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## Effects of age on white matter integrity and negative symptoms in schizophrenia

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### ABSTRACT

The current study examined the relationship between white matter integrity as indexed by diffusion tensor imaging and negative symptom severity in schizophrenia. The current study included statistical controls for age effects on the relationship of interest, a major weakness of the existing literature on the subject. Participants included 59 chronic schizophrenia patients, and 31 first-episode schizophrenia patients. Diffusion-weighted neuroimaging was used to calculate fractional anisotropy (FA) in each major brain region (frontal, temporal, parietal, and occipital lobes). Negative symptoms were measured using the Scale for the Assessment of Negative Symptoms (SANS) in all schizophrenia patients. Significant bivariate correlations were observed between global SANS scores and global FA, as well as in most brain regions. These relationships appeared to be driven by SANS items measuring facial expressiveness, poor eye contact, affective flattening, inappropriate affect, poverty of speech, poverty of speech content, alogia, and avolition. However, upon addition of age as a covariate, the observed relationships became non-significant. Further analysis revealed very strong age effects on both FA and SANS scores in the current sample. The findings of this study refute previous reports of significant relationships between DTI variables and negative symptoms in schizophrenia, and they suggest an important confounding variable to be considered in future studies in this population.

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### 1. Introduction

The negative symptoms of schizophrenia are important to understand at the neural level; they provide the clearest prognostic indication of future quality of life and disease outcomes (Ho et al., 1998; Kwapil, 1998). As assessed by the Scale for the Assessment of Negative Symptoms (SANS), they include diminished emotional expression (unchanging facial expression, poor eye contact, lack of vocal inflection, and a paucity of expressive gestures), alogia (poverty of speech, poor content of speech, blocking, and increased latency to response), avolition (apathy, poor grooming/hygiene, impersistence at work or school, physical anergia), anhedonia (social isolation, loss of interest in recreational and sexual activities, impaired intimacy with others), and attentional impairment (Andreasen, 1982).

A study by our group (Ho et al., 1998) showed that negative symptoms measured by the SANS are the most important indicators of future quality of life in people with schizophrenia. Negative symptom severity was significantly correlated with later occupational impairment, financial dependence, poor social relationships, anhedonia, and low functioning,

whereas positive and disorganized symptoms were not found to be predictive of any element of future quality of life. A further study by Kwapil (1998) showed that disinterest in social contact and social isolation (commonly-described features of schizophrenia) are reliable predictors of the later development of schizophrenia-spectrum disorders in college-aged adults.

The negative symptoms of schizophrenia share some conceptual similarities with the symptoms of major depressive disorder, especially as seen in older adults, which include poor energy, changes in sleep and appetite, apathy, anhedonia, loss of interest in sex, memory and concentration impairments, and depressed mood (Disabato and Sheline, 2012). Researchers have investigated negative symptoms of schizophrenia as they relate to patients with depression. Berenbaum and Oltmanns (1992) examined facial expressiveness and subjective emotional experience in “blunted” and “non-blunted” patients with schizophrenia (ostensibly similar schizophrenia patients with predominant negative symptoms and those without), as compared with patients with major depressive disorder and healthy controls. They found that blunted schizophrenia patients were the least facially expressive group, less so than the depressed and control participants. The findings from Berenbaum and Oltmanns, 1992 suggested subtle differences between schizophrenia and depressed patients in their facial expressiveness and responses to positive-affect-eliciting stimuli.

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Depression in late life is known to correlate with white matter abnormalities, including decreased white matter volumes, increased prevalence of white matter hyperintensity (areas of presumed ischemic damage which appear hyperintense on fluid-attenuated inversion recovery imaging), and decreased fractional anisotropy (Alexopoulos et al., 1997; Krishnan et al., 1997; de Groot et al., 2000; Taylor et al., 2004; Versluis et al., 2006; Murphy et al., 2007). It has been hypothesized that negative symptoms in schizophrenia are similarly related to white matter health. The current study reviews the previous examinations of white matter in schizophrenia and examines whether there are similar correlates between white matter abnormalities and negative symptoms.

