain white matter microstructure using an additive model, while accounting for the non-linear effects of age,. We hypothesized that the *ZNF804A* risk allele (A allele of SNP rs1344706) would be associated with lower FA in psychiatrically healthy individuals.

Methods

Participants

One-hundred seven healthy Caucasians (52% male) between the ages of 8.8 and 68.1 years (mean 31.8 ±16.0; median 26.2) were recruited through local advertisements and by word of mouth. Written informed consent was obtained from participants or if the participant was a minor, from a parent or guardian; all minors provided assent. Participants had no history of a DSM-IV axis I major mood or psychotic disorder as assessed by structured diagnostic interview (Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (Kaufman et al., 1997) or Structured Clinical Interview for DSM-IV disorders Non-Patient Edition (First et al., 2001)). Other exclusion criteria included: (i) intellectual or learning disability; (ii) medications with known adverse cognitive effects; (iii) MRI contraindications; (iv) pregnancy; (v) significant medical illness that could affect brain structure. Mean IQ as estimated from the Wide Range Achievement Test (Reading subtest) was 105.9 ±9.6 (data missing for 14 subjects). Handedness was determined using the Edinburgh Handedness Inventory; median laterality quotient was 0.9

(range -1 to 1). This study was approved by the Institutional Review Board of the North Shore - Long Island Jewish Health System.

Genotyping

Genotyping was performed for ~ 770K genome wide SNPs using Illumina Omni Express arrays according to manufacturers' specifications. SNPs were filtered based on call rate < 98 %, minor allele frequency < 0.025 and Hardy-Weinberg exact test $P < 0.000001$. Samples were filtered based on genotype quality control filtration (sample call rate < 97 %, gender mismatch). Principal component analysis was performed with 98,629 LD pruned ($r^2 > 0.2$) genome wide SNPs to identify the non-Caucasian outliers using ethnicity information. A total 107 individuals passed QC and were identified as Caucasian and considered for further analysis.

DTI Acquisition and Preprocessing

All subjects received a DTI exam at the North Shore University Medical Center, Manhasset, NY, on a GE Signa HDx 3.0 T system (General Electric, Milwaukee, WI). The sequence included volumes with diffusion gradients applied along 31 non-parallel directions (b = 1000 s/mm^2) and 5 volumes without diffusion weighting (TR = 14 s, TE = min., matrix = 128 × 128, FOV = 240 mm). Each volume consisted of 51 contiguous 2.5-mm axial slices acquired parallel to the anterior-posterior commissural line using a ramp sampled, double spin-echo, single shot echo-planar imaging method.

All scans were reviewed by a radiologist and all images were visually inspected to ensure that no gross abnormalities were evident. Image processing was conducted using the Functional Magnetic Resonance Imaging of the Brain Software Library (FSL; Oxford, United Kingdom; http://fsl.fmrib.ox.ac.uk/fsl/). Eddy-current distortions and head-motion displacements were corrected through affine registration of the 31 diffusion volumes to the first b0 volume using FSL's Linear Registration Tool (FLIRT) (Jenkinson and Smith, 2001). The b-vector table (i.e. gradient directions) for each participant was then adjusted according to the rotation parameters of this linear correction. Non-brain tissue was removed using FSL's Brain Extraction Tool. Fractional anisotropy (FA) and diffusivity parameters, putative measures of white matter integrity (Beaulieu, 2002), were then calculated at each voxel of the brain by fitting a diffusion tensor model to the raw diffusion data using weighted least squares in FSL's Diffusion Toolbox.

Tract Based Spatial Statistics

Voxel-wise statistical analysis of the FA data was carried out using TBSS (Tract-Based Spatial Statistics) in FSL (Smith et al., 2006). All subjects' FA data were aligned to the FMRIB58 FA standard brain using the nonlinear registration tool FNIRT (Smith et al., 2006). Next, the mean FA image was created and thinned to create a mean FA skeleton which represents the centers of all tracts common to the group. The FA threshold for the mean FA skeleton was set at 0.2. Each subject's aligned FA data was then projected onto this skeleton.

Prior to statistical testing, studentized residuals were calculated from FA regressions on age and sex using a Poisson model ($c + a*Age*\exp(-b*Age^2)$) for each voxel (Lebel et al., 2012), and the resulting data fed into voxel-wise cross-subject statistics. Studentized age and sex residuals were also calculated for each voxel in the axial and radial diffusivity maps for post-hoc analysis.