### 1.1. Diffusion-weighted neuroimaging in schizophrenia

Diffusion tensor imaging (DTI) is an important method to further study the organization, health, and implicit connectivity of the white matter. Health and organization of white matter fibers constrain the motion of water molecules; hence measurement of water molecular motion is taken as an indicator of white matter health (Basser et al., 1994). The most commonly reported DTI measure is fractional anisotropy (FA), which refers to the degree of directional constraint in diffusion. Higher FA is reported in healthier, more organized white matter tracts, while the converse is true of lower FA. Diffusion-weighted neuroimaging studies in schizophrenia have proliferated dramatically over the past ten years, the majority of which report low FA in schizophrenia (Kanaan et al., 2005; Buchsbaum et al., 2006; Kubicki et al., 2007; Kyriakopoulos et al., 2008; Ellison-Wright and Bullmore, 2009). Ellison-Wright and Bullmore conducted the largest meta-analysis of voxel-based morphometry studies of DTI in schizophrenia, including 15 individual studies, 407 patients with schizophrenia, and 383 healthy controls. They found significant reductions in fractional anisotropy reported by every study in two main areas; the left frontal lobe and the left temporal lobe. The main tracts affected were those connecting the frontal lobe with the thalamus and cingulate cortex, and those connecting the frontal lobe with the insula, hippocampus, amygdala, temporal, and occipital lobes. These and other findings of abnormal white matter corroborate the disconnection hypothesis of schizophrenia (Andreasen et al., 1996a; Andreasen et al., 1997; Friston, 1997), which posits that schizophrenia is related to aberrant connectivity between brain regions, especially in the frontal lobes. This idea is further expanded by Rubinov in 2013, who examined evidence that schizophrenia results from disruption of central/hub brain regions including the prefrontal, limbic, temporal, and parietal regions. Genetic studies (Terwisscha van Scheltinga et al., 2013) show that a subset of schizophrenia genetic risk variants (single nucleotide polymorphisms or “SNPs”) is related to the normal development of white matter, suggesting that disruption in white matter growth increases susceptibility to developing schizophrenia.

Previous reports from our lab showed that FA is significantly lower in schizophrenia patients than controls in the whole brain and in individual regions of interest (frontal lobes, temporal lobes, parietal lobes, occipital lobes, subcortical white matter of the basal ganglia complex). Further examination revealed that these differences were only significant in patients with chronic schizophrenia rather than first episode patients (White et al., 2011). This finding implicated effects of medication and/or the chronic disease state in the development of white matter abnormalities. However, this initial study was limited by using data from multiple study sites with a mix of 1.5 and 3 T scanners. A further study employed more complex analyses of DTI data including studies of manually-placed regions of interest (ROIs), tract-based spatial statistics, and the pothole approach. This study showed multiple ROIs with lower FA in schizophrenia. All patients showed lower FA in the corpus callosum and the posterior thalamic radiations in addition to “potholes” of lower FA throughout the brain (White et al., 2013). This suggested a global low-level decrease in FA.

### 1.2. Neuroimaging and negative symptoms: Previous studies

Several studies have examined the relationship between negative symptoms of schizophrenia and white matter volumetrics, as well as functional neuroimaging. One study reported the global score on the SANS was related to atrophy of orbitofrontal white matter in schizophrenia patients (Sanfilipo et al., 2000). Furthermore, Sanfilipo and colleagues found that when taken individually, significant relationships only emerged with anhedonia-asociality and alogia global scales on the SANS. Findings from functional neuroimaging have implicated low activity of the striatum in the phenomenology of negative symptoms in schizophrenia. Ehrlich et al. (2009) conducted an fMRI experiment in which they showed that negative symptoms were inversely related to striatal activity during a working memory task. Interestingly, patients with high burden of negative symptoms showed much lower striatal activation than patients with minimal negative symptoms. Another study by Juckel et al., 2006, reported an inverse correlation between striatal response magnitude and negative symptom severity in schizophrenia. Finally, two studies report correlations between striatal response magnitude and individual negative symptoms such as anhedonia (Dowd and Barch, 2010) and apathy (Simon et al., 2010).