To test for local associations between genotype and FA, linear regressions of A allele 'dosage' on studentized FA residuals were performed using permutation-based testing. An additive model was tested where A allele dosage was numbered as 0, 1, or 2 alleles and then mean centered. Inference on the statistic maps was carried out using threshold-free cluster enhancement (Smith and Nichols, 2009). The null distribution was built up over 5000 random permutations across the image. The clusters were then thresholded at a level of $P<0.05$, which is corrected for multiple comparisons (i.e. family-wise error). Anatomic location of significant FA clusters was determined with the probabilistic cortical, subcortical and white matter tractography atlases provided in FSL, and an MRI atlas of human white matter anatomy (Mori et al., 2006). For each of the significant FA clusters, the mean studentized residual values for FA, Axial Diffusivity (AD) and Radial Diffusivity (RD) within the clusters were extracted and examined with post-hoc stepwise linear regressions to determine the relative contributions of AD and RD.

Results

Genotyping

Allele frequencies of rs1344706 almost precisely matched those in the original GWAS sample (O'Donovan et al.,). Specifically, overall A-allele frequency was 59.81%. Subjects were grouped according to rs1344706 SNP genotype, yielding 43 AA homozygotes (8 to 60 years old, 34% of whom were younger than 18 years old), 42 AC heterozygotes (14 to 66 years old, 17% < 18 years old) and 22 CC homozygotes (9 to 68 years old, 22% < 18 years old).

TBSS

The TBSS voxelwise analysis revealed that higher A allele dosage significantly predicted lower FA in three regions: right parietal white matter, left forceps minor, and the anterior body/genu of the corpus callosum (Table 2 and Figure 1). There were no regions in which higher A allele dosage predicted higher FA values. Within each of the three clusters, A allele dosage significantly predicted the mean FA, AD and RD studentized residual values, with the exception of AD in the corpus callosum (Figure 1).

In all three clusters, A allele dosage had significant goodness of fit (*R*-squared) to RD for all three ROIs, while goodness of fit to AD was only significant for the left forceps minor (Table 3, columns 2 and 3), with values for RD approaching those for FA (Table 3, column 1). In order to further test the associations of A allele dosage to AD and to RD, two two-stage regressions were performed for each of the clusters assessing the improvement of R-squared when RD was included in addition to AD in the model to predict A allele dosage compared to when RD was not included, as well as the improvement of R-squared when AD

was included in the model in addition to RD compared to when AD was not included. Regressions between AD and A allele dosage was improved by having RD in the model, whereas regressions between RD and A allele was only minimally improved by having AD, further indicating very limited associations between AD and A allele dosage (Table 3, columns 4 and 5).

Discussion

This is the first study that shows statistically significant voxel-wise associations between *ZNF804A* rs1344706 genotype and white matter microstructure, using a stringent TBSS analysis of high-resolution 3T DTI data. Specifically, higher dosage of the schizophrenia risk allele (A) was associated with lower white matter FA and higher RD in the corpus callosum, left forceps minor and right parietal lobe.

The finding in the right parietal lobe is consistent with DTI findings in schizophrenia, which have demonstrated delayed growth trajectories in parietal white matter of patients with childhood-onset schizophrenia (Gogtay et al., 2008) and their siblings (Gogtay et al., 2012), as well as lower parietal white matter FA in adult schizophrenia patients (Ardekani et al., 2003), including never medicated patients with a first psychotic episode (Cheung et al., 2011) and patients with deficit schizophrenia (Rowland et al., 2008). Although the current sample consisted of participants without schizophrenia or other psychotic disorders, the lower FA by increased risk allele dosage is consistent with the observed associations between compromised parietal white matter (micro)structure and schizophrenia, and *ZNF804A* being a risk gene for schizophrenia. Taken together, the current result suggests that ZNF804A may exert its effect on risk for schizophrenia via effects on white matter microstructure in the right parietal lobe.

TBSS analysis also revealed significant regions in the corpus callosum and the forceps minor (Figure 1), showing an association between higher risk allele dosage and lower FA. Lower FA values in the genu and its extensions (i.e. forceps minor) have also been implicated in schizophrenia (Buchsbaum et al., 2006; Kanaan et al., 2006; Lee et al., 2013; Price et al., 2007; Whitford et al., 2010), and found to correlate with degree of reality distortion in patients (Whitford et al., 2010). Our finding also corresponds to the reduced interhemispheric functional connectivity between dorsolatetal prefrontal cortexes (DLPFC) by *ZNF804A* risk genotype carriage during a working memory task (Esslinger et al., 2009). Lower FA by risk allele dosage in the corpus callosum and forceps minor can be interpreted as the structural homologue to this functional connectivity finding, since these structures contain connections between the bilateral DLPFCs.