Very few studies have examined the relationship between negative symptoms and DTI measures in schizophrenia. Those few have limited sample sizes and relatively low-powered neuroimaging parameters. A study of schizophrenia patients with predominantly negative symptoms showed the left hemisphere to be the most affected, especially in frontal and temporal white matter regions (Sigmundsson et al., 2001). In addition, a preliminary report examined ten male patients with chronic schizophrenia, correlating global SANS scores with manually defined regions of interest in the inferior frontal white matter (Wolkin et al., 2003). That study reported significant relationships between FA in the inferior frontal white matter and negative symptoms, especially with affective blunting and anhedonia. Another study reported widespread FA decreases in schizophrenia, where patients with worse outcomes tended to have more severe and widespread FA abnormalities than those with better outcomes (Mitelman et al., 2007). Negative symptom severity was inversely related to FA in the major white matter tracts, especially those of the left hemisphere. Over these few studies there are some common themes; the importance of the left vs the right hemisphere for negative symptoms and significant relationships between FA and negative symptoms in the frontal and temporal lobes. Unfortunately, these previous studies of DTI and negative symptoms were unable to effectively control for the effects of age, due to small samples. This is a confounding factor which must be addressed, given the known relationship between age and DTI measures in schizophrenia as well as in other populations (Pfefferbaum et al., 2005; Salat et al., 2005; Tuch et al., 2005; Rosenberger et al., 2008; Zarei et al., 2009).

The current study aims to examine the relationship between negative symptoms of schizophrenia and fractional anisotropy in the major white matter structures of the brain, while controlling for age. The literature suggests there is likely an effect of age on FA in schizophrenia, given the numerous previous DTI studies in various participant groups that report significant age effects on FA. In addition, the phenomenology of schizophrenia describes persistence or increase of negative symptoms in older age, despite a decrease in positive symptom severity (Gur et al., 1996; Schultz et al., 1997). This suggests there may be a relationship between negative symptom intensity and age in the current sample. We anticipate that strong aging effects on FA and negative symptom scores may overshadow any detectable relationships between negative symptoms and FA.

## 2. Methods

### 2.1. Participants

Participants included 59 patients with chronic schizophrenia, 31 patients with first-episode schizophrenia recruited to participate in

the “Brain imaging in the major psychoses: examining structural and functional connectivity”, [2 RO1 MH 40856-21A1] conducted at the University of Iowa. Participants completed several days of study activities including MRI scans with DTI sequences, measurement of negative symptoms using the Scale for the Assessment of Negative Symptoms (SANS), and careful characterization of their socio-demographic factors. After full description of study procedures, each participant provided informed consent in accordance with the University of Iowa ethical policies and the Declaration of Helsinki.

## 2.2. Demographics

There were no significant differences between the participant groups in terms of sex, but there were significant differences between the groups in age (first episode < chronic), education (first episode < chronic), global SANS scores (chronic < first episode), and medication usage (first episode < chronic) (Table 1). The differences in age reflect that patients experiencing their first episode of schizophrenia are almost always younger than those who are considered to be in the “chronic phase” of the illness. First-episode patients ranged in age from 17 to 33 years; chronic patients ages ranged from 21 to 55 years. Differences in education between first episode and chronic schizophrenia patients may also reflect the effect of age, though they may also reflect the effect of the chronic illness on educational achievement. The effect of medication usage reflects the fact that chronic schizophrenia patients have been treated for a long time, whereas first episode patients are just being diagnosed and beginning their treatment regimens.

## 2.3. Neuroimaging

Participants underwent anatomical and diffusion-weighted imaging using a Siemens TIM Trio 3 T scanner (Erlangen, Germany). T1-weighted anatomical scanning used 256 slices with 1 mm slice thickness and no gap (TE = 2.8 ms, TR = 2530 ms, TI = 909 ms, NEX = 1, echos = 1, flip angle = 10°, field of view = 25.6 cm<sup>2</sup> matrix = 256 × 256). Diffusion-weighted images were gathered on 70 slices with a 2 mm slice thickness and no gap (TE = 82 ms, TR = 8700 ms, flip angle = 90°, b-value = 1000, gradient directions = 30, field of view = 25.6 cm<sup>2</sup>, matrix = 128 × 128, pixel bandwidth = 1395, imaging frequency = 123.255901).

Automated processing of T1 and T2 anatomical images was performed using BRAINS2 (Brain Research: Analysis of Images, Networks, and Systems) software (Magnotta et al., 2002). This software automates AC–PC alignment, image alignment, image intensity standardization, tissue classification, brain extraction, and labeling based on the Talairach atlas (Talairach and Tournoux, 1988). Diffusion-weighted data were first processed using GTRACT software to perform motion correction, co-registration, tensor decomposition, and calculation steps (Cheng et al., 2006). DTI scalar maps (e.g. fractional anisotropy) were then clipped to include only the white matter based on the tissue

classification step from BRAINS2. DTI measures are reported in white matter only in voxels where FA was greater than 0.1, in order to exclude regions containing susceptibility artifacts and areas with imprecise registration. Measures of FA were then obtained in each Talairach-defined lobe, using an automated tool within the BRAINS2 software. The Talairach-defined subcortical box region reported in the current study comprises only a small amount of white matter contained within the basal ganglia complex (Andreasen et al., 1996b).

## 2.4. Scale for the Assessment of Negative Symptoms (SANS)

The SANS (Andreasen, 1982) is a clinician-rated instrument to assess objective behavioral indices in patients with schizophrenia. Five symptom complexes are defined by the scale: affective flattening, avolition, anhedonia, and attentional impairment. In this study a global SANS score was calculated by summing the global scores for each of the five symptom complexes. A higher SANS score indicates more severe negative symptoms.

## 2.5. Analyses

Age, education, SANS scores and medication use were compared between groups using Student's t-tests. Medication use is reported in dose-years. Sex was compared between groups using chi-squared test.

Relationships between variables of interest (FA and global SANS score) were calculated using non-parametric Spearman's correlations. Next, the relationship between individual items from the SANS and FA were calculated using Spearman's correlations. After the discovery of several significant correlations between FA and SANS variables, these relationships were re-examined using partial correlations to control for the effects of age. Spearman's correlations were also used to examine the relationship between age and individual variables of interest (FA and SANS scores). Then, all these correlations were re-calculated in the first-episode and chronic groups individually. Finally, medication effects were also examined using partial correlations.

## 3. Results

In the current large sample of schizophrenia patients (chronic and first episode), we saw significant relationships between higher SANS global scores (as described in methods) and higher FA in the white matter of the cerebrum (Spearman's rho = 0.300,  $p = .004$ ,  $N = 90$ ). Significant relationships were also observed between higher SANS global scores and higher FA in Talairach-defined frontal, parietal, and occipital lobes (Table 2). This effect appeared to be driven by SANS items measuring facial expressiveness, poor eye contact, overall affective flattening, inappropriate affect, poverty of speech, poverty of speech content, overall avolition, and overall avolition/apathy (Table 2). Notably, all these correlations were stronger with the left cerebral FA than with the right cerebral FA (data not shown).