Post-hoc analysis of axial and radial diffusivities revealed that the associations between A allele dosage and RD were greater than the associations between A allele dosage and AD. It has been shown that dysmyelination results in increased RD (Song et al., 2003; Song et al., 2002). The association between RD and A allele dosage may therefore suggest that *ZNF804A* rs1344706 schizophrenia risk genotype contributes to poorer myelination in these white matter regions (Davis et al., 2003). However, this interpretation should be considered tentative since we do not have direct evidence in humans that increased RD solely reflects

dysmyelination. Although the biological functions of *ZNF804A* remain largely unknown, functional brain connectivity studies have shown associations with rs1344706 risk genotype (Esslinger et al., 2011; Esslinger et al., 2009; Paulus et al., 2013; Rasetti et al., 2011; Walter et al., 2011). In these studies, prefrontal and prefrontal-temporal connectivity have been consistently reported to be lower in rs1344706 risk allele carriers, which at least partly corresponds to our findings in the corpus callosum and forceps minor. Functional connectivity between DLPFC and parietal regions has also been found to be reduced in risk genotype carriers (Esslinger et al., 2011), which may be interpreted as a functional counterpart to our structural finding in the right parietal white matter, corpus callosum and forceps minor altogether.

In sum, the present study indicates that the *ZNF804A* schizophrenia risk gene may affect regional white matter microstructure, including white matter regions that have been found compromised in schizophrenia patients. Our results may provide insight into the relationship between *ZNF804A* rs1344706 and pathophysiology of schizophrenia. Further studies are needed to shed light on the causative mechanisms by which the *ZNF804A* gene affects white matter microstructure in healthy individuals and patients with schizophrenia.

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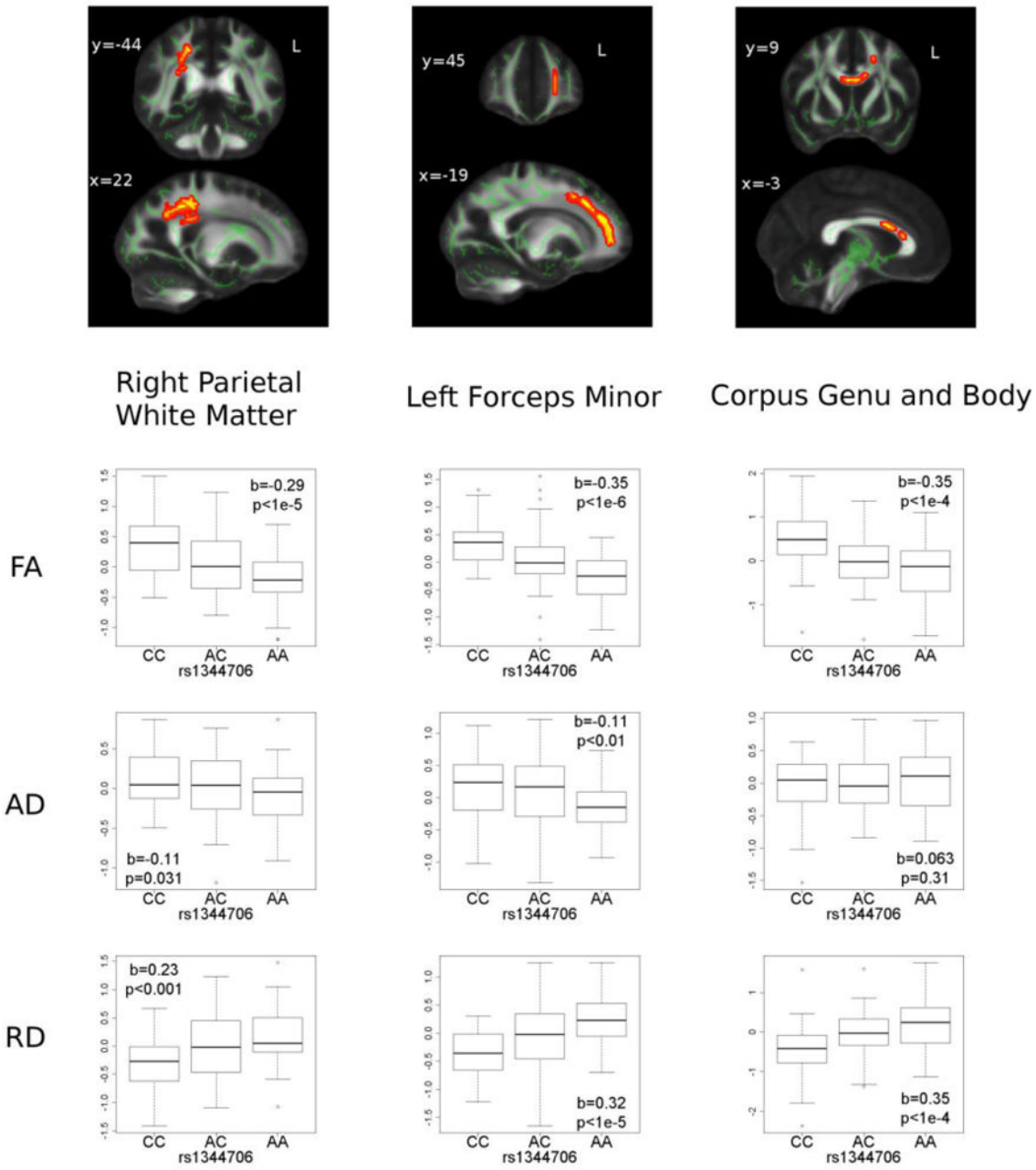