**Table 1**  
Demographic profiles of chronic and first episode schizophrenia groups.

	Chronic SZ mean (SD)	FE SZ mean (SD)	Statistic	Degrees of freedom	Sig.
Sample	59	31	–	–	–
Age (years)	37.7 (8.7)	23.1 (4.4)	$t = 10.530$	87.9	$p < .001$
Sex	12F:47M	6F:25M	$\chi^2 = .012$	1	$p = .912$
Education (years)	13.9 (2.1)	12.4 (4.3)	$t = 1.676$	88	$p = .097$
Paternal Education (years)	14.1 (3.3)	13.65 (3.9)	$t = .607$	88	$p = .473$
Maternal Education (years)	13.8 (2.6)	13.68 (3.6)	$t = .128$	88	$p = .898$
Parental SES	2.8 (0.7)	2.81 (0.9)	$t = -.349$	88	$p = .728$
Global SANS	8.7 (4.7)	12.6 (4.6)	$t = -3.76$	88	$p < .001$
Typical Neuroleptic Dose Years	23.9 (46.6)	0.3 (1.1)	$t = 3.877$	58	$p < .001$
Atypical Neuroleptic Dose Years	33.4 (27.0)	1.2 (2.8)	$t = 9.084$	60	$p < .001$

Footnote: SZ = Schizophrenia; FE = First Episode; SES = Socioeconomic Status; Sig. = Significance; SANS = Scale for the Assessment of Negative Symptoms. Age, education, SANS, and medication use compared using Student's t-tests, sex compared using chi-squared test. Age and medication usage data violated Levene's test for equality of variances, so equal variances were not assumed.

**Table 2**  
Significant relationships with regional FA and SANS items.

	Spearman's rho	Sample	Sig.	Partial r <sup>a</sup>	Sig.
<i>A. Brain Region</i>					
Cerebrum	0.300	90	$p = .004$	0.123	$p = .251$
Frontal lobe	0.284	90	$p = .007$	0.068	$p = .528$
Temporal lobe	0.169	90	$p = .112$	0.115	$p = .285$
Parietal lobe	0.259	90	$p = .014$	0.049	$p = .645$
Occipital lobe	0.340	90	$p = .001$	0.236	$p = .026$
<i>B. SANS item</i>					
Facial expressiveness	0.238	90	$p = .024$	0.128	$p = .233$
Poor eye contact	0.232	90	$p = .028$	0.143	$p = .183$
Total affective flattening	0.275	90	$p = .009$	0.155	$p = .146$
Inappropriate affect	0.223	90	$p = .035$	0.023	$p = .830$
Poverty of speech	0.276	90	$p = .008$	0.153	$p = .153$
Poverty of speech content	0.289	90	$p = .006$	0.124	$p = .246$
Total alogia	0.382	90	$p < .001$	0.182	$p = .087$
Total avolition/apathy	0.235	90	$p = .026$	0.094	$p = .383$

Note: In A, non-parametric bivariate correlations calculated between global scores on the Scale for the Assessment of Negative Symptoms (SANS) and fractional anisotropy (FA) measured in the brain regions listed. In B, non-parametric bivariate correlations calculated between fractional anisotropy (FA) in the entire cerebrum and individual items from the Scale for the Assessment of Negative Symptoms (SANS). The sample included 59 chronic schizophrenia patients and 31 first-episode schizophrenia patients. Higher SANS scores indicate more severe negative symptoms, and positive correlations indicate a direct relationship where higher FA is related to more severe negative symptoms on SANS.

<sup>a</sup> Partial correlation coefficient including age as a covariate with 87 degrees of freedom.

Because of the known relationship between age and FA in DTI studies, we examined this effect in the current sample of chronic and first episode schizophrenia patients. The significant bivariate relationship between global SANS score and FA became non-significant when controlled for age ( $r = .123$ ,  $p = .251$ ,  $df = 87$ ). This effect is logical given that age was significantly correlated with both cerebral FA ( $\rho = -.458$ ,  $df = 90$ ,  $p < .001$ ) and global SANS scores ( $\rho = -.465$ ,  $df = 90$ ,  $p < .001$ ) in the current sample. The strong effect of age on both variables of interest makes it unclear whether there is any true relationship between the two (cerebral FA and negative symptoms), or if there is merely the semblance of a relationship because both are driven by age (younger schizophrenia patients tend to have both higher cerebral FA and more negative symptoms, but the two may not be directly related).

Further analysis examined the first-episode and chronic patients separately. Non-significant bivariate Spearman correlations between global FA in the cerebral white matter and SANS scores were found in both groups (chronic:  $r = .133$ ,  $p = .315$ ,  $N = 59$ ; first-episode:  $r = .244$ ,  $p = .185$ ,  $N = 31$ ). This was also true of partial correlations controlled for age (chronic:  $r = .141$ ,  $p = .291$ ,  $df = 56$ ; first-episode:  $r = .061$ ,  $p = .750$ ,  $df = 28$ ). Though non-significant, both correlations are in the same direction as the finding in the combined group, where higher FA (healthier white matter) is correlated with higher SANS score (more severe negative symptoms).

We examined the possibility that the observed effects were driven by antipsychotic medication use and found that the effect of age on each variable of interest remains after statistical control for medication use. The relationship of SANS score with age remained significant after controlling for the dose years of typical and atypical antipsychotic medications used (which was also strongly collinear with age) ( $r = -.376$ ,  $p = .000$ ,  $df = 85$ ). The relationship of cerebral FA with age was similarly significant after control for medication use ( $r = -.272$ ,  $p = .011$ ,  $df = 85$ ). The converse was not true; medication use was non-significantly correlated with SANS score and cerebral FA when controlled for age.

#### 4. Discussion

The current results from the largest DTI study of chronic and first episode schizophrenia patients at first seemed to suggest a significant relationship between white matter integrity and negative symptomatology in schizophrenia. However, this relationship was rendered non-significant when the effects of age were taken into account. These findings suggest age is a critical covariate to be examined in all studies of negative symptoms in schizophrenia.

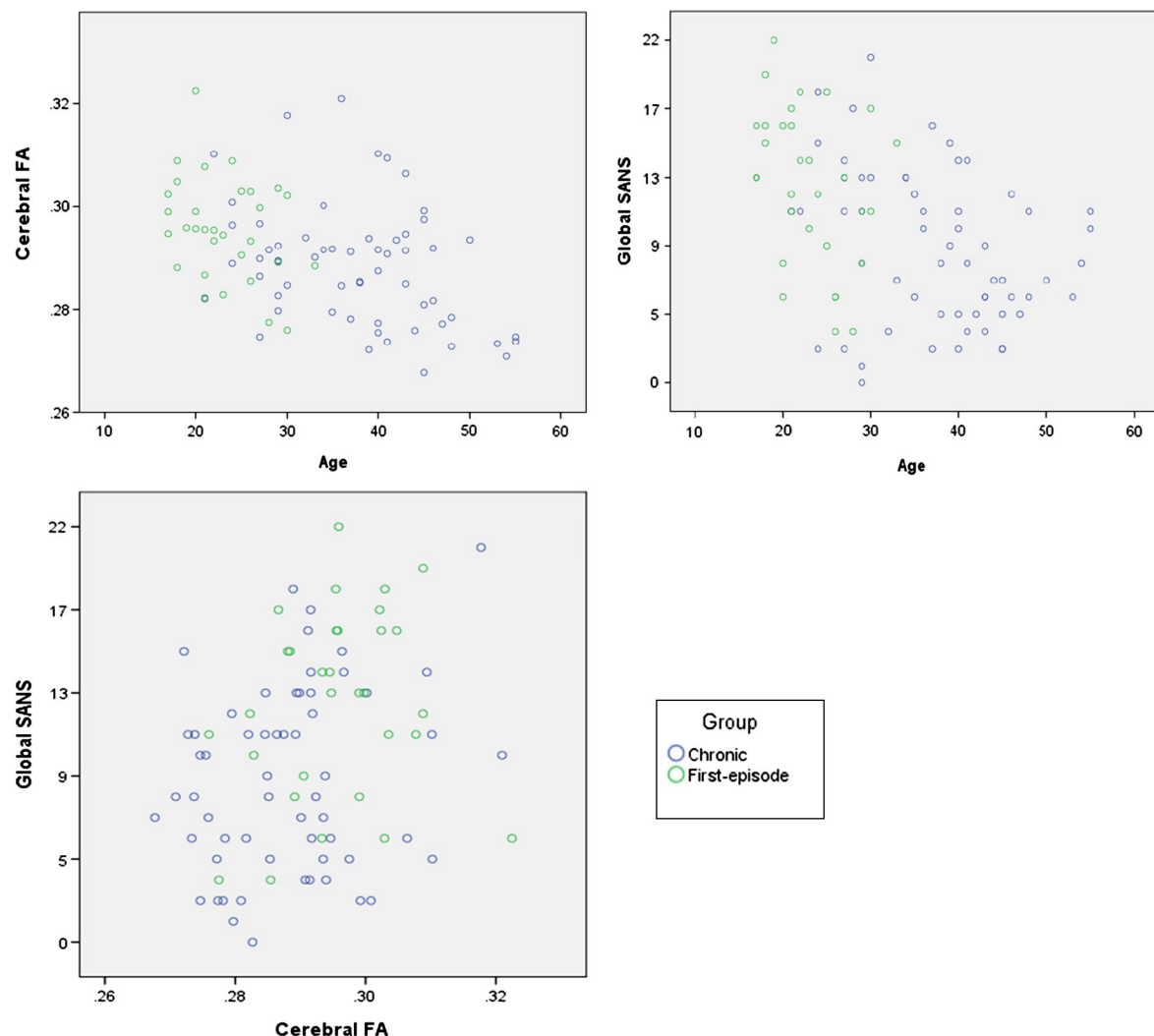
The current study may represent further evidence that though negative symptoms of schizophrenia share heuristic similarities with depression, their neural correlates are very different. Depression is characterized by white matter abnormalities, including decreased white matter volumes and decreased fractional anisotropy (Alexopoulos et al., 1997; Krishnan et al., 1997; de Groot et al., 2000; Taylor et al., 2004; Versluis et al., 2006; Murphy et al., 2007), whereas in the current study, there were no significant relationships between negative symptoms of schizophrenia and fractional anisotropy measures.