Figure 1. Three white matter regions in which lower fractional anisotropy (FA) was associated with *ZNF804A* rs1344706 risk allele (A) dosage

The significant clusters (red-yellow) in the FA images in the top row are 'thickened' into the local tracts by the *tbss-fill* tool for display purposes, and overlaid on the TBSS (tract-based spatial statistics analysis) skeleton in green. The mean studentized residuals from regressions on age and sex were determined for FA, Axial Diffusivity (AD) and Radial Diffusivity (RD) for each of the three clusters. The beta coefficients (*b*) of linear regressions with A allele dosage are shown in each boxplot.

Table 1

Previous DTI studies on *ZNF804A* rs1344706 genotype and white matter microstructure

First Author	Year	MRI	N	Age	Method	Result
Voineskos (Voineskos et al., 2011)	2011	1.5T	62 HC	37.4±12.7	Tractography	Negative
Kuswanto (Kuswanto et al., 2012)	2011	3T	64 HC89 SZ	31.9±10.1 34.3±9.2	ROI	Positive
Sprooten (Sprooten et al., 2012)	2012	1.5T	50 HC83 HC84 sib-BP84 sib-BP	22.7±1.7 21.1±2.4 21.4±2.8	TBSS	Negative
Wei (Wei et al., 2013)	2013	1.5T	69 HC100 SZ	25.4±5.7 26.5±6.9	TBSS	Negative

Kuswanto et al. (Kuswanto et al., 2012): Patients with AA genotype (homozygous for risk allele) showed lower FA in parietal lobes than patients with AC or CC genotype. HC with AA genotype showed higher FA in the right temporal lobe than HC with AC or CC genotype.

DTI = diffusion tensor imaging; FA = fractional anisotropy; HC = healthy controls; SZ = schizophrenia; Sib = sibling; BP = bipolar disorder; ROI = region of interest; TBSS = tract-based spatial statistics.

Table 2

Higher dosage of *ZNF804A* rs1344706 risk allele (A) predicted lower white matter FA in tract-based spatial statistics analysis (p<0.05, corrected)

Tract	Size (voxels)	Peak *p*-value (corrected)	Peak Coordinates		
			X	Y	Z
Right Parietal White Matter	822	0.033	22	-44	39
Left Forceps Minor	346	0.036	-19	45	5
Corpus Genu and Body	186	0.041	-3	9	23

FA = fractional anisotropy

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Table 3

Comparisons of linear regressions on A allele dosage

	Adjusted *R*-squared (p-value)				
	FA∼A	**RD∼A**	**AD∼A**	**(A∼AD+RD) - (A∼AD)**	**(A∼RD+AD) - (A∼RD)**
Right Parietal White Matter	**0.18 (<0.01)**	**0.09 (<0.01)**	**0.03 (0.03)**	**0.12**	**0.07**
Left Forceps Minor	**0.22 (<0.01)**	**0.17 (<0.01)**	**0.06 (<0.01)**	**0.16**	**0.04**
Corpus Genu and Body	**0.13 (<0.01)**	**0.13 (<0.01)**	0.00 (0.32)	**0.13**	0.00

FA∼A = Linear FA regression on A allele dosage;
AD∼A = Linear AD regression on A allele dosage;
RD∼A = Linear RD regression on A allele dosage;
A∼AD+RD = Linear A allele dosage regression on AD and RD
A∼AD = Linear A allele dosage regression on AD
(A∼AD+RD) - (A∼AD) = Improvement of the model by including RD in the model in addition to AD to predict A allele dosage
A∼RD+AD = Linear A allele dosage regression on RD and AD
A∼RD = Linear A allele dosage regression on RD
(A∼RD+AD) - (A∼RD) = Improvement of the model by including AD in the model in addition to RD to predict A allele dosage
FA = fractional anisotropy
AD = Axial Diffusivity
RD = Radial Diffusivity.

Bold Face = significant association or improvement of the model