Not only were the relationships between negative symptoms and FA non-significant after controlling for age, they were in the opposite direction from those previously reported in schizophrenia patients (Sigmundsson et al., 2001; Wolkin et al., 2003). The current study found that patients with more severe negative symptoms also tended to have higher FA values (often interpreted as healthier or more organized white matter). As became clear by examining the scatter plots of the current data (Fig. 1), this tendency was driven by age effects; younger schizophrenia patients tended to have higher FA as well as higher SANS scores.

We further examined the relationship of negative symptoms with FA and age in the first-episode and chronic schizophrenia groups separately, observing non-significant correlations in the same direction as the finding in the combined group, where higher FA (healthier white matter) is associated with higher SANS score (more severe negative symptoms). We suspect the lack of power in these smaller groups may be part of the reason for non-significance, but also the restriction of range in the individual samples (Fig. 1).

It is important to note that the effects of age on white matter are not necessarily linear (Raz et al., 2005), especially in schizophrenia (Andreasen et al., 2011). Our group has previously shown that white matter volume decrease advances in a nonlinear fashion and more rapidly in schizophrenia patients than controls. Our group has further shown that brain volume maturation trajectories in schizophrenia patients vary as a function of antipsychotic medication use, where those who have used more medications have decreased white matter volume over time, while those who use the least medications had white matter volume increases over time (Ho et al., 2011). Antipsychotic use in schizophrenia is ubiquitous and highly inter-related with aging as well as duration of illness. To that end, the current study examined the possibility that medication use might be a stronger mediator of the relationship between negative symptoms and FA than age in the current sample. Interestingly, we found that the effect of age on each variable of interest remains after statistical control for medication use. The converse was not true; medication use was non-significantly correlated with SANS





**Fig. 1.** FA = Fractional Anisotropy; SANS = Scale for the Assessment of Negative Symptoms. Higher SANS scores indicate more severe negative symptoms of schizophrenia. Figure 1 demonstrates the separation of schizophrenia patients by age; first-episode patients are significantly younger than chronic patients. First-episode patients also tend to have higher cerebral FA and higher SANS scores.

score and cerebral FA when controlled for age, suggesting that age is the strongest mediator of the reported relationships.

#### 4.1. Limitations and future directions

A criticism to the interpretation of DTI data is cogently articulated by Assaf and Pasternak (2008), who noted that abnormalities in FA are known to occur in a variety of conditions: normal aging, cognitive dysfunction, multiple sclerosis, schizophrenia, HIV, cerebral ischemia, head trauma, development, dementia, and vascular disease (Grieve et al., 2007; Alexopoulos et al., 2008). Studies of DTI data have further shown that alterations of anisotropy can occur under a variety of circumstances including but not limited to edema and inflammation (Sotak, 2002), demyelination (Huppi et al., 1998), and gliosis, which suggests that FA is not a specific measure, but rather that FA reflects dysfunction at the cellular level without exact identification of the cause (Assaf and Pasternak, 2008). There have been many questions raised about the validity of interpreting DTI data in terms of anisotropy within voxels, where the questions mainly focused on the cellular structure of the brain and implications for water diffusivity. Under those circumstances it is difficult to describe the exact contribution of any particular cellular aspect (such as myelin integrity) to FA because so many other factors can contribute to the signal (Assaf and Pasternak, 2008).

Diffusion tensor imaging also makes several statistical assumptions which may not necessarily be accurate. For example, the method assumes that diffusion follows a Gaussian distribution, but this may not be true in compartmentalized and diffusion-restricted regions (like white matter). In addition, DTI analysis assumes it is appropriate to characterize an entire voxel with a single diffusion tensor, which may not be accurate because within each voxel are tens of thousands of axons and glial cells which are unlikely to form a single, unified structure (Assaf and Pasternak, 2008). This can be especially problematic at tissue interfaces where a voxel may contain multiple types of tissue, such as gray and white or white and CSF, or at branching points where two or more fiber tracts may converge or cross within a single voxel (Jones et al., 2013). Future studies of diffusion-weighted data in schizophrenia should aim to overcome these shortcomings, either by using stronger magnetic fields, more diffusion-sensitizing gradients, and/or using multi-tensor models to examine the data. In addition, the use of cerebral white matter FA and average FA in Talairach-bounded lobes is a rather gross-grained analysis which should be followed up by more sensitive analyses on the order of individual white matter tracts or fibers. Furthermore, given the nonlinear relationship of age with white matter, especially in schizophrenia, future studies should aim to use more elegant statistical controls for age than were presented in the current manuscript.

Diffusion tensor imaging is a powerful technique, and it has the potential to give unprecedented insights into the structure and function of cerebral white matter. As studies proliferate in this area, researchers must use caution in designing and executing experiments that address the major concerns with the methodology, especially in its use to study negative symptoms of schizophrenia. With careful study design and thoughtful data analysis, the many strengths of DTI analysis may be harnessed and the potential for misinterpretation may be avoided.

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#### Contributors

Kelly Rowe Bijanki drafted the manuscript, performed all statistical analyses, performed 85% of the literature search, and guided the design of the neuroimaging analyses. Brendan Hodis implemented the neuroimaging analyses, performed 10% of the literature search, and significantly revised the manuscript. Vincent Magnotta designed the DTI data processing pipeline and supported the revisions of the manuscript. Eugene Zeien provided extensive support throughout the neuroimaging process, gave statistical feedback and revised the manuscript. Nancy C. Andreasen designed the parent study and the original neuroimaging protocols, revised the manuscript, and performed 5% of the literature search. All authors contributed to and have approved the final manuscript.

#### Conflicts of interest

All authors reported they had no conflicts of interest.

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